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ORIGINAL ARTICLE

Degenerative changes of the aortic valve during left ventricular assist device support

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Abstract

Aims Donor heart shortage leads to increasing use of left ventricular assist device (LVAD) as bridge-to-transplant or destination therapy. Prolonged LVAD support is associated with aortic valve insufficiency, representing a relevant clinical problem in LVAD patients. Nevertheless, the impact of LVAD support on inflammation, remodelling, and chondro-osteogenic differentiation of the aortic valve is still not clearly understood. The aim of the study is to evaluate the impact of LVAD support on structural and molecular alterations of the aortic valve.

Methods and results During heart transplantation, aortic valves of 63 heart failure patients without (n = 22) and with LVAD support (n = 41) were collected and used for analysis. Data on clinical course as well as echocardiographic data were analysed. Calcification and markers of remodelling, chondro-osteogenic differentiation, and inflammation were evaluated by computed tomography, by mRNA analysis and by histology and immunohistochemistry. Expression of inflammation markers of the LVAD group was analysed with regard to levels of C-reactive protein and driveline infections. Calcium accumulation and mRNA expression of determined markers were correlated with duration of LVAD support. Data were also analysed relating to aortic valve opening and aortic valve insufficiency. There was no difference in the frequency of cardiovascular risk factors or comorbidities between the patient groups. Expression of matrix metalloproteinase-9 (P = 0.007), alpha-smooth muscle actin (P = 0.045), and osteopontin (P = 0.003) were up-regulated in aortic valves of LVAD patients. Histological appearance of the aortic valve was similar in patients with or without LVAD, and computed tomography-based analysis not yet revealed significant difference in tissue calcification. Expression of interferon gamma (P = 0.004), interleukin-1 beta ($P < 0.0001$), and tumour necrosis factor alpha (P = 0.04) was up-regulated in aortic valves of LVAD patients without concomitant inflammatory cell infiltration and independent from unspecific inflammation. Expression of matrix metalloproteinase-2 (P = 0.038) and transforming growth factor beta (P = 0.0504) correlated negatively with duration of LVAD support. Presence of aortic valve insufficiency led to a significantly higher expression of interferon gamma (P = 0.007) in LVAD patients. There was no alteration in the determined markers in relation to aortic valve opening in LVAD patients.

Conclusions Left ventricular assist device support leads to signs of early aortic valve degeneration independent of support duration. Thus, the aortic valve of patients with LVAD support should be closely monitored, particularly in patients receiving destination therapy as well as in the prospect of using aortic valves of LVAD patients as homografts in case of bridge-to-transplant therapy.

Keywords Aortic valve; Left ventricular assist device; Remodelling; Inflammation; Degeneration

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Introduction

Mechanical circulatory support by left ventricular assist device (LVAD) implantation has been established as a valuable option in heart failure (HF) therapy in times of donor heart shortage and concomitant improvements of established device systems.[1] Today, the annual number of patients receiving LVAD has surpassed the corresponding numbers for cardiac transplantation in most countries.[2,3] The development of aortic valve regurgitation due to structural alterations of the aortic valve is a frequent phenomenon complicating long-term LVAD therapy.[4,5] Reoperation on the aortic valve after previous LVAD implantation is expected to have a relevant negative impact on the outcome of affected patients. Furthermore, aortic valves of LVAD patients eventually receiving heart transplantation (HTx) are regularly harvested for homografts.[6] In the latter circumstance, the question arises whether LVAD therapy may trigger structural remodelling or degeneration of the aortic valve and whether this process is depending on a certain duration of LVAD support. However, knowledge about alterations of aortic valve leaflets during LVAD support, including structural and molecular changes is still limited. Only few studies with mostly small sample sizes have described morphological changes in terms of valve thickening and collagen accumulation in aortic valve tissue of LVAD recipients.[7,8] Furthermore, statements concerning infiltration of inflammatory cells have been inconsistent,[9,10] whereas an increased activation of valvular interstitial cells by increasing amounts of alpha-smooth muscle actin (α-SMA) has been described consistently.[9,11] A very recent study has analysed aortic valves by mass spectrometry revealing up-regulation of proteins associated with transforming growth factor β (TGFβ), the actin/myosin, and the immune system in LVAD patients.[11] Thus, the aim of this work is to investigate the impact of LVAD therapy on inflammation, remodelling, and chondro-osteogenic differentiation of aortic valves with emphasis on LVAD duration using a more robust sample number. A detailed research on LVAD-induced alterations of the aortic valve may contribute to the development of an optimized management of patients treated with LVAD as bridge-to-transplant or destination therapy.

Methods

Human aortic valve tissue

Aortic valve cusps of the diseased heart of patients undergoing HTx were freshly collected in with patients' informed written consent obtained at time of listing for HTx. The investigation conforms to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Institutional Ethics Board of the Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany (reference number: 4567). Immediately after excision, aortic valve cusps were photographed, and individual cusps were snap-frozen and stored in liquid nitrogen as described before.[12]

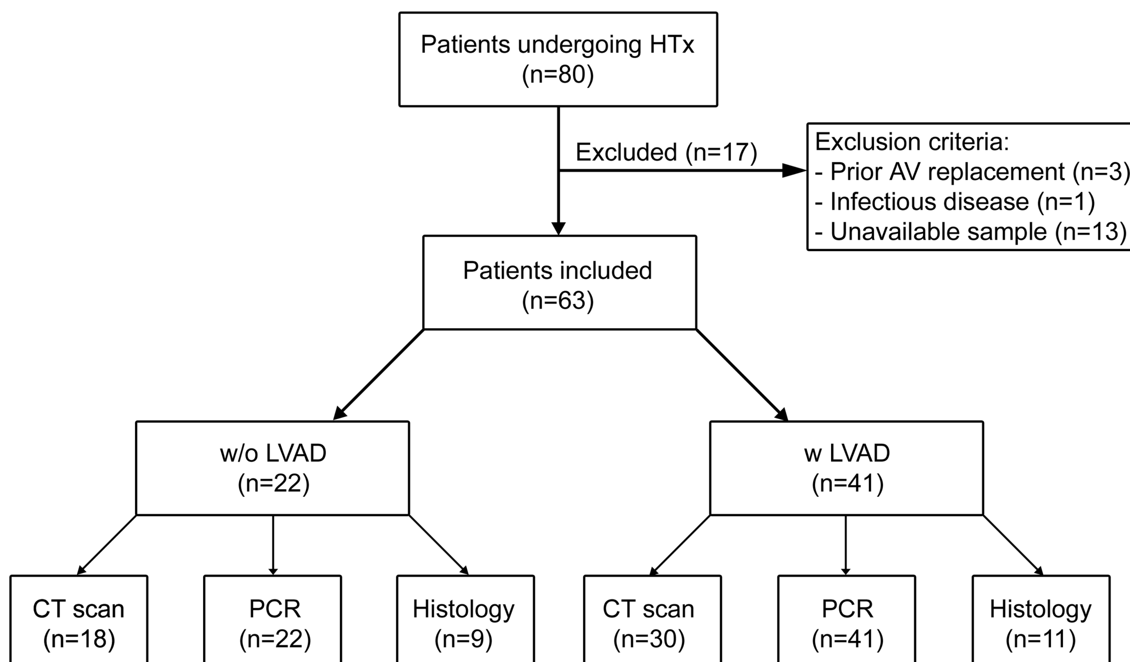
Patients

A total of 80 HF patients receiving HTx at a single centre between December 2011 and April 2018 were initially included. During this period, a standardized collection of tissue samples from patients undergoing HTx was applied with a dedicated team of trained postdocs, technicians, and medical students harvesting the tissue intraoperatively. Patients with previous aortic valve replacement or infectious disease (human immunodeficiency virus, a history of hepatitis B/C or endocarditis) and patients of whom the aortic valves could not be collected due to various reasons or of whom the aortic valves have been used for other studies were excluded from the further study (n = 17). Finally, samples of 63 patients were used for analyses involved in this study (refer to *Figure 1*). Demographic data, primary indication for HTx, cardiovascular risk factors, comorbidities, medication and laboratory values, and duration of LVAD support were collected. According to institutional standard of care, follow-up echocardiography was performed at 3 months post-LVAD implantation or at an earlier time point if clinically indicated. Echocardiographic data on valve function were collected with respect to the presence of aortic valve regurgitation as well as opening movement.

Computed tomography scan for calcium detection

Calcium deposition of available aortic valve cusps (n = 18 without and n = 30 with LVAD) was evaluated by computed tomography performed on a dual-source scanner (Somatom Definition Flash, Siemens Healthineers). In order to preserve tissue quality for later gene expression analysis, computed tomography scans were performed on frozen tissue kept on dry ice during the scan process. In prior test runs, it was confirmed that results of computed tomography scans were comparable for the same tissue sample either in a frozen state or after thawing process (data not shown). Equivalent mass of calcium hydroxyapatite, based on Agatston score measurements was determined using an established software programme (*syngo.via* software version VB30A, Siemens Healthineers).

Figure 1 Flow chart of patient enrolment and experimental setting. Patient enrolment depicting initially screened patients, exclusion criteria, and included patients with subsequent experimental setting. AV, aortic valve; CT, computed tomography scan; HTx, heart transplantation; LVAD, left ventricular assist device; PCR, polymerase chain reaction; w, with; w/o, without.



RNA isolation and semi-quantitative real-time PCR analysis

Analysis of gene expression was performed on aortic valve tissue of all patients enrolled in this study (n = 63). Therefore, total RNA from the cusps was isolated using TRIzol reagent and QIAGEN RNeasy Mini Kit according to the manufacturer's instructions. Complementary DNA was synthesized by using QIAGEN QuantiTect Reverse Transcription Kit. Semi-quantitative real-time PCR and subsequent analysis of gene expression was performed as described before.[12,13] The mRNA of the following targets was analysed using specific primers: interferon gamma (IFNγ), interleukin-1 beta (IL1β), tumour necrosis factor alpha (TNFα), matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), osteopontin (OPN), TGFβ1, alkaline phosphatase (ALP), osteocalcin (OCN), and ribosomal protein L13a as housekeeper (primer sequences are listed in Supporting Information, *Table* S1).

Histology and immunohistochemistry

In a representative subset of aortic valve cusp specimen, histological analyses were performed. Aortic valves of patients without (n = 9) or with LVAD (n = 11) were snap-frozen in cryo compound, and 5 μm sections were prepared. Sections were stained according to standard protocols for haematoxylin/eosin, Movat's pentachrome and von Kossa staining as previously described.[14] Immunohistochemical staining for detection of α-SMA (Sigma) and vimentin as well as staining for CD3, CD86, elastin, collagen type 1 (Abcam), and biglycan (Santa Cruz) were performed as previously described.[15] Analyses and documentation were performed in a blinded fashion using pseudonyms for sample labelling with a Leica DM2000 and Leica LAS software Version 3.8.0.

Statistical analyses

GraphPad Prism Version 7.0 (GraphPad Software, San Diego, CA, USA) was used for graphics and statistical analyses. Because experimental data were non-parametric, median with interquartile range is depicted in graphical presentation, and statistical analysis was performed using unpaired two-tailed Mann–Whitney U-test. Categorical variables were analysed using Fisher's exact test. Correlations were performed using a two-tailed Spearman test, reporting the Spearman correlation coefficient r and the according P value. In general, P values < 0.05 were considered as statistically significant. Data are presented as mean ± standard error of mean.

Results

Aortic valves and data of HF patients were analysed after HTx to evaluate the impact of LVAD support on structural and molecular alterations.

Patient characteristics

Initially, 80 HF patients who underwent HTx were screened. Because of exclusion criteria like prior aortic valve replacement, infectious disease, and unavailable aortic valve samples, 22 patients without LVAD and 41 with LVAD support at the time of transplantation were included in the analyses. Assignment of aortic valves to computed tomography, histology and semi-quantitative real-time PCR analysis is depicted in *Figure 1*. Patient enrolment and patient characteristics are shown in *Figure 1* and *Tables 1* and *2*. Descriptive statistics of patient data show that patients did not differ in their sex, age, and body mass index (*Table 1*). LVAD patients were significantly more often diagnosed with ICM as primary indication for HTx compared with patients without LVAD ($P = 0.0068$). Nevertheless, other indications, for example, arrhythmogenic right ventricular cardiomyopathy, were diagnosed more often in the group without LVAD ($P = 0.0464$), whereas for DCM, there was no difference. There was no

Table 1 Patient characteristics

	All patients (n = 63)	W/o LVAD (n = 22)	LVAD (n = 41)	P value
Sex (male), n (%)	48 (76)	18 (82)	30 (73)	0.5442
Age (years)	53 ± 1.4	54 ± 2.2	53 ± 1.9	0.9060
Body mass index (kg/m^2)	26 ± 0.6	25 ± 0.7	27 ± 0.9	0.1191
Primary indication, n (%)				
Primary DCM	31 (49)	14 (64)	17 (41)	0.1172
ICM	27 (43)	4 (18)	23 (56)	**0.0068**
Other	5 (8)	4 (18)	1 (2)	**0.0464**
LVEF (%)	23 ± 1.1	26 ± 2.1	22 ± 1.2	0.1327
NYHA classification, n (%)				
I	0 (0)	0 (0)	0 (0)	n/a
II	5 (8)	3 (14)	2 (5)	0.3327
III	19 (30)	4 (18)	15 (37)	0.1588
IV	31 (49)	15 (68)	16 (39)	**0.0360**
Cardiovascular risk factors, n (%)				
History of smoking	29 (46)	8 (36)	21 (51)	0.2987
Arterial hypertension	36 (57)	10 (45)	26 (63)	0.1922
Diabetes mellitus	18 (29)	5 (23)	13 (32)	0.5642
Thereof IDD	4 (6)	2 (9)	2 (5)	0.6063
Dyslipoproteinaemia	34 (54)	8 (36)	26 (63)	0.0629
Comorbidities, n (%)				
Chronic kidney disease	40 (63)	13 (59)	27 (66)	0.5972
Coronary artery disease	34 (54)	9 (41)	25 (61)	0.1853
Extracardiac vascular disease	10 (16)	2 (9)	8 (20)	0.4718
Inflammatory disease	8 (13)	1 (5)	7 (17)	0.2425
Medication, n (%)				
Statins	27 (43)	8 (36)	19 (46)	0.5943
Antiplatelet medication	40 (63)	7 (32)	33 (80)	**0.0003**
ACE inhibitor	37 (59)	15 (68)	22 (54)	0.2961
PD5 inhibitor	24 (38)	2 (9)	22 (54)	**0.0004**
Oral anticoagulation[a]	39 (62)	10 (45)	29 (71)	0.0610
β-adrenergic blocker	56 (89)	17 (77)	39 (95)	**0.0449**
Antiarrhythmic drugs	23 (37)	11 (50)	12 (29)	0.1691
Calcium channel blocker	11 (17)	2 (9)	9 (22)	0.3017
Diuretics	50 (79)	19 (86)	31 (76)	0.5148
Oral antidiabetic drugs	8 (13)	2 (9)	6 (15)	0.7020
Allopurinol	11 (17)	6 (27)	5 (12)	0.1703
Catecholamines	3 (5)	3 (14)	0 (0)	**0.0388**
Laboratory values				
Serum creatinine (mg/dL)	1.24 ± 0.052	1.35 ± 0.080	1.19 ± 0.066	0.0895
Platelets (×1000/μL)	237 ± 12.2	235 ± 26.2	237 ± 12.6	0.3761
Leucocytes (×1000/μL)	8.9 ± 0.39	8.6 ± 0.51	9.0 ± 0.54	0.9914
C-reactive protein (mg/dL)	2.8 ± 0.73	3.1 ± 1.77	2.6 ± 0.66	**0.0331**

DCM, dilated cardiomyopathy; ICM, ischaemic cardiomyopathy; IDD, insulin-dependent diabetes mellitus; LVEF, left ventricular ejection fraction; n/a, not applicable; NYHA, New York Heart Association; w/o, without.
Reported data are represented as total number and proportion of whole (%) or as mean ± standard error of mean. Depicted P values were obtained by using Fisher's exact test for categorical and unpaired two-tailed Mann–Whitney U-test for continuous variables; P values < 0.05 were considered as statistically significant. Reported data are represented as mean ± standard error of mean.
[a]Oral long-term anticoagulation includes vitamin K antagonists, dabigatran, or rivaroxaban.

Table 2 Left ventricular assist device data

	LVAD (n = 41)
LVAD type, n (%)	
HeartWare	29 (71)
Heartmate II	8 (20)
Heartmate III	3 (7)
ReliantHeart, HeartAssist5	1 (2)
LVAD duration (days)	485 ± 59
Driveline infection, n (%)	8 (20)
Aortic valve opens, n (%)[a]	26 (81)
Aortic valve insufficiency, n (%)[b]	12 (32)
Additional device support, n (%)[c]	12 (29)

LVAD, Left ventricular assist device.
Reported data are represented as total number and proportion of whole (%) or as mean ± standard error of mean.
[a]Information is based on the last echocardiography prior to HTx; information could not be retrieved for nine patients (22%), thus percentage refers to 32 patients.
[b]Information is based on the last echocardiography prior to HTx; information could not be retrieved for four patients (10%), thus percentage refers to 37 patients. Information could not be retrieved due to HTx within 3 months after LVAD implantation, LVAD implantation, and follow-up care in other centres or lacking documentation.
[c]Extracorporeal membrane oxygenation or right ventricular assist device.

difference in LVEF between the two groups. New York Heart Association stages did not differ between the two groups, except for New York Heart Association stage IV that was significantly more often present in patients of the LVAD group (P = 0.0360). Moreover, the patient groups showed no difference concerning major cardiovascular risk factors and comorbidities. Patients with LVAD significantly more often received antiplatelet drugs (P = 0.0003), PD5 inhibitors (P = 0.0004), and β-adrenergic blockers (P = 0.0449). Patients without LVAD significantly more frequently needed catecholamines in the immediate period prior to transplantation (P = 0.0388). Laboratory values did not vary except for C-reactive protein that was significantly higher in the group without LVAD, however with a rather small numeric difference of 0.5 mg/dL (P = 0.0331).

Concerning the type of LVAD, 71% of LVAD patients had a HeartWare system (Medtronic), 27% had a HeartMate II or III system (Abbott), and one patient had a HeartAssist5 (ReliantHeart; *Table 2*). LVAD duration ranged from 14 to 1452 days (485 ± 59 days) with 20% of the patients suffering from driveline infection. Analysis of the available echocardiography data revealed that in 81% of the LVAD patients, the aortic valve showed regular opening movements. Here, information about aortic valve opening could not be retrieved for nine patients (refer to *Table 2*). In 32% of the LVAD patients, an aortic valve regurgitation was present. Here, information about aortic regurgitation could not be retrieved for four patients (refer to *Table 2*). However, unavailable information concerning aortic valve opening and aortic valve regurgitation was due to lacking documentation, HTx within 3 months after LVAD implantation, or LVAD implantation and follow-up care in other centres. Finally, additional device support like right ventricular assist device or extracorporeal membrane oxygenation was implanted in 29% of the LVAD patients.

Up-regulation of inflammatory markers in aortic valves of left ventricular assist device patients

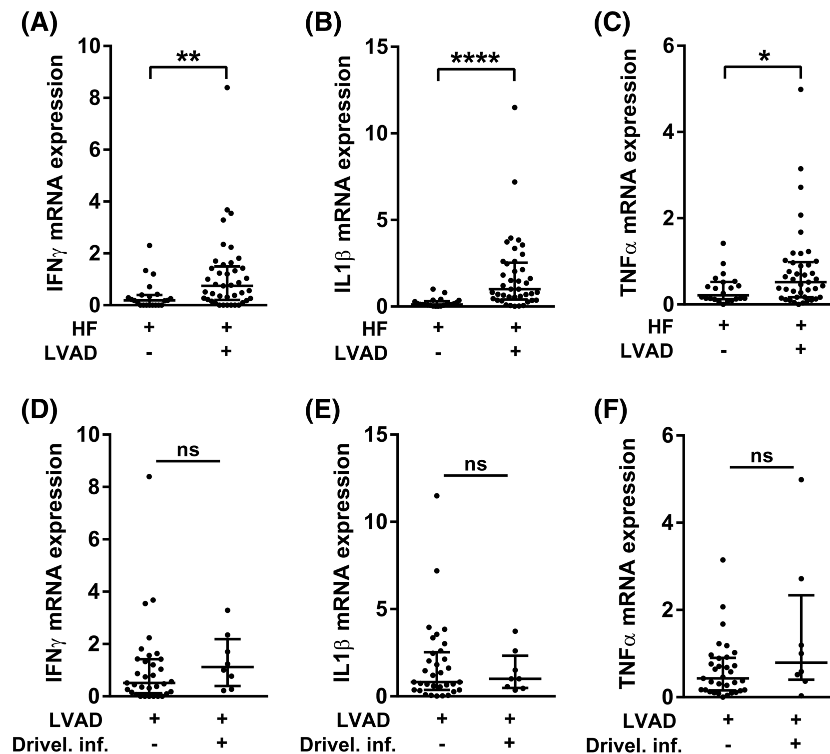
Analysis of mRNA expression showed a significant up-regulation of the inflammatory markers IFNγ (P = 0.0038), IL1β ($P < 0.0001$), and TNFα (P = 0.0395) in aortic valves of patients with LVAD (*Figure 2*A–C). Nevertheless, driveline infections, which occurred in 20% of the patients during LVAD support, did not account for higher mRNA expression of those markers (*Figure 2*D–F; IFNγ: P = 0.195; IL1β: P = 0.961; TNFα: P = 0.176). C-reactive protein levels did also not correlate with mRNA expression of these inflammatory markers (refer to *Figure* S1). Moreover, immunostaining for CD3 and CD68 showed no infiltration of the valve cusps by leukocytes or macrophages (not shown).

Left ventricular assist device induces MMP9 mRNA expression and valvular interstitial cell activation in aortic valves

In order to evaluate whether LVAD-induced changes affect the gross morphology of valvular tissue, we analysed a subset of aortic valves by histology and immunohistochemistry (n = 9 without and n = 11 with LVAD). In this subgroup, LVAD duration was 512 ± 123 days. Haematoxylin/eosin and Movat's pentachrome staining revealed a heterogeneous morphology of the valves (*Figure 3*). Scoring of relevant factors, such as leaflet thickness, tissue compactness, or distribution of extracellular matrix components, thus showed no differences between the two groups. Immunostaining for elastin, collagen type 1, and biglycan also revealed a heterogeneous morphology with no obvious differences between the groups (not shown).

Analysis of mRNA expression of MMP2 and MMP9 revealed that MMP2 expression did not vary (P = 0.931), whereas MMP9 mRNA expression was significantly higher in aortic valves of LVAD patients (P = 0.0071; *Figure 4*A and B). Moreover, α-SMA-staining of tissue sections was performed to evaluate whether the present valvular interstitial cells (VIC) are activated. Here, aortic valves of LVAD patients significantly more often showed VIC activation by α-SMA-positive staining (P = 0.0445, *Figure 4*C and D).

Figure 2 LVAD induces expression of inflammatory markers in aortic valve tissue. Aortic valves of LVAD patients showed a significantly higher IFNγ (A), IL1β (B), and TNFα (C) mRNA expression compared with aortic valves of HF patients without LVAD support. mRNA expression of IFNγ (D), IL1β (E), and TNFα (F) in aortic valves of LVAD patients showed no difference between patients with and without driveline infection. Each data point reflects an individual biological replicate. Drivel. inf., driveline infection; HF, heart failure; LVAD, left ventricular assist device; ns, not significant.**P* value < 0.05; ***P* value < 0.01; *****P* value < 0.0001.



Left ventricular assist device induces early chondro-osteogenic differentiation without full progression to calcification of aortic valves

We further analysed the mRNA expression of OPN, TGFβ1, ALP, and OCN to investigate the impact of LVAD on chondro-osteogenic differentiation in aortic valves. Here, only OPN mRNA expression was significantly up-regulated in the aortic valves of LVAD patients (P = 0.0026), whereas TGFβ1 (P = 0.122), ALP (P = 0.983), and OCN (P = 0.161) showed no regulation (*Figure 5*A–D).

Because von Kossa staining revealed a quite heterogeneous occurrence of calcification ranging from no staining to severe calcification (*Figure 5*E), an independent analysis was employed to quantify the level of calcification. Using the examiner-independent software-based measurement of equivalent calcium mass in three-dimensional image data of computed tomography scans, we observed no relevant difference in the calcium content of aortic valves of HF patients with or without LVAD support (P = 0.429; *Figure 5*F).

MMP2 and TGFβ mRNA expression negatively correlate with the duration of left ventricular assist device support

To evaluate whether duration of LVAD support influences markers of inflammation, remodelling, and differentiation, mRNA expression of IFNγ, IL1β, TNFα, MMP2, MMP9, OPN, TGFβ1, ALP, and OCN as well as calcium mass was correlated with the duration of LVAD support for each patient whose aortic valve cusp underwent computed tomography scan (n = 30). Interestingly, there was a significant negative correlation of MMP2 mRNA expression and LVAD support duration (P = 0.038; r = 0.4424; *Figure 6*A) as well as a strong trend to a negative correlation between TGFβ mRNA expression and LVAD support duration (P = 0.0504; r = −0.3077; *Figure 6*B). There was no correlation between TNFα (P = 0.061), IFNγ (P = 0.107), IL1β (P = 0.201), MMP9 (P = 0.668), ALP (P = 0.077), OPN (P = 0.669), and OCN (P = 0.139) as well as between calcium mass and LVAD support duration, respectively (P = 0.258; not shown).

Figure 3 LVAD does not influence gross morphology of aortic valves. Representative images of a subset of aortic valves of three different patients with or without LVAD show haematoxylin/eosin (H&E) and Movat's pentachrome staining. Morphology in representative valves is heterogeneous, and compactness of tissue as well as distribution of extracellular matrix components shows no differences between aortic valves of patients without or with LVAD. LVAD, left ventricular assist device; w/o, without; bars = 400 μm.

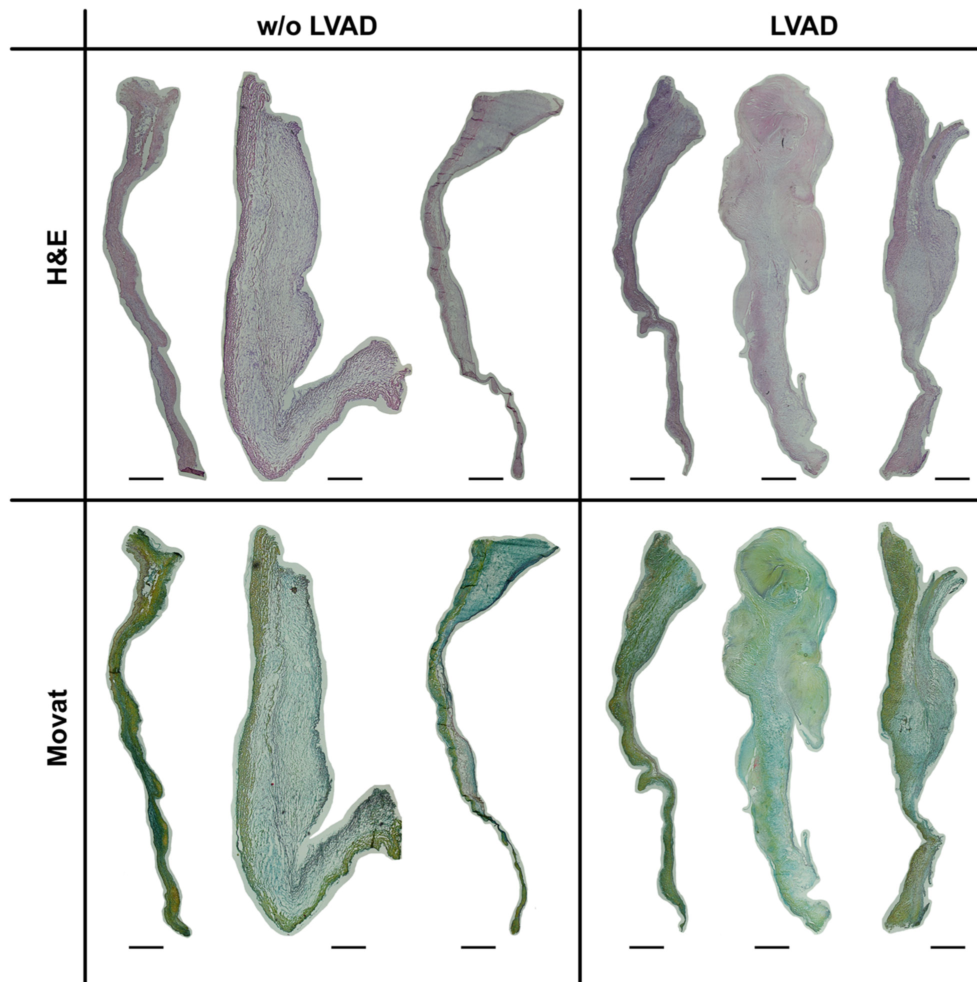


Figure 4 LVAD induces remodelling of aortic valve cusp tissue. Aortic valves of LVAD patients show no difference in MMP2 mRNA expression (A), but a significantly higher MMP9 mRNA expression compared with those of HF patients without LVAD. Representative images of immunohistology staining show vimentin-positive valvular interstitial cells with higher amounts of α-SMA-positive cells in aortic valves of patients with LVAD compared with those of HF patients without LVAD (C and D). Each data point reflects an individual biological replicate. HF, heart failure; LVAD, left ventricular assist device; ns: not significant; w/o, without. ***P* value < 0.01; bars = 50 μm.

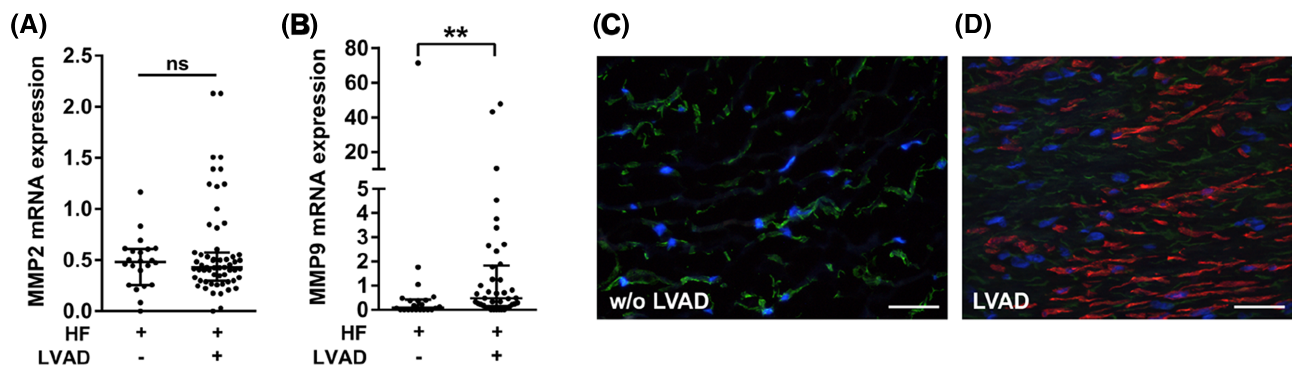


Figure 5 LVAD leads to early chondro-osteogenic differentiation. LVAD support led to a significantly higher mRNA expression of OPN (A) in aortic valves, whereas TGFβ1 (B), ALP (C), and OCN (D) mRNA expression remains unaltered. Quantification of von Kossa staining revealed a heterogeneous distribution and severity of calcification (E). LVAD support did not alter calcium mass in aortic valves (F) as determined by standardized evaluation of computer tomography scans. Each data point reflects an individual biological replicate. HF, heart failure; LVAD, left ventricular assist device; ns, not significant; w/o, without. $^{**}P$ value < 0.01.

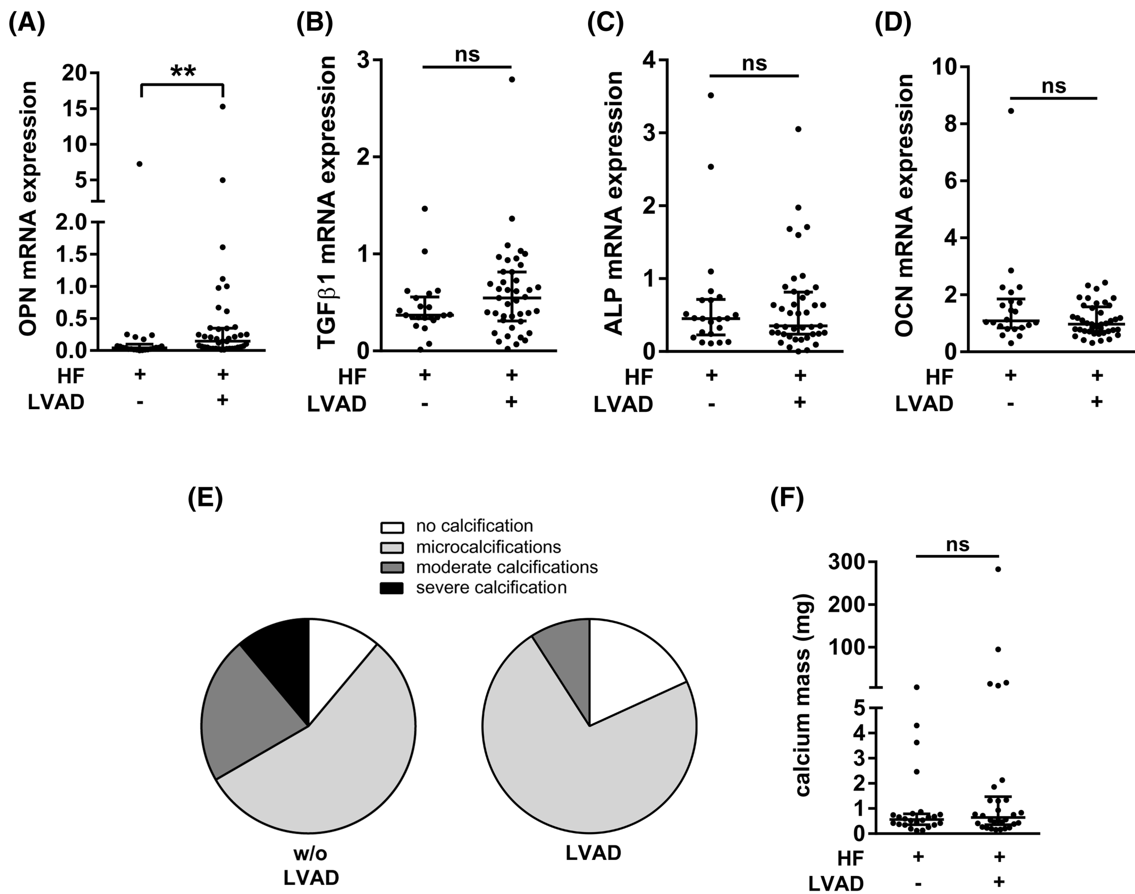
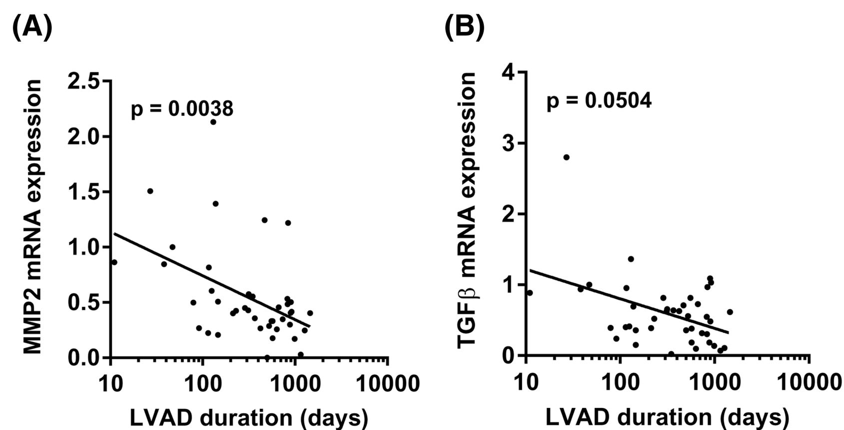


Figure 6 Negative correlation of MMP2 and TGFβ mRNA expression with the duration of LVAD support. Spearman correlations were run to determine the relationship between investigated markers and the duration of LVAD support. MMP2 (A) and TGFβ (B) mRNA expression showed a negative correlation with LVAD support duration. Each data point reflects an individual biological replicate. LVAD, left ventricular assist device.



Influence of aortic valve opening and aortic valve insufficiency on molecular alterations

We evaluated mRNA expression patterns and calcium accumulation levels discriminating between aortic valve tissue samples of patients with documented and regular opening as opposed to a mostly closed aortic valve under LVAD support. Here, determined markers showed no difference between these subgroups (*Figure* **S2**). Using the aforementioned data set to discriminate between aortic valve samples of LVAD patients with or without documented aortic valve insufficiency, respectively, a significantly higher mRNA expression of IFNγ (P = 0.0074) was observed in patients with aortic valve insufficiency (*Figure* **S3**).

Discussion

Left ventricular assist device-induced up-regulation of inflammatory markers in the aortic valve

Studies about inflammatory processes in aortic valves due to haemodynamic changes associated with LVAD support are scarce and to some extent inconsistent. The present work shows an up-regulation of IFNγ, IL1β, and TNFα mRNA expression in aortic valves of patients with LVAD support. To our knowledge, this is the first study reporting this effect of LVAD support on valvular cusp tissue.

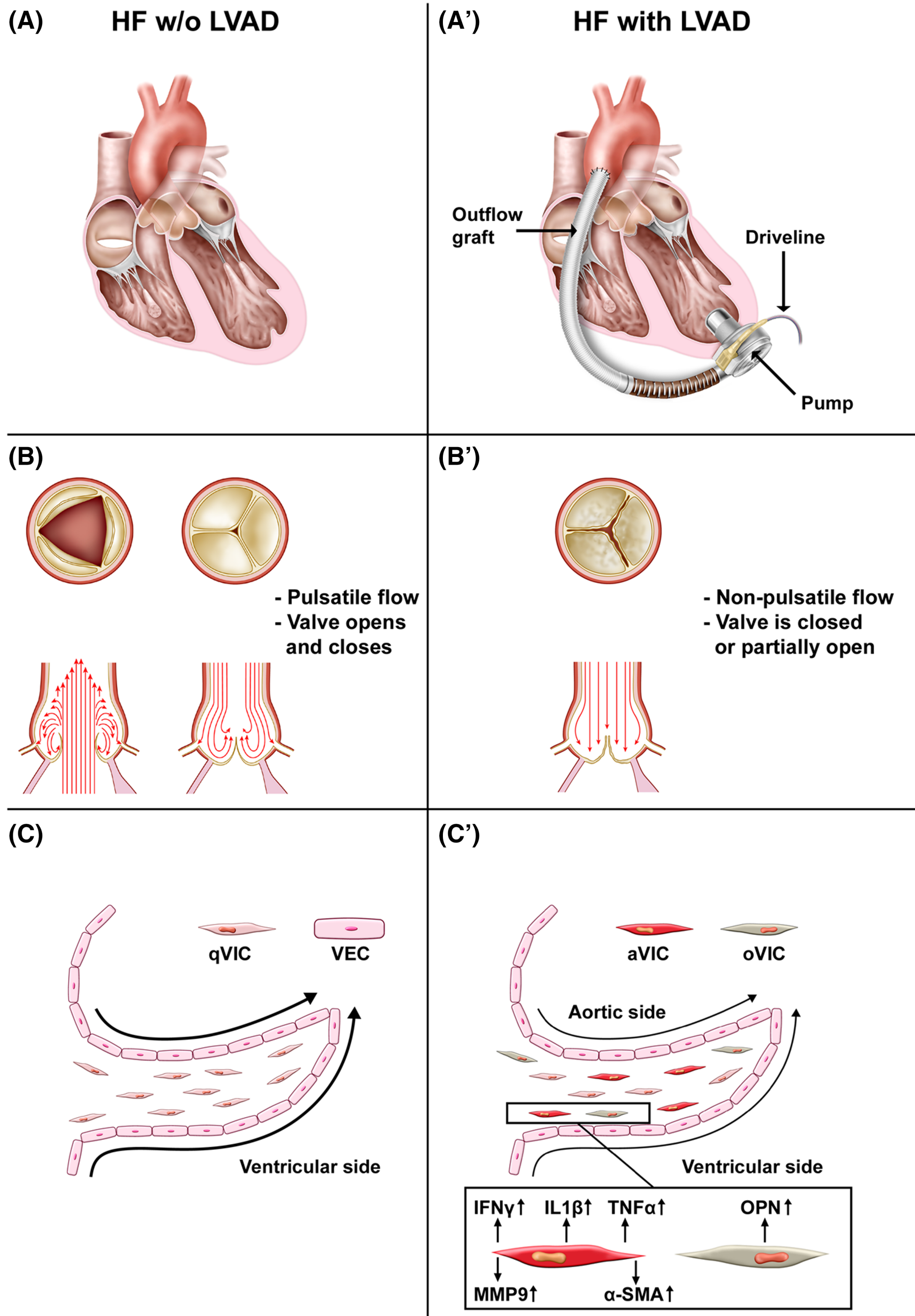
Similar to our findings, previous reports focusing on myocardial tissue changes have shown elevated TNFα as well as elevated IL1β expression after LVAD implantation,[16] whereas also contradictory reports on an adverse effect for IL1β have been published.[17] Stephens *et al*. refer to increased protein expression of immunoglobulin complexes in aortic valves of LVAD patients.[11] However, in our cohort, aggravated expression of inflammatory markers of the LVAD group was not related to driveline infections. As also reported in a longitudinal study,[18] CRP levels of patients in the present study proved to be significantly lower in the LVAD group and expression of inflammatory markers did not correlate with CRP levels, thus unspecific inflammation might not be the reason for the observed findings. Moreover, we found only rarely CD68+ or CD3+ inflammatory cells with no differences in the frequency between the two patient groups, which is in line with previous literature.[9,10] Mudd *et al*. even have reported the absence of any inflammatory cells.[10]

Thus, other inflammatory cells or VIC themselves are likely the source of increased inflammatory markers. It is known that valvular fibroblasts or myofibroblasts contribute to degenerative processes *in vitro*[19] as well as in the heart valve tissue by secretion of cytokines and chemokines (reviewed in Singh and Torzewski[20] and Li *et al*.[21]). Inflammation plays a crucial role in the initiation and progression of calcific aortic valve disease.[22] Here, IL1β and TNFα are key factors in this scenario by activating the canonical NF-κB pathway in VIC inducing remodelling and mineralization.[23] Moreover, IFNγ is associated with progression of calcific aortic valve disease[24,25] and atherosclerotic changes (reviewed in Andersson *et al*.[26]). Taken together, considering the herein observed up-regulation of IL1β, TNFα, and IFNγ, it seems likely that aortic valves of LVAD patients might suffer from abnormal remodelling and consecutive degeneration.

Remodelling and degeneration of aortic valves during left ventricular assist device support

Left ventricular assist device patients are at risk for developing aortic valve insufficiency or for suffering from a worsening of a pre-operatively present insufficiency, which may significantly impair the long-term outcome.[27] In the present study, we could not observe gross differences concerning leaflet thickness and overall extracellular matrix morphology of the aortic valve tissue of LVAD carriers compared with other HF patients undergoing HTx, confirming previous reports.[8,9,11] VIC activation has been consistently described in the literature and is widely accepted as a crucial step towards early changes in valvular tissue homeostasis.[9,11] However, comprehensive studies on cardiac remodelling in context of LVAD support have entirely focused on myocardial tissue. Here, expression of MMP2 and MMP9 has shown no differences in LVAD carriers vs. non-LVAD patients.[28] For better interpretation of these findings, it is important to highlight the fundamentally different changes on myocardial vs. valvular physiology that are induced by LVAD support. On the myocardial level, LVAD support leads to an unloading of the left ventricle, thus to a significant decrease of myocardial wall stress, which among others leads to a decrease of pro-inflammatory tissue activation.[29,30] In contrast, on the valvular level, LVAD support-mediated blood circulation represents a bypass of the aortic valve, which results in a pathological decrease of shear stress at the level of valvular endothelial cells (*Figure 7*). Shear stress is known to represent a crucial signal regulating valvular tissue homeostasis.[31,32] Moreover, the action of the herein used LVADs significantly decreases the pulsatility of the systemic blood flow and reduces the amplitude of mechanical movement of the aortic valve cusps, which substantially interferes with the physiological mechanotransduction at the level of the aortic valve.[33,34] In front of this background, the herein reported findings provide a further piece of the puzzle depicting the whole range of changes induced by implementation of LVAD support. In contrast to myocardial tissue, here we observe an

Figure 7 Impact of LVAD support on the aortic valve. Hearts of heart failure patients (A) experience a pulsatile flow through the aortic valve with frequent opening and closure of the valve during systole and diastole (B). The pulsatile blood sustains homeostasis of the aortic valve characterized by quiescent VIC (qVIC; C). Left ventricular unloading as treatment for heart failure bypasses the aortic valve (A′) leading to non-pulsatile blood flow and partial or permanent closure of the valve (B′). Pathological decrease of the shear stress along the valve cusp leads to a change of the VIC phenotype towards activated VIC (aVIC) up-regulating inflammatory markers, α-SMA and MMP9 as well as towards osteogenic VIC (oVIC) up-regulating osteopontin (C′). α-SMA: alpha-smooth muscle actin; HF, heart failure; IFNγ, interferon gamma; IL1β, interleukin-1 beta; LVAD, left ventricular assist device; MMP9, matrix metalloproteinase 9; OPN, osteopontin; TNFα, tumour necrosis factor alpha; VIC, valvular interstitial cells.



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increase in MMP9 expression in aortic valves of LVAD patients, whereas MMP2 remains unchanged. Moreover, OPN expression increases in aortic valves of LVAD patients, in contrast to findings on the myocardial level.[28,35,36] There was no regulation of TGFβ, ALP, and OCN expression due to LVAD treatment in our setting, whereas elevated TGFβ expression is reported for myocardial tissue under LVAD support.[36] These differences certainly underline the diverse impact of LVAD support on myocardial tissue as opposed to the aortic valve tissue.

The aortic valves examined in the present study showed a quite large heterogeneity in the presence of calcification. With OPN being the only up-regulated chondro-osteogenic marker, a substantial amount of calcification, or calcium accumulation, respectively, was not expectable. This is in accordance with previous findings identifying only minor atherosclerotic lesions with no difference between the patient groups.[9] In summary, up-regulation of inflammatory markers together with an up-regulation of MMP9 and OPN suggest a first step towards initiation of aortic valve disease.[37,38,39] Nevertheless, the pattern of regulated markers is not yet as pronounced as we have reported recently for different stages of calcific aortic valve degeneration. In the latter instance, ALPL, OPN, and biglycan are clearly up-regulated already in early stages of aortic valve disease, that is, fibrotic aortic valves.[12]

Impact of left ventricular assist device support duration on aortic valve biology

It has been shown that aortic insufficiency progresses over time during LVAD support[40] and that expression of remodelling and degenerative markers in myocardial tissue may vary in relation to duration of LVAD support.[36] In our study, MMP2 and TGFβ mRNA expression correlated negatively with LVAD support duration, the latter varying between 14 and 1452 days. These findings suggest that the speed of remodelling or degeneration of the aortic valve may be decreasing in patients with long-term LVAD treatment. It has to be emphasized that although a robust factor in extracellular matrix remodelling, MMP2 is not the only enzyme regulating matrix remodelling in the aortic valve.[41] In contrast to OPN, TGFβ is not a ‘hard’ marker for degeneration but rather a transmitter in different pathways, which might be involved in degenerative processes. Moreover, TGFβ is also known as survival factor,[42] and recent studies have questioned a merely pro-degenerative role for this molecule.[43] However, inflammatory markers seem to be expressed constantly high without negative correlation with LVAD support duration, which might become clinically relevant at some point in bridge-to-transplant or destination therapy patients.

Influence of aortic valve opening and aortic valve insufficiency on molecular alterations

Collectively, our results suggest a significant impact of haemodynamic changes that are associated with LVAD support to become active on the level of aortic valve cusp remodelling. It has been shown that a permanently closed aortic valve under LVAD support is associated with a higher risk to develop aortic valve insufficiency[44] and that a closed valve is an independent predictor of aortic insufficiency.[40] These previous findings underline the role of biomechanics in the functional outcome of the aortic valve in LVAD patients. In our cohort, 81% of the LVAD patients had a documented opening of the aortic valve, and only 32% of the patients had an aortic insufficiency. Thus, a relatively small fraction of LVAD patients might have been ‘at risk’ for developing calcific aortic valve disease according to the driving factor of valve opening activity. This might be responsible for the stable expression pattern of remodelling and degeneration markers in our LVAD patient cohort over time. Nevertheless, a significantly higher expression of inflammatory markers like IFNγ in the aortic valve tissue of patients with aortic insufficiency underlines the clinical relevance of careful monitoring the aortic valve under long-term LVAD support.

Limitations of the study

In contrast to studies on cardiac tissue, analysis of aortic valve tissue and the impact of LVAD treatment do not allow a longitudinal analysis. Thus, differences between aortic valves of patients with and without LVAD support might be inherently biased by the patient cohort diversity. We sought to minimize these potential effects by employing a robust cohort size in our key analyses. The present study does not allow causal interpretation due to its descriptive setting, although the now present findings serve as a sound basis for further mechanistic studies.

Conclusions

Long-term LVAD support may lead to molecular changes of the aortic valve promoting aortic valve disease, but these changes are not necessarily linked to the duration of LVAD support. Nevertheless, LVAD treatment as bridge-to-transplant or destination therapy might require a closer monitoring of the aortic valve for early detection and timely treatment of functional deterioration. Caution is mandatory when heart valves of LVAD carriers become available at time of HTx as possible homografts, and further analysis is needed to better identify possibly irreversible remodelling events in the aortic valves of patients under LVAD support.

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Conflict of interest

M.B., L.M., N.N., J.I.S., U.B., Y.S., N.K., P.H., R.W., P.K., and H.A. have no conflict of interest to declare. P.A. receives speaker honoraria from Medtronic, Abbott, Edwards, Ascyrus Medical, and Abiomed. A.L. and P.A. have received research grants from Abbott and Edwards outside the submitted work.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Correlation of C-reactive protein levels with inflammation markers.
Figure S2. mRNA expression in relation to opening of the aortic valve under LVAD support.
Figure S3. mRNA expression in relation to aortic valve insufficiency of the aortic valve under LVAD support.
Table S1. Primer sequences.

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