Myocardial fibrosis and pro-fibrotic markers in end-stage heart failure patients during continuous-flow left ventricular assist device support

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Abstract

OBJECTIVES: During support with a left ventricular assist device (LVAD), partial reverse remodelling takes place in which fibrosis plays an important role. In this study, we analysed the histological changes and expression of fibrotic markers in patients with advanced heart failure (HF) during continuous-flow LVAD (cf-LVAD) support.

METHODS: In 25 patients, myocardial tissue at the time of LVAD implantation (pre-LVAD) was compared with tissue from the explanted left ventricle (post-LVAD). Interstitial fibrosis and cardiomyocyte size were analysed pre- and post-LVAD. Plasma was obtained from all patients before and during LVAD support. Plasma levels, cardiac mRNA and protein expression of brain natriuretic peptide (BNP), galectin-3 (Gal-3), connective tissue growth factor (CTGF), osteopontin (OPN) and transforming growth factor β-1 were determined.

RESULTS: Fibrosis increased during cf-LVAD unloading (P < 0.05). Cardiomyocytes elongated (P < 0.05), whereas cross-sectional area did not change. BNP, Gal-3, CTGF and OPN were significantly elevated pre-LVAD in comparison with controls. BNP decreased significantly after 1 month of cf-LVAD support (P < 0.001) to near-normal levels. Pro-fibrotic markers remained elevated in comparison with controls.

CONCLUSIONS: cf-LVAD support is associated with lengthening of cardiomyocytes, without alterations in diameter size. Remarkably, myocardial fibrosis increased as well as circulating pro-fibrotic markers. Whether the morphological changes are a direct effect of reduced pulsatility during cf-LVAD support or due to HF progression requires further investigation.

Keywords: Heart failure • LVAD • Reverse remodelling • Biomarkers • Fibrosis

INTRODUCTION

Left ventricular assist devices (LVADs) are being used as bridge to transplantation (BTT) in end-stage heart failure (HF) patients. LVADs provide profound left ventricular pressure and volume unloading, reductions in right ventricular afterload and subsequent pulmonary vascular resistance. Moreover, by normalizing blood pressure and cardiac output, LVAD support improves organ perfusion, which results in improved autonomic function and normalization of the neurohormonal and cytokine milieu, resulting in partial reverse remodelling of the heart [1]. Achieving sustained myocardial recovery, allowing LVAD removal (bridge to recovery, BTR), is one of the most desirable goals as this could reduce the need for heart transplantation (HTx). The mechanisms of reverse remodelling remain poorly understood. Much knowledge has emerged from studies of pulsatile-flow LVADs (pf-LVADs), but currently these devices have been replaced by continuous-flow LVADs (cf-LVADs). cf-LVADs have the advantages of smaller size, better durability, higher energy efficiency and less surgical trauma compared with pf-LVAD support. Concern exists regarding the effects of chronic, diminished-pulsatile flow of cf-LVADs on cardiovascular architecture, end-organ perfusion and long-term outcome. From a haemodynamic perspective, cf-LVADs unload the left ventricle constantly throughout the cardiac cycle, whereas pulsatile devices unload the ventricle only during a selected portion of this cycle. End-organ function and clinical outcomes may be equivalent and perhaps superior during cf-LVAD compared with pf-LVAD support. cf-LVADs seem to be similarly effective or even better in transplant rates and post-transplant outcome, and show fewer LVAD-related complications when compared with pf-LVADs [2]. Despite these potential advantages of cf-LVAD support, volume unloading appears more pronounced in

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pf-LVAD–supported patients, which may be translated into more favourable outcomes in terms of BTR. Studies are warranted to investigate which LVAD design is superior in achieving myocardial recovery: pulsatile- or continuous-flow LVAD. In addition, lack of validated biomarkers for recovery aggregates uncertainties about ideal timing for LVAD explantation.

The current era of genomics, proteomics and metabolomics has led to the discovery of innumerable novel candidate biomarkers. The American Heart Association emphasized the critical appraisal of novel markers to determine their clinical utility [3]. As fibrosis is thought to play an important role in the chance of successful recovery [4], the purpose of this study was to establish the amount of fibrosis and cardiomyocyte size during cf-LVAD support and to relate this to pro-fibrotic biomarkers in BTT patients. We evaluate the change in brain natriuretic peptide (BNP) levels during cf-LVAD support, since BNP reflects myocardial wall stress and has direct effect on cardiac fibroblasts to inhibit fibroic responses [5]. In addition, the present study analyses the change in novel pro-fibrotic markers, such as galectin-3 (Gal-3; member of the lectin family whose expression is associated with inflammatory cells), connective tissue growth factor (CTGF; a secreted matricellular protein), osteopontin (OPN; glycoprotein that is expressed in cardiomyocytes and fibroblasts) and transforming growth factor β-1 (TGFβ-1; a member of a superfamily of homologues that regulate cell growth and differentiation).

MATERIALS AND METHODS

Patient characteristics

From March 2006 until December 2011, 85 patients received a cf-LVAD (Heart-Mate II, Thoratec, CA, USA) as BTT at the University Medical Center in Utrecht, Netherlands. Of these, 25 patients with a non-ischaemic dilated cardiomyopathy (DCM) were selected. Plasma was collected before LVAD implantation, 1, 3 and 6 months after implantation and prior to HTx. Control plasma was gathered from 10 healthy individuals. Of the 25 patients, 17 underwent HTx. The remaining 8 patients were still awaiting HTx. Myocardial tissue at the time of LVAD implantation (pre-LVAD; apical core) was collected, and compared with the tissue from the explanted left ventricle (post-LVAD), remote from the suture area of the inflow cannula. To rule out the effect of implantation of the inflow cannula on this fibrosis, we also examined longitudinal sections of the complete left ventricle. Control myocardial tissue was obtained from three unmatched organ donors whose hearts were not suitable for HTx for various reasons (e.g. cancer), but had normal ventricular function and no structural heart disease. Written informed consent was obtained from all LVAD patients. Approval has been obtained from the institutional review board.

Cardiomyocyte size and fibrosis

Myocardial tissue was fixed in 10% formalin and embedded in paraffin. After staining, the slides (5 µm; size: ±1 × 1 cm) were analysed at ×40 magnification using an Aperio XT slide scanner (Aperio, Vista, CA, USA), and measured with Aperio Image Scope. Cardiomyocyte length was measured between the intercalated discs after staining the specimens with N-cadherin. To determine the diameter of the cardiomyocytes, specimens were stained with modified Azan, and point-to-point perpendicular lines were drawn across the cross-sectional area of the cell at the level of the nucleus. Results were expressed as the average size of 200 cardiomyocytes per slide. For the evaluation of fibrosis, sections were stained with Masson Trichrome, and 15 random fields were selected. Automated image analysis was done using a colour deconvolution algorithm [6], and myocardial fibrosis was calculated with the use of Image J (National Institute of Health, Bethesda, USA), a commonly used technique [7]. Any field containing a large scar area was excluded so that quantification reflected interstitial fibrosis only. Three independent researchers performed the measurements. The percentage of fibrosis was expressed as the average ratio of the total fibrotic area divided by the total myocardial area of the whole slide. Comparison between groups was performed within the same staining procedure.

Biomarkers

Plasma levels. Gal-3, CTGF, OPN and TGFβ-1 were analysed in EDTA plasma by ELISA according to the description of the manufacturer: Gal-3 (BG Medicine, Inc., Waltham, USA); CTGF (FibroGen, Inc., San Francisco, USA); OPN (Assay Design, Inc., Ann Arbor, USA); TGF-β1 (eBioscience, San Diego, USA). BNP was analysed on a Dxl 800 immunochemistry system (Beckman Coulter, Brea, CA, USA).

mRNA. mRNA expression of all biomarkers was determined by Q-PCR on the LightCycler 480 (Roche Diagnostics BV, Almere, Netherlands) as described previously [8]. Total RNA was isolated out of 20 slides of 10 µm frozen myocardial tissue using the miRNeasy Mini Kit (Qiagen, Inc., Austin, USA). Copy DNA (cDNA) was synthesized with the use of superscript III, oligo-dT and random primers (Invitrogen, Oslo, Norway).

Immunochemistry of Gal-3, OPN and CTGF. Formalin-fixed, paraffin-embedded myocardial tissue samples were cut at 5-µm thickness. Endogenous peroxidase was blocked with H2O2 for 30 min. Sections were pre-treated with either citrate (Gal-3 and CTGF) or pepsin (OPN), and incubated with primary antibody diluted in PBS/1% BSA for 1 h. For CTGF, slides were incubated with primary antibody in PBS/3% BSA overnight (4° C), followed by a washing step in PBS/Tween-20. PowerVision poly-anti-rabbit IgG (ImmunoVision Technologies, Duiven, Netherlands), PowerVision poly-anti-mouse IgG (ImmunoVision Technologies) and RagPo polyclonal rabbit-anti-goat IgG (Dako, Heverlee, Belgium) were used as secondary antibodies for OPN, Gal-3 and CTGF, respectively. The slides were developed with DAB solution for 10 min. For CTGF, three-step immunohistochemistry (IHC) was carried out. As a tertiary antibody, 150 µl of peroxidase-labelled anti-rabbit BrightVision IgG (ImmunoLogic, Duiven, Netherlands) was used for 30 min. After a 5-min washing step of PBS, the slides were developed with 200 µl of NovaRed solution for 10 min. The nuclei were counterstained with Mayer’s Haematoxylin.

Statistical analysis

Categorical data are presented by number (%) and continuous data by median (interquartile range, IQR, i.e. 25th–75th percentile) or by mean ± standard error, where appropriate. Mann–Whitney U-tests and Fisher’s exact tests were performed to compare differences between patients and healthy controls. The
pre- and post-LVAD differences were evaluated with the Wilcoxon signed-rank test. The interobserver variability is indicated by the interclass correlation coefficient. Plasma data were evaluated using a random intercept model that adjusts for within-subject correlation between repeated measurements. A P-value of <0.05 was considered as statistically significant. All analyses were performed using SPSS, version 18 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Clinical setting

Demographics. Patient demographics are given in Table 1. All patients presented initially with NYHA IV functional class despite optimal medical therapy, including intravenous inotropic therapy (Table 2). Median age was 46 (Q1–Q3 31–54) years, 72% (n = 18) was male and 44% (n = 11) had a familial cause of DCM. A total of 64% (n = 16) of the patients had a biventricular pacemaker or an implantable cardioverter defibrillator (bvPM/ICD) prior to cf-LVAD implantation. The median duration of mechanical support, based on the patients who already underwent HTxs, was 279 (Q1–Q3 193–505) days.

Prior to LVAD implantation, only 9 (36%) and 15 (60%) patients received beta-blockade and angiotensin-converting enzyme (ACE) inhibition, respectively (Table 2). Beta-blockers and ACE inhibitors often had to be stopped because of deterioration of haemodynamics and after initiation of inotropic therapy. After LVAD implantation, ~50% of the patients were treated with ACE inhibition, and the majority of the patients (~70%) received aldosterone antagonists (Table 2).

Change in fibrosis during cf-LVAD support. Biopsies from patients with end-stage HF prior to cf-LVAD implantation showed 18% fibrosis (±IQR 13–32), whereas healthy controls demonstrated 13% (±IQR 8–15; P < 0.05). After cf-LVAD support, fibrosis increased from 18 to 32% (±IQR 23–43; P < 0.05), with some individual variation (Fig. 1A and B). The interobserver variability was 0.937, indicating a high interobserver reliability. Representative examples of the myocardium of pre-cf-LVAD (Fig. 1C), post-cf-LVAD (Fig. 1D) and healthy controls (Fig. 1E) are shown with arrowheads pointing to interstitial fibrosis. The amount of fibrosis was diffusely present throughout the whole left ventricle. Areas with almost normal structure were neighboured by areas, showing severe morphological disorganization. Figure 2 represents the change in fibrosis in relation to the length of support, suggesting that the change is not related to cf-LVAD duration.

Change in cardiomyocyte size during cf-LVAD support. In end-stage HF patients, the cross-sectional area of the cardiomyocytes prior to cf-LVAD was 649 ± 71 µm² (mean ± SEM), and decreased slightly to 588 ± 71 µm² (P = 0.33). In controls, the cross-sectional area was significantly smaller (242 ± 59 µm²; P < 0.01; Fig. 3A).

The length of the cardiomyocytes before cf-LVAD implantation was 74 ± 3.5 µm, significantly longer than controls (53 ± 5.9 µm; P < 0.05; Fig. 3B). After cf-LVAD support, cardiomyocyte length increased significantly (P < 0.05) to 82 ± 2.7 µm. There was no relation between the length of cardiomyocytes and the duration of cf-LVAD support (data not shown).

Biomarkers

B-type natriuretic peptide

Plasma. BNP was severely elevated in patients with end-stage HF before cf-LVAD (1925 ± 163 pg/ml; mean ± SEM), but decreased rapidly after implantation to almost normal levels (P < 0.001) and remained stable thereafter (Fig. 4A).

mRNA. mRNA of BNP in myocardial tissue decreased significantly during cf-LVAD support, but remained higher compared with controls (pre: 1.7 ± 0.5; post: 0.5 ± 0.14; mean ± SEM; P = 0.01; Fig. 5A).

Table 1: Patient demographics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>(n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46 (31–54)</td>
</tr>
<tr>
<td>Male</td>
<td>18 (72%)</td>
</tr>
<tr>
<td>Duration of HF (days)</td>
<td>1754 (416–2301)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.0 (20.3–26.2)</td>
</tr>
<tr>
<td>NYHA classification IV</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>bvPM/ICD</td>
<td>16 (64%)</td>
</tr>
<tr>
<td>Non-ischaemic DCM</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>Aetiology of non-ischaemic DCM</td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>9 (36%)</td>
</tr>
<tr>
<td>Familial</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Peripartum</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Toxic (drugs/medication)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>cf-LVAD support</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>Days of LVAD support</td>
<td>279 (193–505)</td>
</tr>
</tbody>
</table>

NYHA: New York Heart Association; bvPM/ICD: biventricular pacemaker/implantable cardioverter defibrillator; HF: heart failure; DCM: dilated cardiomyopathy; cf-LVAD: continuous-flow left ventricular assist device.

* Categorical data are presented as number (%) and continuous data as median (25–75% percentile).
* Familial DCM is defined if the patient has one or more family members who are diagnosed with idiopathic DCM and/or has a first-degree relative with an unexplained sudden death under the age of 35 years.
* Days of LVAD support are based on the patients who already underwent heart transplantation.

Table 2: Medication use before and during cf-LVAD support

<table>
<thead>
<tr>
<th></th>
<th>Pre-LVAD (n = 25)</th>
<th>1 month (n = 25)</th>
<th>3 months (n = 25)</th>
<th>6 months (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inotropic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amiodarone</td>
<td>12 (48%)</td>
<td>17 (68%)</td>
<td>17 (68%)</td>
<td>16 (70%)</td>
</tr>
<tr>
<td>ACE inhibitor/ ARB</td>
<td>15 (60%)</td>
<td>13 (52%)</td>
<td>14 (56%)</td>
<td>10 (43%)</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>9 (36%)</td>
<td>1 (4%)</td>
<td>3 (12%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>5 (2%)</td>
<td>2 (8%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>23 (92%)</td>
<td>15 (60%)</td>
<td>11 (44%)</td>
<td>10 (43%)</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>21 (84%)</td>
<td>17 (68%)</td>
<td>18 (72%)</td>
<td>15 (65%)</td>
</tr>
</tbody>
</table>

ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blockers.
Galectin-3

Patients before cf-LVAD support demonstrated substantially elevated Gal-3 levels (35.5 ± 3.7 ng/ml) in comparison with controls (P < 0.001), which temporarily decreased after implantation (21.2 ± 3.7 ng/ml; P < 0.001), but rose again prior to HTx (32.1 ± 3.9 ng/ml; P < 0.05 vs 6 months of cf-LVAD support; Fig. 4B). mRNA. mRNA levels were significantly elevated prior to and after LVAD implantation compared with controls (pre 1.2 ± 0.18; post 1.2 ± 0.16; Fig. 5B).

IHC. Gal-3 stained weak to moderate in the cardiomyocytes, and did not change during mechanical support. Capillaries and stromal cells stained stronger pre-LVAD compared with post-LVAD (arrowheads). Inflammatory cells, when present, stained even more intensely (Fig. 6A and B).

Connective tissue growth factor

Plasma. In HF patients, the CTGF concentration was significantly higher (23.6 ± 2.7 ng/ml) compared with controls (13.1 ± 1.1 ng/ml; P = 0.02), and remained stable during cf-LVAD support (Fig. 4C).

mRNA. mRNA levels in cardiac tissue were elevated in comparison with controls, but, like the plasma levels, did not change significantly during mechanical support (Fig. 5C).

IHC. CTGF staining of cardiomyocytes decreased during cf-LVAD support. The majority of stromal cells were negative (arrowheads; Fig. 6C and D).

Osteopontin

Plasma. In HF patients, the OPN concentration prior to cf-LVAD implantation was severely elevated (99.5 ± 11.8 ng/ml) compared with controls (26.7 ± 3.7 ng/ml). Directly after implantation, OPN increased even further, but tended to decrease, and at the time of HTx it was back on its pre-implantation level.
mRNA. The mRNA of OPN in cardiac tissue pre-LVAD decreased significantly (pre 0.14 ± 0.07, post 0.02 ± 0.004; \( P < 0.01 \)), and reached the level of healthy controls (Fig. 5D).

IHC. OPN showed a moderate staining in the cardiomyocytes pre- and post-LVAD (Fig. 6E and F). Endothelial tissue and stromal cells were negative for OPN (arrowheads). In post-LVAD, the extracellular matrix stained strongly.

**Transforming growth factor β-1**

Plasma. TGFβ-1 levels in HF patients were lower than in controls, and a large interindividual variation was seen. Plasma levels of TGFβ-1 did not alter during mechanical support (data not shown).

mRNA. During LVAD support, there was a significant increase in TGFβ-1 mRNA (pre: 0.10 ± 0.008, post: 0.19 ± 0.03; \( P < 0.001 \); Fig. 5E).

**DISCUSSION**

This study presents a comprehensive analysis of myocardial fibrosis and plasma levels and cardiac mRNA of several fibrotic markers in patients with end-stage HF during cf-LVAD support as BTT. The principal findings are that cf-LVAD support is associated with an overall increase in myocardial fibrosis and lengthening of cardiomyocytes, accompanied by a persistent high level of pro-fibrotic markers. Our data could not answer whether the increase in fibrosis is a direct effect of reduced pulsatility during cf-LVAD support or due to HF progression, and warrants further investigation.

**Cardiomyocyte size and fibrosis**

As has been reported previously, pf-LVAD support results in partial reverse remodelling of the heart, characterized by a substantial decrease in cardiomyocyte diameter and length [9]. However, in our study with cf-LVAD patients, the diameter of the cardiomyocytes did not change, and the length increased. This difference in response of cardiomyocyte size between devices might be consistent with less ventricular unloading during cf-LVAD support, although the almost complete normalization of BNP in our study does not seem to support that. It might be that cardiomyocyte size in cf-LVAD patients is influenced by the physiological consequences of long-term continuous flow on the peripheral vasculature.

Fibrosis is recognized as a major component of cardiac remodelling in HF, and occurs as the result of the unbalance between the enhanced syntheses of collagen and unchanged (or reduced) collagen degradation by matrix metalloproteinases. The net effect of cardiac fibrosis is exaggerated mechanical stiffness, disorganized contraction caused by cardiomyocyte separation and worsening tissue hypoxia. The response of collagen concentration during LVAD support remains controversial. Some studies have found a reduction in collagen during LVAD support [10], whereas most studies showed an increase [11, 12], which is in agreement with the present study. Differences in techniques, concomitant medication and duration of support could account for some of these discrepancies in total collagen measurement. In our study, a large microscopic field was examined, scar tissue was excluded and three independent investigators performed the measurements with a strong interobserver correlation coefficient, thereby minimizing the effects of the heterogeneous nature of fibrosis.

Clinical data have shown that treatment with ACE inhibitors and aldosterone antagonists reduces myocardial collagen turnover in patients with HF [13]. The individual variation, which we noted in the alterations of fibrosis, appeared not to be due to the use of pharmacological therapy and/or to the time of cf-LVAD support. The use of ACE inhibitors and aldosterone antagonists are in essence not changed before and after LVAD therapy. Before LVAD therapy, 88% of the patients were treated with intravenous inotropic therapy. After LVAD, this treatment was stopped in all. Six months after LVAD implantation, 13% of our patients received beta-blockers. It seems unlikely that the overall increase in myocardial fibrosis is caused by the low incidence of beta-blockade in combination with persistent activity of the sympathetic system in our cohort. The LVAD implantation resulted in a complete reversal of HF as assessed by the clinical improvement and the sustained normalization of BNP was accompanied by decreased sympathetic activity.

**Biomarkers**

Plasma biomarkers to predict myocardial recovery during mechanical support are needed in order to assess the feasibility and
ideal timing for LVAD explantation. As fibrosis is thought to play an important role in successful recovery, levels of circulating fibrotic biomarkers (BNP, Gal-3, CTGF, OPN and TGFβ-1) were evaluated. Moreover, cardiac mRNA of all biomarkers was analysed by Q-PCR and protein expression by IHC.

BNP, a well-known HF marker, is related to wall stress of the left ventricle, and is known to have an anti-fibrotic function [5]. Plasma and mRNA of BNP decrease during mechanical support [14], as was confirmed in our study. This decrease confirms the impressive effects of LVAD support on the decompensated state. In this regard, BNP seems not suitable as a load-independent predictive marker of myocardial function in patients on cf-LVAD support.

Gal-3 promotes cardiac fibroblast proliferation and collagen deposition [15], and levels can predict mortality in acute [16] and chronic HF [17]. In a study by Milting et al. [18], circulating levels of Gal-3 remained stable at 1 month after LVAD implantation. Yet, elevated Gal-3 seems to be a risk factor for death on device after LVAD implantation [19]. In our study, and in agreement with the literature, circulating Gal-3 is significantly elevated in advanced HF patients compared with healthy individuals. During cf-LVAD support, a temporarily decline of Gal-3 levels is noted, followed by an increase to pre-LVAD levels after longer periods of support, accompanied by an increase in myocardial fibrosis.

CTGF, a downstream modulator of the TGFβ pathway, promotes fibroblast proliferation and modulates the activity of growth factors in the extracellular matrix, thereby promoting myocardial fibrosis [20]. Plasma levels of CTGF are upregulated in patients with chronic HF [7, 20], which was confirmed in our study. During cf-LVAD support, circulating levels of CTGF as well as myocardial mRNA remain stable. Nevertheless, the expression of CTGF by IHC shows a diminished expression in cardiomyocyte post-LVAD compared with pre-LVAD.

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**Figure 4:** Circulating biomarkers during continuous-flow LVAD (cf-LVAD) support. Brain natriuretic peptide (BNP, A); galectin-3 (Gal-3, B); connective tissue growth factor (CTGF, C) and osteopontin (OPN, D) before, 1, 3 and 6 months after LVAD implantation and prior to HTx or explantation (n = 25). Data are presented as mean ± standard error. The asterisk represents $P < 0.05$. 
OPN, almost undetectable in healthy myocardium, is required for the differentiation and activation of myofibroblasts, and directly regulates collagen-I deposition [21]. Clinically, previous studies have suggested a role for OPN as a diagnostic marker in HF patients [22], irrespective of HF origin. Moreover, OPN predicts adverse right ventricular remodelling and dysfunction in pulmonary hypertension [23], and adds a prognostic value to NT-proBNP with regard to long-term mortality and re-hospitalization in HF patients [22]. In this study, directly after cf-LV AD implantation OPN increases, later on tends to decrease, but at the time of HTx it is back on its pre-implantation level. The initial increase might be explained by the surgical procedure of cf-LV AD implantation, whereas the final increase might be related to the pro-fibrotic response during mechanical support. The decrease in mRNA and stable protein expression of OPN in this study was consistent with our previous work, where we examined OPN levels in pf-LVAD-supported patients [24]. The lack of parallel changes in mRNA and circulating OPN might be explained by post-transcriptional regulators within the myocardium.

TGFβ-1 is a pro-fibrotic cytokine that stimulates the production of extracellular matrix proteins in the heart [25]. TGFβ-1 mRNA increases significantly during cf-LVAD support, whereas circulating TGFβ-1 remains stable, but is lower in comparison with controls. This may indicate that the increase in myocardial TGFβ-1 stimulates fibrosis, but the myocardial levels are not reflected in the circulation. The increase in mRNA expression of TGFβ-1 is in contrast to a previous study by Felkin et al. [25], which did not detect changes during mechanical support. Since TGF-β1 showed variation between individuals, and circulating levels did not alter, circulating TGF-β1 does not seem to be a suitable marker for reverse remodelling during cf-LVAD support.

From this study, it seems plausible that the increase in myocardial fibrosis after cf-LVAD implantation might be the result of the increase in Gal-3, the persisting high levels of CTGF and the increase in myocardial levels of TGFβ-1. The recurrence of high levels of OPN at the end of cf-LVAD support might be additive.

**Limitations**

We acknowledge a number of limitations to this study. The small number of patients constitutes a limitation. Of the total number of patients with LVAD as BTT (n = 25), a minority (n = 3) had a relatively short history of HF duration (between 50 and 100 days). Excluding these 3 patients, however, did not influence the outcome. Additionally, since the duration of LVAD support is variable until HTx, we corrected for this time difference for circulating biomarkers by a linear mixed model with time as a continuous variable, and similar results were found. Also, due to regional heterogeneity of biomarkers, some degree of sample error may have occurred. Due to ethical reasons, myocardial tissue of a control group consisting of end-stage HF patients without LVAD support is unavailable. Hence, it is not possible to determine whether the increase in fibrosis is a manifestation of further progression of...
cardiac remodelling or a direct result of lesser degree of ventricular unloading during cf-LVAD support. Finally, histological analysis is restricted to the left ventricle only, due to the location of the inflow cannula. Due to these limitations, we consider our study mainly as hypothesis generating, and our results need to be confirmed in a larger cohort of patients.

CONCLUSION

The present study demonstrates lengthening of cardiomyocytes and an increase in fibrosis during cf-LVAD support, together with the persistence of several pro-fibrotic circulating markers. At this moment, it is not clear whether this pro-fibrotic response is caused by the lesser degree of ventricular unloading by the cf-LVAD, the potential pathophysiological alterations induced by chronic less pulsatile support or due to HF itself. In our study, the complete normalization of BNP levels suggests that lesser unloading by cf-LVADs does not seem to be an explanation. Whatever the cause, it may have substantial impact on the chance of successful recovery in these patients.

Conflict of interest: Jaap R. Lahpor is a member of the Thoratec European Advisory Board.

REFERENCES
