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The Journal of
Heart and Lung
Transplantation

http://www.jhltonline.org

RESEARCH CORRESPONDENCE

Reverse histologic remodeling after mechanical unloading of failing hearts in children with dilated cardiomyopathy

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Heart failure (HF) is associated with adverse cellular, structural and functional changes in the myocardium, referred to as "remodeling." There is now compelling evidence that prolonged unloading of the left ventricle (LV) with the use of a left ventricular assist device (LVAD) in adults with HF is associated with structural reverse remodeling that can be accompanied by significant functional improvement.[1] Mohapatra et al showed that short-term LVAD therapy in children with severe HF can reverse molecular remodeling with normalization of dystrophin in the LV after 8 to 16 days from LVAD implantation.[2] Canseco et al reported that prolonged mechanical unloading induces adult human cardiomyocyte proliferation.[3] As children retain more proliferative and regenerative potential, it seems likely that those children with end-stage HF due to dilated cardiomyopathy (DCM) have theoretical advantages, over adults, for recovery with LVAD support.

The primary purpose of the study was to analyze the effect of mechanical unloading on echocardiographic parameters of LV, including end-diastolic diameter (LVDD), and z-score, and fractional shortening (LVSF), serum N-terminal pro-brain-type natriuretic peptide (NT-BNP) and histologic changes.

We retrospectively studied 17 pediatric patients (Table 1) receiving LVAD support (pulsatile LVAD [Berlin Heart, Berlin, Germany], P-LVAD, $n = 8$; and continuous-flow LVAD, [HeartWare, HeartWare, Inc., Framingham, MA], CF-LVAD, $n = 9$) for end-stage HF due to DCM. All patients underwent successful heart transplantation, except 1 who died of multi-organ failure. We analyzed echocardiograms before LVAD implantation (pre-LVAD), immediately after implantation and at the time of heart transplantation (post-LVAD). We also analyzed the biopsy tissue from formalin-fixed, paraffin-embedded sections of 17 matched LV samples, their pre-VAD core biopsy from apex and post-explant heart specimens (except 1 patient at autopsy). We used a digital pathology slide scanner (Aperio; Leica Biosystems) for morphometric analysis of cardiac myocytes. Cardiomyocytes were counted in 4 1-cm^2 areas ($\times$400 magnification) of cardiac tissue and reported per high-power field (HPF). The percentage interstitial fibrosis was analyzed using a computerized semi-quantitative method and reported as percent fibrosis per HPF.

In total, there were 12 boys (70%), with a median age of 9.3 years (range 0.15 to 17.5 years), median weight 36 kg (range 4.8 to 75 kg), median body surface area 1.2 m^2 (range 0.26 to 1.97 m^2), median time from diagnosis of HF to LVAD implantation 3 months (range 0.5 to 6 months) and median duration of LVAD support 71 days (range 8 to 223 days). All patients had severe LV dilation. Their LVDD values (in millimeters) and z-scores are given in Table 2. Immediately after LVAD implantation, LVDD decreased from 56.7 $\pm$ 18.8 mm to 46.1 $\pm$ 20 mm ($p = 0.0003$) and z-score of LV in diastole decreased from 5.9 $\pm$ 2.7 to 2.2 $\pm$ 3.6 ($p = 0.001$). However, when we analyzed the echocardiographic change at the time of explantation, there were no significant changes in LVDD from those obtained before LVAD implantation (Table 2). Our finding is in agreement with an earlier report on the impact of chronic unloading of LV by LVAD and the accompanying echocardiographic changes.[4]

We found a statistically significant decrease in serum NT-BNP ($p = 0.007$) and histologic improvement in post-LVAD hearts, with a 64% increase in number of cardiomyocytes ($p = 0.004$) and 56% decrease in interstitial fibrosis ($p = 0.003$) (Figure 1 and Table 2). We compared age at LVAD implant, duration of LVAD and duration of heart failure with change in number of myocytes per HPF and reduction in percent fibrosis, and the results depicted in Figure 2. Only young age at LVAD implant correlated with reduction in percent fibrosis per HPF ($R = 0.52$, $p = 0.03$). There was no significant change in number of myocytes per HPF between P-LVAD and CF-LVAD (69 $\pm$ 64 and 21 $\pm$ 19, respectively, $p = 0.51$), whereas reduction in the percent of interstitial fibrosis was statistically significant after P-LVAD when compared with CF-LVAD (28 $\pm$ 23% vs 15 $\pm$ 7%, $p = 0.02$). Young age at LVAD implant and P-LVAD were major factors for higher reduction in percent fibrosis in our analysis. Our findings are similar to those

1053-2498/$ - see front matter
http://dx.doi.org/10.1016/j.healun.2017.06.010

Table 1 Summary of Children With HF, Type of VAD Support and Histologic Changes

Patient	Age (years)	Gender	Diagnosis	Duration of HF (months)	Duration of LVAD (days)	Type of LVAD	Number of myocytes/HPF (pre- vs post-LVAD)	Fibrosis (%) (pre- vs post-LVAD)
1	0.15	M	DCM	0.5	11	P-LVAD	169 vs 297	49 vs 6
2	17.5	M	DCM, ventricular tachycardia	6	12	CF-LVAD	57 vs 81	10 vs 6
3	14	M	DCM, ventricular tachycardia	3	62	CF-LVAD	73 vs 61	59 vs 26
4	12.8	M	DCM	2	40	CF-LVAD	61 vs 93	9 vs 8
5	9.5	F	DCM	2	72	CF-LVAD	86 vs 90	9 vs 9
6	9.3	F	DCM	6	92	CF-LVAD	39 vs 77	13 vs 29
7	0.45	M	LVNC, DCM	4	210	P-LVAD	54 vs 70	69 vs 6
8	0.2	M	DCM	2	17	P-LVAD	139 vs 62	14 vs 8
9	2.1	M	Congenital heart block, DCM	1	223	P-LVAD	75 vs 112	71 vs 8
10	0.3	F	DCM, ventricular tachycardia	2.5	16	P-LVAD	81 vs 266	51 vs 6
11	15.5	M	DCM	5	57	CF-LVAD	34 vs 57	19 vs 4
12	1.3	M	DCM, ventricular tachycardia	3	8	P-LVAD	107 vs 127	35 vs 21
13	8	M	DCM	2	188	P-LVAD	42 vs 58	13 vs 20
14	14.3	M	Familial DCM	6	43	CF-LVAD	20 vs 67	86 vs 57
15	17.5	F	DCM	8	28	CF-LVAD	34 vs 72	12 vs 4
16	16	M	DCM	1	16	CF-LVAD	58 vs 62	15 vs 13
17	1.8	F	DCM	3	18	P-LVAD	62 vs 139	49 vs 17

CF-LVAD, continuous-flow left ventricular assist device (HeartWare); DCM, dilated cardiomyopathy; F, female; HF, heart failure; M, male; P-LVAD, pulsatile left ventricular assist device (Berlin Excor).

obtained in a previous study on idiopathic DCM.[5] The functional differences between P-LVADs and CF-LVADs are crucial, especially for the evaluation of reverse remodeling, but this is beyond the scope of the current study. Further studies should investigate whether pulsatility by itself or the different degrees of LV unloading by 2 types of VAD (P-LVAD and CF-LVAD) play a role in myocardial recovery.

Another similar study demonstrated a significant reduction in size of hypertrophic myofibers and cardiomyocyte proliferation after unloading, but there was only partial recovery of function calculated by LV ejection fraction and myocyte contractility.[6] The decrease in percent fibrosis in our study coincides with findings from the study by Bruckner et al, who showed a decrease in collagen deposition from time of LVAD implantation to the time of transplantation.[7]

Short-term mechanical unloading has been associated with normalization of cytoskeletal integrity and inhibition of intramyocardial pro-apoptotic cytokinesis, suggesting that molecular reverse remodeling in failing hearts may begin very early during mechanical unloading.[2] Molecular reverse remodeling was accompanied by a significant decrease in BNP level.[2] Mohapatra et al also analyzed the pre- and post-LVAD expression of profiles of genes; 109 of the 326 genes were differentially expressed, with 59 changing by more than 2-fold. However, the changes were not consistent between genes of related functions, and a portion of the gene expression levels changed toward disease-associated states rather than healthy states. These differential gene regulations indicate that LVAD remodeling does not indicate uniform reversal of molecular disease traits. It is possible

Table 2 Summary of Echocardiographic and Histopathologic Changes

Variables	Pre-LVAD[a]	Immediately after implantation of LVAD	Post-LVAD[a]	*p*-value[a]
LVDD (mm)	56.7 ± 18.8[b]	46.1 ± 20[b]	55.4 ± 20	0.22
LVDD *z*-score	5.9 ± 2.8[b]	2.2 ± 3.6[b]	4.8 ± 4	0.17
LVSF (%)	12 ± 0.05		14 ± 0.1	0.23
Serum BNP (pg/ml)	17,038 ± 2,068		1,963 ± 2,829	0.007
Average number of cardiomyocytes/HPF	62 ± 38		102 ± 75	0.004
Interstitial fibrosis (%)	34 ± 26		15 ± 13	0.003

BNP, brain natriuretic peptide; HPF, high-power field; LV, left ventricular; LVAD, left ventricular assist device; LVDD, left ventricular end-diastolic diameter; LVSF, left ventricular fractional shortening.

[a]Pre-LVAD vs post-LVAD.

[b]Pre-LVAD vs immediately after LAVD implantation (*p*-value = 0.0003 and 0.001, respectively).