Purpose: Inflammatory pathways have been widely implicated as major players in the pathogenesis of heart failure. We hypothesized that the inflammatory profile of patients requiring left ventricular assist device (LVAD) support is associated with the improvement in structure and function with chronic mechanical unloading.

Methods: From 2008–2014, tissue and serum samples were obtained in 105 patients receiving durable LVADs for stage D heart failure. Turn-down echocardiography was serially performed to assess criteria for recovery. Responders (R) were defined as having a relative increase in the left ventricular ejection fraction (LVEF) > 50%, a final resulting LVEF > 40 %, and a left ventricular end diastolic diameter (LVEDD) < 60mm. Protein levels of cytokines were measured in both myocardial tissue and in serum.

Results: Of the 105 patients analyzed, 20 were Responders (R) and 85 were Non-Responders (NR). Average duration of LVAD support was 474.8 days for Responders and 267.4 days for Non-Responders. Circulating cytokines GM-CSF, IL-1β, IL-5, and IL-2 were significantly higher in Responders (Table 1). In left ventricular tissue, GM-CSF, IL-10, and IL-7 were also significantly higher in Responders (Table 1). Ratios of pro- (TNFα and IL-1β) to anti-inflammatory (IL-10) cytokines were analyzed. Circulating TNFα/IL-10 and IL-1β/IL-10 were higher in Responders. Inversely, left ventricular tissue TNFα/IL-10 and IL-1β/IL-10 ratios were lower in Responders.

Conclusion: Patients that exhibit functional recovery after mechanical unloading appear to present within an inflammatory milieu. Pro-inflammatory and counteracting, anti-inflammatory cytokines are more highly expressed. Moreover, these data suggest that both serum and tissue factors can be used to target patients that might benefit from focused clinical efforts aimed at bridging to recovery rather than defaulting straight to transplantation or destination therapy.

	Serum		Left Ventricular Tissue	
Cytokine – mean ± SEM (pg/ml)	Non-Responders (N = 85)	Responders (N = 20)	Non-Responders (N = 85)	Responders (N = 20)
GM-CSF	**2.7 ± 0.8***	**62.8 ± 35.9***	**1.7 ± 0.6***	**5.8 ± 1.9***
IFNγ	8.8 ± 1.1	21.3 ± 12.9	0.6 ± 0.1	0.5 ± 0.3
IL-10	81.9 ± 21.9	58.9 ± 25.1	**91.3 ± 46.1***	**393.9 ± 157***
IL-12	30.8 ± 19.8	6.9 ± 2.2	0.3 ± 0.03	0.2 ± 0.03
IL-13	5.3 ± 2.9	7.3 ± 2.4	2.5 ± 0.4	1.7 ± 0.7
IL-1β	**0.5 ± 0.1***	**2.4 ± 0.8***	1.3 ± 0.1	1.2 ± 0.2
IL-2	**1.9 ± 0.4***	**4.2 ± 1.3***	2.9 ± 2.3	0.8 ± 0.2
IL-4	3.3 ± 1.2	7.4 ± 4.4	0.3 ± 0.05	0.4 ± 0.09
IL-5	**0.9 ± 0.2***	**2.4 ± 0.7***	0.4 ± 0.06	0.5 ± 0.2
IL-6	44.4 ± 6.9	30.6 ± 12.7	5.5 ± 0.5	5.2 ± 0.9
IL-7	5.6 ± 1.0	6.6 ± 1.9	**4.4 ± 0.9***	**11.4 ± 3.4***
IL-8	25.1 ± 8.2	30.4 ± 8.9	0.4 ± 0.08	0.2 ± 0.05
TNFα	15.5 ± 2.3	16.6 ± 4.2	0.8 ± 0.06	0.7 ± 0.1

*Table 1 * p <0.05*

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LVAD Support Is Associated with Reduced Serum VEGF-D Levels

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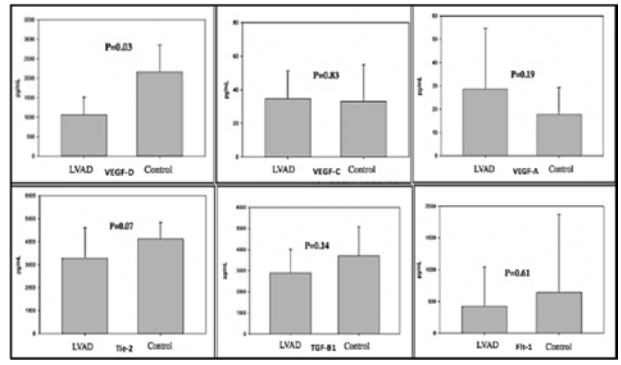
Purpose: LVADs are associated with an increased incidence of GI bleeding of unclear etiology. This study is designed to investigate differences in the biochemical angiogenesis profiles of patients with heart failure with and without LVAD support.

Methods: Twenty-one subjects were included in the study - 10 LVAD subjects and 11 subjects with heart failure without LVAD support as controls. Each subject provided serum and plasma for analysis via an angiogenesis panel multiplex ELISA (MSD catalogue #K15190G-1) and TGF-β1 ELISA (R&D Systems catalogue #DB100B).

Results: Mean duration of device support was 378 days in the LVAD subjects. LVAD and control subject groups did not differ in age (51 vs. 56.3 years, p=0.34). LVAD subjects had a lower mean creatinine (1.35 vs. 1.74 mg/dL, p=0.006) and lower mean NYHA class (1.73 vs. 3, p=0.002). Four of the LVAD subjects (40%) had a history of GI bleeding. One control patient had a history of GI bleeding. As shown in figure 1, serum levels of VEGF-D were significantly lower in LVAD subjects (1058 + 457 vs. 2154 + 697 pg/ml, p=0.03). LVAD and non-LVAD subjects had similar TGF-β1 plasma levels (2888 + 1123 vs. 3701 + 11378 pg/mL, p=0.14). Levels of flt-1, VEGF-A, and VEGF-C were also not different. There was a trend toward a lower level of serum Tie2 in LVAD subjects (3283 + 1328 vs. 4093 + 695 pg/mL, p=0.07).

Conclusion: LVAD subjects have reduced serum VEGF-D compared to non-LVAD heart failure subjects. VEGF-D is responsible for lymphangiogenesis, and thus may be elevated in states of high interstitial hydrostatic pressure (such as heart failure). Tie2 may be lower in LVAD subjects compared to non-LVAD heart failure subjects. Tissue Tie2, when stimulated by angiopoeitin-1, acts as a "brake" on angiogenesis - promoting stabilization and stasis of blood vessel formation. Thus a reduction in Tie2 signaling may result in augmented angiogenesis. Future investigation should focus on alterations in the Tie2/angiopoetin pathway in LVAD subjects, especially those with GI bleeding.



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Relationship of Myocardial Fibrosis with the Potential of Mechanical Unloading to Induce Favorable Cardiac Structural and Functional Response

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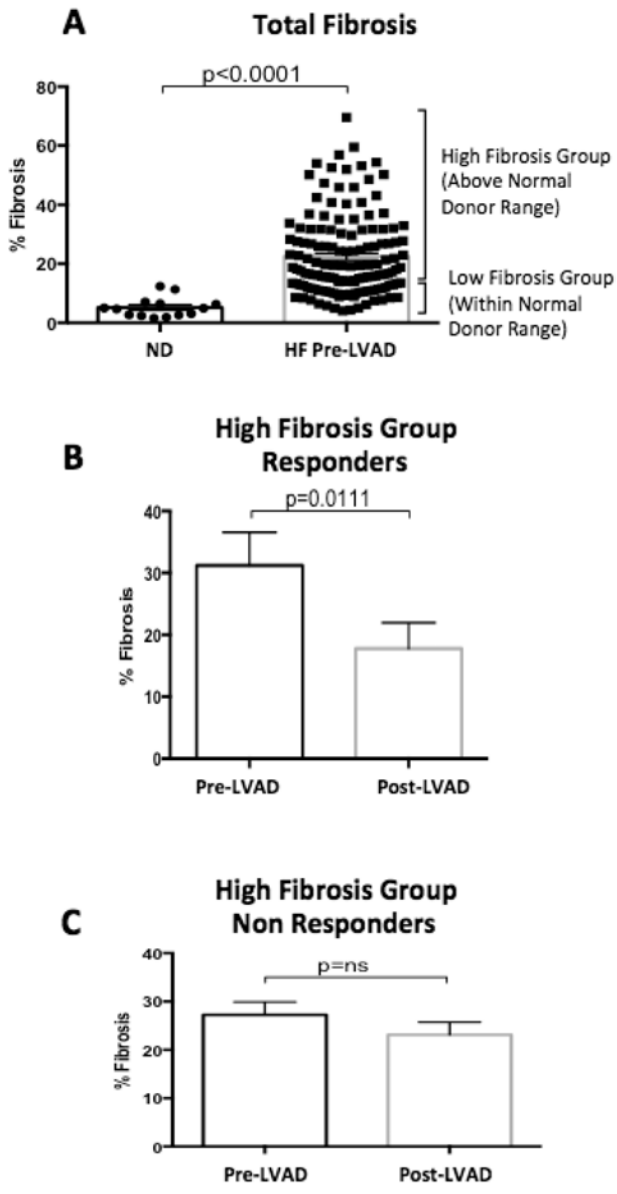
Purpose: Previous studies of fibrosis changes after mechanical unloading in LVAD patients yielded conflicting results. Those pulsatile era LVAD studies were small-scale and rarely correlated their findings with post-LVAD cardiac functional response.

Methods: We prospectively evaluated 142 dilated cardiomyopathy patients who required durable LVAD and 14 normal donors (ND). Tissue was obtained from the LV apex at LVAD implant and transplant and evaluated as previously validated (infarct related scars excluded). "Responders" were classified by serial post-LVAD echocardiography as: relative increase in LVEF >50%, a final resulting LVEF >40%, and a final LVEDD <60 mm.

Results: The pre-LVAD degree of interstitial fibrosis varied significantly and revealed that advanced heart failure (HF) patients had fibrosis levels both within and above the fibrosis range of the normal donor hearts (Low Fibrosis Group: n=53, High Fibrosis Group: n=89, Figure A). The percentage of Responders was similar between the Low and High Fibrosis groups (23% vs. 19%, p=ns, respectively). Responders from the High Fibrosis Group had a significant reduction in total collagen after LVAD unloading (from 31% to 18%, p=0.01, Figure B), whereas no post LVAD collagen changes were identified in the Non Responders of the High Fibrosis Group (Figure C) and in the Low Fibrosis Group regardless of response.

Conclusion: In this large-scale human tissue study, advanced HF patients appear to have significant variation in the degree of interstitial fibrosis despite being clinically similarly ill. Increased pre-LVAD interstitial fibrosis does not appear to preclude patients from developing post-LVAD favorable functional and structural response. This unexpected result is consistent with the finding that Responders who had increased fibrosis pre-LVAD decreased collagen content post-unloading. Future investigations are warranted to elucidate the mechanisms driving these phenomena.



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Soluble ST2 Levels in End-Stage Heart Failure and during LVAD Support

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Purpose: The interleukin 33 (IL-33)/suppressor of tumorigenicity 2 (ST2) pathway might play an important role in the progression of heart failure (HF) and is related to cardiac fibrosis. Although the exact source of the ST2 protein is not clarified, increased serum levels (sST2) are associated with adverse outcome in HF. Left ventricular assist device (LVAD) patients offer a clinical model to investigate sST2 both during severe end-stage HF as well as after LV unloading. In this study sST2 measurements were performed sequentially before and after LVAD support in the same patients. Furthermore we analyzed which clinical factors were associated with sST2 levels during HF.
Methods: Serial serum measurements of sST2 were performed in EDTA plasma pre-implantation and 1, 3 and 6 months after LVAD-implantation in 38 patients, using the high-sensitive Presage ST2 assay. The overall effect of LVAD on sST2 levels was analyzed with the Friedman test. Comparison at different time points was done with Wilcoxon-signed-rank test, corrected for multiple testing. Several clinical factors were analyzed for their relation with sST2 levels using Mann Whitney U tests.
Results: sST2 levels were significantly elevated in end-stage HF just before LVAD implantation (74.2 ng/ml (IQR 54.7 -116.9; normal < 30 ng/ml) and decreased substantially during LVAD support, to 29.5 ng/ml (IQR 24.7-46.6) (p< 0.001), normalizing in most patients. This normalization was complete at 3 months post-LVAD. The variation in sST2 levels at baseline could not be correlated to any of the clinical factors tested (gender, HF etiology, duration of HF, right ventricular function and kidney function).
Conclusion: LVAD support results in a significant drop in sST2 levels with normalization within 3 months post implantation. This suggests that even in patients with end-stage HF, sST2 may be used as a biomarker to monitor therapy. The great variance in sST2 levels at baseline cannot be explained by differences in gender, HF etiology or duration, right ventricular function and kidney function.

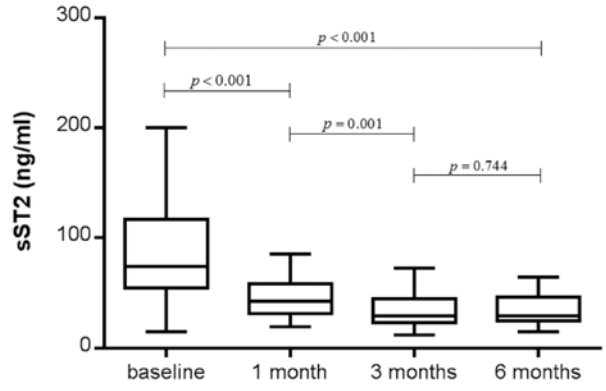

Figure 1. Boxplot showing median sST2 levels during LVAD support with IQR (25-75%), p <0.05 was considered significant.

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Interleukin-1 Receptor Antagonist for the Treatment of Heart Failure in Patients with Left Ventricular Assist Devices

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Purpose: Patients with left ventricular assist devices (LVADs) that experience sufficient myocardial recovery can become candidates for device explantation. Inflammation promotes adverse cardiac remodeling, and antagonism of the Interleukin-1 (IL-1) receptor using Anakinra has been shown to decrease inflammation and reduce adverse myocardial remodeling. We hypothesized that the incidence and magnitude of myocardial recovery in patients with LVADs can be significantly improved through the administration of Anakinra.
Methods: A prospective, pre-post design, pilot investigation of the use of Anakinra in patients with newly implanted LVADs was begun. Beginning four weeks after LVAD implant, patients received 100 mg of Anakinra daily for two weeks. Biologic efficacy was assessed through serial monitoring of C-reactive protein (CRP), tumor necrosis factor (TNF)-alpha, neutrophil counts, and interleukins 1-beta, 6, and 10. Clinical efficacy was assessed by echocardiographic evaluation of ejection fraction (EF), and left ventricular end diameter in diastole and systole (LVEDd and LVEDs, respectively). To maximize the opportunity for recovery, patients were followed for six months.
Results: Seven patients (of an intended 10) have been enrolled thus far, and five have completed the trial. Among patients completing the trial, CRP was 2.7 ± 0.7 mg/dL prior to Anakinra, which reduced to 1.0 ± 0.8 mg/dL ($p = 0.078$) after Anakinra and 1.0 ± 0.6 mg/dL at 6 months ($p = 0.042$). Neutrophil count was 6.7 ± 2.2 k/μL before Anakinra, compared to 5.1 ± 0.9 k/μL after Anakinra ($p = 0.043$) and 4.2 ± 0.8 k/μL at 6 months ($p = 0.043$). EF prior to Anakinra was 18 ± 7%, 21 ± 15% after Anakinra ($p = 0.317$), and 34 ± 11% at 6 months ($p = 0.068$). There were no significant differences over the course of the study with regard to LVEDd, LVEDs, TNF-alpha, or interleukins 1-beta, 6, or 10.
Conclusion: Anakinra use in newly implanted LVAD patients is associated with a reduction in inflammatory markers. Myocardial recovery, as measured by EF, trended towards significance over the course of the study. Additional