Journal of the American Heart Association

ORIGINAL RESEARCH

Circulating and Myocardial Cytokines Predict Cardiac Structural and Functional Improvement in Patients With Heart Failure Undergoing Mechanical Circulatory Support

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BACKGROUND: Recent prospective multicenter data from patients with advanced heart failure demonstrated that left ventricular assist device (LVAD) support combined with standard heart failure medications, induced significant cardiac structural and functional improvement, leading to high rates of LVAD weaning in selected patients. We investigated whether preintervention myocardial and systemic inflammatory burden could help identify the subset of patients with advanced heart failure prone to LVAD-mediated cardiac improvement to guide patient selection, treatment, and monitoring.

METHODS AND RESULTS: Ninety-three patients requiring durable LVAD were prospectively enrolled. Myocardial tissue and blood were acquired during LVAD implantation, for measurement of inflammatory markers. Cardiac structural and functional improvement was prospectively assessed via serial echocardiography. Eleven percent of the patients showed significant reverse remodeling following LVAD support (ie, responders). Circulating tumor necrosis factor alpha, interleukin (IL)-4, IL-5, IL-6, IL-7, IL-13, and interferon gamma were lower in responders, compared with nonresponders ($P<0.05$, all comparisons). The myocardial tissue signal transducer and activator of transcription-3, an inflammatory response regulator, was less activated in responders ($P=0.037$). Guided by our tissue studies and a multivariable dichotomous regression analysis, we identified that low levels of circulating interferon gamma (odds ratio [OR], 0.06; 95% CI, 0.01–0.35) and tumor necrosis factor alpha (OR, 0.05; 95% CI, 0.00–0.43), independently predict cardiac improvement, creating a 2-cytokine model effectively predicting responders (area under the curve, 0.903; $P<0.0001$).

CONCLUSIONS: Baseline myocardial and systemic inflammatory burden inversely correlates with cardiac improvement following LVAD support. A circulating 2-cytokine model predicting significant reverse remodeling was identified, warranting further investigation as a practical preintervention tool in identifying patients prone to LVAD-mediated cardiac improvement and device weaning.

Key Words: biomarkers ■ cardiac recovery ■ growth factors/cytokines ■ inflammation ■ left ventricular assist device

Left ventricular assist devices (LVADs) are an established therapeutic option for patients with advanced heart failure (HF) refractory to standard medical therapy and are increasingly used either as a bridge to heart transplantation or as a lifetime destination therapy.[1] LVAD-induced pressure and volume unloading promotes the reversal of stress-related compensatory responses of the overloaded

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.020238

For Sources of Funding and Disclosures, see page 10.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- By investigating the myocardial and systemic inflammatory burden in patients with advanced heart failure before mechanical circulatory support, this study directly connects and correlates the potential for structural and functional cardiac improvement with the underlying biological derangements.

What Are the Clinical Implications?

- A circulating 2-cytokine model could serve as a practical clinical tool to identify patients with advanced heart failure prone to improve cardiac structure and function after left ventricular assist device support and thus guide patient selection and clinical management.

Nonstandard Abbreviations and Acronyms

NFkB	nuclear factor kappa-light-chain-enhancer of activated B cells
STAT3	signal transducer and activator of transcription 3

myocardium.[2–11] Based on single center and multicenter studies (eg, INTERMACS [Interagency Registry for Mechanically Assisted Circulatory Support]) approximately 5% of ischemic and 20% of patients with nonischemic chronic cardiomyopathy improve significantly their cardiac structure and function following LVAD support.[12,13]

The RESTAGE-HF (Remission from Stage D Heart Failure) multicenter US trial was recently published.[16] It enrolled 40 patients with nonischemic cardiomyopathy, left ventricular ejection fraction (LVEF) <25% and cardiomegaly, age <60, and duration of chronic HF less than 5 years. Standard HF pharmacological regimen was implemented, and regular echocardiograms were performed at reduced LVAD speed to test underlying cardiac function. Overall, 40% of all enrolled (16/40) patients achieved the primary end point (P<0.0001) of sufficient improvement of cardiac function to reach criteria for LVAD explantation with sustained remission from HF (freedom from transplant/VAD/death) at 12 months, whereas from the 36 patients who actually received the study protocol 19 were explanted (52.3%). Postexplantation survival, free from LVAD or transplantation, was 90% at 1 year and 77% at 2 and 3 years. The investigators concluded that in this multicenter prospective study, the strategy of LVAD support combined with a standardized pharmacologic and cardiac function monitoring protocol resulted in a high rate of LVAD removal and was feasible and reproducible with device explantations taking place in all 6 participating sites.[16]

Although the role of tissue biomarkers predicting LVAD-mediated cardiac recovery has been examined in a few studies,[14–17] circulating biomarkers are yet to be associated with cardiac recovery. Given that cytokines play a central role in the pathophysiology of HF,[18] with several studies highlighting their importance as a prognostic tool,[16,19] we sought to investigate whether baseline circulating proinflammatory cytokines can predict post-LVAD cardiac improvement.

METHODS

Data Sharing

The data, analytic methods, and study materials will be made available to other researchers upon reasonable request. Please contact the corresponding author.

Study Population

We enrolled consecutive patients with advanced chronic HF requiring circulatory support with durable continuous-flow LVAD, as bridge-to-transplant or destination therapy, from 2008 to 2013, at 1 of the institutions comprising the Utah Transplantation Affiliated Hospitals (U.T.A.H.) Cardiac Transplant program (University of Utah Health Sciences Center, Intermountain Medical Center, and the George E. Wahlen VA Medical Center, all in Salt Lake City, Utah). The study was approved by the institutional review board of the participating institutions, and written informed consent was obtained from all patients (University of Utah Institutional Review Board 30622 Effects of Mechanical Unloading on Myocardial Function and Structure in Humans). Patients who required LVAD support because of acute HF (acute myocardial infarction, acute myocarditis, postcardiotomy cardiogenic shock, etc.), were prospectively excluded.

Echocardiographic Evaluation

Echocardiographic examinations were performed at the echocardiography laboratories of the participating institutions and stored digitally. The echocardiograms were performed within 2 weeks preceding LVAD implantation and then at months 1, 2, 3, 4, 6, 9, and 12 after implantation, as previously described.[20]

After serial turn-down echocardiographic evaluation (for more details refer to Data S1), the patients were categorized into 2 groups based on the change in left ventricular (LV) function after LVAD unloading. We defined as *responders*, patients who demonstrated

either a final LVEF ≥40% or a final LVEF 35% to 40% with ≥50% relative improvement. The remaining patients were defined as *nonresponders*.

Pre-LVAD Clinical Data

Clinical and laboratory data were collected within 24 hours preceding LVAD implantation. Right heart catheterization was performed within the week before LVAD implantation.

Post-LVAD Clinical Management

After device implantation, the device speed was adjusted to achieve adequate flows and LV decompression. The pump speed during the postimplantation hospitalization and at subsequent outpatient clinic visits was adjusted under echocardiographic guidance to achieve a midline position of the interventricular and interatrial septum and minimum mitral valve regurgitation. Intermittent aortic valve opening was desirable but not always achieved.

Patients were medically managed at the discretion of the treating physicians, with the goal to achieve maximum doses of guideline-directed HF medications as tolerated by the patient.

Laboratory Measurements

Tissue and Serum Acquisition

Myocardial tissue was prospectively collected from the LV apical core at LVAD implantation, immediately frozen in liquid nitrogen, and subsequently stored in –80°C to be used for protein and gene expression analysis. Blood was also collected at the time of LVAD implantation. The blood was centrifuged, and the serum was aliquoted and stored in –80°C.

Western Blotting

Protein was extracted using lysis buffer supplemented with protease and phosphatase inhibitor. Total protein lysate was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Antibodies against glyceraldehyde 3-phosphate dehydrogenase, STAT3 (signal transducer and activator of transcription 3), phospho-STAT3, p65, and phospho-p65 (cell signaling) were used for immunoblotting. Western blots were developed using the ECL Plus western blotting reagent (GE Healthcare) and Kodak Biomax MR film.

Cytokine Measurement

We selected to measure the levels of cytokines that have been implicated in cardiovascular disease.[21–24] Cytokines in serum and cardiac tissue were measured using Multiplex Immunoassays (Millipore), which are comparable to traditional enzyme-linked immunosorbent assay in terms of specificity and sensitivity[25] (for more details refer to Data S1). The immunoassay signal was detected using the Luminex 200 Multiplexing Instrument. After identifying a differential cytokine expression between responders and nonresponders, we measured the expression of transcription factors that are known to be activated by these specific cytokines. Indeed, interferon gamma (IFNγ) is 1 of the main activators of the JAK/STAT signaling pathway, whereas tumor necrosis factor alpha (TNFα) is known to activate the p65 pathway.[26,27]

Statistical Analysis

For descriptive purposes, categorical variables were summarized as frequencies and percentages and compared using the Pearson chi-square test or the Fisher's exact test, as appropriate. Continuous variables were summarized as mean±standard error. Comparisons between responders and nonresponders were performed using the Student's *t* test. A 2-tailed *P* value of 0.05 was used to test significance. Univariate predictors of cardiac recovery were identified using logistic regression analysis that included preimplant clinical, echocardiographic, hemodynamic, and serum laboratory variables.

For the development of the multivariable model, tissue variables were dichotomized using the highest C-statistic to define the optimal cut point for each variable. Given the large number of cytokine variables considered for inclusion in the multivariable model, to limit the number of false positive findings to at most 5%, we applied the Benjamini-Hochberg method of false discovery rate adjusted *P* values, using a q=0.20 to permit a more liberal adjustment, and then corrected so that when an adjusted *P* value is compared with alpha=0.05, it is actually an adjusted comparison to alpha=0.20. Any variable with a false discovery rate-adjusted *P*<0.20 was considered for inclusion in the multivariable model, as were variables suggested to be significant in previous studies.[28] Collinearity among candidate variables was assessed, resulting in variables associated with cardiac recovery: sex; HF duration; LV end-systolic diameter; dichotomized tissue variables TNFα, interleukin (IL)-6, and IL-13; and dichotomized serum variables TNFα, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p70, IL-13, and IFNγ.

Once these variables were identified, a bootstrap resampling technique was used to evaluate the stability of the models resulting from the inclusion of variables mentioned. The frequency of variables selected in each sample, called bootstrap inclusion fractions, were calculated for each potential variable. Bootstrap inclusion fractions are defined as the percentage

of time that each variable would be retained in the model as a significant predictor in a large number of bootstrap resamples in which the variable selection is repeated.[29,30] Variables with bootstrap inclusion fractions <50% were dropped from the model as unreliable, as these would not likely remain significant predictors in future data sets. After removal of unreliable variables, our model included 2 variables: serum TNFα and serum IFNγ.

The final prediction model was then internally validated with bootstrapping, allowing for the use of the entire study group to validate the model. An optimism coefficient was generated and subtracted from the initial area under curve. All analyses were performed using STATA software, version 15 (StataCorp LP, College Station, TX).

RESULTS

Study Population

Ninety-three patients were included in the final study population. For the patients to be included, adequate echocardiographic follow-up and serum and tissue samples collected before LVAD implantation were required.

Baseline demographic, laboratory, and echocardiographic data are presented in Table 1. There were no differences in the demographics between the 2 study groups. In Table 2, it is evident that the baseline hemodynamic profile was also similar between the 2 groups, and it was indicative of advanced HF, whereas all hemodynamic indices were improved in both groups at 2 months post-LVAD implantation. As indicated by the 2 months post-LVAD hemodynamics both groups underwent significant pressure unloading following LVAD support. Notably, the responder group had higher LVEF and less dilated LV cavity at the time of LVAD implantation. Echocardiographic assessment following LVAD implantation, revealed that responders (n=10), as expected, had more pronounced cardiac functional and structural improvement as depicted by LVEF (45±3 vs 19±1; $P<0.001$), LV end-diastolic diameter (4.5±0.3 vs. 6.3±0.1; $P<0.001$) and LV end-systolic diameter (3.7±0.3 vs. 5.7±0.1; $P<0.001$), compared with nonresponders (n=83). Of note, 9 out of 10 responders achieved maximal cardiac structural and functional improvement within 5 months post-LVAD unloading, with the remaining responder achieving it 12 months after circulatory support.

With regard to the pharmacologic management of these patients before LVAD support and at 2 months post-LVAD implantation, no differences were observed in the HF medications used in the 2 study groups, both in terms of the proportion of patients using these agents, as well as the drug dosage (Table 3).

Cardiac Tissue Cytokines

The expression pattern of each cytokine varies significantly in the failing human heart. We found that IL-6, IL-7, IL-1β, and IL-13 are detected in at least 2 times higher levels compared with TNFα, IL-2, IL-5, IL-8 (Table 4). TNFα levels were significantly lower in the cardiac tissue of responders compared with nonresponders (Table 4). These data suggest an association between the low TNFα expression in human heart tissue and the potential for structural and functional cardiac improvement.

Inflammation-Associated Transcription Factor Levels in Cardiac Tissue

To further investigate the effect of cytokines on the reverse remodeling of the failing human heart, we measured the levels of activated STAT3 and NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells), in the cardiac tissue of responders and nonresponders. In response to cytokines, such as TNFα, IL-6, and IFNγ, transcription factors STAT3 and NFkB get phosphorylated and translocate to the nucleus, in order to regulate genes that play important role in inflammation, fibrosis, and hypertrophy. Our results demonstrated lower levels of phosphorylated STAT3 in the myocardium of responders at the pre-LVAD intervention time point, suggesting that lower baseline STAT3 activation may be associated with favorable response to subsequent mechanical unloading and circulatory support (Figure 1a). Interestingly, the levels of phosphorylated p65, the activated NFkB subunit, were similar in the 2 study groups (Figure 1b).

Circulating Cytokines

In accordance with these myocardial tissue cytokine expression patterns, the serum cytokine results also demonstrated variable detection levels. IL-6, IL-8, and IL-10 were the most highly detected cytokines in the serum of patients with advanced HF (Table 5). When we compared the serum cytokine levels between the 2 groups, we found significantly lower TNFα, IL-5, IL-6, IL-7, IL-13, and IFNγ levels in the serum of responders, suggesting that lower systemic inflammatory burden is associated with LVAD-mediated cardiac structural and functional improvement (Table 5).

Circulating Cytokines Predictive Model

From a clinically practical point of view, identifying baseline, preintervention circulating biomarkers predictive of the potential of subsequent cardiac improvement, can affect patient care and is currently an unmet need. In order to identify such predictors and guided by our myocardial tissue and blood findings, we initially performed a univariate analysis of selected circulating cytokines. Individual cytokine levels dichotomized at their optimal cut points, were

Table 1. Demographic, Laboratory, and Echocardiographic Parameters in Responders and Nonresponders before LVAD Implantation

	Responders (n=10)	Nonresponders (n=83)	*P* Value
Men, n (%)	6 (60%)	70 (84%)	0.06
White, n (%)	9 (90%)	67 (81%)	0.68
Age, y	57±7	60±1	0.54
Heart failure etiology, n (%)			
Ischemic cardiomyopathy	4 (40%)	38 (46%)	0.73
Nonischemic cardiomyopathy	6 (60%)	45 (54%)	
Diabetes mellitus, n (%)	3 (30%)	33 (40%)	0.53
Hypertension, n (%)	5 (50%)	40 (48%)	0.91
New York Heart Association functional class			
III, n (%)	4 (40%)	22 (27%)	0.37
IV, n (%)	6 (60%)	61 (73%)	
Interagency Registry for Mechanically Assisted Circulatory Support profile			
1, n (%)	1 (10%)	5 (6%)	0.58
2, n (%)	2 (20%)	12 (15%)	
3, n (%)	3 (30%)	40 (48%)	
4–7, n (%)	4 (40%)	26 (31%)	
Duration of heart failure, mo	64±24	89±8	0.30
Inotrope-dependent, n (%)	6 (60%)	52 (68%)	0.64
Temporary mechanical circulatory support pre-LVAD, n (%)	1 (10%)	1 (1%)	0.07
Device therapy			
Cardiac resynchronization therapy-defibrillator, n (%)	9 (90%)	51 (61%)	0.09
Implantable cardioverter-defibrillator, n (%)	10 (100%)	76 (92%)	0.99
LVAD implantation strategy			
Bridge to decision, n (%)	0 (0%)	2 (3%)	0.46
Bridge to transplant, n (%)	7 (70%)	41 (49%)	
Destination therapy, n (%)	3 (30%)	40 (48%)	
LVAD type			
HeartMate II, n (%)	10 (100%)	57 (69%)	0.42
HeartWare, n (%)	0 (0%)	14 (17%)	
Jarvik, n (%)	0 (0%)	10 (12%)	
Levacor, n (%)	0 (0%)	1 (1%)	
Ventrassist, n (%)	0 (0%)	1 (1%)	
Duration of LVAD support, d	691±253	472±59	0.42
Laboratory measurements			
White blood cells, $\times 10^9$/L	7.9±1.3	8.1±0.3	0.86
Neutrophils, $\times 10^9$/L	5.9±1.2	6.0±0.4	0.97
Neutrophils, %	70±3	70±1	0.99
Lymphocytes, $\times 10^9$/L	1.5±0.2	1.5±0.1	0.91
Lymphocytes, %	21±3	19±1	0.54
Neutrophils/lymphocytes ratio	4.0±0.5	4.8±0.4	0.45
Hemoglobin, g/dL	12.3±0.7	12.5±0.2	0.72
International normalized ratio	1.2±0.1	1.3±0.0	0.34
Sodium, mmol/L	133±2	135±1	0.32
Creatinine, mg/dL	1.6±0.3	1.4±0.1	0.22
Total bilirubin, mg/dL	1.8±0.5	1.4±0.1	0.51
Alkaline phosphatase, mg/dL	115±14	110±6	0.81
Aspartate transaminase, mg/dL	51±15	58±8	0.77

(*Continued*)

Table 1. Continued

	Responders (n=10)	Nonresponders (n=83)	*P* Value
Alanine transaminase, mg/dL	54±23	79±21	0.68
Total protein, g/dL	6.9±0.3	7.1±0.1	0.62
Albumin, g/dL	3.8±0.2	3.8±0.1	0.86
B-type natriuretic peptide, pg/mL	2118±720	1329±107	0.33
Echocardiographic measurements			
Left ventricular ejection fraction, %	22±3	17±1	0.034*
Left ventricular end-diastolic diameter, cm	6.0±0.4	7.0±0.1	0.013*
Left ventricular end-systolic diameter, cm	5.3±0.4	6.3±0.1	0.01*

LVAD indicates left ventricular assist device.
*$P<0.05$.

associated with cardiac improvement after LVAD support (Table S1). Multivariable regression analysis adjusting for all confounding variables (see statistical section for details), identified TNFα and IFNγ as independent predictors of recovery (odds ratio, 0.06; 95% CI, 0.01–0.35 and 0.05, 0.00–0.43 respectively) (Table 6). The combination of TNFα and IFNγ resulted in a 2-cytokine model with high performance in predicting LVAD-mediated cardiac improvement (area under curve 0.903, $P<0.0001$) (Figure 2a). Internal validation of the selected serum model (ie, validation accounting for variability due to parameter estimation) was performed using a bootstrap approach to create an optimism-corrected area under curve of 0.908.

Comparison Between "Circulating Cytokines" and "Clinical Variables" Predictive Models

Finally, we examined the role of baseline clinical characteristics as predictors of structural and functional cardiac improvement. The univariate analysis identified baseline LVEF, LV end-diastolic diameter, and LV end-systolic diameter as predictors of cardiac recovery. After

Table 2. Hemodynamic Measurements in Responders and Nonresponders before LVAD Implantation and at 2 Months after LVAD Implantation, Indicating that Both Groups Underwent Significant Pressure Unloading Following LVAD Support

	Responders (n=10)	Nonresponders (n=83)	*P* Value
Hemodynamic measurements before LVAD implantation			
Systolic arterial pressure (mm Hg)	100±4	106±2	0.32
Diastolic arterial pressure (mm Hg)	65±3	70±1	0.25
Heart rate (bpm)	87±5	87±2	0.96
Mean right atrial (mm Hg)	10±2	12±1	0.42
Pulmonary capillary wedge (mm Hg)	26±2	25±1	0.59
Systolic pulmonary arterial (mm Hg)	57±4	57±2	0.94
Diastolic pulmonary arterial (mm Hg)	27±3	26±1	0.86
Pulmonary vascular resistance (Wood units)	4.2±1.2	4.4±0.3	0.87
Cardiac output	3.7±0.5	3.5±0.1	0.47
Cardiac index ($L{\cdot}m^{-2}{\cdot}min^{-1}$)	2.0±0.3	1.7±0.1	0.30
Hemodynamic measurements at 2 mo post-LVAD Implantation			
Systolic arterial pressure (mm Hg)	98±5	103±2	0.43
Diastolic arterial pressure (mm Hg)	82±6	81±2	0.85
Heart rate (bpm)	78±6	82±2	0.48
Mean right atrial (mm Hg)	9±1	10±1	0.58
Pulmonary capillary wedge (mm Hg)	9±1	15±1	0.02*
Systolic pulmonary arterial (mm Hg)	31±5	40±2	0.16
Diastolic pulmonary arterial (mm Hg)	13±2	17±1	0.15
Pulmonary vascular resistance (Wood units)	2.6±0.6	2.6±0.2	0.98
Cardiac output	4.9±0.8	4.5±0.1	0.34
Cardiac index ($L\ m^{-2}\ min^{-1}$)	2.0±0.1	2.2±0.1	0.49

LVAD indicates left ventricular assist device.
*$P<0.05$.

Table 3. Pharmacologic Management in Responders and Nonresponders before LVAD Implantation and at 2 Months after LVAD Implantation

	Responders (n=10)	Nonresponders (n=83)	*P* Value
Medications before LVAD Implantation			
Beta blockers, n (%)	8 (80)	58 (70)	0.72
Beta blockers, dose	1.2±0.3	0.9±0.1	0.35
ACE inhibitors, n (%)	3 (30)	35 (42)	0.52
ACE inhibitors, dose	1.0±0.5	1.2±0.2	0.93
Angiotensin II receptor blockers, n (%)	1 (10)	16 (20)	0.68
Angiotensin II receptor blockers, dose	n/a*	n/a*	n/a*
Aldosterone antagonists, n (%)	5 (50)	53 (64)	0.49
Aldosterone antagonists, dose	1.3±0.3	1.1±0.1	0.64
Diuretics, n (%)	10 (100)	82 (99)	0.99
Diuretics, dose	2.1±0.4	2.7±0.2	0.36
Medications at 2 mo post-LVAD implantation			
Beta blockers, n (%)	7 (70)	39 (48)	0.32
Beta blockers, dose	0.8±0.2	0.7±0.1	0.78
ACE inhibitors, n (%)	3 (30)	24 (30)	0.99
ACE inhibitors, dose	1.8±0.8	1.1±0.2	0.31
Angiotensin II receptor blockers, n (%)	3 (30)	6 (8)	0.06
Angiotensin II receptor blockers, dose	1.0±0.5	0.7±0.1	0.47
Aldosterone antagonists, n (%)	3 (30)	24 (30)	0.99
Aldosterone antagonists, dose	1.5±0.5	1.0±0.1	0.15
Diuretics, n (%)	8 (80)	69 (86)	0.63
Diuretics, dose	1.1±0.4	1.5±0.2	0.38

Medication dosage normalization: 1 dose of beta blocker=carvedilol 25 mg, 1 dose of ACE inhibitor=lisinopril 10 mg, 1 dose of angiotensin ii receptor blocker=losartan 50 mg, 1 dose of aldosterone blocker=spironolactone 25 mg, 1 dose of diuretic=furosemide 40 mg.

ACE indicates angiotensin-converting enzyme; and LVAD, left ventricular assist device.

*n/a indicates non applicable, *t* test cannot be performed as only 1 subject in the responders' group is on angiotensin II receptor blockers pre-LVAD implantation.

adjusting for all clinical characteristics, comorbidities, and hemodynamic, echocardiographic, and laboratory values, the combination of baseline LV end-systolic diameter and sodium was shown to be the best clinical predictive model (area under curve 0.795, *P*=0.006). However, when directly compared with the cytokine model, the latter remains a numerically superior model for prediction of cardiac improvement (Figure 2b).

DISCUSSION

The recently held National Institutes of Health/National Heart, Lung, and Blood Institute working group has identified that a critical shortcoming in the field of cardiac recovery with mechanical circulatory support is that most studies to date have failed to directly connect and correlate functional cardiac changes with the underlying biological derangements.[3] Although several studies have investigated potential tissue biomarkers, this is the first study to assess the role of circulating biomarkers as predictive of LVAD-mediated cardiac improvement. By performing a relatively large-scale human tissue and serum analysis, we demonstrated decreased TNFα protein in the myocardium of responders. In addition, lower cytokine levels were measured in the serum of patients who improved their cardiac function after LVAD unloading and circulatory support. These findings suggest that lower cardiac and systemic inflammatory burden is associated with higher likelihood of cardiac improvement after mechanical support (Figure 3). We identified a 2-cytokine model that could serve as a novel predictor of LVAD-mediated cardiac structural and functional improvement.

Inflammatory cytokines have been associated with the severity and progression of HF.[31,32] Whether the activation of inflammatory pathways plays a causative role in the development of HF syndrome or just represents an epiphenomenon and a marker of disease severity is a subject of ongoing investigation.[33] Previous observational human LVAD studies are characterized by a small sample size, limited access to human cardiac tissue and blood, and a limited number of measured cytokines. In our study, we took advantage of the multiplex technology that allows the detection of multiple cytokines, and we applied this assay to cardiac tissue and serum obtained from a cohort of 93 patients with advanced HF at the time of device implantation. Baseline, preintervention cytokine levels were correlated with LV function after LVAD unloading, as defined by serial echocardiographic assessment. The goal of our study

Table 4. Comparison of Cardiac Tissue Cytokine Levels Between Responders and Nonresponders at the Time of LVAD Implantation

	Responders (n=10)	Nonresponders (n=83)	*P* Value*
Tumor necrosis factor alpha	0.29 (0.22–0.36)	0.56 (0.34–1.01)	0.03
IL-2	0.32 (0.21–1.01)	0.24 (0.02–0.96)	0.16
IL-5	0.18 (0.13–0.39)	0.18 (0.06–0.32)	0.19
IL-6	6.14 (3.06–10.13)	4.84 (3.68–6.41)	0.19
IL-7	1.02 (0.67–1.37)	0.95 (0.49–5.20)	0.25
IL-8	0.23 (0.17–0.29)	0.20 (0.12–0.30)	0.20
IL-1β	1.30 (1.22–1.85)	1.25 (0.05–1.88)	0.16
IL-13	0.63 (0.18–1.44)	0.94 (0.55–5.01)	0.16

IL indicates interleukin; and LVAD, left ventricular assist device.

*Benjamini-Hochberg false discovery rate adjusted *P* values, using a q=0.20.

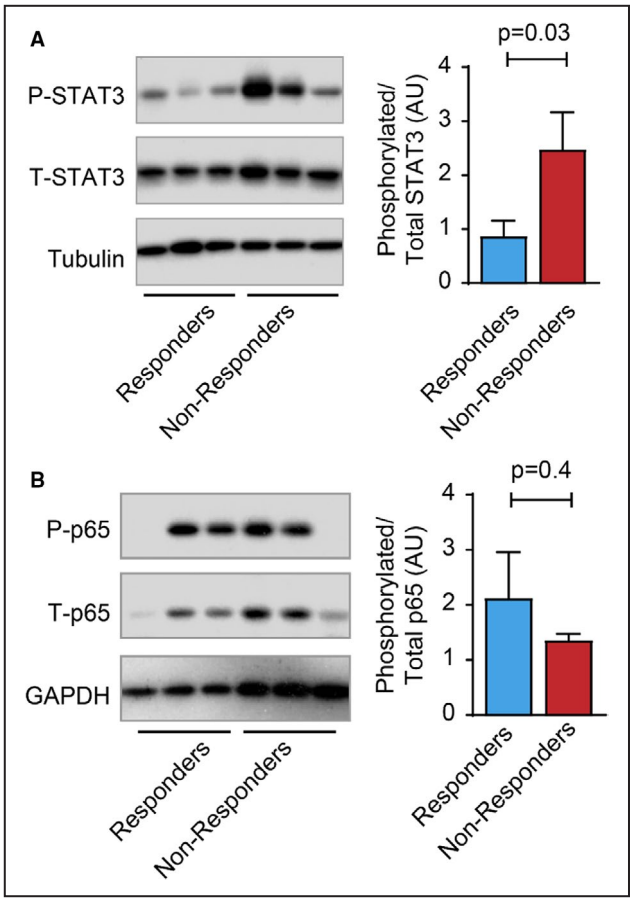

Figure 1. Inflammation-associated transcription factor levels in cardiac tissue of responders and nonresponders. **A**, Decreased ratio of phosphorylated/total signal transducer and activator of transcription 3 (P-STAT3/T-STAT3) in the cardiac tissue of responders. **B**, The ratio of phosphorylated/total p65 does not differ significantly between responders and nonresponders. AU indicates Arbitrary Units.

was to identify cytokine profiles that could predict improvement of LV function after mechanical unloading and circulatory support. Our tissue analysis demonstrated that IL-6, IL-1β, IL-7, and IL-13 are detected in higher levels in the human myocardium compared with IL-8, TNFα, IL-5, and IL-2. Of note, the protein levels of TNFα were significantly lower in responders compared with nonresponders. There was no difference in the tissue levels for the rest of the cytokines between the 2 study groups. These findings suggest that despite the low expression levels, TNFα may play a more important role in the cellular mechanisms mediating cardiac recovery compared with other cytokines.

As mentioned, there is a paucity of LVAD studies looking into the predictive ability of baseline circulating cytokines in terms of subsequent cardiac improvement; however, prior studies did investigate the relationship between pre-LVAD cytokines and post-LVAD adverse events and clinical outcomes (other than cardiac improvement). In a study of 32 patients with advanced

Table 5. Comparison of Serum Cytokine Levels Between Responders and Nonresponders at the Time of LVAD Implantation

	Responders (n=10)	Nonresponders (n=83)	*P* Value*
Tumor necrosis factor alpha	6.04 (3.61–7.85)	11.89 (6.70–16.29)	0.005
IL-2	0.48 (0.05–1.67)	0.94 (0.30–1.93)	0.09
IL-4	0.01 (0.01–0.02)	0.21 (0.01–1.98)	0.02
IL-5	0.18 (0.09–0.33)	0.41 (0.20–0.87)	0.02
IL-6	3.75 (1.52–9.91)	20.42 (7.07–59.6)	0.005
IL-7	0.67 (0.27–1.09)	2.74 (1.02–5.65)	0.005
IL-8	6.89 (4.47–27.42)	13.33 (6.93–24.47)	0.08
IL-1β	0.05 (0.04–0.41)	0.11 (0.05–0.30)	0.10
IL-13	0.02 (0.02–0.02)	0.48 (0.05–3.46)	0.005
IL-12p70	0.30 (0.10–0.90)	0.91 (0.23–4.25)	0.05
IL-10	13.76 (11.33–65.72)	34.47 (21.46–97.96)	0.05
Interferon gamma	1.81 (0.26–2.19)	4.87 (2.65–10.86)	0.006

IL indicates interleukin; and LVAD, left ventricular assist device.
*Benjamini-Hochberg false discovery rate adjusted *P* values, using a q=0.20.

HF, serum cytokine levels were similar before LVAD implantation and did not correlate with post-LVAD implantation adverse events.[34] This study included patients who were supported with intracorporeal and extracorporeal, pulsatile and nonpulsatile devices. In contrast, in another study of 41 patients undergoing implantation of intracorporeal continuous-flow device, higher preimplantation IL-6 levels were associated with longer intensive care unit stay and development of postoperative multiorgan failure.[35] Our serum cytokine analysis was more robust and included multiple targets. In agreement with the latter study, we found significantly higher levels of IL-6 in the serum of nonresponders, whereas there was no difference in IL-8 levels. Furthermore, we found higher levels of TNFα, IL-4, IL-5, IL-7, IL-13, and IFNγ levels, suggesting that higher systemic inflammatory burden could negatively affect LVAD-mediated cardiac improvement. After performing a dichotomous multivariable analysis, we identified a 2-cytokine model (IFNγ and TNFα) that was highly predictive of cardiac improvement.

In our study we provide evidence that TNFα could be used as a marker of disease severity and consequently as a prognostic tool for early identification of

Table 6. Serum Cytokines Multivariable Analysis

	Cut point (pg/mL)	Odds ratio	95% CI
Interferon gamma	2.25	0.06	0.01–0.35
Tumor necrosis factor alpha	8.31	0.05	0.00–0.43

The final model was limited to these 2 variables.

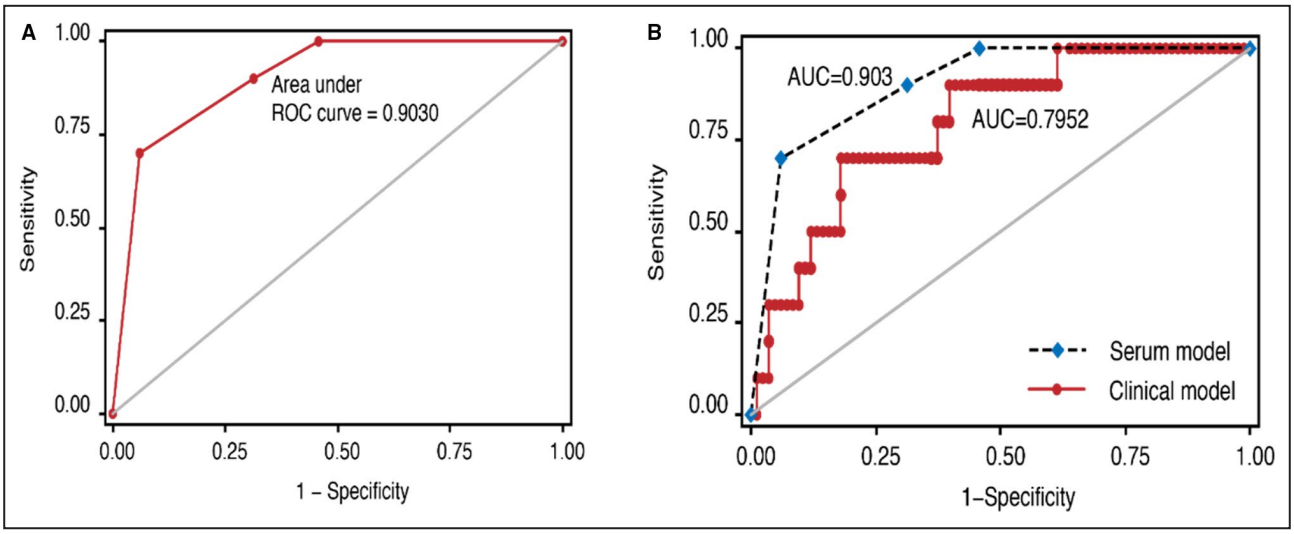

Figure 2. Serum cytokine model compared with clinical model in predicting structural and functional cardiac improvement. **A**, A 2-cytokine model that combines pre-left ventricular assist device serum levels of tumor necrosis factor alpha and interferon gamma and has high sensitivity and specificity in prediction of cardiac recovery. **B**, The 2-cytokine model ("circulating biomarker model") has better performance in predicting myocardial improvement compared with a "clinical variables model" that combines left ventricular end-systolic diameter and serum sodium. AUC indicates area under curve; and ROC, receiver operating characteristic.

responders to mechanical unloading. An important question that could shed light on the role of TNFα in the pathophysiology of HF is the origin of the TNFα detected in the serum. The corresponding TNFα up-regulation in the heart suggests that human myocardium is a source of TNFα production. However, the disproportionate higher serum levels, compared with cardiac levels, suggest that other organs such as skeletal muscle may also contribute to the serum TNFα pool.

The second cytokine that was found to play an important role as a predictor of LVAD-mediated cardiac improvement is IFNγ. Prior animal studies have reported conflicting results regarding the role of IFNγ in HF. In a

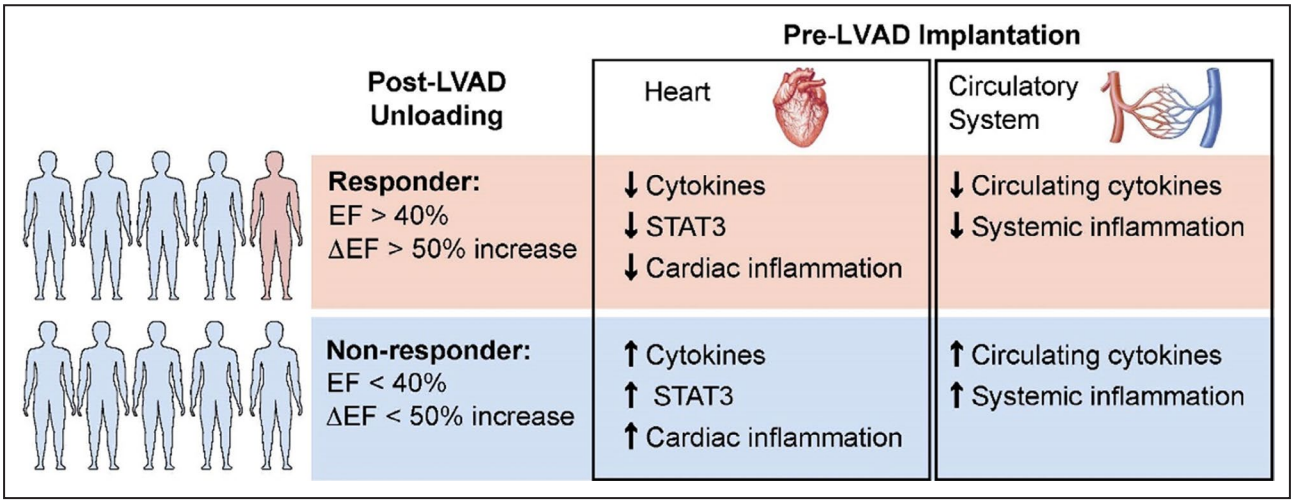

Figure 3. The baseline myocardial and systemic inflammatory burden inversely correlates with myocardial improvement following mechanical circulatory support.
More specifically, tissue and circulating levels of TNFα, as well as circulating IL-5, IL-6, IL-7, IL-13, and IFNγ were lower in responders compared with nonresponders. Additionally, the STAT3, an inflammatory response regulator, was less activated in the myocardial tissue of responders. Guided by our findings, we identified a pre-LVAD circulating 2-cytokine model (IFNγ and TNFα), effectively predicting post-LVAD significant cardiac reverse remodeling. The evaluation of preintervention inflammatory burden of advanced HF candidates could further refine patient selection for advanced HF therapies. Δ indicates delta/change; EF, ejection fraction; HF, heart failure; IFNγ, interferon gamma; IL, interleukin; LVAD, left ventricular assist device; STAT3, signal transducer and activator of transcription 3; and TNFα, tumor necrosis factor alpha.

rat model of liver overexpression of IFNγ, investigators found increased myocardial inflammation and fibrosis along with decreased LV systolic function.[36] However, in another rat study, IFNγ infusion attenuated pressure overload-induced hypertrophy.[37] In patients with HF, IFNγ serum levels were increased compared with controls. Lower IFNγ serum levels have also been associated with less severe peripartum cardiomyopathy and higher chances of cardiac recovery.[38] Our dichotomous analysis showed that IFNγ has a good prognostic value in detecting patients with potential for significant cardiac improvement after mechanical unloading. Again, our data could not distinct whether elevated serum IFNγ is a marker of disease severity or is involved in the direct activation of remodeling pathways of the diseased heart.

Another finding of our study is that systemic and cardiac inflammatory cytokine changes do not necessarily follow the same pattern. Although multiple cytokines were found to be elevated in the serum of our advanced HF population, only TNFα was significantly increased in the cardiac tissue. To further characterize the inflammatory response of the failing human heart, we measured the levels of activated transcription factors, STAT3 and NFkB, that play a central role in the cellular inflammatory processes. STAT3 was less activated in the tissue of responders compared with nonresponders, whereas p65 activation was similar between the 2 study groups. The STAT3 activation is known to be induced by multiple different cytokines, including IL-6, TNFα, and IFNγ,[39] and this finding is in agreement with the elevated levels of the aforementioned cytokines in the serum of nonresponders. Despite the fact that a similar group of cytokines could induce NFkB activation, the phosphorylated p65 levels did not differ between responders and nonresponders. These findings led us to hypothesize that both circulating and cardiac cytokines might induce cardiac inflammatory response specifically through STAT3 but not through NFkB.

Limitations

Despite being one of the largest studies to simultaneously evaluate human cardiac tissue and serum for identification of cardiac improvement predictors, the study population remains relatively small. The enrollment took place in a consortium program (ie, U.T.A.H. Cardiac Transplant Program), which allowed us to better control the quality of the biological samples and echocardiographic and clinical phenotyping of the enrolled patients, but this poses limitations on the generalizability of the results. In addition, the 2-cytokine model was internally validated by bootstrapping. The lack of a separate validation cohort remains a limitation and larger prospective studies are warranted to validate our findings.

CONCLUSIONS

In summary, we demonstrate that baseline, preintervention myocardial and circulating cytokine levels correlate with the potential of the failing human heart to recover after LVAD unloading. A dichotomous multivariable analysis resulted in a 2-cytokine circulating biomarker model with high sensitivity and specificity in predicting cardiac improvement. In the light of the recently published RESTAGE-HF prospective trial, showing that reverse cardiac remodeling during LVAD unloading can be achieved in very high rates and in a reproducible way in many centers, the proposed *cardiac improvement biomarker* could have an impact on clinical practice and warrants further investigation. Specifically, this 2-cytokine circulating biomarker model could be tested as a practical decision aid tool for prognostication in the triage of patients with advanced HF, to the most appropriate therapeutic intervention; either LVAD implantation as bridge-to-recovery or bridge-to-transplant and destination/lifetime therapy. Whether the cytokine upregulation is a marker of disease severity or a driver of the adverse remodeling process through STAT3 activation also warrants further translational and clinical investigations.

ARTICLE INFORMATION

Received November 19, 2020; accepted June 4, 2021.

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Sources of Funding

This work was supported by the American Heart Association Heart Failure Strategically Focused Research Network, 16SFRN29020000 (Drs. Drakos, Stehlik, and Selzman), National Heart, Lung, and Blood Institute R01 HL135121-01 (Dr. Drakos), National Heart, Lung, and Blood Institute R01 HL132067-01A1 (Dr. Drakos), Nora Eccles Treadwell Foundation (Dr. Drakos), and National Heart, Lung, and Blood Institute T32HL007576 (Dr. Taleb).

Disclosures

None.

Supplementary Material

Data S1
Table S1
Reference 40

REFERENCES

1. Molina EJ, Shah P, Kiernan MS, Cornwell WK, Copeland H, Takeda K, Fernandez FG, Badhwar V, Habib RH, Jacobs JP, et al. The society of thoracic surgeons intermacs 2020 annual report. *Ann Thorac Surg*. 2021;111:778–792. doi: 10.1016/j.athoracsur.2020.12.038

2. Birks EJ, Tansley PD, Hardy J, George RS, Bowles CT, Burke M, Banner NR, Khaghani A, Yacoub MH. Left ventricular assist device and drug therapy for the reversal of heart failure. *N Engl J Med*. 2006;355:1873–1884. doi: 10.1056/NEJMoa053063
3. Drakos SG, Pagani FD, Lundberg MS, Baldwin JT. Advancing the science of myocardial recovery with mechanical circulatory support: A working group of the national, heart, lung, and blood institute. *JACC Basic Transl Sci*. 2017;2:335–340.
4. Wever-Pinzon O, Drakos SG, McKellar SH, Horne BD, Caine WT, Kfoury AG, Li DY, Fang JC, Stehlik J, Selzman CH. Cardiac recovery during long-term left ventricular assist device support. *J Am Coll Cardiol*. 2016;68:1540–1553.
5. Maybaum S, Mancini D, Xydas S, Starling RC, Aaronson K, Pagani FD, Miller LW, Margulies K, McRee S, Frazier OH, et al. Cardiac improvement during mechanical circulatory support: a prospective multicenter study of the LVAD Working Group. *Circulation*. 2007;115:2497–2505. doi: 10.1161/CIRCULATIONAHA.106.633180
6. Dandel M, Weng Y, Siniawski H, Potapov E, Drews T, Lehmkuhl HB, Knosalla C, Hetzer R. Prediction of cardiac stability after weaning from left ventricular assist devices in patients with idiopathic dilated cardiomyopathy. *Circulation*. 2008;118:S94–105. doi: 10.1161/CIRCULATIONAHA.107.755983
7. Birks EJ, George RS, Hedger M, Bahrami T, Wilton P, Bowles CT, Webb C, Bougard R, Amrani M, Yacoub MH, et al. Reversal of severe heart failure with a continuous-flow left ventricular assist device and pharmacological therapy: a prospective study. *Circulation*. 2011;123:381–390. doi: 10.1161/CIRCULATIONAHA.109.933960
8. Lamarche Y, Kearns M, Josan K, Bashir J, Ignaszewski A, Kaan A, Kealy J, Moss R, Cheung A. Successful weaning and explantation of the Heartmate II left ventricular assist device. *Can J Cardiol*. 2011;27:358–362. doi: 10.1016/j.cjca.2011.01.005
9. Liden H, Karason K, Bergh CH, Nilsson F, Koul B, Wiklund L. The feasibility of left ventricular mechanical support as a bridge to cardiac recovery. *Eur J Heart Fail*. 2007;9:525–530. doi: 10.1016/j.ejheart.2006.12.003
10. Simon MA, Primack BA, Teuteberg J, Kormos RL, Bermudez C, Toyoda Y, Shah H, Gorcsan J 3rd, McNamara DM. Left ventricular remodeling and myocardial recovery on mechanical circulatory support. *J Card Fail*. 2010;16:99–105. doi: 10.1016/j.cardfail.2009.10.018
11. Drakos SG, Terrovitis JV, Anastasiou-Nana MI, Nanas JN. Reverse remodeling during long-term mechanical unloading of the left ventricle. *J Mol Cell Cardiol*. 2007;43:231–242. doi: 10.1016/j.yjmcc.2007.05.020
12. Wever-Pinzon J, Selzman CH, Stoddard G, Wever-Pinzon O, Catino A, Kfoury AG, Diakos NA, Reid BB, McKellar S, Bonios M, et al. Impact of ischemic heart failure etiology on cardiac recovery during mechanical unloading. *J Am Coll Cardiol*. 2016;68:1741–1752.
13. Topkara VK, Garan AR, Fine B, Godier-Furnémont AF, Breskin A, Cagliostro B, Yuzefpolskaya M, Takeda K, Takayama H, Mancini DM, et al. Myocardial recovery in patients receiving contemporary left ventricular assist devices: Results from the interagency registry for mechanically assisted circulatory Support (INTERMACS). *Circ Heart Fail*. 2016;9:1–11. doi: 10.1161/CIRCHEARTFAILURE.116.003157
14. Barton PJ, Felkin LE, Birks EJ, Cullen ME, Banner NR, Grindle S, Hall JL, Miller LW, Yacoub MH. Myocardial insulin-like growth factor-I gene expression during recovery from heart failure after combined left ventricular assist device and clenbuterol therapy. *Circulation*. 2005;112:I46–50. doi: 10.1161/01.CIRCULATIONAHA.105.525873
15. Birks EJ, Hall JL, Barton PJR, Grindle S, Latif N, Hardy JP, Rider JE, Banner NR, Khaghani A, Miller LW, et al. Gene profiling changes in cytoskeletal proteins during clinical recovery after left ventricular-assist device support. *Circulation*. 2005;112:I57–64. doi: 10.1161/CIRCULATIONAHA.104.526137
16. Torre-Amione G, Stetson SJ, Youker KA, Durand JB, Radovancevic B, Delgado RM, Frazier OH, Entman ML, Noon GP. Decreased expression of tumor necrosis factor-alpha in failing human myocardium after mechanical circulatory support : A potential mechanism for cardiac recovery. *Circulation*. 1999;100:1189–1193.
17. Seidel T, Navankasattusas S, Ahmad A, Diakos NA, Xu WD, Tristani-Firouzi M, Bonios MJ, Taleb I, Li DY, Selzman CH, et al. Sheet-like remodeling of the transverse tubular system in human heart failure impairs excitation-contraction coupling and functional recovery by mechanical unloading. *Circulation*. 2017;135:1632–1645. doi: 10.1161/CIRCULATIONAHA.116.024470
18. Dick SA, Epelman S. Chronic heart failure and inflammation: what do we really know? *Circ Res*. 2016;119:159–176. doi: 10.1161/CIRCRESAHA.116.308030
19. Parissis JT, Farmakis D, Nikolaou M, Birmpa D, Bistola V, Paraskevaidis I, Ikonomidis I, Gaitani S, Venetsanou K, Filippatos G, et al. Plasma B-type natriuretic peptide and anti-inflammatory cytokine interleukin-10 levels predict adverse clinical outcome in chronic heart failure patients with depressive symptoms: a 1-year follow-up study. *Eur J Heart Fail*. 2009;11:967–972. doi: 10.1093/eurjhf/hfp125
20. Drakos SG, Wever-Pinzon O, Selzman CH, Gilbert EM, Alharethi R, Reid BB, Saidi A, Diakos NA, Stoker S, Davis ES, et al. Magnitude and time course of changes induced by continuous-flow left ventricular assist device unloading in chronic heart failure: insights into cardiac recovery. *J Am Coll Cardiol*. 2013;61:1985–1994. doi: 10.1016/j.jacc.2013.01.072
21. Clarke R, Valdes-Marquez E, Hill M, Gordon J, Farrall M, Hamsten A, Watkins H, Hopewell JC. Plasma cytokines and risk of coronary heart disease in the PROCARDIS study. *Open Heart*. 2018;5:e000807. doi: 10.1136/openhrt-2018-000807
22. Anroedh SS, Akkerhuis KM, Oemrawsingh RM, Garcia-Garcia HM, Brankovic M, Regar E, van Geuns R-J, Serruys PW, Daemen J, van Mieghem NM, et al. Associations of 26 circulating inflammatory and renal biomarkers with near-infrared spectroscopy and long-term cardiovascular outcome in patients undergoing coronary angiography (ATHEROREMO-NIRS Substudy). *Curr Atheroscler Rep*. 2018;20:52. doi: 10.1007/s11883-018-0752-8
23. Wodsedalek DJ, Paddock SJ, Wan TC, Auchampach JA, Kenarsary A, Tsaih SW, Flister MJ, O'Meara CC. IL-13 promotes in vivo neonatal cardiomyocyte cell cycle activity and heart regeneration. *Am J Physiol Heart Circ Physiol*. 2019;316:H24–H34. doi: 10.1152/ajpheart.00521.2018
24. Bartekova M, Radosinska J, Jelemensky M, Dhalla NS. Role of cytokines and inflammation in heart function during health and disease. *Heart Fail Rev*. 2018;23:733–758. doi: 10.1007/s10741-018-9716-x
25. Knight PR, Sreekumar A, Siddiqui J, Laxman B, Copeland S, Chinnaiyan A, Remick DG. Development of a sensitive microarray immunoassay and comparison with standard enzyme-linked immunoassay for cytokine analysis. *Shock*. 2004;21:26–30. doi: 10.1097/01.shk.0000101668.49265.19
26. Elyasi A, Voloshyna I, Ahmed S, Kasselman LJ, Behbodikhah J, De Leon J, Reiss AB. The role of interferon-gamma in cardiovascular disease: an update. *Inflamm Res*. 2020;69:975–988.
27. Gordon JW, Shaw JA, Kirshenbaum LA. Multiple facets of NF-kappaB in the heart: to be or not to NF-kappaB. *Circ Res*. 2011;108:1122–1132.
28. Yin MY, Ruckel S, Kfoury AG, McKellar SH, Taleb I, Gilbert EM, Nativi-Nicolau J, Stehlik J, Reid BB, Koliopoulou A, et al. Novel model to predict gastrointestinal bleeding during left ventricular assist device support. *Circ Heart Fail*. 2018;11:e005267. doi: 10.1161/CIRCHEARTFAILURE.118.005267
29. Royston P, Sauerbrei W. Bootstrap assessment of the stability of multivariable models. *Stata J*. 2009;9:547–570. DOI: 10.1177/1536867X0900900403.
30. Vittinghoff E, Glidden DV, Shiboski SC, Regression MCE. *Methods in Biostatistics: Linear, Logistic, Survival, and Repeated Measures Models*, 2nd: ed. New York: Springer-Verlag; 2012.
31. Kaur K, Sharma AK, Singal PK. Significance of changes in TNF-alpha and IL-10 levels in the progression of heart failure subsequent to myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2006;291:H106–H113.
32. Kubota T, Miyagishima M, Alvarez RJ, Kormos R, Rosenblum WD, Demetris AJ, Semigran MJ, Dec GW, Holubkov R, McTiernan CF, et al. Expression of proinflammatory cytokines in the failing human heart: comparison of recent-onset and end-stage congestive heart failure. *J Heart Lung Transplant*. 2000;19:819–824. doi: 10.1016/S1053-2498(00)00173-X
33. Heidenreich P. Inflammation and heart failure: therapeutic or diagnostic opportunity? *J Am Coll Cardiol*. 2017;69:1286–1287. doi: 10.1016/j.jacc.2017.01.013
34. Caruso R, Campolo J, Verde A, Botta L, Cozzi L, Parolini M, Milazzo F, Nonini S, Martinelli L, Paino R, et al. Relationship between early inflammatory response and clinical evolution of the severe multiorgan failure in mechanical circulatory support-treated patients. *Mediators Inflamm*. 2014;2014: 281790. doi: 10.1155/2014/281790
35. Caruso R, Botta L, Verde A, Milazzo F, Vecchi I, Trivella MG, Martinelli L, Paino R, Frigerio M, Parodi O. Relationship between pre-implant interleukin-6 levels, inflammatory response, and early outcome in patients

supported by left ventricular assist device: a prospective study. *PLoS One*. 2014;9:e90802. doi: 10.1371/journal.pone.0090802
36. Reifenberg K, Lehr HA, Torzewski M, Steige G, Wiese E, Kupper I, Becker C, Ott S, Nusser P, Yamamura K, et al. Interferon-gamma induces chronic active myocarditis and cardiomyopathy in transgenic mice. *Am J Pathol*. 2007;171:463–472.
37. Jin H, Li W, Yang R, Ogasawara A, Lu H, Paoni NF. Inhibitory effects of interferon-gamma on myocardial hypertrophy. *Cytokine*. 2005;31:405–414.
38. Forster O, Hilfiker-Kleiner D, Ansari AA, Sundstrom JB, Libhaber E, Tshani W, Becker A, Yip A, Klein G, Sliwa K. Reversal of IFN-gamma, oxLDL and prolactin serum levels correlate with clinical improvement in patients with peripartum cardiomyopathy. *Eur J Heart Fail*. 2008;10:861–868.
39. Haghikia A, Stapel B, Hoch M, Hilfiker-Kleiner D. STAT3 and cardiac remodeling. *Heart Fail Rev*. 2011;16:35–47. doi: 10.1007/s10741-010-9170-x
40. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005;18:1440–1463. doi: 10.1016/j.echo.2005.10.005

Supplemental Material

Data S1.

Supplemental Methods

Echocardiographic Evaluation:

The first set of echocardiographic images was obtained while the LVAD was providing full support. Subsequently, the speed of the LVAD was gradually reduced to the lowest setting recommended by the manufacturer, and a second set of echocardiographic images was obtained approximately 30 minutes later ("turn-down study"). The turn-down echocardiographic studies were performed only on therapeutic international normalized ratio (2.0 to 3.0). Turn-down echocardiographic studies were not performed in patients with a history of stroke/transient ischemic attack, LVAD thrombosis, hemolysis, difficulties in achieving optimal anticoagulation, or during subtherapeutic international normalized ratio. LV wall thickness, internal dimensions and their derivatives LV mass and fractional shortening were obtained from two-dimensional echocardiographic images in accordance with current American Society of Echocardiography (ASE) guidelines [1]. Assessment of LV volumes and LV Ejection Fraction was performed using the apical 4- and 2-chamber views. The endocardial border at both end-systole and end-diastole was manually traced and the systolic and diastolic volumes were determined using the biplane modified Simpson formula (40). All the echocardiograms were interpreted by 2 independent readers (M.Y.Y., S.G.D.).

Multiplex Immunoassay

Cytokines in cardiac tissue and serum were measured using Multiplex Immunoassays (Millipore). This assay detects free cytokines. The immunoassay signal was detected using the Luminex 200 Multiplexing Instrument. The intra-assay % coefficient variation is <5%. The assay sensitivity range is 0.11-8.17 pg/ml.

Table S1. Serum cytokines univariate analysis.

	Cut point (pg/ml)	Odds Ratio	95% CI
TNFa	8.31	0.05	0.01-0.42
IL2	0.06	0.12	0.03-0.53
IL4	0.14	0.15	0.03-0.75
IL5	0.24	0.20	0.05-0.82
IL6	9.92	0.12	0.02-0.61
IL7	1.1	0.09	0.02-0.47
IL8	7.84	0.24	0.06-0.93
IL1b	0.06	0.30	0.08-1.17
IL13	0.05	0.05	0.01-0.26
IL 12p70	0.44	0.20	0.05-0.82
IL10	14.08	0.14	0.03-0.54
IFNg	2.25	0.06	0.01-0.33

IFN: interferon, IL: interleukin, TNF: Tumor Necrosis Factor