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Abstract

Purpose: Left ventricular assist device (LVAD) support is associated with peripheral vascular abnormalities beyond those associated with heart failure (HF). These abnormalities are associated with persistent functional impairments that adversely impact quality of life (QoL). Methods for measuring peripheral vascular function in this population are needed. **Methods:** This pilot study investigated the use of contrast-enhanced ultrasound (CEUS) using standardized protocols to estimate changes in peripheral (quadriceps) muscle perfusion among HF patients (INTERMACS profile 3) undergoing LVAD implantation (n=7). Patients were then stratified by those who did ("responders", n=4) and did not ("non-responders", n=3) report QoL improvement with LVAD support. **Results:** Serial measurements obtained pre-operatively and 3 months following LVAD implantation showed no significant change ($p>0.23$) in muscle perfusion by all CEUS-based measures at rest or with exercise stimulus for the overall population. Responders exhibited improved muscle perfusion at rest ($p=0.043$) and decreased time to peak contrast enhancement ($p=0.010$) at 3 months compared to baseline, suggesting improved delivery of blood to the extremities post-LVAD. Non-responders exhibited unchanged resting muscle perfusion ($p>0.99$), time to peak contrast enhancement ($p=0.59$), and response to exercise stimulus ($p>0.99$) following LVAD therapy. **Conclusion:** Our findings suggest that CEUS evaluation is a promising non-invasive, quantitative modality for real-time assessment of peripheral vasculature and muscle perfusion as an indication of treatment response in LVAD recipients, and that this modality may capture perfusion measures important to QoL following LVAD implantation.

Introduction

Peripheral abnormalities, including those related to vascular function and skeletal muscle, contribute to functional impairment and poor quality of life (QoL) in advance heart failure (HF) patients, and have been implicated in frailty [1-3]. Left ventricular assist devices (LVADs), while effective at correcting central hemodynamic derangements associated with advanced left HF, may not correct these peripheral abnormalities. In fact, recent studies suggest continuous flow LVADs are associated with additional peripheral vascular dysfunction, and such dysfunction may contribute to persistent functional impairment and frailty, as well as GI bleeding [4-9].

While many frail LVAD recipients experience improvement in QoL with LVAD support, up to half remain frail following LVAD implantation and report persistent poor QoL despite LVAD support [4, 10-13]. These poorly-responding patients have similar pre-operative profiles as those in whom frailty and QoL improve, and the mechanisms related to this variable response to LVAD support are not known. We hypothesized that peripheral vascular abnormalities adversely impact muscle perfusion, contributing to persistent frailty, functional impairment, and poor QoL experienced by some HF patients following LVAD implantation.

In an effort to develop methods to study this further, this pilot study sought to investigate the novel application of contrast-enhanced ultrasound (CEUS) to quantify skeletal muscle perfusion in frail HF patients pre- and post-LVAD implantation. CEUS is a safe, non-invasive technique that utilizes ultrasound contrast agents to provide image enhancement [14-16]. These contrast agents are shell-stabilized, gas-filled, non-toxic microbubbles that, when injected intravenously, act as harmonic oscillators within the ultrasound beam to increase the impedance mismatch and enhance the resulting ultrasound image [14, 16]. CEUS is particularly useful in imaging perfusion, improving differentiation between normal and abnormal flow conditions and

tissue delineation [16, 17]. While CEUS has been shown to provide valuable information regarding limb perfusion in patients with peripheral artery disease [18, 19], this technology has not yet been applied to muscle perfusion in the LVAD population.

Methods

Enrollment and Clinical Evaluation

Advanced HF patients approved for HeartMate II continuous flow LVAD (Thoratec, Pleasanton, CA) implantation at Thomas Jefferson University Hospital (from April 2015 through May 2017) were considered for enrollment in this IRB-approved study, provided that they were ambulatory and not dependent on temporary mechanical circulatory support (INTERMACS profile 3 or higher) at the time of pre-operative baseline testing. Nine patients agreed to participate and provided signed informed consent, and seven of these patients completed follow up assessments and are included in the present analysis (study n = 7).

CEUS exams (detailed below) and other clinical assessments were performed according to standardized protocols by trained study personnel prior to LVAD implantation (baseline) and 3 months post-implantation. The clinical assessments included clinical frailty evaluation [20], knee extensor strength, and QoL based on the Kansas City Cardiomyopathy Questionnaire (KCCQ).

Ultrasound Evaluation

CEUS exams were conducted on a standardized portion of the quadriceps muscle, measured as half the distance between the patient's femoral head and patella. The scan was first performed in a resting state. A second exam was then performed in a fatigue state after an exercise stimulus consisting of knee extension while an examiner provided resistance of approximately

50% of the patient's maximum knee extensor strength with a handheld MicroFET 2 dynamometer (Hoggan Scientific, Salt Lake City, UT) until exhaustion (i.e., until the participant was no longer able to generate at least 50% of maximum knee extensor force).

CEUS scanning was performed using a Siemens S3000 Helx Evolution scanner (Siemens Healthineers, Mountain View, CA) equipped with a C6 probe. For each of the CEUS exams, a bolus injection of 0.3 mL of the UCA Definity (Lantheus Medical Imaging, N. Billerica, MA) was administered intravenously. Definity is approved by the FDA for cardiac imaging, and this study represents an off-label use for estimation of skeletal muscle perfusion. Definity has been shown to effectively measure muscle perfusion in various applications [19, 21, 22]. CEUS imaging of muscle perfusion can be augmented in patients with reduced blood flow, such as patients with peripheral artery disease, by utilizing repetitive UCA destruction-replenishment sequences to analyze muscle reperfusion at the resonant frequency of the microbubbles [19]. Therefore, during each CEUS exam, three destruction-reperfusion sequences were acquired to quantitatively evaluate muscle perfusion. These sequences consisted of destructive US pulses at a mechanical index (MI) of 1.06, which was generated to rupture the UCA within the area of the muscle being imaged, followed by nonlinear imaging at a lower intensity (MI of 0.04) to allow monitoring of the UCA re-perfusion into the muscle.

Time-intensity curves and parametric maps were generated offline using Matlab (MathWorks, Natick, MA) to estimate perfusion over the quadriceps muscle by calculating the slope of the curve from the time contrast was first visualized to the peak intensity [17, 23, 24]. These data were used to calculate estimated muscle perfusion, contrast arrival time, and time to peak contrast enhancement, which is defined as the time from contrast infusion to the point at

which maximum pixel intensity is reached. Muscle area was measured using the calipers feature on the scanner on grayscale images acquired during each scanning session.

Statistical Analysis

Muscle perfusion analyses comparing baseline to 3-months post-LVAD were performed for the population as a whole and stratified by change in KCCQ. An increase of 5 or more points in the KCCQ is considered clinically meaningful [25, 26]. Patients reporting an increase ≥5 points in the KCCQ at the 3 month examination were classified as "responders" to LVAD therapy, and those who did not report improved QoL were classified as "non-responders".

Statistical analysis was performed with GraphPad Prism 8 (GraphPad Software, La Jolla, CA), using 2-way ANOVA and paired t-tests to compare the data within and between response groups and observational time points with a 95% confidence interval. Vital statistics were compared using paired Student's t-tests. Results were collected in triplicate then averaged, and error is reported as standard deviation (SD).

Results

Cohort-Based Evaluation

Baseline patient demographics and characteristics are reported in Table 1. Administration of Definity contrast agent and subsequent CEUS examination were well-tolerated in all patients, with no adverse effects, and provided substantial muscle enhancement imaging (Figures 1 and 2).

When evaluating the cohort as a whole, we found that muscle strength significantly improved from baseline to 3 months post-LVAD (182.8 ± 67.3 dyn vs. 230.1 ± 62.3 dyn, $p <$

0.0001). However, there were no changes in any of the other clinical or physical function parameters ($p > 0.67$).

Patients demonstrated response to exercise demand at baseline, exhibiting reduced time to contrast arrival following exercise (-12.3 ± 7.7 seconds, $p = 0.018$) as well as reduced time to peak contrast following exercise (-20.9 ± 38.4 seconds, $p = 0.036$). Patients retained response to exercise demand at 3 months post-LVAD, with similar average decrease in contrast arrival time following exercise (-14.9 ± 12.0 seconds, $p = 0.003$). However, there were no significant differences in any of the CEUS parameters from baseline to 3 months post-LVAD ($p > 0.09$, Table 1).

Cohort Stratified by Change in QoL

When stratified based on QoL improvement, four patients were classified as responders (17.25 ± 11.87 point increase in KCCQ score) and three were classified as non-responders (6.00 ± 8.19 point decrease in KCCQ score). Clinical evaluation outcomes and vitals for each subgroup are shown in Table 1. Responders were more likely to have ischemic heart failure etiology ($p < 0.0001$) and higher body mass index (BMI) ($p = 0.021$) than those classified as non-responders. Other baseline clinical characteristics were similar between responders and non-responders ($p > 0.06$).

At the 3-month evaluation, responders and non-responders showed no difference in LVAD pump parameters ($p > 0.14$, Table S1), body weight and BMI ($p > 0.12$; Table S1). However, responders exhibited a significantly lower Doppler blood pressure (79.5 ± 3.4 mmHg) compared to non-responders (105.0 ± 5.0, $p = 0.0002$) despite similar blood pressure at baseline. Responders also had a lower pulsatility index (5.9 ± 1.2) compared to non-responders (7.9 ± 0.6, $p = 0.049$). Both groups exhibited similar ($p = 0.70$) improvements in muscle strength from baseline to 3

months post-LVAD (responders increased 46.0 ± 49.9 dyn, p = 0.009; non-responders increased 37.9 ± 40.8 dyn, p = 0.024). Non-responders exhibited slightly decreased quadriceps muscle diameter at 3 months (-0.6 ± 0.5 cm, p = 0.004 compared to baseline), but no change in overall muscle area (-0.5 ± 1.4 cm^2, p = 0.39). Responders exhibited no change in either quadriceps muscle diameter (+0.3 ± 1.5 cm, p = 0.58) or muscle area (+0.5 ± 3.0 cm^2, p = 0.62) following LVAD implantation. Muscle area was similar between responders and non-responders (Table S1, p > 0.28).

Summarized results from CEUS examinations are shown in Table 2. Baseline images from a patient classified as a responder are shown in Figure 1, while images from this same patient at 3-months post-LVAD are shown in Figure 2. There is a noticeable difference in muscle perfusion between the resting state and the fatigue state as seen by the difference in peak contrast enhancement (compare Figs. 1B and 1D), indicating some baseline capacity to meet exercise-induced demand in these peripheral muscles. Responders showed clear contrast enhancement during the resting state 3 month post-LVAD examination (Fig. 2B), suggesting improved peripheral blood flow in this population.

Quantitatively, responders exhibited significantly increased resting quadriceps muscle perfusion from baseline to 3 months post-LVAD (12.5 ± 3.6 mL/s*mg vs. 41.7 ± 31.6 mL/s*mg, p = 0.004), but not fatigue post-exercise (p = 0.70). Non-responders showed no significant difference in quadriceps muscle perfusion from baseline to 3 months post-LVAD at rest or fatigue (p > 0.54, Table 2).

Compared to baseline, time to peak contrast enhancement at 3 months post-LVAD was significantly faster in responders at rest (85.1 ± 42.9 s vs. 51.2 ± 17.9 s, p = 0.010). However, similar improvement was not observed in fatigue (p > 0.99). Among non-responders, the change

in time to peak contrast between baseline and 3 months post-LVAD was numerically longer at rest (+12.9 ± 22.4 s) and fatigue (+8.0 ± 23.1 s), but neither of these differences was significant ($p > 0.59$).

At baseline, responders showed significant reduction in the time needed for contrast to appear in the fatigued quadriceps muscle compared to resting (24.8 ± 8.3 s vs. 42.5 ± 5.1 s, $p = 0.005$). Similarly at 3 months post-LVAD, fatigue state contrast arrival time was significantly less than at rest for responders (24.0 ± 1.8 s vs. 37.5 ± 6.5 s, $p = 0.029$). Non-responders showed no significant differences in contrast arrival time between resting and fatigue state at baseline ($p > 0.99$) nor at 3 months post-LVAD ($p = 0.20$; Table 2). There were no significant differences in resting or fatigue contrast arrival time between baseline and 3 months post-LVAD for either response group ($p > 0.82$).

Discussion

To our knowledge, this is the first study to investigate the use of CEUS to assess skeletal muscle perfusion in advanced HF patients receiving LVAD support. We found CEUS to be feasible and well tolerated at rest and following exercise stimulus even in this advanced HF population. Interestingly, there was no improvement in muscle perfusion for the overall cohort following LVAD implantation. However, in the subgroup reporting improved QoL ("responders"), there were consistent markers of improved delivery of blood to the periphery post-LVAD, including improved resting muscle perfusion and decreased time to peak contrast enhancement compared to baseline. No such improvements were present in LVAD recipients with no improvement in QoL at 3 months ("non-responders"). We also identified baseline differences in CEUS measures following exercise stimulus in responders vs. non-responders. In combination,

these pilot findings support CEUS assessment of muscle perfusion as feasible and clinically meaningful in LVAD recipients.

The lack of overall improvement in skeletal muscle perfusion is surprising given the increase in cardiac output provided by LVAD support. However, the validity of this finding is supported by prior studies finding persistent and worsening peripheral vascular dysfunction with continuous flow LVADs, possibly related to further compromised vasodilation from the lack of pulsatility [4-6]. Some abnormalities have the potential to compromise muscle performance, manifesting as persistent physical frailty and functional impairment [4, 11], with peak oxygen consumption (VO_2) often remaining $\leq$ 50% of predicted despite LVAD support [4, 10, 27].

There are several potential clinically impactful applications of CEUS supported by these promising pilot findings. A recent study by Teigen et al. with computed tomography (CT) found pectoralis muscle size and attenuation could predict clinical outcomes after LVAD implantation, supporting the potential value of including skeletal muscle assessment when considering a patient for LVAD implantation [3]. CEUS evaluation of skeletal muscle perfusion provides real-time assessment of peripheral vascular performance not captured by other measures and with the potential to enhance prognostic models [28].

Improvement in HF-related QoL, such as those measured by the KCCQ, is expected following LVAD implantation. However, the response to LVAD support can be variable, especially in older, frail recipients, in whom up to 50% remain frail and without significant improvement in QoL following LVAD implantation [13, 29]. To date, there is no reliable method to predict this lack of response to LVAD therapy prior to implantation [13, 28, 30-33]. Baseline differences in CEUS measures of response to exercise stimulus in the “responder” vs. “non-

responders" warrant further investigation as potential predictors of subsequent response to LVAD support [28].

Recent studies support the importance of peripheral vascular function and skeletal muscle performance to health status in patents post-LVAD [34, 35]. Muscle strength and peak oxygen uptake are independently associated with KCCQ outcomes, and knee extensor strength specifically appears important to the health status of patients post-LVAD implantation [29, 34, 35]. Our findings support peripheral vascular performance and related muscle perfusion as plausible mechanisms for persistent frailty, functional impairments and poor QOL despite LVAD support. If validated in a larger cohort, CEUS assessments could also potentially be used to assess response to interventions targeting peripheral vasculature and skeletal muscle performance, such as exercise interventions, medical therapies or optimization of LVAD settings [36-38].

This study has several limitations, including the small sample size, with only seven LVAD recipients completing the 3-month follow-up assessments. In addition, all patients were treated with the HeartMate II LVAD; patients receiving the newer HeartMate 3 were not included as this was still an investigational device at the time of the study. Patients were only followed for 3 months following LVAD implantation, at which point many LVAD recipients may still experience active recovery from operative stress and long-standing clinical heart failure [13, 39]. Observations from this study, while encouraging regarding the value of CEUS to assess muscle perfusion, require confirmation in a larger sample size, over a longer duration (e.g. 6 months to a year) and in patients with newly commercially available LVADs, such as the HeartMate 3.

Finally, we limited our pilot study to only the UCA Definity, although *in vitro* and animal *in vivo* studies show that other UCAs are also effective in intermittent destruction-replenishment CEUS perfusion imaging [19, 40]. Calculation of muscle perfusion rates is a useful tool for

estimating blood velocity, especially when assessing peripheral vascular behavior and adaptive response to exercise demand [17, 18, 41]. We also found that skeletal muscle perfusion in these patients increased with successive CEUS destruction-reperfusion pulses together with infusion of UCAs, in agreement with other studies regarding patients with peripheral artery disease [18, 19]. Consequently, the use of CEUS may influence subsequent perfusion measures. However, the CEUS protocol was applied consistently to all study participants at each assessment and this phenomenon is unlikely to account for the study observations.

Conclusion

In this pilot study, we found that CEUS estimation of peripheral muscle perfusion was feasible and well tolerated in advanced HF patients receiving LVAD support. Furthermore, we found that preoperative changes in CEUS measures in response to exercise stimulus and improvements in resting muscle perfusion following LVAD implantation were limited to those patients reporting improved QoL. Although based on a limited sample size, these findings are supported by prior studies reporting peripheral vascular dysfunction and persistent frailty associated with poor QoL in up to 50% of frail LVAD recipients. Based on our findings, we suggest that CEUS evaluation is a promising non-invasive, well-tolerated, quantitative modality for assessment of peripheral vasculature and skeletal muscle perfusion that warrants further study as a potential marker of prognosis and an indication of treatment response in LVAD recipients.

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Tables

Table 1: Pre-LVAD demographics of patient population (n=7), separated into responders (n=4) and non-responders (n=3). Data is reported as mean ± standard deviation.

	Whole Cohort (n=7)	**Responders (n=4)**	**Non-Responders (n=3)**
Pre-Implant Data			
Age, (years)	60.9 ± 5.2	60.8 ± 6.4	61.0 ± 4.6
Sex			
Male, (%)	87.5	75.0	100.0
Race			
Non-Hispanic White, (%)	57.1	75.0	33.3
Non-Hispanic Black, (%)	42.9	25.0	66.7
Heart Failure Etiology			
Ischemic, (%)	42.9	75.0	0.0
Non-ischemic, (%)	57.1	25.0	100.0
Ejection Fraction, (%)	15.0 ± 5.8	15.0 ± 7.1	15.0 ± 5.0
Baseline Vitals			
BMI, (kg/m^2)	27.9 ± 5.5	31.3 ± 4.5	23.4 ± 2.6
Systolic Blood Pressure, (mmHg)	105.4 ± 7.6	108.0 ± 9.0	102.0 ± 4.6
Diastolic Blood Pressure, (mmHg)	73.9 ± 6.0	76.3 ± 6.7	70.7 ± 3.8
Muscle Strength, (dyn)	182.8 ± 67.3	220.5 ± 53.7	168.8 ± 48.4
Muscle Diameter, (cm)	6.3 ± 0.8	5.9 ± 0.8	6.8 ± 0.3
Muscle Area, (cm^2)	9.0 ± 1.1	8.5 ± 0.6	9.7 ± 1.3
Characteristic Data			
INTERMACS Profile, (1-7)	3 ± 0	3 ± 0	3 ± 0
Fried Frailty Score, (1-5)	3 ± 1	3 ± 1	4 ± 1
KCCQ Score, (0-100)	70 ± 16	66 ± 19	77 ± 10

*Table 2: CEUS results for whole cohort (n=7), broken down into responders (n=4) and non-responders (n=3). Data is reported as mean ± standard deviation, *p < 0.05, **p < 0.01.*

	Baseline		3 Months Post Implant		Degree of Change Post-LVAD	
Whole Cohort (n=7)						
	Resting	**Fatigue**	**Resting**	**Fatigue**	**Resting**	**Fatigue**
Muscle Perfusion, (mL/s*mg)	31.6 ± 105.8	37.9 ± 33.2	33.7 ± 26.0	34.0 ± 27.6	+2.2 ± 47.4	-3.9 ± 24.7
	+ 6.3 ± 44.8		*+ 0.3 ± 18.7*			
Contrast Arrival Time, (s)	38.6 ± 6.7	26.3 ± 7.3	36.4 ± 8.4	21.6 ± 6.7	- 2.1 ± 10.7	- 4.7 ± 8.9
	*- 12.3 ± 7.7**		*- 14.9 ± 12.0***			
Time to Peak Contrast, (s)	65.7 ± 39.5	44.8 ± 13.2	51.8 ± 21.2	44.5 ± 19.6	- 13.9 ± 51.5	- 0.2 ± 22.3
	*- 20.9 ± 38.4**		*- 7.4 ± 5.5*			
Responders (n=4)						
	Resting	**Fatigue**	**Resting**	**Fatigue**	**Resting**	**Fatigue**
Muscle Perfusion, (mL/s*mg)	12.5 ± 3.6	36.8 ± 44.3	41.7 ± 31.6	43.1 ± 33.1	+ 29.2 ± 36.4**	+ 6.4 ± 28.7
	+ 24.3 ± 44.9		*+ 1.5 ± 25.3*			
Contrast Arrival Time, (s)	42.5 ± 5.1	24.8 ± 8.3	37.5 ± 6.5	24.0 ± 1.8	- 5.0 ± 10.0	- 0.8 ± 8.6
	*- 17.8 ± 4.8***		*- 13.5 ± 5.7**			
Time to Peak Contrast, (s)	85.1 ± 42.9	49.9 ± 13.5	51.2 ± 17.8	43.5 ± 17.9	- 34.0 ± 58.6**	- 6.4 ± 20.4
	*- 35.3 ± 47.8***		*- 8.0 ± 7.0*			
Non-Responders (n=3)						
	Resting	**Fatigue**	**Resting**	**Fatigue**	**Resting**	**Fatigue**
Muscle Perfusion, (mL/s*mg)	57.0 ± 163.4	39.3 ± 7.5	23.2 ± 9.6	21.8 ± 10.4	- 33.8 ± 36.8	- 17.5 ± 10.4
	- 17.7 ± 38.7		*- 1.3 ± 9.3*			
Contrast Arrival Time, (s)	33.3 ± 4.9	28.3 ± 6.8	35.0 ± 12.0	18.3 ± 10.0	+ 1.7 ± 12.5	- 10.0 ± 7.2
	- 5.0 ± 2.0		*- 16.7 ± 19.4*			
Time to Peak Contrast, (s)	39.8 ± 7.1	38.0 ± 9.7	52.6 ± 26.1	46.0 ± 22.7	+ 12.9 ± 22.4	+ 8.0 ± 23.1
	- 1.8 ± 5.7		*- 6.7 ± 4.1*			

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Figure Legends

Figure 1: CEUS images of quadriceps muscle at baseline from a patient classified as a responder. A) Pre-infusion image taken in the resting state, muscle and bone are delineated on the image. B) Image taken during peak contrast enhancement in the resting state, showing little change in contrast enhancement, and therefore little muscle perfusion. C) Pre-infusion image taken in the fatigue state. D) Image taken during peak contrast enhancement in the fatigue state, showing a more noticeable increase in contrast and therefore increased muscle perfusion.

Figure 2: CEUS images of quadriceps muscle at 3 months post-LVAD implantation for the same patient as in Figure 1, who was classified as a responder. A) Pre-infusion image taken in the resting state, muscle and bone are delineated on the image. B) Image taken during peak contrast enhancement in the resting state, showing increased contrast enhancement and therefore increased muscle perfusion (compared to baseline, 1B). C) Pre-infusion image taken in the fatigue state. D) Image taken during peak contrast enhancement in the fatigue state, showing greater contrast enhancement and therefore greater muscle perfusion.