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Pulsatility is a Predictive Marker of Improved Cardiac Function in Patients with Liquid Matrix-treated Left Ventricular Assist Devices

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Abstract

Objective: Left ventricular assist devices (LVADs) are utilized as a bridge to transplant or as destination therapy for patients with end-stage heart failure. Although cardiac offloading from these devices rarely leads to complete remodeling and functional recovery, the use of mesenchymal cells to modulate heart failure has been explored in recent years due to its intrinsic regenerative properties. Current methods of evaluating cardiac function have too much variability, difficulty of access, or require too frequent follow up to create universal weaning protocols. We hypothesized that the administration of amniotic allograft liquid matrix (LM) containing amnion-derived mesenchymal stem cells (aMSCs) in patients with LVADs could improve left ventricular function and be positively associated with pulsatility.

Methods: Flow cytometry, mass spectroscopy, and enzyme-linked immunoassays were used to characterize aMSCs and LM that were administered to 9 patients with LVADs. Results were compared to samples from 7 control patients with LVADs that did not receive aMSC and LM.

Results: Patients who received aMSCs and LM (n=9) demonstrated a significant increase in standardized pulsatility at 30 (P = .007), 90 (P = .02), and 180 (P = .05) days post-implant when compared to control patients who did not receive the treatment (n=7).

Discussion: We conclude that the use of aMSCs and LM in patients with LVADs could be a promising treatment strategy, and pulsatility can be a reproducible and consistent diagnostic metric to evaluate left ventricular function without intra- or inter-observer variability.

Introduction

Heart failure (HF) remains a growing epidemic and a leading healthcare cost. Recent estimates indicate approximately 6.2 million Americans over 20 years of age have HF, and 42.3% of these patients have a 5-year fatality rate after hospitalization. This has resulted in an increased economic burden exceeding $30.7 billion.[1] Although cardiac transplantation is the optimal therapy for end-stage HF, the limitation of donor organs has shifted the need, and increased the use, of left ventricular assist devices (LVADs).

Many regenerative treatment strategies have been attempted to augment and improve functional recovery in patients with LVADs. The application of mesenchymal stem cells (MSCs) has demonstrated significant interest in recent years due to their pluripotency, anti-inflammatory, and immunosuppressive capabilities. In the PROMETHEUS and TAC-HFT clinical trials, MSC-treated cardiac tissue improved contractile function and perfusion, and the treatment reduced infarct size.[2,3]

The addition of extracellular liquid matrix (LM) containing adhesion and signaling proteins is hypothesized to improve regeneration and function through improved cell survival and localization. The feasibility of combining LVAD support and particulate extracellular matrix (P-ECM) through intramyocardial injections was explored in a bovine model.[4] P-ECM+LVAD treatment provided the most significant increase in ejection fraction (EF) and reduced cardiac fibrosis 60 days post-treatment.[4] A similar study was performed in patients and revealed delivery of MSCs allowed for successful weaning 90 days after LVAD implantation compared to the control group.[5] Although these effects have not been consistently reproducible in other studies, this is perhaps due to inconsistent methods and inter-observer bias in evaluating cardiac function and recovery.Thus, our goal was to utilize on-board LVAD diagnostics to identify functional differences between patients administered with amniotic MSC (aMSCs) and LM at the time of LVAD implantation compared to control patients (LVAD only).

A universal and reproducible marker of left ventricular (LV) contractility is needed to determine the patient's response to therapeutic treatments as current methods of determining LV function are prone to intra- and inter-observer variability, are too expensive, or require frequent follow up.[7] Pulsatility is the difference between end-diastolic flow from the peak systolic flow during a single cardiac cycle and indirectly measures contractility. As contractility increases, end-systolic volume decreases, thereby increasing stroke volume. Our group has previously used an in vitro model with a Total Artificial Heart (Syncardia Systems, Inc.) and Donovan Mock Circulation System to accurately correlate pulsatility with cardiac contractility through the variation of preload, afterload, and LV pumping force.[6] Therefore, we retrospectively studied 16 patients who underwent an LVAD implantation to validate pulsatility as a marker of improved cardiac function with myocardial aMSCs+LM treatment.

Methods

Study Design

Internal review board approval was attained for this investigation (#1507990305). Waiver of informed consent was attained to gather information and analyze samples from patients, and no patient's identifiers were used or recorded to protect privacy. HVAD serial numbers and implant dates were used during data collection for analysis.

This single-center, retrospective study included patients who underwent a Heartware Ventricular Assist Device (HVAD, Medtronic) implantation as a bridge to cardiac transplant between 2013-2015. Patients were placed in two groups: Controls (HVAD only), and Treatment (patients received aMSC+LM with HVAD placement at time of chest closure). Every patient who consented to receiving aMSCs+LM (n=9) at the time of HVAD implant received the therapy. Patients who received their HVAD prior to availability of aMSCs+LM (n=7) or did not consent to receiving the additional therapy were placed in the control group.

The treatment (1.2 million aMSCs+LM, 1ml) was delivered via a 22G needle into the LV myocardium through the LV anterior, inferior, and lateral wall. Additionally, the aMSCs+LM mixture was injected off-pump (2.4 million, 2ml mixed with 5ml normal saline) into the right atrium via the right atrial appendage with a 22G needle, steadily over 5 minutes. The aMSCs+LM were derived from human birth tissue donated under informed consent following Cesarean sections. Allografts were processed and packaged at an FDA registered and AATB accredited facility in accordance with Current Good Manufacturing Practice standards.

Flow Cytometry Analysis

Flow cytometric analysis of the aMSCs+LM was performed to identify the cell phenotypes and characterize surface antigen markers including CD90, CD44, CD105, and CD73. Data acquisition and analysis used the 488 nm, 532 nm, and 640 nm laser lines on a BD™ LSR II flow cytometer with FACSDiva™ software (BD Biosciences). Spectral compensation was adjusted using a cell population with a single staining and an unstained control.

Characterization of Membrane and Flow Proteins

Lysates of aMSCs membrane were prepared and underwent SDS-PAGE. After staining with Coomassie Blue, sections were excised, digested, and analyzed by a Velos™ Orbitrap mass spectrometer (Thermo Scientific) after in-line fractionation by a C18 column using Proxeon liquid chromatography. The same method was used to measure protein concentration from the aMSCs+LM.

Acquisition of Log Files

The log files from the HVAD devices were obtained while caring for the patient. Once steady-state was achieved in device variability following HVAD implant (~5-10 days post-implant), a baseline log file data was collected and the exported log files were analyzed in STATA (StatCorp, LLC) and MATLAB (MathWorks). Parameters that were evaluated included the following: time, speed (RPM), average flow values recorded (mLPM), minimum flow values recorded (mLPM), and pulsatility (mLPM). Pulsatility was calculated by a device algorithm that records the differential between peak systolic flow velocity and minimum diastolic flow velocity over a 3-second window every 15 minutes (Figure 1); this method has been validated in another study.[8]

Log File Pulsatility (L/min) = HVAD Estimated Flow $_{max}$ – HVAD Estimated Flow $_{min}$

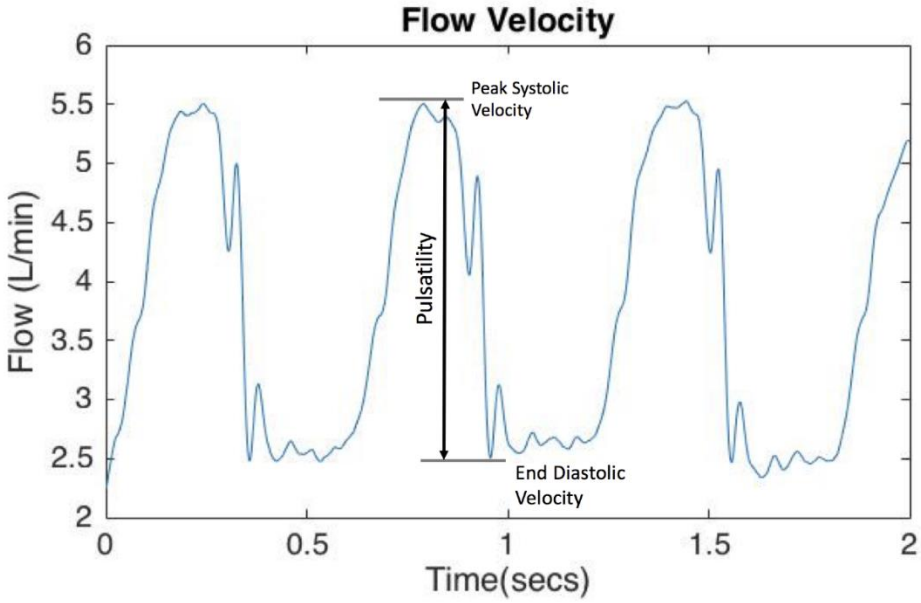

Figure 1. *Pulsatility is the difference between the peak systolic and end-diastolic flow velocities during a single cardiac cycle. Data shown was acquired from a Transonic Flow Meter during modeling with the Donovan Mock Circulation loop, Total Artificial Heart (Syncardia Systsems, Inc), and Heartware Ventricular Assist Device (Medtronic).*

Echocardiography

Echocardiograms were obtained as part of clinical care and interpreted following standard of care protocols.[9] EF was measured and verified by two cardiologists to limit inter-observer bias. Echocardiograms were not available for all patients at every time point. Reported EF values were standardized to baseline EF.

Statistical Methods

Baseline patient characteristic differences were tested using a Fisher's exact test. The standardized pulsatility (SP) for each patient was calculated by using the ratio of pulsatility to baseline pulsatility. We used data from the device log files and the entirety of the pulsatility data within each 5-day window. Differences between control and treatment groups, with respect to SP, were analyzed using a Mann-Whitney nonparametric test with aMSCs+LM administration as the independent variable.

Results

Clinical Characteristics

Baseline characteristics of the control and treated patients were similar (Table 1). At the time of HVAD implantation, 16 patients were screened for therapy. The majority (6/7) of the control patients received cardiopulmonary bypass support during their LVAD implantations, as only one patient was implanted off-pump. However, about half (5/9) of the aMSCs+LM patients patients received cardiopulmonary bypass support, and the four other patients were off-pump. There was no significant difference in hospitalization times (P = .7), bypass times (P = .07), sex (P = .3), renal failure (P = .3), diabetes (P = 1.0), hypertension (P = .6), or previous cardiac surgeries (P = .6). There were no significant differences in medications at baseline, 3-, or 6-months post-LVAD implantation (Supplementary Table A). Similarly, no significant differences in mean arterial pressure (MAP) was found between the groups at any point (Supplementary Table B). Of note, in the treatment group (aMSCs + LM), 6 patients had concomitant procedures (2 tricuspid valves, 1 aortic valve, 2 left atrial appendage ligation, and 1 left ventricular vryoablation) while the control group did not have any.

Table 1. **Demographics and Clinical Characteristics of Study Population.**
Data is presented as median (interquartile range) or number (frequency).

Characteristic	aMSCs+LM (n=9)	LVAD only (n=7)
Age (years)	58.0 (50.0 – 64.0)	59.0 (50.0 -66.0)
Male (%)	8 (88.9%)	4 (57.1%)
Renal Failure	4 (44.4%)	1 (14.3%)
Diabetes	3 (33.3%)	3 (42.9%)
Hypertension	6 (66.7%)	3 (42.9%)
Previous Cardiac Surgery	6 (66.7%)	4 (57.1%)
Mini-thoracotomy/ Full sternotomy	5/4	5/2
Hospitalization (days)	32.0 (21.0-59.0)	30.0 (19.0-78.5)
Bypass Time (mins)	108.0 (107.0-109.0)	98.0 (73.0-117.0)

Pulsatility

There was no statistically significant difference in pulsatility between the aMSCs+LM and control groups at baseline. Over time, the aMSCs+LM group did demonstrate significant increases in pulsatility, whereas the control group did not (Figure 2). Using a Wilcoxon rank-sum test and normalizing pulsatility to baseline, the aMSCs+LM group demonstrated significant increases in pulsatility at 30- (P = .007), 90- (P = .02), and 180- (P = .05) days post-LVAD implantation when compared to the control group (Table 2).

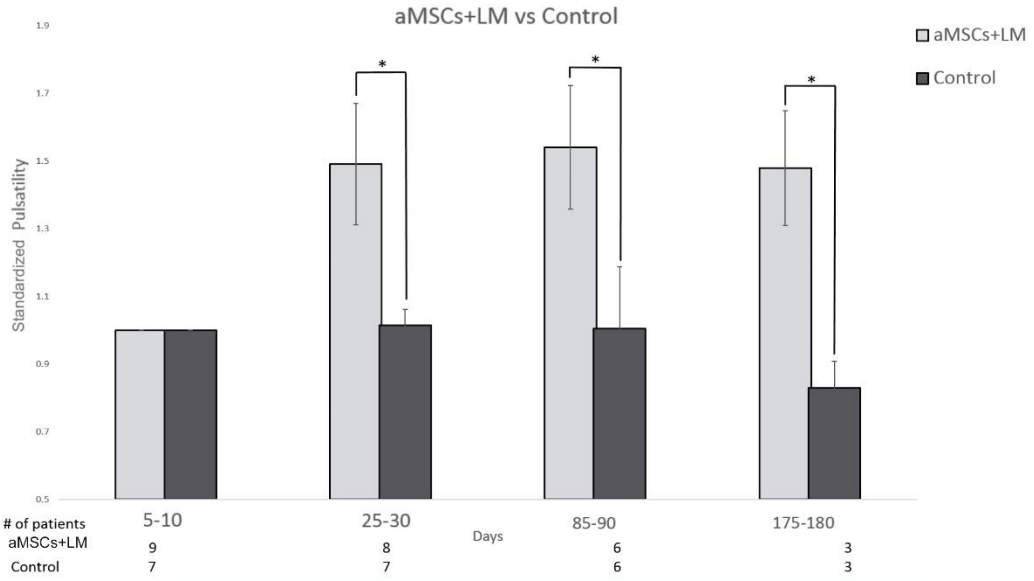

Figure 2. *Standardized pulsatility of treatment and control patients from Day 5 to Day 180 after implantation of left ventricular assist device. Significant differences in pulsatility were found with the treatment group at 30 (P = .007), 90 (P = .02), and 180 (P = .05) days after implantation.*

Echocardiography

At baseline, five (55.5%) from the aMSCs+LM group and seven LVAD only patients had echocardiograms. Subsequent echocardiograms were limited due to lack of follow-up, and three patients received a heart transplant within the follow-up period. There was a non-significant trend toward an increased normalized EF and left ventricular inner diameter (LVID) in the aMSCs+LM patients when compared to the control group (Figure 3).

Adverse Events

No adverse events related to device implantation or aMSC+LM administration including acute device failure, thrombosis, immune reactions, stroke, or

anaphylaxis were reported. There were no device replacements or device-related deaths in either group.

Additionally, the therapy's effects on anti-HLA antibody presence (Panel Reactive Antibody, PRA) were retrospectively analyzed in these patients before and periodically after aMSC+LM injection. Baseline and postoperative class I and II PRA screenings were analyzed, along with peri- and postoperative blood product administration for each patient. Compared to baseline values, all patients' postoperative HLA sensitization either remained the same or decreased.

Table 2. Standardized pulsatility from baseline to 180 days after LVAD implantation is presented alongside significance comparisons between groups

Days	aMSCs+LM			LVAD only			P-value
	N	SP	SD	N	SP	SD	
5-10	9	1.000	0	7	1.000	0	N.S.
15-20	8	1.380	0.517	7	1.014	0.101	0.0270
25-30	8	1.491	0.508	7	1.014	0.126	0.0070
35-40	7	1.516	0.544	6	1.031	0.167	0.0111
45-50	7	1.384	0.607	6	1.016	0.115	0.0688
55-60	8	1.295	0.454	5	0.958	0.085	0.0637
65-70	7	1.380	0.554	5	0.939	0.193	0.1717
75-80	5	1.549	0.628	5	0.905	0.167	0.1230
85-90	6	1.540	0.449	6	1.005	0.198	0.0206
95-100	6	1.358	0.488	6	1.064	0.151	0.0660
105-110	5	1.078	0.222	6	1.094	0.156	0.3961
115-120	5	1.106	0.272	3	1.029	0.211	0.5000
125-130	2	1.156	0.463	2	0.928	0.129	0.5000
135-140	3	1.506	0.492	2	0.947	0.088	0.2000
145-150	6	1.151	0.442	3	1.091	0.187	0.3571
155-160	6	1.302	0.407	2	1.022	0.366	0.2143
165-170	5	1.345	0.409	4	0.925	0.218	0.0952
175-180	3	1.479	0.294	3	0.830	0.136	0.0500

** N, number of patients; SP, standardized pulsatility; SD, standard deviation*

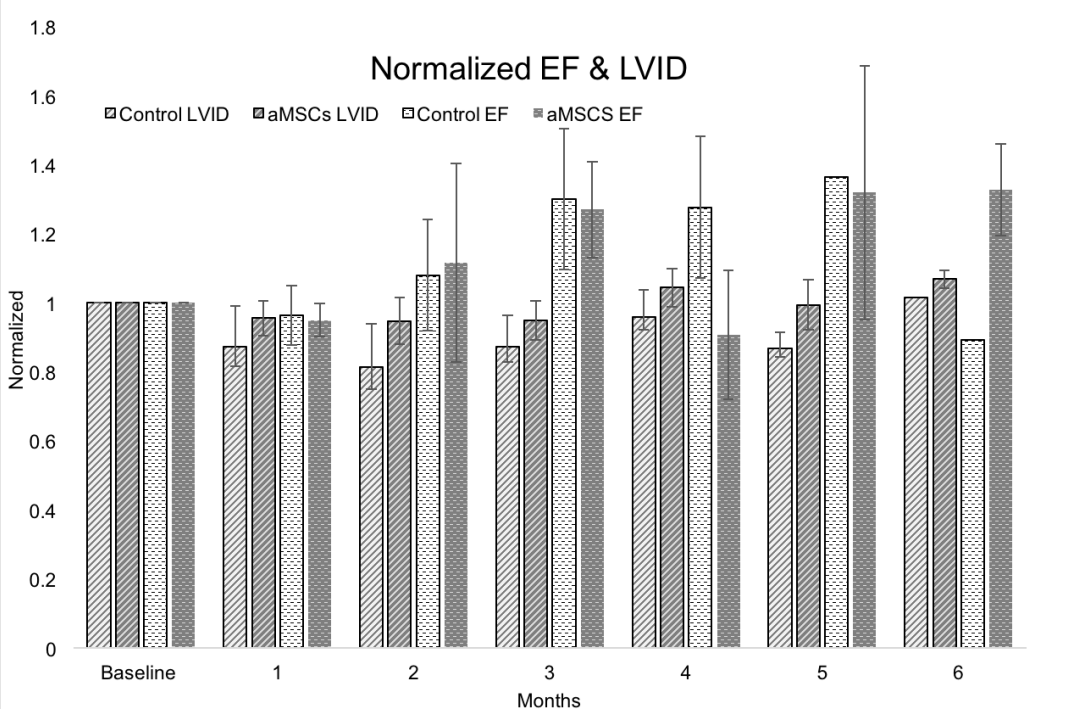

Figure 3. *Comparison of normalized ejection fraction (EF, %) and left venricular internal diameter (LVID, cm) to baseline of the mesenchymal stem cell/liquid matrix (aMSC) group and Control group (left ventricular assist device only) between baseline and six months. The treatment group demonstrated a trend toward increased normalized EF and LVID over the 6-month time period.*

Discussion

Pulsatility is a useful, indirect measure of contractile function in patients with an implanted LVAD. Prior reports of stem cell administration in patients show improvements in EF and infarct size, and stem cell therapy shows the potential for VAD weaning and explantation.[5] Yet, these studies do not have enough consistency to support widespread adoption. Though prior studies found a positive trend in VAD weaning success with stem cell therapy, they did not demonstrate significant improvements.[5] This may be attributed to the administration of mesenchymal precursor cells rather than the mature heterogeneous mixture of aMSCs+LM presented here. Other studies use the primary endpoints of EF or infarct size to determine cardiac status.[2,3] These diagnostic methods are prone to intra-observer and inter-observer variability.[7] Additionally, echocardiograms are taken at discrete time points where the patient's results may not reflect their long-term status. Hence, it is difficult to compare the published studies. Pulsatility offers the advantage of being an objective, reproducible, and consistent method through device diagnostics while providing continuous data, unlike discrete data gathered from other HF diagnostics. This analysis method can be used across different therapy types and implanting centers to create a universal weaning protocol.

This retrospective analysis found that pulsatility, a derivative of stroke volume, increased in patients with advanced HF receiving aMSCs+LM therapy during LVAD implantation.Patients who were treated with aMSCs+LM demonstrated significant increases in pulsatility from baseline values compared to control patients at 30, 90, and 180 days postoperatively. Importantly, the mean standardized pulsatility for the aMSCs+LM group did not fall below 1.00, suggesting contractile function only improved during the study. In comparison, the control (LVAD only) group did not demonstrate improved pulsatility; many patients (4/7) demonstrated reductions in pulsatility over time after implantation. We found no significant differences in MAP between the groups at any point. Afterload was directly proportional to MAP, and our group has previously demonstrated that if afterload remains constant, an increase in pulsatility will only occur with improved cardiac contractility and increased preload.[6,10] This suggests all patients experiencing an improvement in pulsatility also improved their stroke volume and stroke work.

Some hearts may be unable to restore contractile function or remodel even in unloaded conditions. The observed reduction in pulsatility contradicts current literature that suggests unloading the LV increases contractility for patients with end-stage HF.[11] Similarly, the aMSCs+LM group demonstrated a larger positive trend in EF and LVID compared to control. In contrast, EF in the control group returned to baseline within six months. These data support the hypothesis that use of aMSCs+LM improves heart function in contrast to LVAD alone. These data also correspond with the data attained from an in vitro model that showed anticipated pulsatility increase with improved cardiac contractility.[6]

Characterization of aMSCs through flow cytometry confirmed the presence of surface antigens CD73+, CD90+, and CD105+, which is consistent with aMSC lineage (Figure 4, Supplemental Table C). The aMSCs+LM therapy also contained markers that previous studies have demonstrated to have roles in cell survival and proliferation in cardiac models (Supplementary Table D).[12,13] Others proteins notable in the aMSCs+LM therapy included collagen VI and fibronectin, which have shown an increase in endogenous stem cell self-renewal, muscle regeneration, and activation of satellite cells (Supplementary Table D).[14,15]

A substantial portion of the proteins, cytokines, gene expression, and growth factors identified within the aMSCs+LM samples aid in cell-cell interactions, cellular retention, growth proliferation, extracellular matrix integrity, cell migration, angiogenesis, oxidative homeostasis, and cardiomyocyte differentiation. This may explain why patients receiving aMSCs+LM therapy demonstrated significant increases in pulsatility and improved EF compared to LVAD only patients. Improvement of pulsatility in the aMSCs+LM group may be attributed to the administered allograft's immunomodulatory and paracrine factors. Additionally, proteins that synergistically exhibit antimicrobial effects, such as inducible gene-H3 (IG-H3) and natriuretic peptides B precursor (NPPB), are in aMSCs+LM (Supplementary Figure A). This may assist in stem cell retention by inducing an anti-inflammatory environment, particularly in patients supported by VADs.

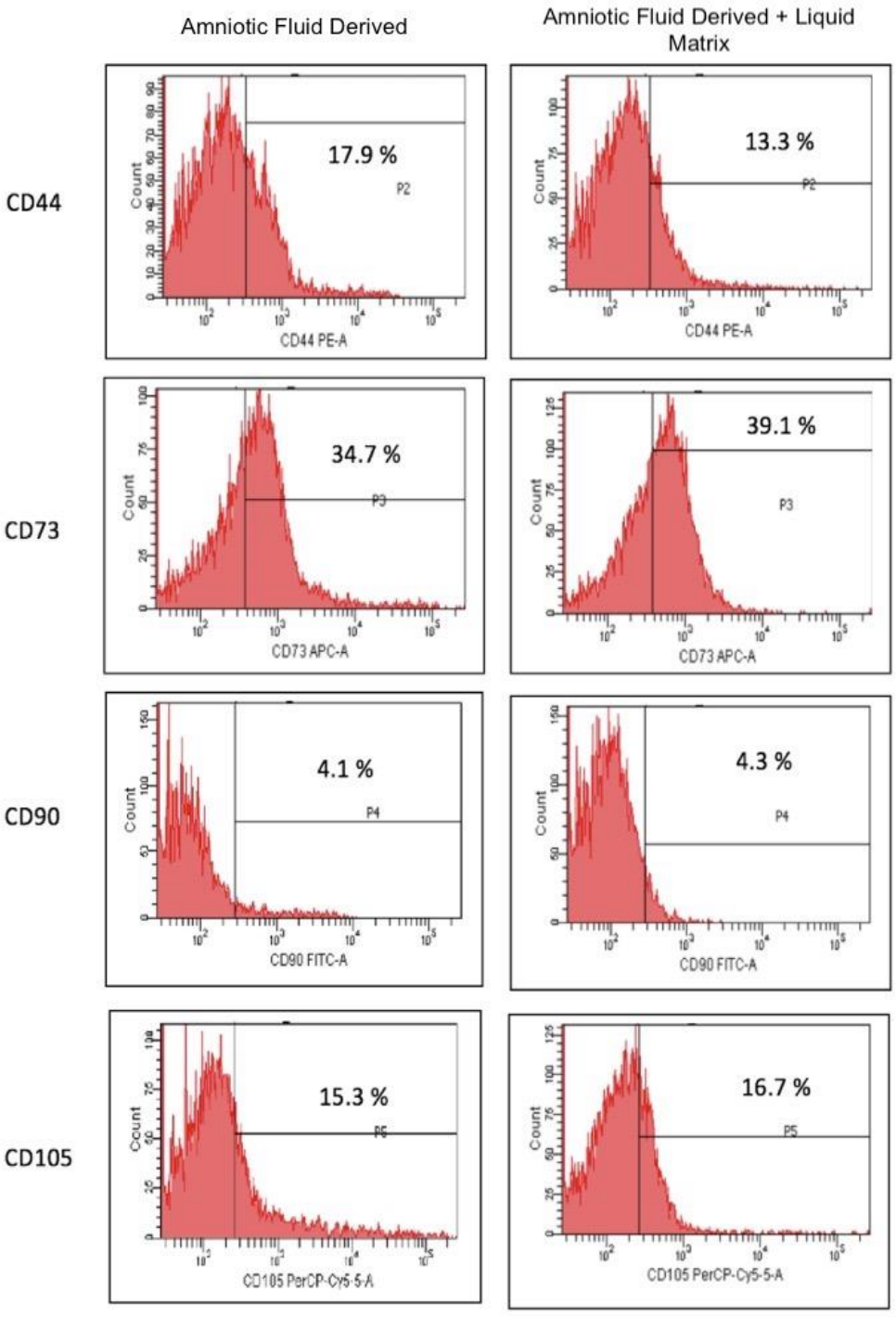

Figure 4. *Histogram of flow cytometric analysis of amnion-derived mesynchymal stem cells confirmting the presence of CD44+, CD73+, CD90+, and CD105+ cells.*

Moreover, a high concentration of NPPB is suggestive of heart failure and functions as a paracrine antifibrotic factor in the heart.

Though the results of this study are promising, it has limitations. Due to the nature of a retrospective study, the groups were non-randomized. The data collection decreased over time due to patients lost to follow up, which limited data analysis and statistical power at later time points. The HVAD data retention was limited to only 31 days due to device specifications. Therefore, there were gaps in collected pulsatility data from patients who were not subsequently evaluated. So while results from this study suggest cardiac status improvement with aMSCs+LM therapy, a larger, prospective study would be necessary to draw meaningful conclusions of the therapy efficacy on a larger scale.

In conclusion, the findings demonstrate the administration of aMSCs+LM improved cardiac function in this limited cohort of patients, but a large-scale study is needed to determine if widespread improvements are observed. The HVAD diagnostic metric, pulsatility, was demonstrated to be a meaningful measure of cardiac contractility in patients, consistent with our group's prior findings from an in vitro model. This suggests pulsatility could be used as an objective endpoint in determining when a patient has recovered sufficiently to warrant HVAD explant, independent of the therapy used to restore LV contractility.

With the discontinuation of the HVAD, it would be beneficial to explore the addition of the pulsatility index in similar HF devices. The results from this initial study provide support for a prospective study to determine widespread response and to create an LVAD weaning protocol dependent on improvements in pulsatility.

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Philemon Mikail is a current Medtronic employee in the pacemaker division. He performed the research in this manuscript prior to employment with Medtronic, received no compensation or benefits from Medtronic, and has never been affiliated with the HVAD division. Janny Garcia is an employee of Medtronic's Heartware HVAD division but solely provided expert knowledge on the HVAD functions and not on analysis or conclusions.

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