Plasma Levels of MicroRNA-155 Are Upregulated with Long-Term Left Ventricular Assist Device Support

Teresa Wang,* Emily C. O'Brien,† Joseph G. Rogers,‡§ Daniel L. Jacoby,¶ Michael E. Chen,¶ Jeffrey M. Testani,¶ Dawn E. Bowles,‖ Carmelo A. Milano,‖ G. Michael Felker,‡§ Chetan B. Patel,‡§ Pramod N. Bonde,# and Tariq Ahmad¶

Left ventricular assist device (LVAD) therapy unloads the failing heart but exposes the human body to unique pathophysiologic demands such as continuous blood flow and complete univentricular support, which are associated with increased risk of adverse clinical outcomes. MicroRNAs (miRNAs) are 22–23 nucleotide RNAs involved in regulation of multiple biologic processes including the pathogenesis of heart failure (HF). Thus, measurement of miRNAs may have potential in both diagnostics as circulating biomarkers and in therapeutics for targeted interventions. We examined 23 distinct miRNAs that have previously been shown to play a role in HF pathogenesis and measured them in 40 individuals both before continuous-flow LVAD implantation and at a median of 96.5 days after implantation. Quantitative real-time polymerase chain reaction was performed for miRNA amplification, and 19 miRs were included in statistical analysis. Wilcoxon signed-rank tests were used to compare within-patient median relative quantification values pre- and post-LVAD placement. The median age of patients was 67 years, and 57.5% were at Interagency Registry for Mechanically Assisted Circulatory Support level 1–2. After LVAD support, only miR-155 was found to be statistically significant ($p < 0.002$), with an upregulation in plasma expression levels with LVAD support, which persisted regardless of the direction of change in serial HF biomarker levels. MicroRNA-155, which has been shown to play a central role in inflammation and neovascularization, was upregulated with long-term LVAD support. If validated by future studies, miR-155 may help further inform on underlying LVAD physiology and has a role as a therapeutic target in this patient population. *ASAIO Journal* 2017; 63:536–541.

Key Words: ventricular assist device, microRNA

End-stage heart failure (HF) patients are refractory to usual medical therapies and face a risk of death that is greater than most cancers.[1] At present, the most effective therapy is cardiac transplantation, but this option is limited by the lack of suitable donor organs and strict eligibility criteria.[2] Left ventricular assist devices (LVADs) provide an alternative means of treatment and are increasingly being used as either a bridge to transplantation or destination therapy. Even though it provides circulatory support for the failing heart, LVAD therapy also exposes the body to pathophysiologic changes that are unprecedented in human evolution: continuous blood flow, an external power source, and univentricular support. As a result, patients supported with VADs experience a unique variety of clinical conditions such as gastrointestinal bleeding, strokes, right ventricular failure, and infections, the pathobiology of which remains imprecisely understood.[3] Because of the limitations of usual cardiovascular assessments such as imaging in VAD patients, biomarker assessments have the potential to uncover the unique pathophysiologic changes that occur with VAD support.[4]

MicroRNAs (miRNAs) have emerged as bona fide regulators of physiologic and pathologic cardiovascular function.[5] MicroRNAs are 22–23 nucleotide single-stranded noncoding RNAs that bind messenger RNAs at their 3′ untranslated regions and negatively regulate their expression through translational repression or messenger RNA degradation. The importance of miRNAs comes from their ability to repress hundreds of target genes, often regulating biologic networks entirely.[6,7] MicroRNAs are stable and readily detectable in peripheral circulation and amenable to silencing; therefore, they have been postulated to play an important role as circulating biomarkers and potential therapeutic targets in HF.[8,9] Therefore, we sought to examine baseline levels and long-term changes in the expression of 23 distinct miRNAs in 40 patients who underwent LVAD placement at Duke University Medical Center (DUMC).

Methods

Study Population

Details outlining the patient population have been previously outlined.[4] From January 1, 2011 to October 30, 2012, a total of 40 patients aged 18 and older who required mechanical circulatory support with continuous-flow LVAD as a bridge to transplantation or destination therapy were enrolled at DUMC. Patients agreed to collection and banking of peripheral blood samples for the

From the *Department of Internal Medicine, Hospital of University of Pennsylvania, Philadelphia, Pennsylvania; †Department of Cardiology, Duke Clinical Research Institute, Durham, North Carolina; ‡Department of Internal Medicine, Duke University Medical Center, Durham, North Carolina; §Division of Cardiology, Department of Cardiology, Duke University Medical Center, Durham, North Carolina; ¶Department of Cardiology, Section of Cardiovascular Medicine, Yale University School of Medicine, New Haven, Connecticut; ‖Division of Cardiac Surgery, Department of Cardiology, Duke University Medical Center, Durham, North Carolina; and #Department of Cardiology, Section of Cardiovascular Surgery, Yale University School of Medicine, New Haven, Connecticut.

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Correspondence: Tariq Ahmad, Department of Cardiology, Section of Cardiovascular Medicine, Yale University School of Medicine, New Haven, CT. Email: tariq.ahmad@yale.edu.

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purpose of research and had paired long-term (>60 days) samples available. Blood samples were collected within 24 hours before LVAD implantation and at >60 days postimplant *via* peripheral vein into ethylenediaminetetraacetic acid containing tubes, centrifuged immediately, and stored at −80°C before analysis. Both miRNA and biomarker levels were measured from blood samples at the time points specified above. Clinical data at baseline, including demographics, medications, comorbidities, and laboratory data, were collected up to 24 hours before LVAD implantation. Data on more detailed assessments performed before LVAD placement for clinical purposes, such as cardiopulmonary exercise testing, invasive hemodynamics, echocardiographic parameters, and usual laboratories, were evaluated. As a control, plasma was collected from seven healthy control subjects. This study was approved by the DUMC Institutional Review Board, performed in accordance with the ethical guidelines of the declaration of Helsinki, and all patients provided written informed consent.

MicroRNA Measurements

Reverse transcription reactions were performed using the Life Technologies TaqMan MicroRNA Reverse Transcription Kit (Part number 4366596) (Thermo Fischer Scientific). An Applied Biosystems 9700 PCR System (Thermo Fischer Scientific) was used to carry out the reverse transcription polymerase chain reactions. An *a priori* list of 23 miRNAs purported to be associated with the pathogenesis of HF were selected for investigation: miRNA-1, miRNA-10a, miRNA-15b, miRNA-16, miRNA-21, miRNA-24, miRNA-27a, miRNA-27b, miRNA-29a, miRNA-92a, miRNA-103, miRNA-126, miRNA-133a, miRNA-146a, miRNA-146b, miRNA-155, miRNA-159a, miRNA-195, miRNA-221, miRNA-222, miRNA-320, miRNA-423, and miRNA-872. Nineteen miRNAs (miR-15b, miR-16, miR-21, miR-24, miR-27a, miR-29a, miR-92a, miR-103, miR-126, miR-133a, miR-146a, miR-146b, miR-155, miR-159a, miR-195, miR-221, miR-222, miR-320, and miR-423) were ultimately included for further analysis as they demonstrated adequate expression in >80% of patient samples. Quantitative real-time polymerase chain reactions were performed in quadruplicate for all samples, and threshold cycle (Ct values) were detected. Ct values were computed with Life Technologies/Applied Biosystems ExpressionSuite Software (version 1.0.3) (Thermo Fischer Scientific). The data were analyzed with automatic settings for assigning the baseline. The Ct was defined as the fractional cycle number at which the fluorescence exceeded the given threshold. MicroRNA expression levels were normalized to a global normalization value, defined as the mean of all Ct values computed. Relative quantification (RQ) was obtained using the $2^{-\Delta\Delta Ct}$ method,[10] by which the normalized fold change was determined as follows: 1) normalization of the Ct of the target miRNA to that of the global normalization value for all pre- and post-LVAD samples. To compute the pre-LVAD RQ ratios, we calculated 2) $\Delta Ct^{pre} = Ct^{pre,\ target} - Ct^{global}$, and $\Delta Ct^{control} = Ct^{control,\ target} - Ct^{global}$ for all pre-LVAD samples, 3) differential expression ($\Delta\Delta Ct^{pre}$) of each miRNA was calculated (and expressed as a $2^{-\Delta\Delta Ct}$ ratio) by subtracting mean $\Delta Ct^{control}$ from mean ΔCt^{pre}. To compute the post-LVAD RQ ratios, we calculated 4) $\Delta Ct^{post} = Ct^{post,\ target} - Ct^{global}$, and $\Delta Ct^{control} = Ct^{control,\ target} - Ct^{global}$ for all post-LVAD samples, 5) differential expression ($\Delta\Delta Ct^{post}$) of each miRNA was calculated (and expressed as a $2^{-\Delta\Delta Ct}$ ratio) by subtracting mean $\Delta Ct^{control}$ from mean ΔCt^{post}. In the formulas above, "control" refers to the samples of the seven healthy patients, "global" is the global normalization value, and "target" is that of the target miRNA of interest.

Statistical Analysis

Baseline characteristics of the study cohort are expressed as medians (interquartile ranges [IQRs]) for continuous variables and percentages for categorical variables. We examined miR levels in several ways: 1) baseline levels stratified by pre-LVAD N-terminal pro-brain natriuretic peptide (NT-proBNP) tertile levels, 2) changes in miRs *via* median pre/post ratio overall, and 3) changes in miRs *via* median pre/post ratio according to improvement in levels of three validated HF biomarkers after LVAD placement: galectin-3, NT-proBNP, and soluble ST2 (sST2). In the absence of clear guidelines for clinically important degrees of improvement in biomarker levels after LVAD placement, we *a priori* defined this as a >25% decrease from baseline levels for each of these markers, as this degree of change approximates the suggested threshold for biologic variation in established HF.[4,11] We used Wilcoxon signed-rank tests to compare differences in miRNA levels before and

Table 1. Baseline Characteristics of Entire Cohort (N = 40)

Characteristic	Median
Age (years)	67 (51–74)
BMI (kg/m^2)	30.2 (25.6–35.3)
Length of stay (days)	14.5 (11–22)
Sodium (mmol/L)	136 (132–138)
Potassium (mmol/l)	4.2 (3.8–4.6)
Blood urea nitrogen (mg/dl)	28 (16.5–40.5)
Creatinine (mg/dl)	1.4 (1.2–1.9)
Albumin (g/dl)	3.3 (2.9–3.6)
ALT (U/L)	21 (17–25)
AST (U/L)	26 (22–32.5)
INR	1.3 (1.1–1.4)
RDW (%)	16.7 (14.8–18.7)
Total cholesterol (mg/dl)	120.5 (97–145)
LDL (mg/dl)	62 (47–78.5)
HDL (mg/dl)	30.5 (23–41.5)
LVEF (%)	20 (15–20)
LVEDD (cm)	6.9 (6.3–7.3)
RAP (mm Hg)	12 (7–19)
PCWP (mm Hg)	25 (19–31)
Cardiac index (L/min/m^2)	1.9 (1.7–2.2)
Peak VO_2 (ml/kg/min)	12.7 (11.6–13.7)
Ve-VO_2, slope	42 (34.6–47.3)
NT-proBNP (pg/ml)	3162 (1696–4811)
Galectin-3 (ng/ml)	24 (16–35)
Soluble ST2 (ng/ml)	67 (42–113)
GDF-15 (ng/L)	3232 (2250–5862)
CRP (mg/L)	22 (6–29)
Copeptin (pmol/L)	101 (84–128)
NGAL (ng/ml)	132 (112–185)

ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; GDF, growth differentiation factor; HDL, high-density lipoprotein; INR, international normalized ratio; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; RDW, red cell distribution width; Vo2, measured maximal oxygen uptake.

Table 2. Baseline MiRNA Levels According to Tertiles of Baseline NT-proBNP

	Tertile 1				Tertile 2				Tertile 3				
MicroRNA	N	Median	Q1	Q3	N	Median	Q1	Q3	N	Median	Q1	Q3	*p*
16	13	0.57	0.33	1.18	13	0.54	0.37	0.90	12	0.63	0.36	0.88	0.9810
21	10	1.17	1.06	1.86	12	1.32	1.13	1.64	12	1.29	1.07	1.56	0.9434
24	12	1.36	0.81	2.26	12	1.43	1.03	1.84	12	1.34	1.17	1.91	0.9091
103	11	1.23	1.01	1.74	12	1.27	0.83	1.41	11	0.75	0.31	1.32	0.1778
126	12	0.84	0.52	1.37	12	0.69	0.50	0.83	12	0.68	0.35	1.33	0.6283
155	13	0.92	0.77	1.40	13	0.71	0.68	0.88	12	0.94	0.65	1.45	0.3996
195	12	0.63	0.47	1.20	12	0.60	0.37	1.12	12	0.72	0.35	0.81	0.8487
221	12	1.04	0.93	1.55	12	1.09	1.01	1.39	12	1.09	0.94	1.52	0.9043
222	13	0.27	0.23	0.63	13	0.37	0.28	0.41	12	0.53	0.33	0.80	0.2025
320	13	1.04	0.44	4.03	13	1.01	0.58	1.09	12	1.19	0.58	1.45	0.8032
423	10	1.97	1.54	2.48	11	2.60	1.25	4.76	12	2.15	1.79	3.52	0.4719
133a	13	0.53	0.24	4.98	12	0.61	0.48	1.19	12	0.45	0.28	1.00	0.7118
146a	12	1.85	1.32	2.23	13	1.33	1.04	1.71	12	1.67	1.15	2.86	0.2410
146b	12	1.29	1.10	1.47	12	1.00	0.82	1.26	12	1.00	0.85	1.72	0.2860
159a	10	0.34	0.18	0.60	12	0.29	0.15	0.62	12	0.18	0.05	0.76	0.7755
15b	12	0.76	0.45	1.12	13	0.69	0.40	0.95	11	0.65	0.50	0.91	0.7713
27a	11	0.73	0.55	1.05	11	0.49	0.36	0.82	11	0.34	0.24	0.44	0.0071
29a	13	0.74	0.53	1.38	12	0.64	0.42	0.74	12	0.68	0.38	0.86	0.2881
92a	13	1.00	0.70	1.51	13	1.45	0.91	1.61	12	1.50	1.10	2.01	0.3836

Tertiles ranges for NT-proBNP are as follows: Tertile 1 (<2151 pg/ml), tertile 2 (2151–4577 pg/ml), tertile 3 (≥4578 pg/ml).
**p* value from Kruskal–Wallis test.
NT-proBNP, aminoterminal pro B-type natriuretic peptide.

after LVAD placement. Two-tailed *p* values were computed for all statistical tests. We applied a Bonferroni correction by dividing the original critical value ($p = 0.05$) by the number of hypotheses being tested (the number of candidate miRs: n = 19). This created a new critical value of $p = 0.0026$, which was used to determine statistical significance. Statistical analysis was performed using SAS version 9.3 statistical software (SAS Institute Inc., Cary, NC).

Results

Patient Characteristics

Table 1 shows the baseline characteristics of the entire cohort of 40 individuals who underwent LVAD placement. The median age was 67 years (IQR: 51–74); N = 26 patients (65%) were Caucasian, and N = 29 (72.5%) were male. The majority of patients (62.5%) were implanted with a Heartmate II LVAD, with the remaining implanted with a HeartWare LVAD. Overall, median left ventricular ejection fraction was 20% (15–20), left ventricular end diastolic dimension was 6.9 cm (6.3–7.3), right atrial pressure was 12 mm Hg (7–19), pulmonary capillary wedge pressure was 25 mm Hg (19–31), and cardiac index was 1.9 L/min/m^2 (1.7–2.2). Cardiopulmonary exercise testing parameters also revealed a high degree of exercise intolerance, with median peak measured maximal oxygen uptake (VO_2) of 12.7 (11.6–13.7) ml/kg/min and minute ventilation-carbon dioxide production relationship ($VeVCO_2$) slope of 42 (34.6–47.3). Median concentrations of commonly measured electrolytes were within normal limits. Blood urea nitrogen, creatinine levels, and red cell distribution width were slightly elevated (28 mg/dl, 1.4 mg/dl, 16.7%, respectively), whereas albumin levels were slightly decreased (3.3 g/dl). As compared with the expected ranges, HF biomarkers were markedly elevated as follows: NT-proBNP (3162 pg/ml [upper limit of normal 1200]), galectin-3 (24 ng/ml [25.9]), sST2 (67 ng/ml [35]), growth differentiation factor (GDF)-15 (3232 ng/L [1200]), C-reactive protein (22 mg/L [3]), copeptin (101 pmol/L [40]), and neutrophil gelatinase-associated lipocalin (NGAL) (132 ng/ml [117]).

Baseline MicroRNA Levels Stratified by Brain Natriuretic Peptide Levels

Given the predominance of natriuretic peptides as the objective biochemical measure of HF, we examined miRNA levels shown in **Table 2**, stratified by tertiles of NT-proBNP. Subjects in the highest tertile of NT-proBNP had quantitatively longer length of stay (16 *vs.* 11 days), higher degrees of renal insufficiency (creatinine 1.8 *vs.* 1.4mg/dl), and poorer cardiac index (1.9 *vs.* 2.1 L/min/m^2). Additionally, the highest tertile cohort had greater elevations

Table 3. Median Pre–Post MicroRNA Ratios in the Overall Cohort

MicroRNA	N	Median Pre/Post Ratio	Lower Quartile	Upper Quartile	*p*
103	33	1.40	0.76	1.91	0.13
159a	33	1.28	0.48	2.02	0.21
423	33	1.20	0.80	1.87	0.25
21	34	1.10	0.90	1.30	0.26
92a	40	1.09	0.70	1.66	0.99
320	40	1.07	0.74	1.54	0.51
195	38	1.02	0.52	1.94	0.85
146a	38	0.98	0.56	1.24	0.33
24	37	0.95	0.60	1.36	0.76
29a	38	0.94	0.71	1.38	0.64
16	40	0.93	0.56	1.80	0.97
15b	37	0.92	0.68	1.17	0.21
221	35	0.91	0.67	1.18	0.22
27a	32	0.88	0.62	1.26	0.20
133a	39	0.86	0.54	1.94	0.93
146b	36	0.84	0.63	1.07	0.08
222	40	0.84	0.45	1.15	0.02
126	37	0.82	0.54	1.32	0.22
155	40	0.78	0.54	1.01	0.002

in plasma concentrations of biomarkers measured, including galectin-3, sST2, GDF-15, and NGAL. Differences in the baseline miRNA levels of the highest tertile of patients compared with that of the lowest tertile were not statistically significant.

Changes in MicroRNA Levels with left Ventricular Assist Device Support

Serial blood draws were performed at a median of 96.5 (72–150) days post-LVAD placement. **Table 3** and **Figure 1** show the change in RQ ratios from pre- to post-LVAD implantation, demonstrated as a median pre/post ratio. After LVAD implantation, seven miRNAs (miR-21, miR-92a, miR-103, miR-159a, miR-195, miR-320, and miR-423) were downregulated, represented by a pre/post ratio >1. The largest reductions (*i.e.*, highest median pre/post ratio >1) were demonstrated by miR-103 (ratio 1.40 [IQR: 0.76–1.91]) and miR-159a (ratio 1.28 [0.48–2.02]). However, these differences did not reach statistical significance (miR-103, $p = 0.12$; miR-159a, $p = 0.21$). Twelve miRNAs (miR-15b, miR-16, miR-24, miR-27a, miR-29a, miR-126, miR-133a, miR-146a, miR-146b, miR-155, miR-221, and miR-222) were found to be upregulated as shown by a pre/post ratio <1. The largest increase in miRNA levels occurred with miR-126 (ratio 0.82 [0.54–1.32]) and miR-155 (ratio 0.78 [0.54–1.01]). Notably, the change in plasma miRNA level with LVAD implantation was only statistically significant for miR-155 ($p < 0.002$).

MicroRNA Levels According to Biomarker Change

Last, we examined changes in plasma miRNA levels with LVAD implantation according to prespecified changes in serial biomarker (galectin-3, NT-proBNP, and sST2) levels. Median pre/

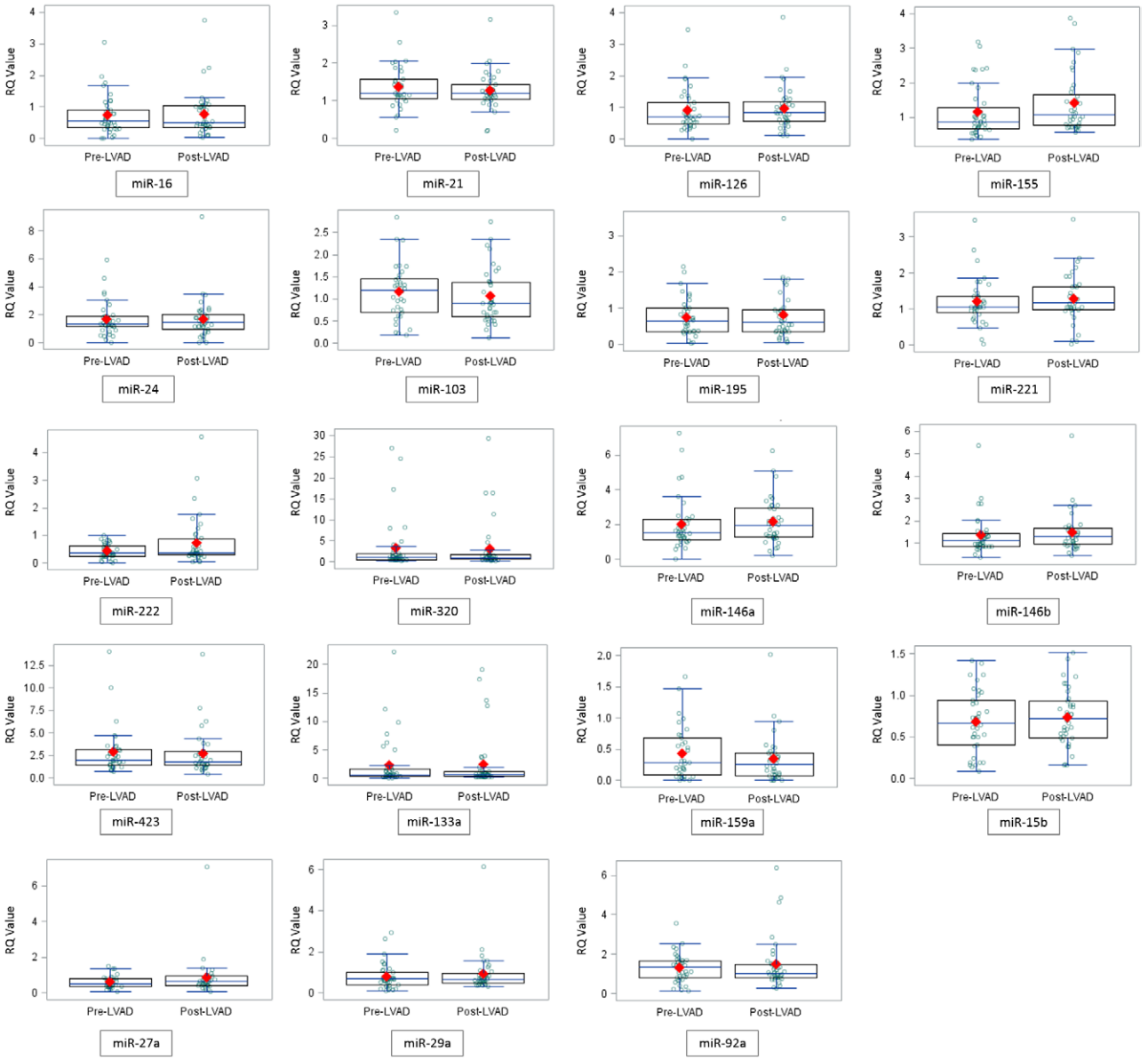

Figure 1. Change in RQ ratios from pre- to post-LVAD implantation. Individual value plots of median (red) microRNA RQ values for patients pre- and post-LVAD, along with interquartile (25–75%) ranges. All plasma levels of the specified miRNAs remained relatively unchanged (not statistically significant) with the exception of miR-155, which was upregulated. LVAD, Left ventricular assist device; RQ, relative quantification. full color online

Table 4. Median Pre–Post Ratios Among Patients with Less Than 25% Decrease in Serial Levels of Galectin-3, NT-proBNP, and sST2 (No Improvement)

MicroRNA	N	Median	Lower Quartile	Upper Quartile
103	23	1.37	0.68	2.25
159a	24	1.34	0.59	2.13
21	24	1.17	0.91	1.30
16	28	1.16	0.61	1.92
92a	28	1.16	0.70	1.75
195	27	1.14	0.56	1.96
320	28	1.11	0.79	1.52
423	23	1.11	0.66	1.87
146a	27	1.04	0.57	1.39
24	27	1.04	0.60	1.40
15b	25	1.02	0.68	1.21
29a	27	0.94	0.69	1.46
27a	23	0.90	0.60	1.31
126	27	0.90	0.62	1.35
221	25	0.90	0.64	1.00
133a	27	0.89	0.56	1.84
146b	26	0.88	0.67	1.07
155	28	0.80	0.60	1.06
222	28	0.79	0.44	1.05

NT-proBNP, aminoterminal pro B-type natriuretic peptide.

post ratios are shown for individuals with a ≤25% decrease (no improvement, **Table 4**) *versus* a >25% decrease (improvement, **Table 5**) in each of serial galectin-3, NT-proBNP, and sST2 levels. Approximately 1/3 of individuals demonstrated an improvement in biomarker levels with LVAD implantation; in this group, a majority of miRs (N = 11) showed an overall upregulation of levels. Notably, miR-155 along with four other miRs (27a, 126, 133a, and 146b) were upregulated in both cohorts of patients with and without improvement in prespecified biomarker levels. Smaller sample size limited formal statistical analysis.

Discussion

Our study evaluated baseline levels and long-term changes in the expression of circulating miRNAs in patients with end-stage HF supported by LVADs. We found that, after correcting for multiple testing, plasma levels of 18 of the 19 cardiac miRNAs previously shown to play a role in HF pathophysiology remained unchanged with LVAD support; only expression of miR-155 was upregulated. This appeared to occur regardless of improvement in serial levels of HF biomarkers. MicroRNA-155 has been recently shown to play a fundamental role in the axis between inflammation and arteriogenesis (formation of collateral arteries), both processes of which are aberrant in LVAD patients.[5,12] It is entirely plausible that increases in expression of miR-155 after VAD support may explain the untoward predisposition toward neovascularization, and therefore gastrointestinal bleeding. If supported by further study, this hypothesis lends itself to testing *via* selective inhibition of miR-155 in subgroups of LVAD patients.

Table 5. Median Pre–Post Ratios Among Patients with Greater than 25% Decrease in Serial Levels of Galectin-3, NT-proBNP, and sST2 (Improvement)

MicroRNA	N	Median	Lower Quartile	Upper Quartile
103	8	1.44	0.90	1.57
423	8	1.38	0.71	3.33
159a	8	1.26	0.46	2.10
29a	8	1.17	0.94	1.54
221	8	1.09	0.81	1.32
222	9	1.03	0.50	1.10
21	8	1.03	0.91	1.38
320	9	1.02	0.73	1.55
24	8	0.95	0.64	1.29
27a	7	0.91	0.60	1.03
15b	9	0.90	0.56	1.02
146b	8	0.90	0.63	1.12
195	8	0.89	0.63	1.11
92a	9	0.88	0.80	1.56
16	9	0.79	0.55	1.12
155	9	0.70	0.50	0.98
146a	9	0.66	0.48	1.02
133a	9	0.64	0.32	1.94
126	8	0.62	0.51	1.28

NT-proBNP, aminoterminal pro B-type natriuretic peptide.

MicroRNAs have emerged as key regulators of multiple biologic pathways; their dominance arising from an ability to control hundreds of target genes, turning on or off the entire pathways simultaneously.[13] Furthermore, they are stable, readily detectable in peripheral circulation, and amenable to silencing; therefore, they have generated considerable excitement as circulating biomarkers and potential therapeutic targets in cardiovascular disease.[7] As a result, over just a brief period of time, several miRNAs have been found to modulate expression levels of genes that govern the process of adaptive or maladaptive cardiac remodeling in HF, viral myocarditis, arrhythmias, and coronary heart disease.[14–21]

There is a significant body of literature in support of miR-155 being a proinflammatory miRNA, serving as a key regulator of both the innate and adaptive immune systems. It has subsequently been implicated in several disease states with an inflammatory undertone, ranging from alcoholic liver disease to arthritis to several malignancies.[22–24] In the realm of cardiology, miR-155 has been extensively studied in terms of its role in vascular dysfunction.[25] A recently published study appears to bridge this gap between inflammation and vascular disease, as it relates to miR-155. In a murine model of neovascularization, Pankratz *et al*.[5] found that miR-155 was a key driver of macrophage-mediated collateral artery formation (arteriogenesis), and mice deficient in miR-155 had attenuated arteriogenesis after femoral artery ligation.[12] This effect was mediated *via* miR-155 modulation of the leukocyte-enriched feedback regulator gene suppressor of cytokine signaling 1 (SOCS1) that antagonizes Jak/Stat activation of chemokines and cytokines.[26] Moreover, Yang *et al*.[27] found that miR-155 mediated angiogenesis and endothelial cell maturation by targeting E2F transcription factor 2 (E2F2) transcription factor. In light of these findings and our previous report of high degrees of inflammation after long-term LVAD support, it is plausible that miR-155 over-activation might be playing a role in neovascularization in the gastrointestinal tract and contribute to the high rates of bleeding seen in this patient population.[4] Furthermore, dysregulation of the innate immune system might contribute to increased risk of infection seen after LVAD placement.

Given the seemingly ubiquitous functions of miR-155 in basic cellular functions, the clinical implications of our findings will require careful validation and study. Indeed, there remains a large gap in translating miR-155 from our study into a clinically relevant and valid tool, and we recognize that larger sample sizes, correlation to clinical parameters, and long-term follow-up will be of the utmost importance. If future studies show that

miR-155 is indeed upregulated after LVAD support, and more so in patients with excessive gastrointestinal bleeding, it may represent a diagnostic predictive tool to identify patients at risk of development of arteriovenous malformation and a therapeutic target for prevention of unwanted arteriogenesis. Moreover, given the heterogeneity of patients receiving LVADs, it would be useful to stratify miR-155 levels according to HF etiology, especially given that hypoxia is a chief feature of ischemic cardiomyopathy and presumably enhances angiogenesis and endothelial cell maturation. Indeed, inhibitors of miR-155 are already in late stages of clinical development for hematologic malignancies and amyotrophic lateral sclerosis; they may be readily testable in patients with LVADs who represent a unique population that may be particularly amenable to early phase clinical research.[28]

Several potential study limitations require consideration. First, and foremost, as previously mentioned, these findings are strictly hypothesis generating, and although buttressed by numerous prior studies, will need validation in other LVAD cohorts. Our study hypothesis was broad in that the miRs measured could change in either direction; thus, even with the Bonferroni correction, we are limited in drawing conclusions based on statistical outcome alone. Second, we did not have granular information about clinical outcomes in our patient population, limiting our ability to demonstrate clear-cut associations between miR-155 and neovascularization. Moreover, our clinical correlation included all patients regardless of HF etiology, duration of HF, or concomitant therapy with HF medications, contributing to greater heterogeneity of our clinical dataset. Third, our measurements of Ct values in the reverse transcription polymerase chain reactions utilized a "global normalization" number as the reference value. However, to date, there is no consensus with respect to miR reference genes that can be used as internal controls for biologic variability.[29]

Conclusions

In summary, we found that plasma levels of 18 of the 19 miRNAs previously associated with cardiovascular pathophysiology remained unchanged with long-term LVAD support; only expression of miR-155 was upregulated. This appeared to occur regardless of improvement in serial levels of HF biomarkers. MicroRNA-155 has been recently shown to play a fundamental role in the axis between inflammation and formation of collateral arteries, both processes of which are aberrant in LVAD patients. If corroborated by further study, miR-155 might represent a mechanism by which untoward neovascularization occurs in LVAD patients and a potential target for therapeutic intervention.

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