SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Measurement of serum creatinine and Cystatin C, and estimation of glomerular filtration rate: Serum creatinine was measured by enzymatic method (Cobas 8000; Roche Diagnostics, Indianapolis, IN). Serum Cystatin C was measured using a particle enhanced immune turbidimetric assay (Cobas Integra 400 plus; Roche Diagnostics, Indianapolis, IN). $eGFR_{Cys}$ and $eGFR_{Cr}$ were calculated using CKD Epidemiology Collaboration (CKD-EPI) CysC equation[25] and Modification of Diet in Renal Disease (MDRD) [26], respectively. We selected the MDRD equation as it was previously used in other studies that investigated renal function in patients with LVAD and after heart transplant[51,52].

Specifically, $eGFR_{Cr}$ was calculated using the following equation: $eGFR_{Cr} = 175 \times S_{cr}^{-1.154} \times age^{-0.203} \times 1.212$ (if patient is black) $\times 0.742$ (if female); $eGFR_{Cys}$ was calculated using the following equation: $eGFR_{Cys} = 133 \times \min(S_{Cys}/0.8, 1)^{-0.499} \times \max(S_{Cys}/0.8, 1)^{-1.328} \times 0.996^{Age} \times 0.932$ [if female]; S_{Cys} is standardized serum cystatin C; min = indicates the minimum of $S_{Cys}/0.8$ or 1; max = indicates the maximum of $S_{Cys}/0.8$ or 1.

Measurements of plasma and serum biomarkers: Trimethylamine-*N*-oxide was measured in plasma using ultra performance Liquid Chromatography-tandem Mass Spectrometry (after protein precipitation using deuterated (D9)-TMAO as the internal standard. LC-MS analysis was performed on a platform comprising Eksigent UPLC 100

integrated to API 4000) mass spectrometer controlled by Analyst 1.6 (ABSciex, Foster City, CA).

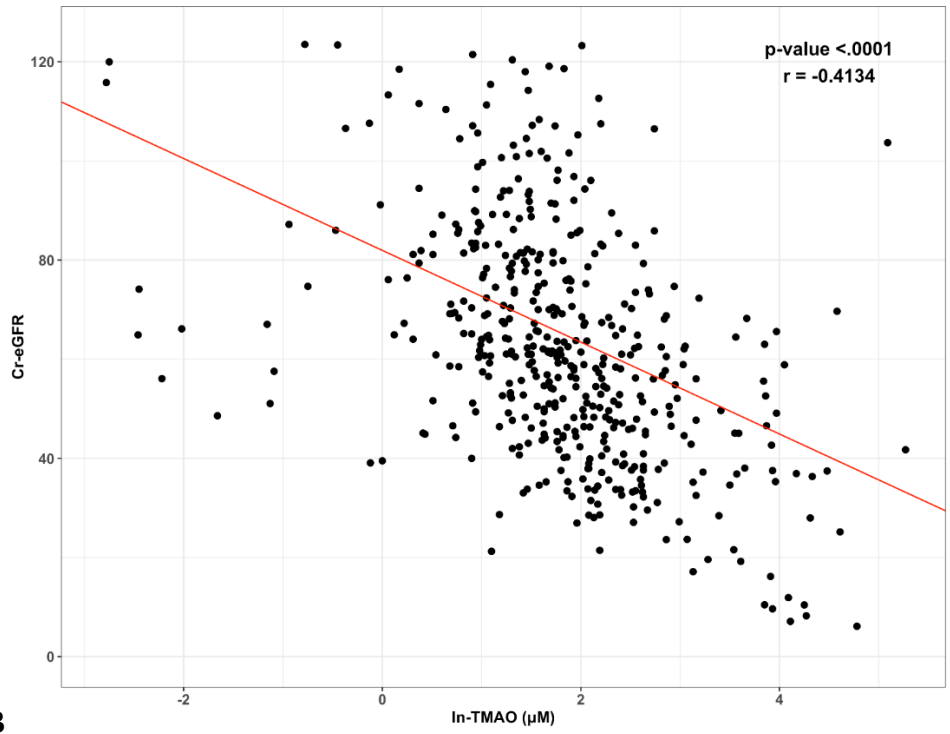
Serum CRP was measured using a high sensitivity (0.3mg/L) particle enhanced turbidimetric assay on the automated analyzer, Integra 400 plus (Roche Diagnostics, Indianapolis, IN). Serum TNF-α was assessed using a high sensitivity Enzyme Linked Immunoassay (ELISA) kit (RD systems, Minneapolis, MN). Plasma LPS was measured using a LAL chromogenic endotoxin quantitation kit (Pierce Thermoscientific, Rockford, IL).

Stool collection: Patients provided non-fasting stool samples in sterile stool hats using a protocol similar to the Human Microbiome Project[53]. Samples were transferred to the Columbia University Irving Medical Center (CUIMC) Microbiome Core Lab, processed, aliquoted and stored at -80°C within 12 hours of collection.

DNA extraction and sequencing: DNA was extracted from stool using the MagAttract PowerSoil DNA Kit on an Eppendorf epMotion 5075 Liquid Handling Workstation. For bacterial sequencing, we amplified the V3-V4 regions of the 16S rRNA gene using standard primers with Illumina Nextera adaptors (Illumina, Madison WI). PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, Jersey City NJ) and quantified using the Quant-iT Broad Range dsDNA Assay kit (Thermo Fisher Scientific, Waltham, MA). Libraries were normalized and pooled with a 10% PhiX spike and sequenced on an Illumina MiSeq with a v3 kit. Negative controls were included on all sequencing runs.

SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

A



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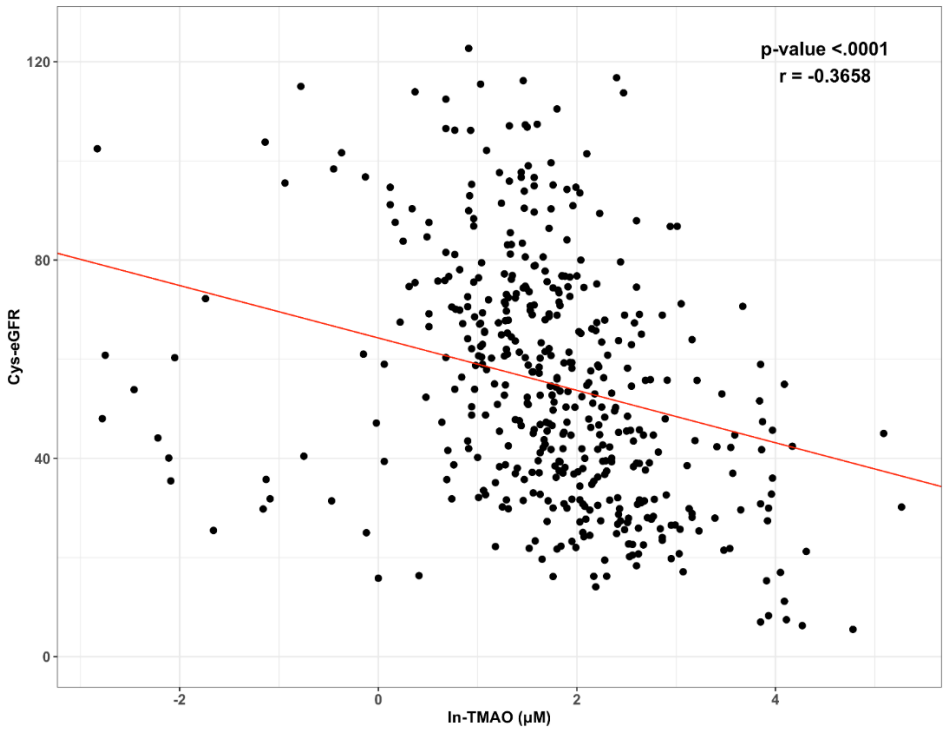
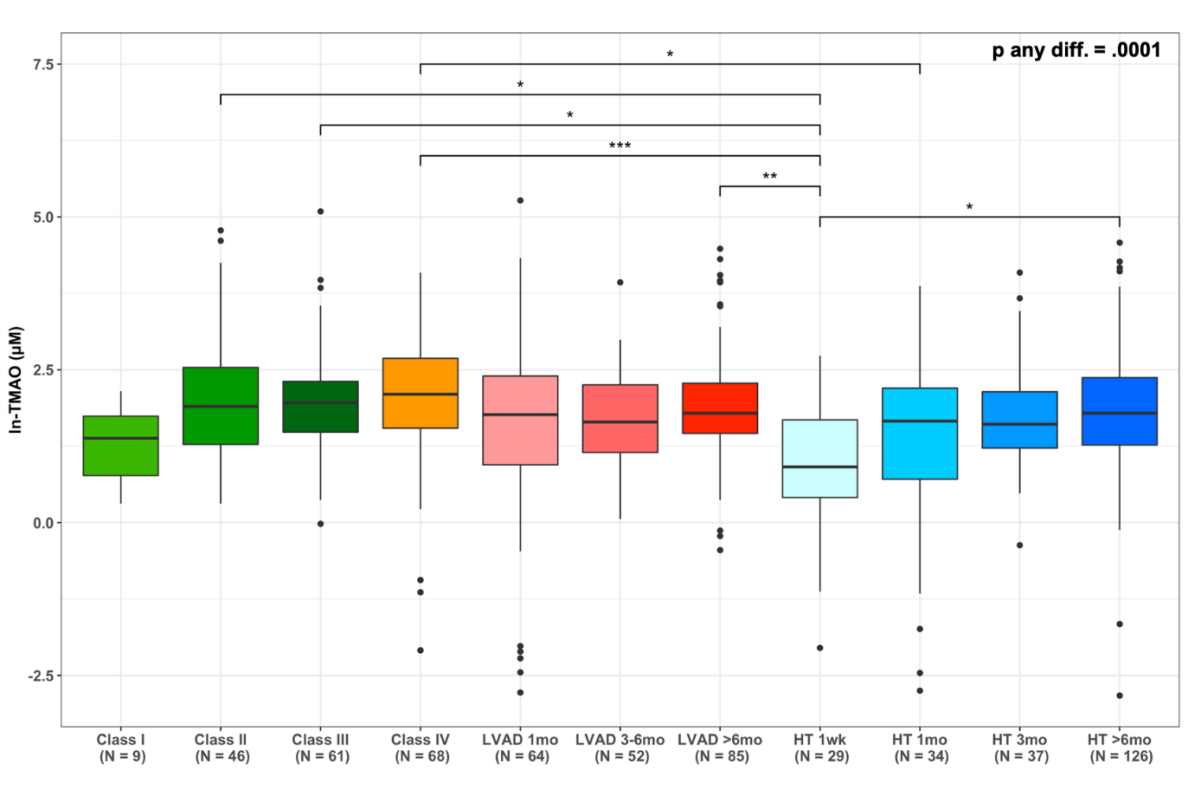
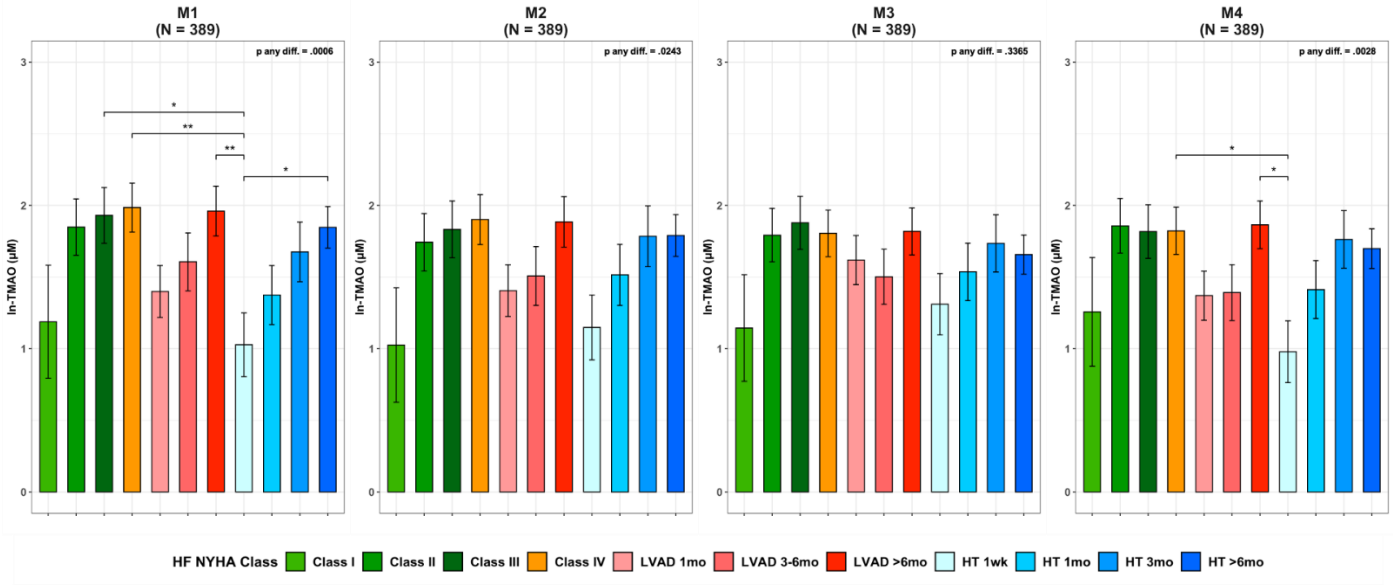


Supplemental Figure I: Correlation between circulating TMAO and indices of renal function (**A**) ln-TMAO and $eGFR_{Cr}$, (**B**) ln-TMAO and $eGFR_{Cys}$, $eGFR_{Cr}$, creatinine based estimated glomerular filtration rate; $eGFR_{Cys}$, cystatin C based estimated glomerular filtration state

A


p any diff. = .0001
ln-TMAO (μM)
7.5
5.0
2.5
0.0
-2.5
*
*
*
***
**
*
Class I (N = 9)
Class II (N = 46)
Class III (N = 61)
Class IV (N = 68)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 85)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 37)
HT >6mo (N = 126)

B

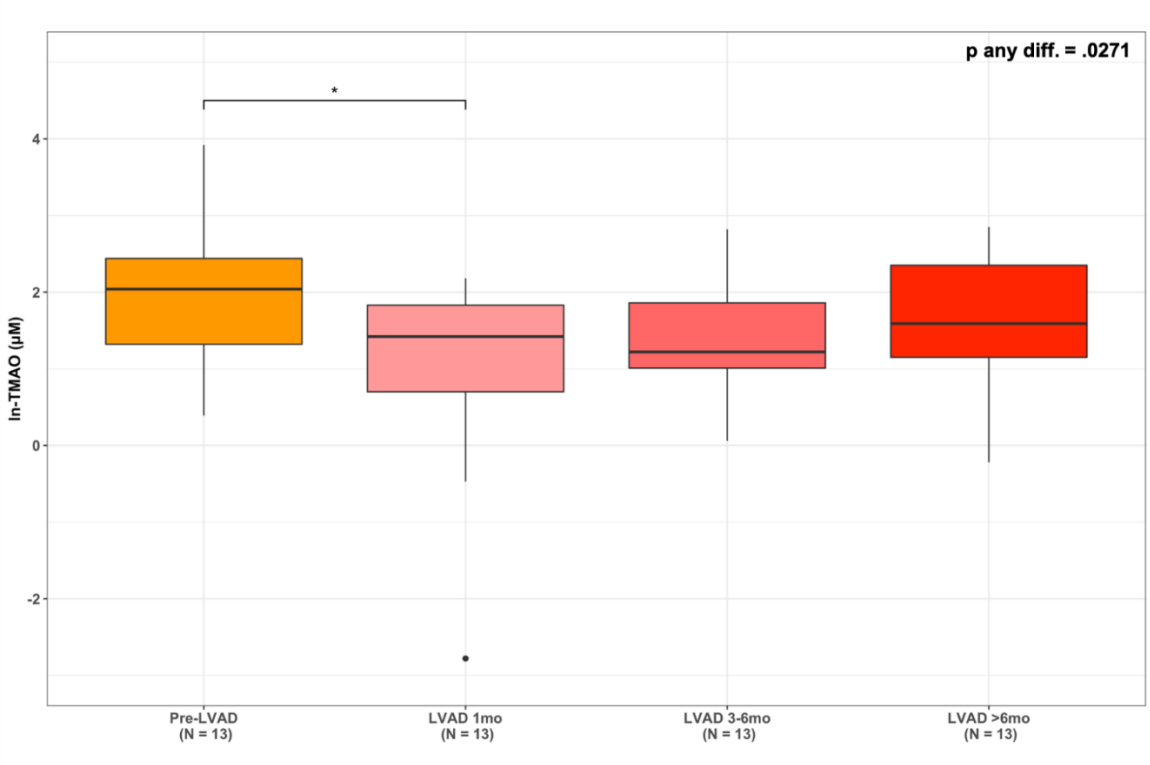

M1 (N = 389)
p any diff. = .0006
M2 (N = 389)
p any diff. = .0243
M3 (N = 389)
p any diff. = .3365
M4 (N = 389)
p any diff. = .0028
ln-TMAO (μM)
HF NYHA Class
Class I
Class II
Class III
Class IV
LVAD 1mo
LVAD 3-6mo
LVAD >6mo
HT 1wk
HT 1mo
HT 3mo
HT >6mo

Supplemental Figure II. Variation in circulating TMAO across disease categories of Heart Failure, LVAD and Heart Transplant patients (only patients with concurrent measure of serum creatinine and Cystatin C were included N=389). **(A)** unadjusted ln-TMAO levels **(M0)**; **(B)** adjusted least squared means: **M1**: adjusted for age, sex, race/ethnicity; **M2**: adjusted for Model 1 and antibiotics use one month prior to stool collection; **M3**: adjusted for Model 2 plus serum creatinine estimated glomerular filtration rate ($eGFR_{Cr}$); **M4**: adjusted for Model 2 plus Cystatin C estimated glomerular filtration rate ($eGFR_{Cys}$);

p-value: * 0.05-0.01; ** 0.01-0.001; *** <0.001;

HF, heart failure; HT, heart transplant; LVAD, left ventricular assist device.

A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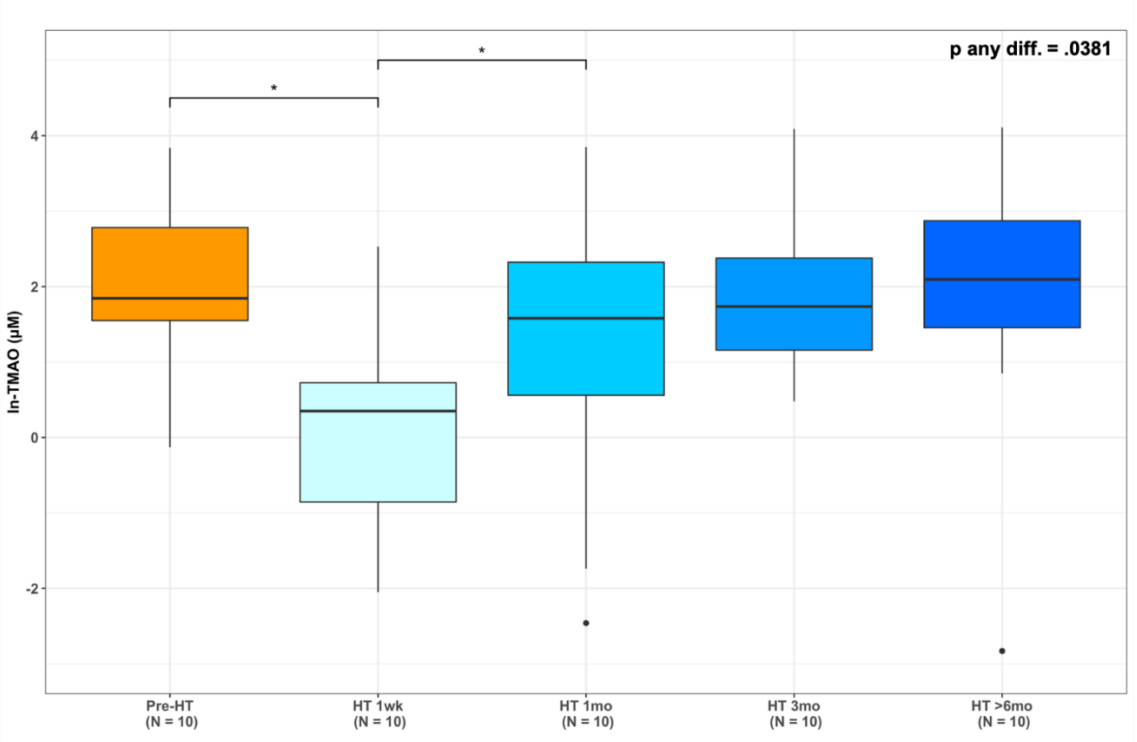
*
ln-TMAO (μM)
4
2
0
-2
Pre-LVAD
(N = 13)
LVAD 1mo
(N = 13)
LVAD 3-6mo
(N = 13)
LVAD >6mo
(N = 13)

B

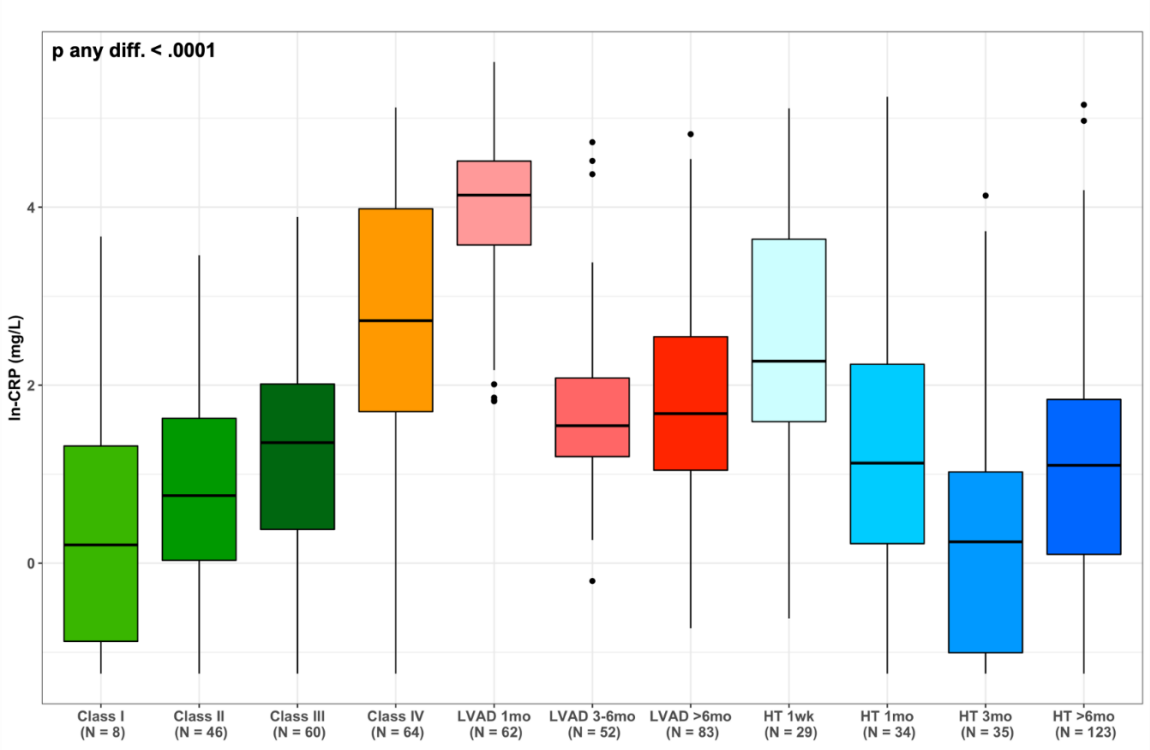

p any diff. = .0381
*
*
ln-TMAO (μM)
4
2
0
-2
Pre-HT
(N = 10)
HT 1wk
(N = 10)
HT 1mo
(N = 10)
HT 3mo
(N = 10)
HT >6mo
(N = 10)

Supplemental Figure III: Variation in circulating TMAO in a subset of patients with longitudinally collected data at all time points (**A**) pre- and post-LVAD (N=13), p-value for any difference based on unadjusted liner mixed model <0.05; and (**B**) pre- and post-HT (N=10); p-value for any difference based on unadjusted liner mixed model < 0.05.

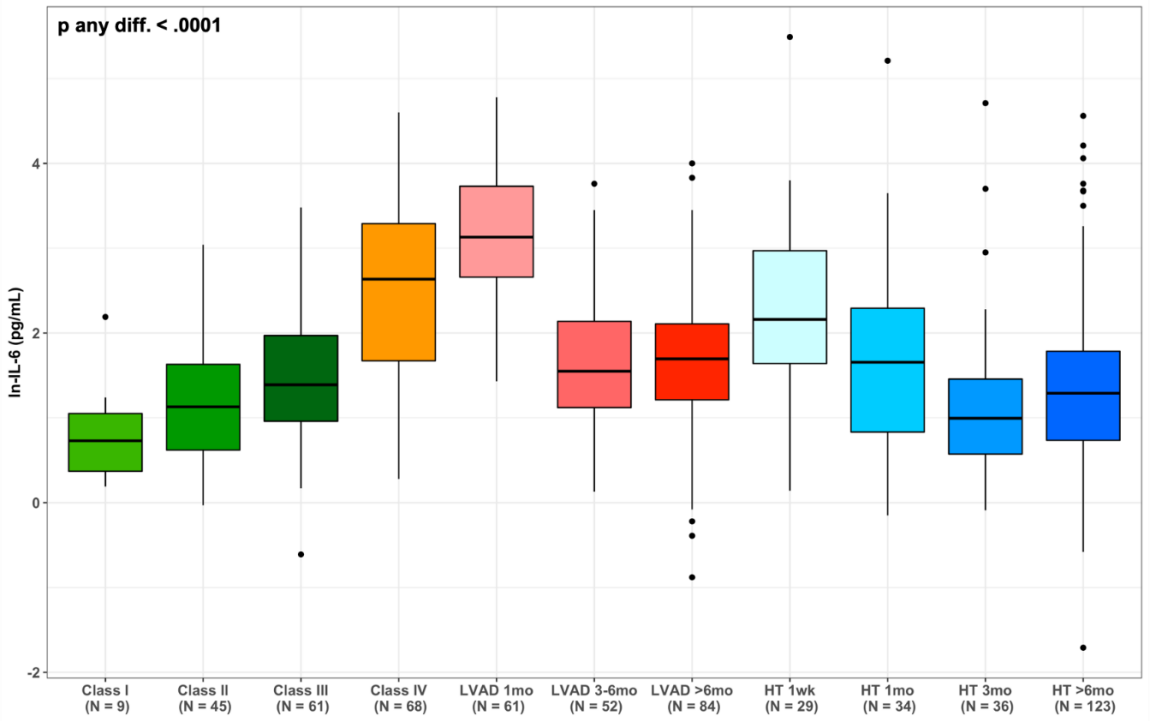
p-value: * 0.05-0.01;

LVAD, left ventricular assist device; HT, heart transplant.

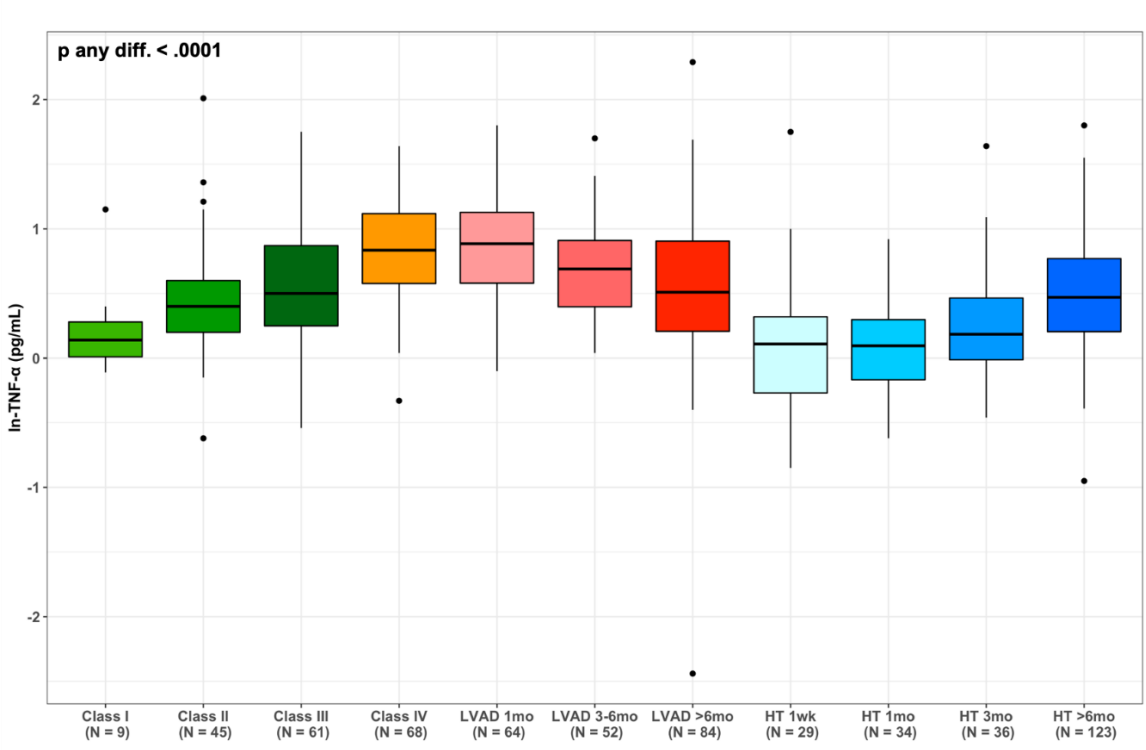
A


p any diff. < .0001
ln-CRP (mg/L)
4
2
0
Class I (N = 8)
Class II (N = 46)
Class III (N = 60)
Class IV (N = 64)
LVAD 1mo (N = 62)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 83)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 35)
HT >6mo (N = 123)

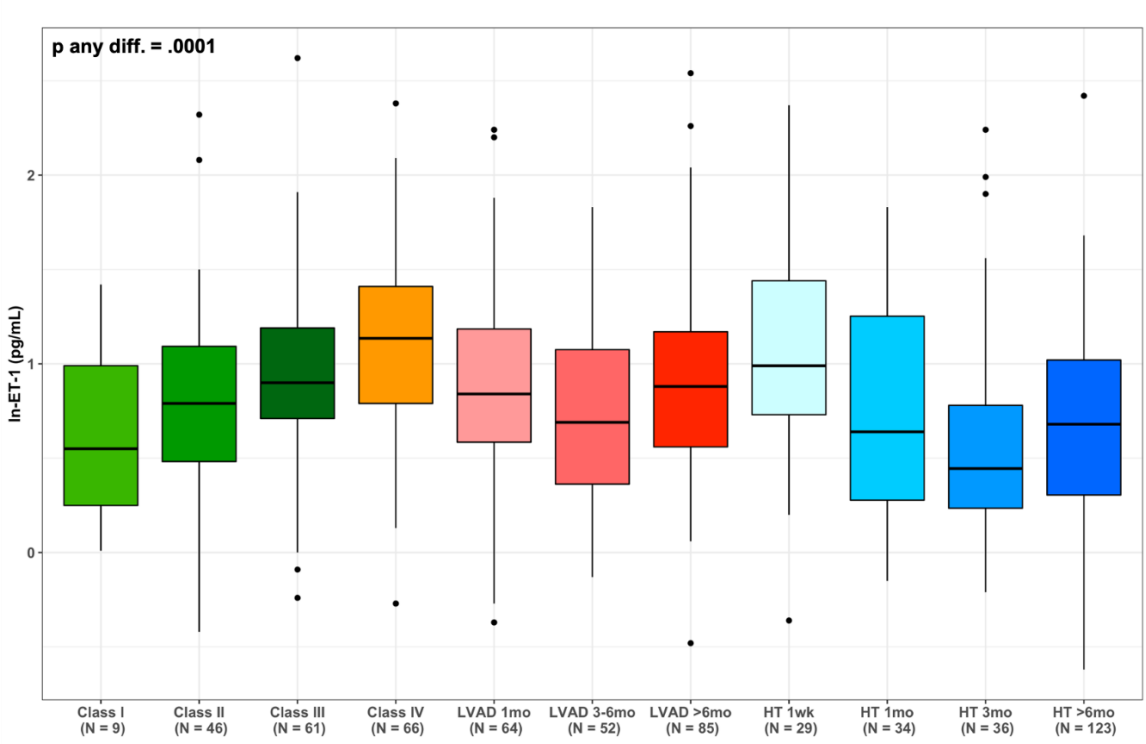
B


p any diff. < .0001
ln-IL-6 (pg/mL)
4
2
0
-2
Class I (N = 9)
Class II (N = 45)
Class III (N = 61)
Class IV (N = 68)
LVAD 1mo (N = 61)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 84)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 123)

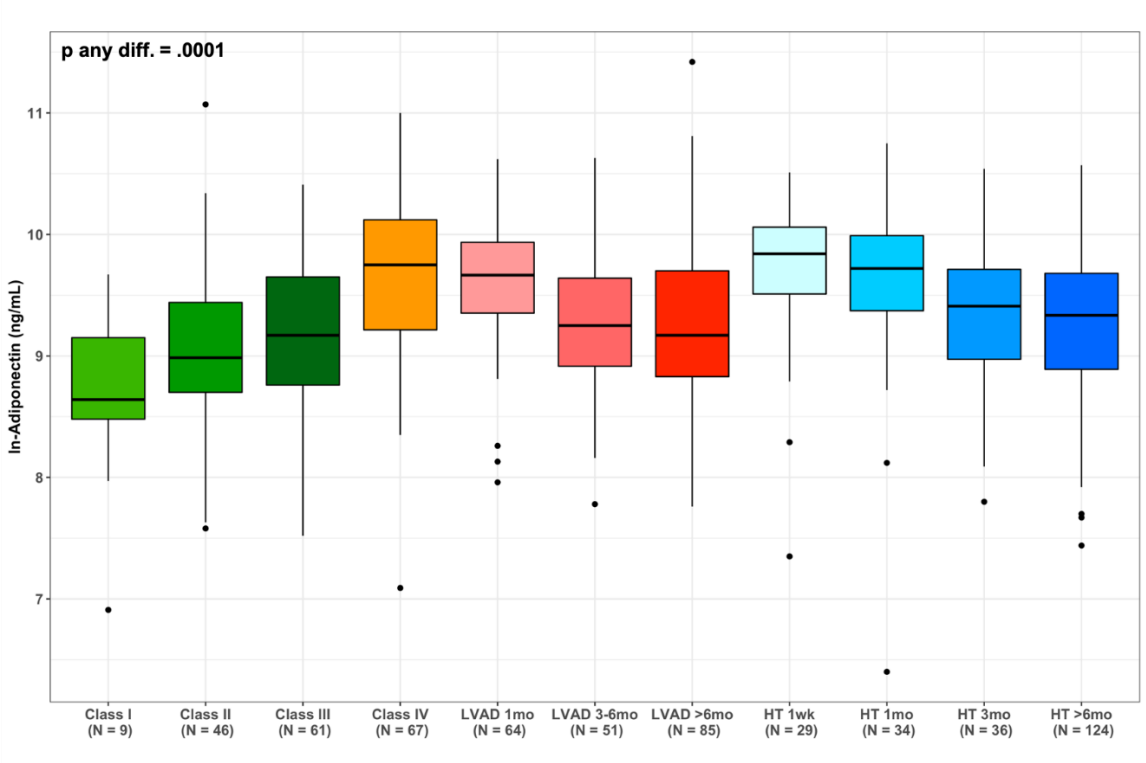
C


p any diff. < .0001
ln-TNF-α (pg/mL)
2
1
0
-1
-2
Class I (N = 9)
Class II (N = 45)
Class III (N = 61)
Class IV (N = 68)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 84)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 123)

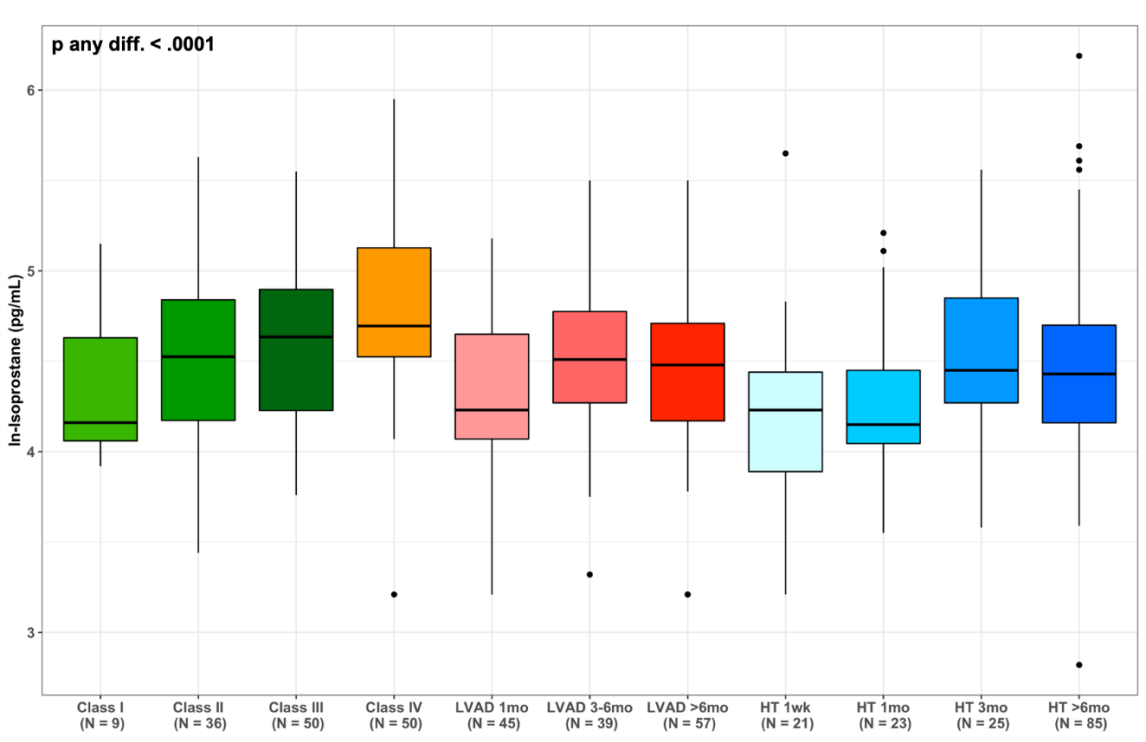
D


p any diff. = .0001
ln-ET-1 (pg/mL)
2
1
0
Class I (N = 9)
Class II (N = 46)
Class III (N = 61)
Class IV (N = 66)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 85)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 123)

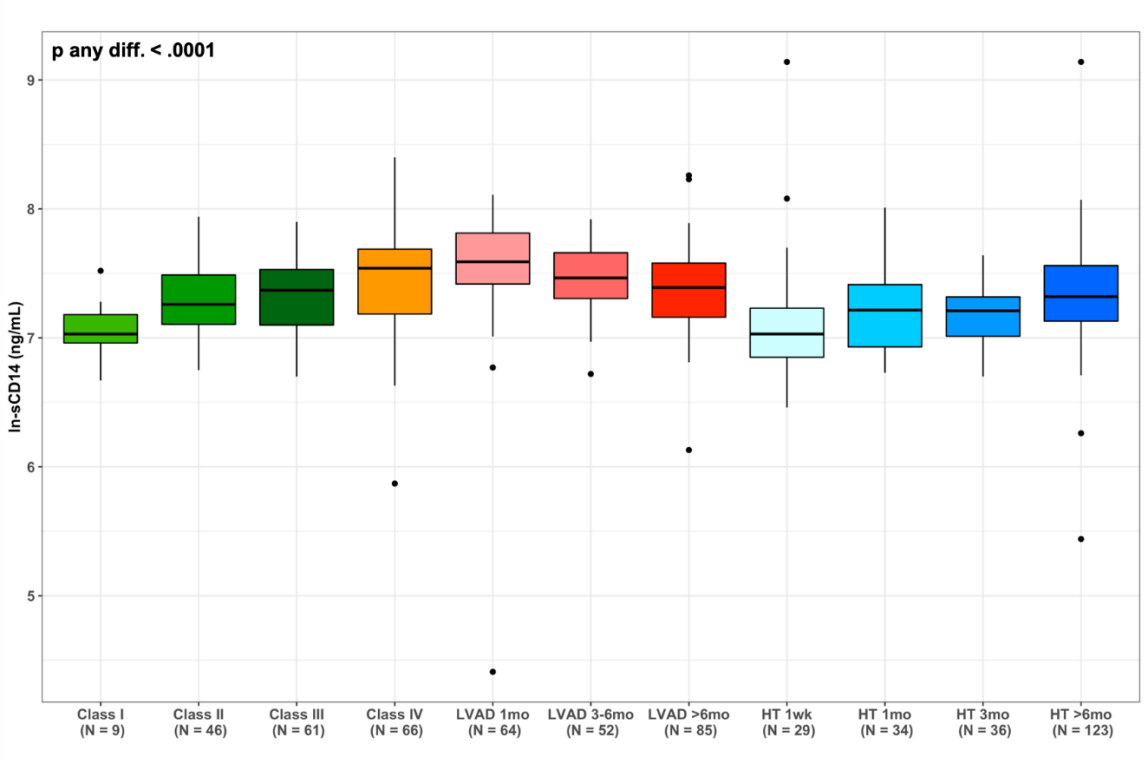
E


p any diff. = .0001
ln-Adiponectin (ng/mL)
11
10
9
8
7
Class I (N = 9)
Class II (N = 46)
Class III (N = 61)
Class IV (N = 67)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 51)
LVAD >6mo (N = 85)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 124)

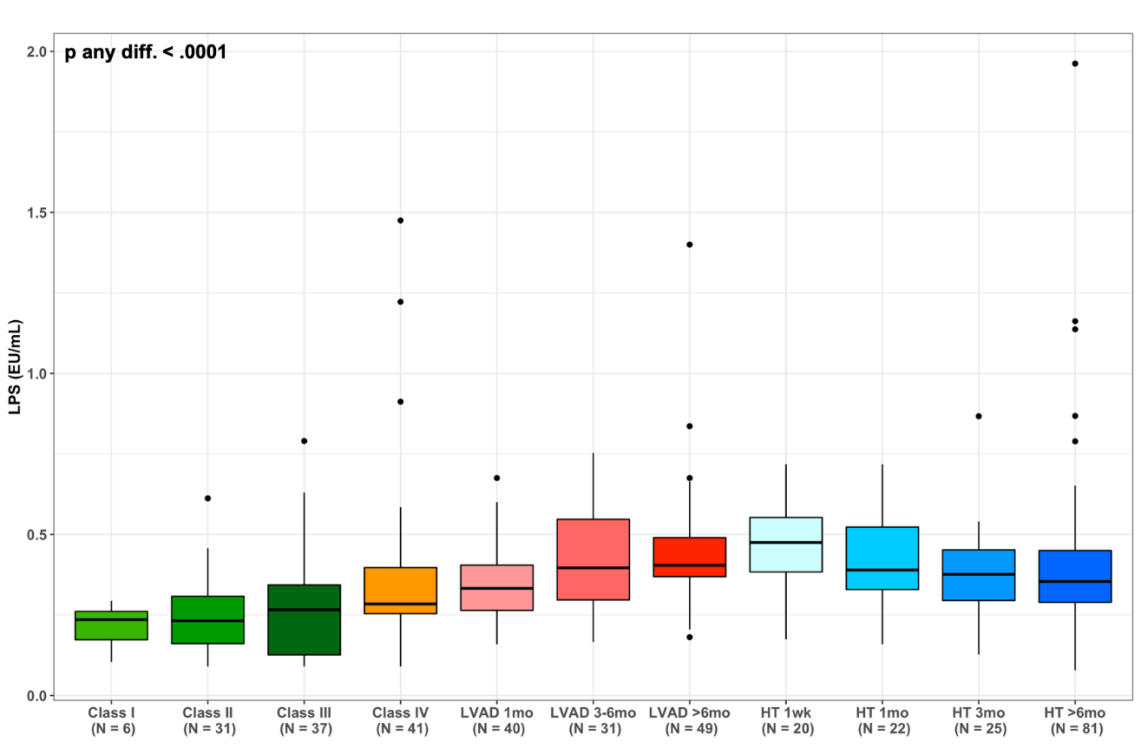
F


p any diff. < .0001
ln-Isoprostane (pg/mL)
6
5
4
3
Class I (N = 9)
Class II (N = 36)
Class III (N = 50)
Class IV (N = 50)
LVAD 1mo (N = 45)
LVAD 3-6mo (N = 39)
LVAD >6mo (N = 57)
HT 1wk (N = 21)
HT 1mo (N = 23)
HT 3mo (N = 25)
HT >6mo (N = 85)

G


p any diff. < .0001
ln-sCD14 (ng/mL)
9
8
7
6
5
Class I (N = 9)
Class II (N = 46)
Class III (N = 61)
Class IV (N = 66)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 85)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 123)

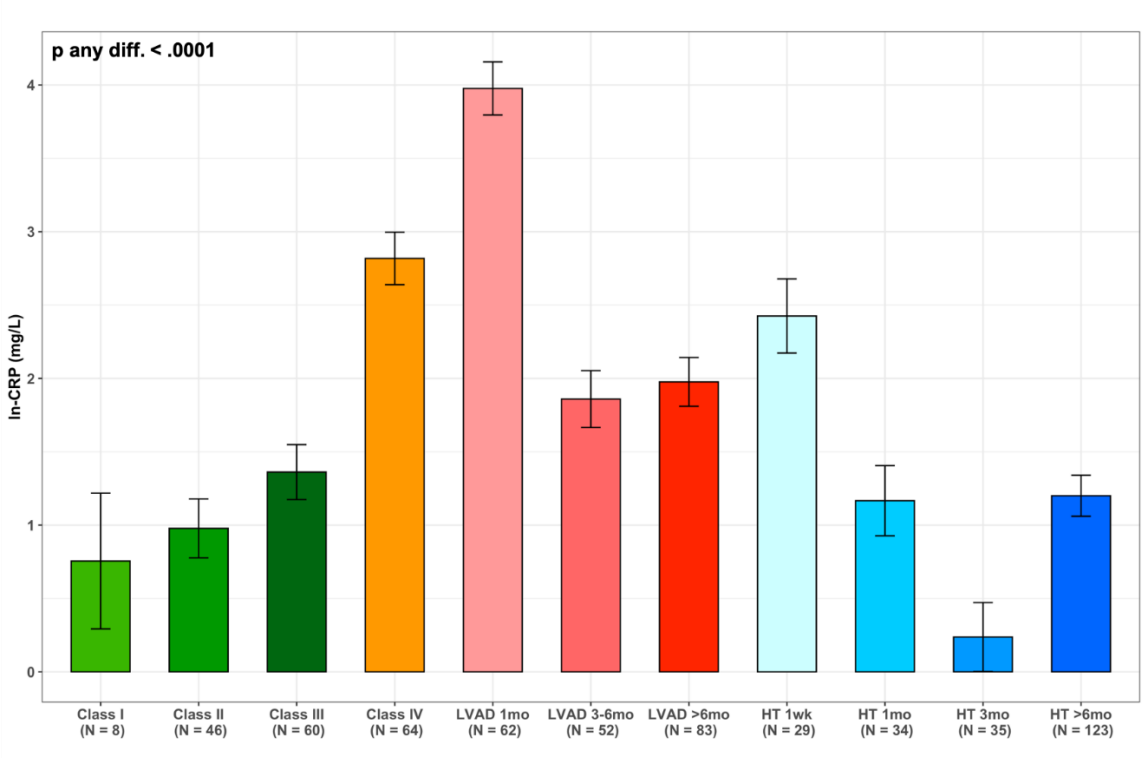
H


p any diff. < .0001
LPS (EU/mL)
2.0
1.5
1.0
0.5
0.0
Class I (N = 6)
Class II (N = 31)
Class III (N = 37)
Class IV (N = 41)
LVAD 1mo (N = 40)
LVAD 3-6mo (N = 31)
LVAD >6mo (N = 49)
HT 1wk (N = 20)
HT 1mo (N = 22)
HT 3mo (N = 25)
HT >6mo (N = 81)

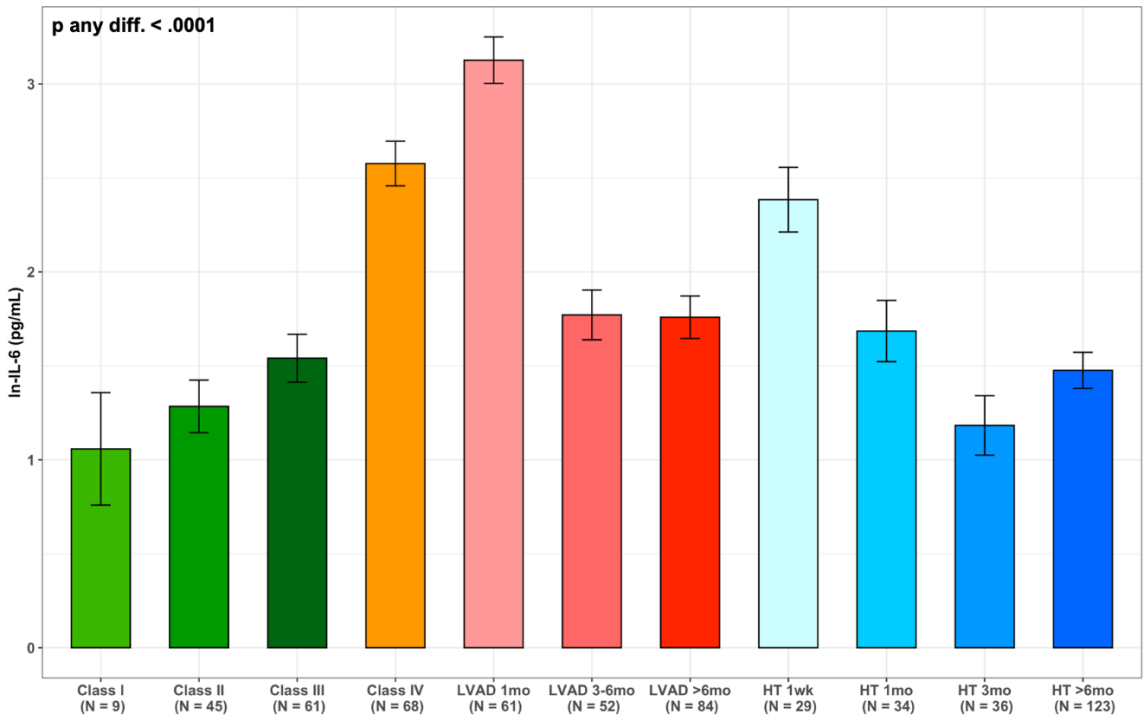
Supplemental Figure IV: Variation in circulating biomarkers of inflammation, oxidative stress and endotoxemia across disease categories (HF Class I, II, III, IV), LVAD (1month, 3-6month, >6 month), HT (1 week, 1 month, 3 month, >6 month). (**A**) CRP. (**B**) IL-6. (**C**) TNF-α. (**D**) ET-1. (**E**) Adiponectin. (**F)** Isoprostane. (**G**) sCD14. (**H**) LPS.

CRP, C-reactive protein; ET-1 endothelin-1; IL-6, interleukin-6; LPS, lipopolysaccharide; sCD14 soluble CD14; TNF- α, tumor necrosis factor alpha. HF, heart failure; LVAD, left ventricular assist device; HT, heart transplantation.

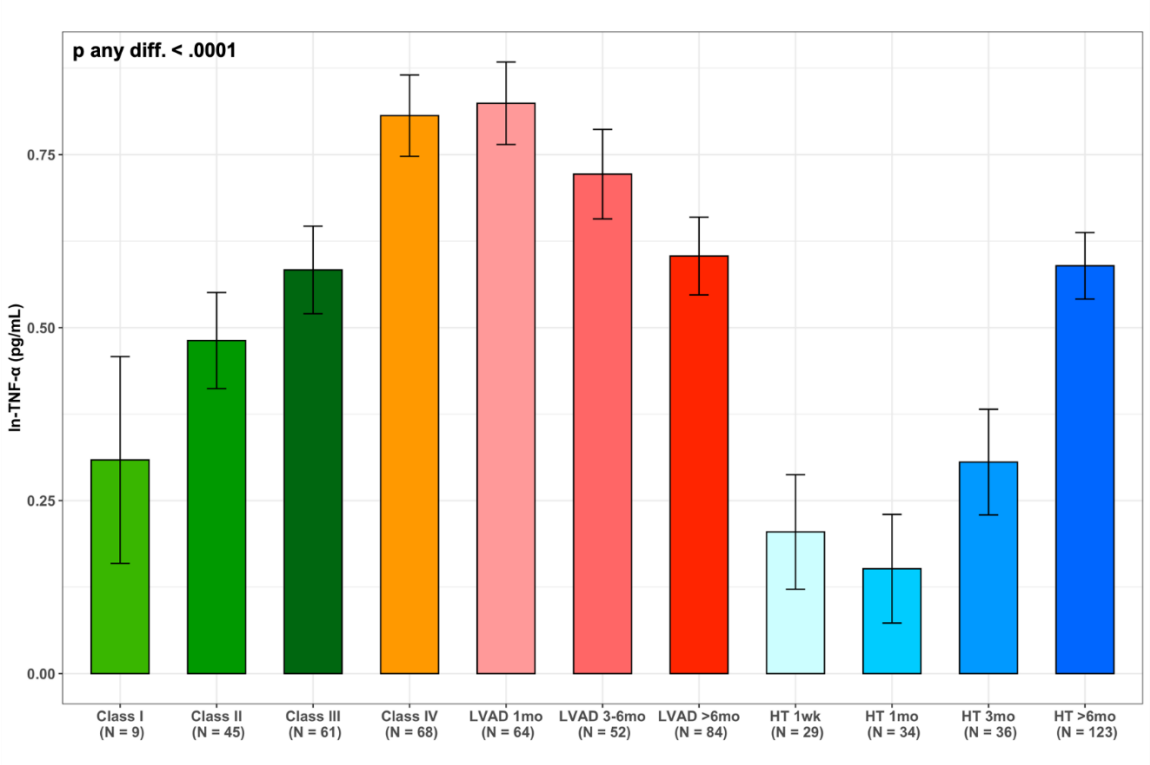
A


p any diff. < .0001
ln-CRP (mg/L)
0
1
2
3
4
Class I (N = 8)
Class II (N = 46)
Class III (N = 60)
Class IV (N = 64)
LVAD 1mo (N = 62)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 83)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 35)
HT >6mo (N = 123)

B


p any diff. < .0001
ln-IL-6 (pg/mL)
0
1
2
3
Class I (N = 9)
Class II (N = 45)
Class III (N = 61)
Class IV (N = 68)
LVAD 1mo (N = 61)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 84)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 123)

C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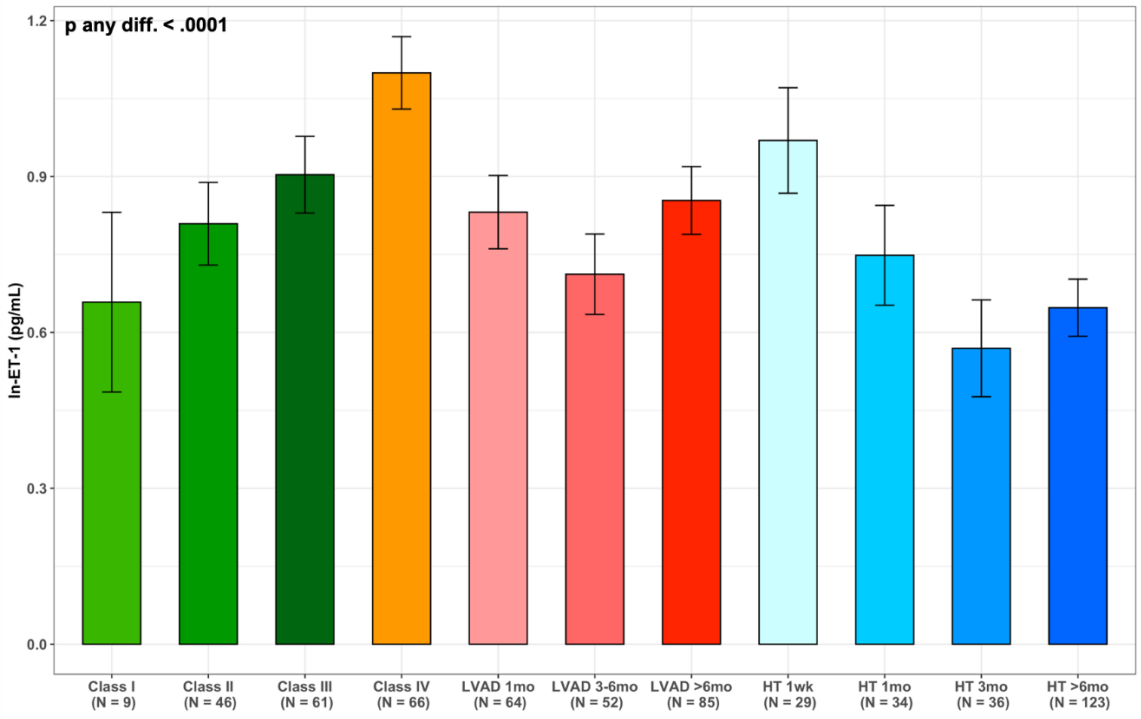
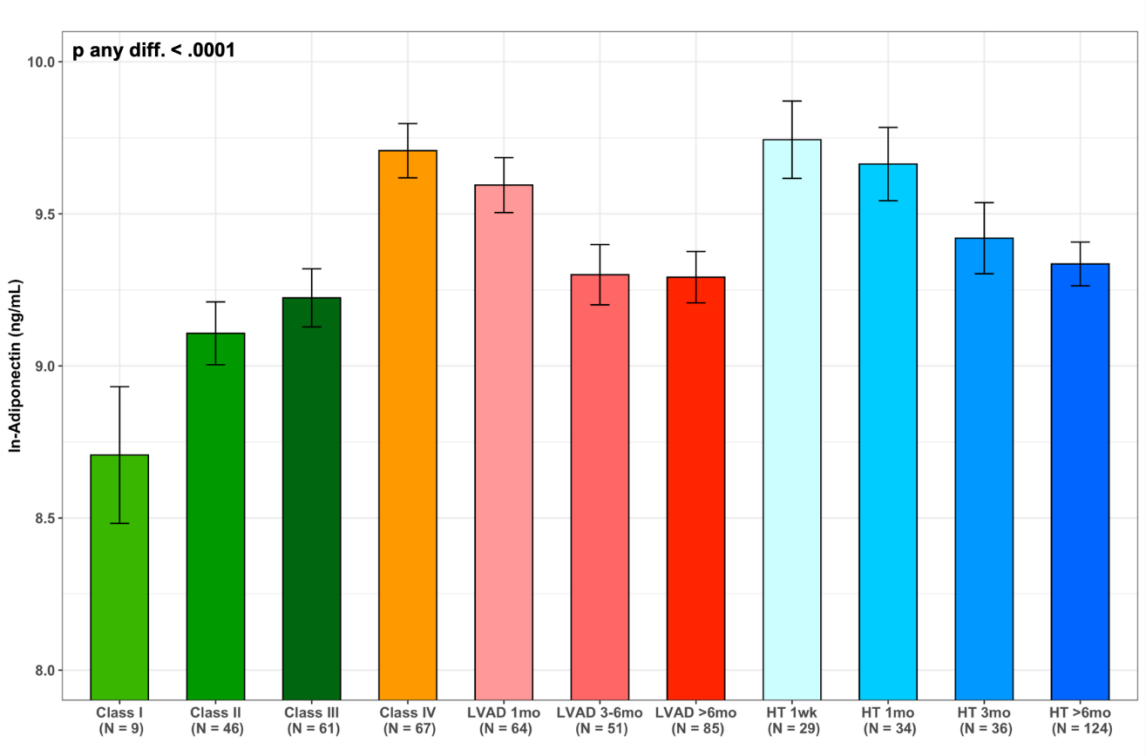
ln-TNF-α (pg/mL)
0.00
0.25
0.50
0.75
Class I (N = 9)
Class II (N = 45)
Class III (N = 61)
Class IV (N = 68)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 84)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 123)

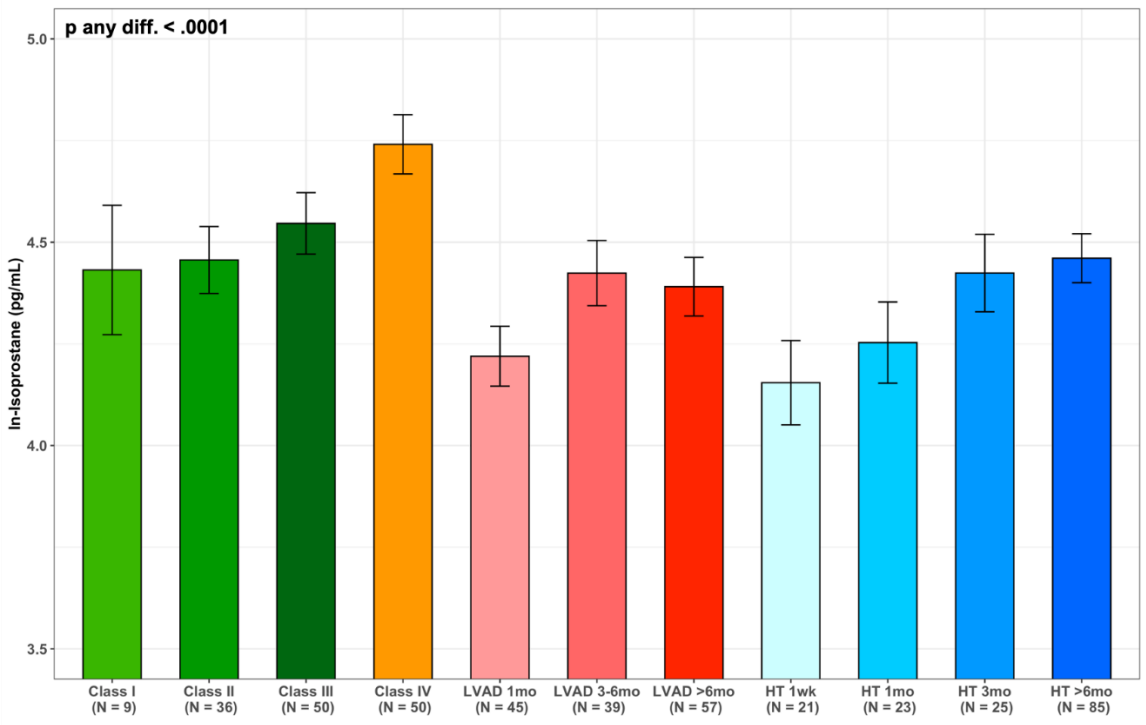
D


p any diff. < .0001
ln-ET-1 (pg/mL)
0.0
0.3
0.6
0.9
1.2
Class I (N = 9)
Class II (N = 46)
Class III (N = 61)
Class IV (N = 66)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 85)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 123)

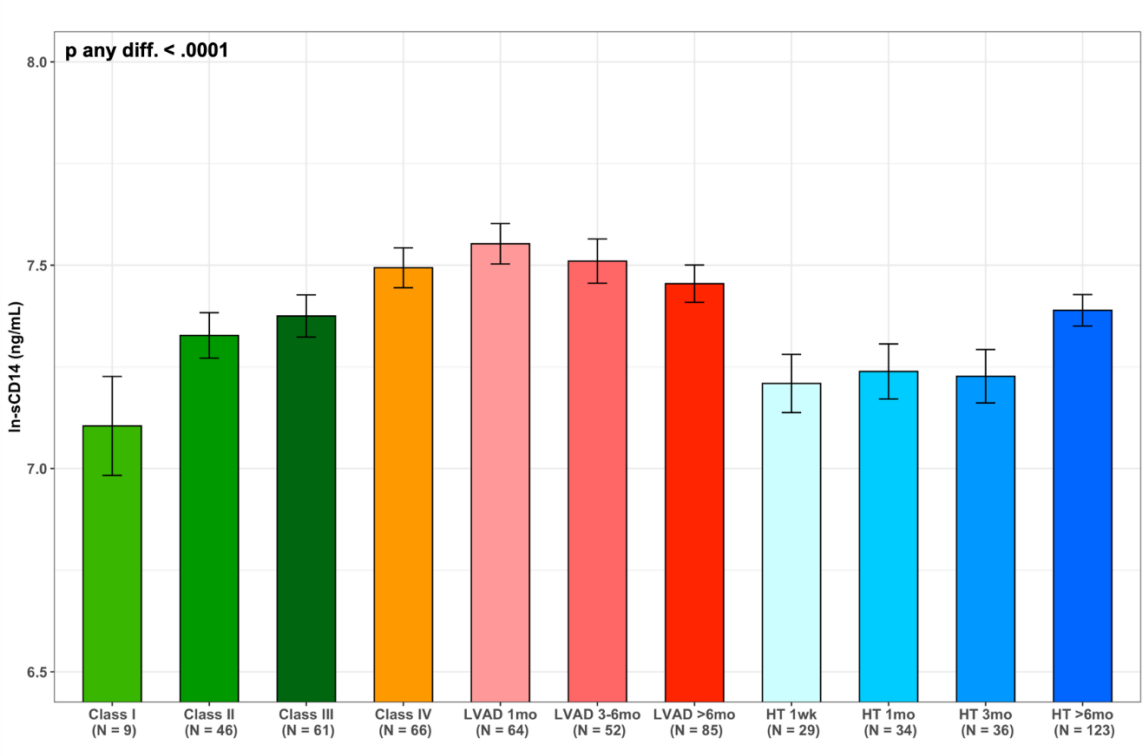
E


p any diff. < .0001
ln-Adiponectin (ng/mL)
10.0
9.5
9.0
8.5
8.0
Class I (N = 9)
Class II (N = 46)
Class III (N = 61)
Class IV (N = 67)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 51)
LVAD >6mo (N = 85)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 124)

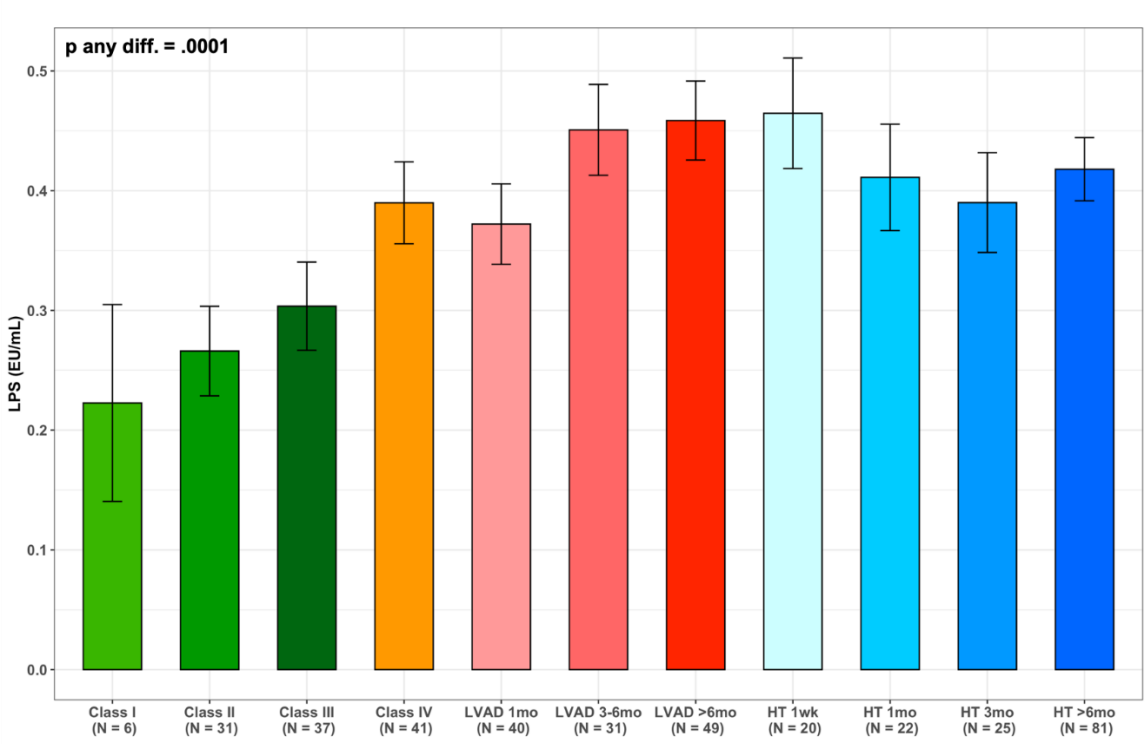
F


p any diff. < .0001
ln-Isoprostane (pg/mL)
5.0
4.5
4.0
3.5
Class I (N = 9)
Class II (N = 36)
Class III (N = 50)
Class IV (N = 50)
LVAD 1mo (N = 45)
LVAD 3-6mo (N = 39)
LVAD >6mo (N = 57)
HT 1wk (N = 21)
HT 1mo (N = 23)
HT 3mo (N = 25)
HT >6mo (N = 85)

G

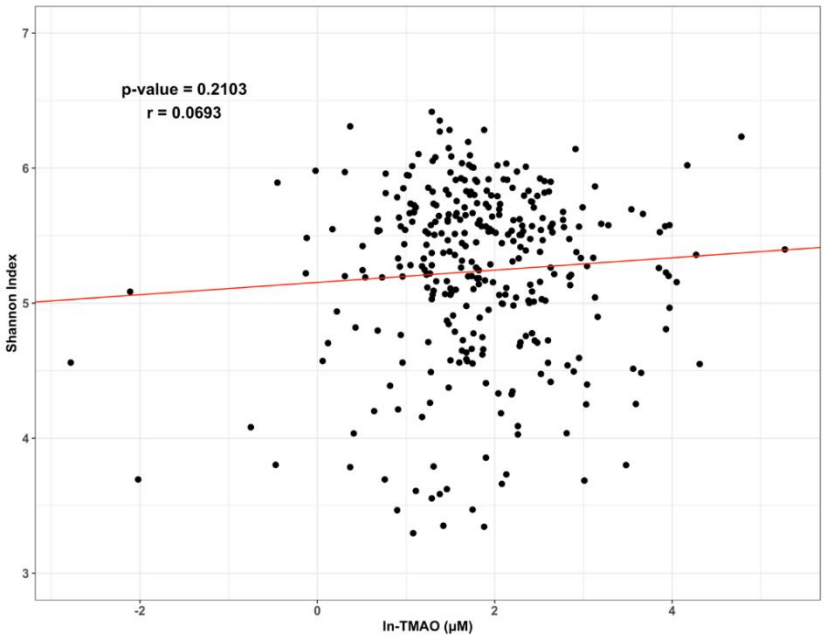

p any diff. < .0001
ln-sCD14 (ng/mL)
8.0
7.5
7.0
6.5
Class I (N = 9)
Class II (N = 46)
Class III (N = 61)
Class IV (N = 66)
LVAD 1mo (N = 64)
LVAD 3-6mo (N = 52)
LVAD >6mo (N = 85)
HT 1wk (N = 29)
HT 1mo (N = 34)
HT 3mo (N = 36)
HT >6mo (N = 123)

H

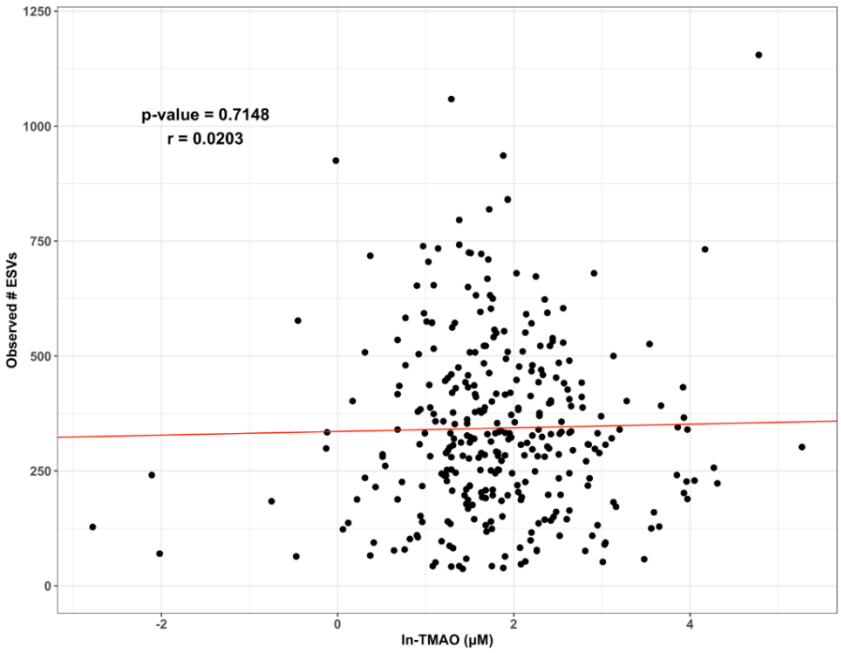

p any diff. = .0001
LPS (EU/mL)
0.5
0.4
0.3
0.2
0.1
0.0
Class I (N = 6)
Class II (N = 31)
Class III (N = 37)
Class IV (N = 41)
LVAD 1mo (N = 40)
LVAD 3-6mo (N = 31)
LVAD >6mo (N = 49)
HT 1wk (N = 20)
HT 1mo (N = 22)
HT 3mo (N = 25)
HT >6mo (N = 81)

Supplemental Figure V: Adjusted least squared means of circulating biomarkers of inflammation, oxidative stress and endotoxemia across disease categories (HF Class I, II, III, IV), LVAD (1month, 3-6month, >6 month), HT (1 week, 1 month, 3 month, >6 month). (**A**) CRP. (**B**) IL-6. (**C**) TNF-α. (**D**) ET-1. (**E**) Adiponectin. (**F)** Isoprostane. (**G**) sCD14. (**H**) LPS; Adjusted for age, sex, race/ethnicity, and antibiotics use one month prior to stool collection **(M2)**; CRP, C-reactive protein; ET-1 endothelin-1; IL-6, interleukin-6; LPS, lipopolysaccharide; sCD14 soluble CD14; TNF- α, tumor necrosis factor alpha. HF, heart failure; LVAD, left ventricular assist device; HT, heart transplantation.

A



B



Supplemental Figure VI: Correlation between TMAO and gut alpha diversity metrics. (**A**) Shannon Index and (**B**) number of observed of ESVs.

SUPPLEMENTAL TABLES

Supplemental Table I: Baseline Characteristics of HF Patients Providing Stool Samples

Study cohorts	NYHA Class I	NYHA Class II	NYHA Class III	NYHA Class IV	P-value
Number of samples (N=97)	7	29	31	30	
Demographic and clinical characteristics					
Age (years)	61.1 ± 15.2	61.2 ± 12.9	63.5 ± 12.9	59.6 ± 13.5	0.71
Male	6 (85.7%)	21 (72.4%)	25 (80.6%)	28 (93.3%)	0.20
Race					0.04
White	1 (14.3%)	15 (51.7%)	20 (64.5%)	14 (46.7%)	
Black	2 (28.6%)	8 (27.6%)	7 (22.6%)	7 (23.3%)	
Hispanic	3 (42.9%)	1 (3.4%)	3 (9.7%)	2 (6.7%)	
Other	1 (14.3%)	5 (17.2%)	1 (3.2%)	7 (23.3%)	
Smoking	3 (42.9%)	15 (51.7%)	16 (51.6%)	14 (46.7%)	0.95
Etiology, Ischemic	2 (28.6%)	14 (48.3%)	20 (64.5%)	12 (40.0%)	0.16
Hypertension	4 (57.1%)	21 (72.4%)	16 (51.6%)	19 (63.3%)	0.42
Diabetes	1 (14.3%)	9 (31.0%)	10 (32.3%)	9 (30.0%)	0.82
Atrial fib/flutter	1 (14.3%)	9 (31.0%)	14 (45.2%)	18 (60.0%)	0.05
Stroke	0 (0.0%)	1 (3.4%)	4 (12.9%)	1 (3.3%)	0.30

Laboratory parameters					
BUN (mg/dl)	16.0 ± 2.4	25.5 ± 12.2	25.9 ± 9.0	30.4 ± 18.3	0.12
Serum Cr (mg/dl)	1.1 ± 0.3	1.6 ± 1.8	1.3 ± 0.3	1.4 ± 0.4	0.64
Serum CysC (mg/liter)	1.1 ± 0.1	1.6 ± 1.4	1.5 ± 0.5	1.7 ± 0.5	0.40
$eGFR_{Cr}$ (ml/min/1.73m^2)	73.8 ± 20.0	63.0 ± 26.0	61.6 ± 18.7	60.2 ± 23.4	0.62
$eGFR_{Cys}$ (ml/min/1.73m^2)	72.2 ± 14.2	57.4 ± 24.0	54.4 ± 22.3	46.9 ± 21.8	0.05
NT-ProBNP (ng/l) Median [IQI]	443 [255, 751]	721 [197, 2267]	2418 [1123,4917]	2945 [1817,4606]	<0.001
Na (mmol/l)	142.3 ± 2.7	140.7 ± 3.5	140.2 ± 3.7	136.7 ± 5.0	0.001
AST (U/L)	26.2 ± 17.1	21.5 ± 9.1	25.8 ± 13.9	53.5 ± 91.5	0.14
ALT (U/L)	24.7 ± 16.4	22.1 ± 13.6	28.5 ± 27.1	83.7 ± 191.1	0.18
Total Bilirubin (mg/dl)	0.5 ± 0.1	0.5 ± 0.3	0.8 ± 0.4	1.0 ± 0.4	<0.001
LDH (U/L)	215 ± 96.1	230 ± 71.8	290 ± 116.4	312 ± 125.0	0.26
Biomarkers					
ln-CRP (mg/L)	0.9 ± 1.9	0.9 ± 1.4	1.3 ± 1.3	2.6 ± 1.5	<0.001
ln-IL-6 (pg/mL)	1.0 ± 0.6	1.2 ± 0.7	1.4 ± 0.7	2.4 ± 1.0	<0.001
ln-TNF-α (pg/mL)	0.3 ± 0.4	0.5 ± 0.4	0.6 ± 0.4	0.8 ± 0.4	0.001
ln-ET-1 (pg/mL)	0.7 ± 0.5	1.0 ± 0.5	1.1 ± 0.5	1.1 ± 0.5	0.27
ln-Adiponectin (ng/mL)	8.6 ± 0.8	8.9 ± 0.7	9.3 ± 0.6	9.7 ± 0.7	<0.001
ln-Isoprostane (pg/mL)	4.4 ± 0.3	4.5 ± 0.5	4.7 ± 0.4	4.8 ± 0.4	0.06
ln-sCD14 (ng/mL)	7.1 ± 0.2	7.3 ± 0.2	7.3 ± 0.3	7.4 ± 0.3	0.02

LPS (EU/mL)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.3	0.04
Medications					
ASA	5 (71.4%)	24 (82.8%)	18 (58.1%)	18 (60.0%)	0.16
Coumadin	0 (0.0%)	2 (6.9%)	8 (25.8%)	3 (10.0%)	0.09
ACEi	3 (42.9%)	10 (34.5%)	9 (29.0%)	2 (6.7%)	0.04
ARB	4 (57.1%)	9 (31.0%)	15 (48.4%)	3 (10.0v)	0.006
Aldosterone Antagonists	4 (57.1%)	18 (62.1%)	23 (74.2%)	20 (66.7%)	0.72
β-blockers	7 (100.0%)	28 (96.6%)	28 (90.3%)	21 (70.0%)	0.011
Statins	4 (57.1%)	14 (48.3%)	17 (54.8%)	19 (63.3%)	0.71
Loop diuretics	5 (71.4%)	23 (79.3%)	27 (87.1%)	24 (80.0%)	0.74
Digoxin	2 (28.6%)	4 (13.8%)	7 (22.6%)	8 (26.7%)	0.63
Antibiotics*	0 (0.0%)	4 (13.8%)	4 (12.9%)	7 (23.3%)	0.40

NYHA, New York Heart Association; BMI, body mass index; AST, aspartate transaminase; ALT, alanine transaminase, LDH, lactate dehydrogenase; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; Cr, Creatinine; CysC, Cystatin C; eGFR, estimated glomerular filtration rate; TNF-α, tumor necrosis factor alpha; sCD14, soluble CD14; IL-6, interleukin-6; CRP, C-reactive protein; ET-1, endothelin-1; LPS, lipopolysaccharide; ASA, acetylsalicylic acid; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker. Data presented n (%) or mean ± SD as appropriate, unless otherwise noted.

* Antibiotic use is defined as any antibiotic use that was indicated for infection that was estimated to have occurred 1 month before stool collection.

Supplemental Table II: Baseline Characteristics of LVAD Patients Providing Stool Samples

Study cohorts	LVAD (1mo)	LVAD (3-6mo)	LVAD (>6mo)	P-value
Number of samples (N=120)	37	31	52	
Demographic and clinical characteristics				
Age (years)	58.6 ± 12.2	59.1 ± 14.3	58.9 ± 14.1	<0.001
Male	32 (86.5%)	26 (83.9%)	43 (82.7%)	0.89
Race				0.19
White	26 (70.3%)	22 (71.0%)	33 (63.5%)	
Black	5 (13.5%)	6 (19.4%)	15 (28.8%)	
Hispanic	1 (2.7%)	2 (6.5%)	3 (5.8%)	
Other	5 (13.5%)	1 (3.2%)	1 (1.9%)	
BMI (kg/m^2) Median [IQI]	28.7 [21.7, 29.4]	26.1 [24.6, 30.1]	28.0 [24.9, 32.2]	0.50
Smoking	24 (64.9%)	19 (61.3%)	27 (51.9%)	0.44
Etiology, Ischemic	23 (62.2%)	13 (41.9%)	19 (36.5%)	0.05
INTERMACS Profile				0.76
1	2 (5.4%)	2 (6.5%)	3 (5.8%)	
2	20 (54.1%)	18 (58.1%)	29 (55.8%)	
3	8 (21.6%)	10 (32.3%)	14 (26.9%)	

4	3 (8.1%)	1 (3.2%)	2 (3.8%)	
5-7 or not provided	4 (10.8%)	0 (0.0%)	4 (7.7%)	
Hypertension	24 (64.9%)	17 (54.8%)	30 (57.7%)	0.68
Diabetes	14 (37.8%)	11 (35.5%)	19 (36.5%)	0.98
Atrial Fib/flutter	18 (48.6%)	14 (45.2%)	26 (50.0%)	0.91
Stroke	0 (0.0%)	2 (6.5%)	6 (11.5%)	0.10
Time after LVAD, months Median [IQR]	0.7 [0.6, 1.1]	5.13 [3.9, 6.2]	11.9 [10.1, 16.2]	<0.001
Laboratory parameters				
BUN (mg/dl)	18.4 ± 10.5	23.3 ± 11.2	24.4 ± 11.3	<0.001
Serum Cr (mg/dl)	1.2 ± 0.5	1.3 ± 0.5	1.5 ± 1.0	<0.001
Serum CysC (mg/l)	1.5 ± 0.5	1.6 ± 0.6	1.6 ± 0.8	0.10
$eGFR_{Cr}$ (ml/min/1.73m^2)	80.0 ± 41.6	63.9 ± 24.0	60.2 ± 24.4	<0.001
$eGFRc_{ys}$ (ml/min/1.73m^2)	53.1 ± 22.0	49.7 ± 22.5	56.0 ± 27.6	0.05
NT-ProBNP (ng/l) Median [IQI]	3116 [2255, 4262]	1289 [580, 2117]	905 [454, 2014]	<0.001
Na (mmol/l)	135.8 ± 4.2	140.4 ± 3.2	140.0 ± 3.0	<0.001
AST (U/L)	26.6 ± 9.5	26.1 ± 11.6	23.5 ± 8.5	0.36
ALT (U/L)	25.8 ± 13.3	20.3 ± 10.8	18.7 ± 8.4	0.02
Total Bilirubin (mg/dl)	0.8 ± 0.8	0.5 ± 0.2	0.6 ± 0.3	0.04
LDH (U/L)	340 ± 108.2	316 ± 97.6	320 ± 121.0	0.43
Biomarkers				

ln-CRP (mg/L)	3.9 ± 0.9	1.6 ± 1.0	1.5 ± 1.1	<0.001
ln-IL-6 (pg/mL)	3.2 ± 0.8	1.7 ± 0.8	1.6 ± 0.8	<0.001
ln-TNF-α (pg/mL)	0.9 ± 0.3	0.7 ± 0.4	0.5 ± 0.5	<0.001
ln-ET-1 (pg/mL)	1.0 ± 0.6	0.8 ± 0.5	0.8 ± 0.5	0.35
ln-Adiponectin (ng/mL)	9.6 ± 0.5	9.3 ± 0.6	9.2 ± 0.8	0.01
ln-Isoprostane (pg/mL)	4.3 ± 0.4	4.5 ± 0.4	4.4 ± 0.5	0.07
ln-sCD14 (ng/mL)	7.6 ± 0.3	7.5 ± 0.2	7.3 ± 0.3	0.004
LPS (EU/mL)	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.01
Medications				
ASA	35 (94.6%)	26 (83.9%)	34 (65.4%)	0.003
Coumadin	17 (45.9%)	29 (93.5%)	44 (84.6%)	<0.001
ACEi	3 (8.1%)	10 (32.3%)	14 (26.9%)	0.04
ARB	1 (2.7%)	3 (9.7%)	15 (28.8%)	0.002
Aldosterone Antagonists	15 (40.5%)	14 (45.2%)	17 (32.7%)	0.50
β-blockers	20 (54.1%)	28 (90.3%)	49 (94.2%)	<0.001
Statin	11 (29.7%)	11 (35.5%)	17 (32.7%)	0.88
Loop diuretics	26 (70.3%)	26 (83.9%)	32 (61.5%)	0.10
Digoxin	9 (24.3%)	11 (35.5%)	20 (38.5%)	0.36
Antibiotics*	23 (62.2%)	8 (25.8%)	12 (23.1%)	<0.001

LVAD, left ventricular assist device; BMI, body mass index; AST, aspartate transaminase; ALT, alanine transaminase, LDH, lactate dehydrogenase; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; Cr, Creatinine; CysC, Cystatin C; eGFR, estimated glomerular filtration rate; TNF-α, tumor necrosis factor alpha; sCD 14, soluble CD14; IL-6, interleukin-6; CRP, C-reactive

protein; ET-1, endothelin-1; LPS, lipopolysaccharide; ASA, acetylsalicylic acid; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker. Data presented n (%) or mean ± SD as appropriate, unless otherwise noted.

*Antibiotic use is defined as any antibiotic use that was indicated for infection that was estimated to have occurred 1 month before stool collection

Supplemental Table III: Baseline Characteristics of HT Patients Providing Stool Samples

Study cohorts	HT (1wk)	HT (1mo)	HT (3mo)	HT (>6mo)	P-value
Number of samples (N=110)	13	12	13	72	
Demographic and clinical characteristics					
Age, (years)	54.9 ± 10.2	54.8 ± 11.0	51.5 ± 12.1	57.4 ± 12.2	<0.001
Male	12 (92.3%)	11 (91.7%)	12 (92.3%)	59 (81.9%)	0.56
Race					0.88
White	7 (53.8%)	5 (41.7%)	8 (61.5%)	37 (51.4%)	
Black	3 (23.1%)	6 (50.0%)	2 (15.4%)	21 (29.2%)	
Hispanic	2 (15.4%)	1 (8.3%)	2 (15.4%)	9 (12.5%)	
Other	1 (7.7%)	0 (0.0%)	1 (7.7%)	5 (6.9%)	
Smoking	9 (69.2%)	6 (50.0%)	5 (38.5%)	33 (45.8%)	0.40
Etiology, Ischemic	6 (46.2%)	4 (33.3%)	5 (38.5%)	35 (48.6%)	0.74
Hypertension	6 (46.2%)	8 (66.7%)	8 (61.5%)	63 (87.5%)	0.003
Diabetes	5 (38.5%)	4 (33.3%)	4 (30.8%)	36 (50.0%)	0.45
Atrial Fib/flutter	5 (38.5%)	4 (33.3%)	5 (38.5%)	21 (29.2%)	0.86
Stroke	4 (30.8%)	4 (33.3%)	3 (23.1%)	10 (13.9%)	0.25
Time after HT, months Median [IQI]	0.30 [0.3,0.3]	0.84 [0.7,1.2]	2.63 [2.23 3.1]	39.4 [9.3, 89.3]	<0.001

Laboratory parameters					
BUN (mg/dl)	32.7 ± 15.6	35.2 ± 13.9	32.0 ± 14.9	26.0 ± 10.0	0.005
Serum Cr (mg/dl)	1.1 ± 0.3	1.4 ± 0.4	1.3 ± 0.3	1.6 ± 1.0	0.09
Serum CysC (mg/l)	1.6 ± 0.6	1.5 ± 0.5	1.3 ± 0.3	1.52 ± 0.8	0.56
$eGFR_{Cr}$ (ml/min/1.73m^2)	80.3 ± 30.5	65.2 ± 24.0	62.0 ± 16.8	59.5 ± 39.7	0.02
$eGFRc_{ys}$ (ml/min/1.73m^2)	52.9 ± 24.1	52.4 ± 22.9	59.5 ± 20.3	55.2 ± 24.7	0.08
Na (mmol/l)	137.3 ± 2.2	139.4 ± 2.9	139.4 ± 3.7	141.7 ± 2.8	<0.001
AST (U/L)	21.6 ± 8.8	24.3 ± 8.4	22.4 ± 9.2	24.6 ± 15.5	0.76
ALT (U/L)	24.2 ± 13.1	44.5 ± 25.2	36.4 ± 33.8	21.7 ± 12.9	<0.001
Total Bilirubin (mg/dl)	0.6 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.4	0.50
LDH (U/L)	707 ± 293.4	315 ± 92.3	308 ± 77.0	249.0 ± 86.6	<0.001
Biomarkers					
ln-CRP (mg/L)	2.3 ± 1.3	0.6 ± 1.4	0.4 ± 1.7	1.1 ± 1.2	<0.001
ln-IL-6 (pg/mL)	2.3 ± 0.7	1.6 ± 0.8	1.3 ± 1.0	1.3 ± 0.9	<0.001
ln-TNF-α (pg/mL)	0.2 ± 0.4	0.1 ± 0.4	0.2 ± 0.5	0.5 ± 0.5	0.002
ln-ET-1 (pg/mL)	1.0 ± 0.4	0.8 ± 0.5	0.9 ± 0.6	0.7 ± 0.5	0.18
ln-Adiponectin (ng/mL)	9.6 ± 0.8	9.7 ± 0.5	9.4 ± 0.7	9.2 ± 0.6	0.005
ln-Isoprostane (pg/mL)	4.1 ± 0.3	4.1 ± 0.3	4.5 ± 0.3	4.4 ± 0.5	0.02
ln-sCD14 (ng/mL)	7.1 ± 0.4	7.2 ± 0.4	7.2 ± 0.3	7.3 ± 0.3	0.17
LPS (EU/mL)	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.13
Medications					
ASA	3 (23.1%)	10 (83.3%)	12 (92.3%)	65 (90.3%)	<0.001

Coumadin	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (5.6%)	0.53
ACE inhibitors	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (6.9%)	0.43
ARB	0 (0.0%)	0 (0.0%)	0 (0.0%)	16 (22.2%)	0.02
Aldosterone Antagonists	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (4.2%)	0.65
β-blockers	0 (0.0%)	2 (16.7%)	0 (0.0%)	17 (23.6%)	0.06
Statin	5 (38.5%)	12 (100.0%)	11 (84.6%)	60 (83.3%)	0.001
Loop diuretics	4 (30.8%)	3 (25.0%)	7 (53.8%)	17 (23.6%)	0.17
Antibiotics*	13 (100.0%)	12 (100.0%)	13 (100.0%)	21 (29.2%)	<0.001
Immunosuppression					
Tacrolimus	12 (92.3%)	12 (100.0%)	13 (100.0%)	53 (73.6%)	0.02
Cyclosporine	0 (0.0%)	0 (0.0%)	0 (0.0%)	15 (20.8%)	0.03
Prednisone	11 (84.6%)	12 (100.0%)	13 (100.0%)	52 (72.2%)	0.03
Sirolimus	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.4%)	0.91
Everolimus	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (4.2%)	0.65
Mycophenolate Mofetil	11 (84.6%)	11 (91.7%)	11 (84.6) %	53 (73.6%)	0.43

HT, heart transplant; BMI, body mass index; AST, aspartate transaminase; ALT, alanine transaminase, LDH, lactate dehydrogenase; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; Cr, Creatinine; CysC, Cystatin C; eGFR, estimated glomerular filtration rate; TNF-α, tumor necrosis factor alpha; sCD 14, soluble CD14; IL-6, interleukin-6; CRP, C-reactive protein; ET-1, endothelin-1; LPS, lipopolysaccharide; ASA, acetylsalicylic acid; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; NA, not available.

Data presented n (%) or mean (SE) as appropriate, unless otherwise noted.

*Antibiotic use is defined as any antibiotic use that was indicated for infection or prophylaxis as per HT protocol that was estimated to have occurred 1 month before stool collection.

Supplemental Table IV: Mean ln-TMAO Values Before and After LVAD Among Patients With Repeated Longitudinal Measures

Variables		LVAD 1 Month	LVAD 3-6 Months	LVAD >6 Months
Crude	**Number of Samples**	49	37	19
	Median Time Pre-LVAD [IQI], days	3 [1, 7]	3 [1, 6]	2 [1, 6]
	Median Time Post-LVAD [IQI], days	19 [15, 25]	143 [105, 188]	315 [291.5, 330.5]
	ln-TMAO Mean (95% CI) Pre-LVAD	1.85 (1.49, 2.22)	1.96 (1.71, 2.21)	1.80 (1.5, 2.09)
	ln-TMAO Mean (95% CI) Post-LVAD	1.41 (1.05, 1.78)	1.65 (1.40, 1.90)	1.79 (1.5, 2.08)
	p-value	0.0669	0.0198	0.9842
$eGFR_{Cr}$ Adjusted	**Number of Samples**	32	27	12
	Median Time Pre-LVAD [IQI], days	3 [1, 9.5]	2 [1, 6]	3 [1, 6.25]
	Median Time Post-LVAD [IQI], days	19 [15, 25]	151 [104.5, 187.5]	315.5 [285.8, 344.3]
	ln-TMAO Mean (95% CI) Pre-LVAD	1.80 (1.37, 2.23)	1.94 (1.69, 2.18)	1.84 (1.47, 2.21)
	ln-TMAO Mean (95% CI) Post-LVAD	1.27 (0.84, 1.70)	1.68 (1.43, 1.93)	1.93 (1.56, 2.31)
	p-value	0.0579	0.0516	0.6414

$eGFR_{Cys}$ Adjusted	**Number of Samples**	27	20	8
	Median Time Pre-LVAD [IQI], days	3 [1, 7.5]	2 [1, 6]	4 [1.75, 6.50]
	Median Time Post-LVAD [IQI], days	20 [16, 25]	117.5 [101.3,156.8]	314 [285.5, 322.8]
	ln-TMAO Mean 95% CI) Pre-LVAD	1.98 (1.49, 2.46)	2.10 (1.79, 2.40)	2.08 (1.66, 2.50)
	ln-TMAO Mean (95% CI) Post-LVAD	1.19 (0.71, 1.67)	1.68 (1.38, 1.99)	1.79 (1.37, 2.22)
	p-value	0.0154	0.0226	0.3051

Means correspond to adjusted least-squared means. IQI, interquartile interval; LVAD, left ventricular assist device; SE, standard error

Supplemental Table V: Mean ln-TMAO Values Before and After HT Among Patients With Repeated Longitudinal Measures

Variables		HT 1 Week	HT 1 Month	HT 3-6 Months	HT >6 Months
Crude	**Number of Samples**	17	20	20	19
	Median Time Pre-HT [IQI], days	57 [22,134]	64 [20.5,140.3]	54 [14.3,140.3]	51 [13.5,160.5]
	Median Time Post-HT [IQI], days	8 [7, 10]	21 [19, 28]	85 [57, 111.5]	147 [141.5,169]
	ln-TMAO Mean (95% CI) Pre-HT	1.94 (1.38, 2.51)	1.98 (1.32, 2.64)	1.99 (1.57, 2.41)	1.98 (1.37, 2.58)
	ln-TMAO Mean (95% CI) Post-HT	0.74 (0.17, 1.31)	1.22 (0.56, 1.88)	1.90 (1.48, 2.33)	1.54 (0.94, 2.14)
	p-value	0.0004	0.0536	0.7075	0.2169
$eGFR_{Cr}$ Adjusted	**Number of Samples**	11	14	13	9
	Median Time Pre-HT [IQI], days	37 [16.5, 67.5]	36.5 [12.3, 79.5]	16 [11, 62]	16 [12, 66]
	Median Time Post-HT [IQI], days	9 [8, 10]	22.5 [19.3, 28.3]	110 [57, 113]	169 [141, 180]
	ln-TMAO Mean (95% CI) Pre-HT	2.22 (1.57, 2.87)	2.00 (1.45, 2.56)	2.23 (1.88, 2.78)	2.19 (1.27, 3.11)

	ln-TMAO Mean (95% CI) Post-HT	0.94 (0.29, 1.59)	0.73 (0.02, 1.44)	1.77 (1.32, 2.23)	1.55 (0.63, 2.47)
	p-value	0.0006	0.0008	0.0773	0.2560
$eGFR_{Cys}$ Adjusted	**Number of Samples**	13	15	15	14
	Median Time Pre-HT [IQI], days	51 [22, 201]	62 [13.5, 160.5]	51 [13, 160.5]	44 [12, 111]
	Median Time Post-HT [IQI], days	9 [7, 10]	21 [19, 28]	59 [56.5, 110.5]	146 [141.25, 167.25]
	ln-TMAO Mean (95% CI) Pre-HT	2.32 (1.58, 3.05)	2.16 (1.49, 2.82)	2.05 (1.53, 2.56)	2.04 (1.54, 2.54)
	ln-TMAO Mean (95% CI) Post-HT	0.39 (-0.34, 1.13)	0.22 (-0.58, 1.02)	1.77 (1.26, 2.29)	1.88 (1.38, 2.37)
	p-value	0.0001	<0.0001	0.2949	0.5097

Means correspond to adjusted least-squared means. IQI, interquartile interval; HT, heart transplant; SE, standard error

Supplemental Table VI: Association Between ln-TMAO Levels and Biomarkers of Inflammation, Endotoxemia and Oxidative Stress

	M0			M3			M4		
Dependent Variables	**Regression Coefficient**	**p-value**	**N**	**Regression Coefficient**	**p-value**	**N**	**Regression Coefficient**	**p-value**	**N**
ln-Adiponectin	0.042	0.0947	606	0.064	0.0465	476	0.022	0.4879	456
ln-TNF-alpha	0.077	<.0001	605	0.024	0.2718	474	0.019	0.3128	457
ln-sCD14	0.053	0.0004	605	0.027	0.1323	475	0.015	0.3610	454
ln-IL6	0.001	0.9721	602	0.007	0.8945	470	-0.092	0.0622	452
ln-Isoprostane	0.041	0.0554	440	-0.039	0.0895	393	-0.027	0.2438	385
ln-CRP	-0.092	0.1285	596	-0.071	0.3514	468	-0.189	0.0124	445
ln-ET-1	0.037	0.0579	605	0.029	0.2366	474	0.014	0.5789	455
ln-LPS	-0.011	0.6482	383	0.029	0.3084	342	0.005	0.8444	334

TNF- α, tumor necrosis factor alpha; sCD14 soluble CD14; IL-6, interleukin-6; CRP, C-reactive protein; ET-1 endothelin-1; LPS, lipopolysaccharide; **Model(M)0**, unadjusted; **M3,** adjusted for age, sex, race/ethnicity, antibiotics use one month prior to stool collection, and serum creatinine estimated glomerular filtration rate ($eGFR_{Cr}$); **M4,** adjusted for age, sex, race/ethnicity, antibiotics use one month prior to stool collection, and Cystatin C estimated glomerular filtration rate ($eGFR_{Cys}$). Mixed models with random intercepts were used to account for repeated sampling. N corresponds to the number of observations included in each model, depending on the availability of $eGFR_{Cr}$, $eGFR_{Cys}$, and blood biomarker data.

Supplemental Table VII: Differences in Unadjusted and Adjusted Mean Values of Shannon Index and Number of Observed ESVs Between Select Groups of Patients

Patient Comparison Groups	A) Shannon Index		B) # Observed ESVs	
	M0	M2	M0	M2
Class I - LVAD 1mo	1.06 (p=0.0009)	0.78 (p=0.0594)	321.69 (p=0.0012)	241.46 (p=0.053)
Class I - HT 1wk	1.41 (p<.0001)	1.00 (p=0.0185)	342.19 (p=0.0036)	235.92 (p=0.1875)
Class I - HT 1mo	1.10 (p=0.0062)	0.72 (p=0.2953)	334.44 (p=0.0063)	235.39 (p=0.2142)
Class II - LVAD 1mo	0.59 (p=0.0035)	0.38 (p=0.2483)	168.58 (p=0.0099)	111.89 (p=0.3112)
Class II - HT 1wk	0.94 (p=0.0002)	0.60 (p=0.1082)	189.09 (p=0.0737)	106.34 (p=0.8305)
Class III - LVAD 1mo	0.55 (p=0.0074)	0.36 (p=0.2986)	189.31 (p=0.0012)	142.46 (p=0.051)
Class III - HT 1wk	0.90 (p=0.0004)	0.58 (p=0.1276)	209.82 (p=0.0237)	136.92 (p=0.4925)
Class III - HT 1mo	0.58 (p=0.1381)	0.31 (p=0.9249)	202.06 (p=0.0484)	136.39 (p=0.5507)
Class IV - HT 1wk	0.70 (p=0.0202)	0.40 (p=0.6528)	131.29 (p=0.5306)	55.06 (p=0.9982)
LVAD 1mo - LVAD 3-6mo	-0.63 (p=0.0008)	-0.47 (p=0.0352)	-168.62 (p=0.0069)	-127.25 (p=0.1025)
LVAD 1mo - LVAD >6mo	-0.55 (p=0.0012)	-0.37 (p=0.1251)	-153.62 (p=0.0051)	-105.14 (p=0.1966)
LVAD 1mo - HT >6mo	-0.48 (p=0.0042)	-0.31 (p=0.2688)	-124.89 (p=0.0325)	-75.95 (p=0.5925)
LVAD 3-6mo - HT 1wk	0.98 (p<.0001)	0.70 (p=0.0213)	189.13 (p=0.067)	121.71 (p=0.6398)

LVAD 3-6mo - HT 1mo	0.66 (p=0.0466)	0.42 (p=0.6123)	181.37 (p=0.1189)	121.18 (p=0.6900)
LVAD >6mo - HT 1wk	0.89 (p=0.0001)	0.59 (p=0.0645)	174.13 (p=0.0782)	99.60 (p=0.8106)
HT 1wk - HT >6mo	-0.82 (p=0.0004)	-0.53 (p=0.1229)	-145.39 (p=0.2279)	-70.41 (p=0.9722)

Only group differences visualized in Figure 4 that were statistically significant ($p<0.05$) are presented. Results are present as the difference in mean A) Shannon Index or B) Observed ESVs between the patient groups in the first column; Results from linear mixed models unadjusted **(M0)** and adjusted for age, sex, race/ethnicity, and antibiotics use one month prior to stool collection **(M2)**;

HF, heart failure; LVAD, left ventricular assist device; HT, heart transplantation.