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ORIGINAL ARTICLE

Cell-Free DNA Profiles End-Organ Injury and Predicts Outcomes in Advanced Heart Failure With Left Ventricular Assist Device Implantation

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BACKGROUND: Adverse events after durable left ventricular assist devices (LVADs) pose a challenge to survival. However, there are limited risk stratification approaches. Plasma cell-free DNA (cfDNA) offers potential as a biomarker for assessing end-organ injury and risk stratification.

METHODS: The study included a multicenter prospective cohort of patients with heart failure with and without LVAD (cohort 1), a separate cohort of patients with heart failure with paired samples before and after LVAD (cohort 2) implantation, and a comparator group of healthy controls. Nuclear cfDNA (ncfDNA) and mitochondrial cfDNA were quantified by digital droplet polymerase chain reaction. Tissue-specific cfDNA was identified using whole-genome bisulfite sequencing. Differences in cfDNA levels by LVAD use were assessed with the Wilcoxon rank-sum test or the paired t test. Outcomes (hemocompatibility-related adverse event–free survival and infection-free survival) by cfDNA tertiles were compared by log-rank tests.

RESULTS: Cohort 1 had 76 patients with LVAD and 144 without LVAD. Cohort 2 had 40 patients with LVAD with samples before and after LVAD. ncfDNA levels were 4-fold higher (9794 versus 2386 copies/mL; $P<0.001$), and mtDNA was 1.5-fold higher (134 707 versus 82 054 copies/mL; $P=0.01$) in cohort 1 compared with healthy controls (n=48). Patients without LVAD had higher ncfDNA levels compared with those with LVAD in cohort 1 (11 423 versus 7912 copies/mL; $P=0.019$). After LVAD placement in cohort 2, ncfDNA nearly halved (18 980 versus 10 228 copies/mL; $P<0.001$), with significant reductions in innate immune, vascular endothelium, gastrointestinal, and liver cfDNA levels. The highest pre-LVAD tertile of ncfDNA was associated with worse infection-free (HR, 2.94 [95% CI, 1.31–6.56]; $P=0.006$) and hemocompatibility-related adverse event–free (HR, 3.24 [95% CI, 1.03–10.3]; $P=0.034$) survival.

CONCLUSIONS: LVAD implantation was associated with reductions in systemic and tissue-specific cfDNA levels. cfDNA levels offer promise for improving risk stratification of LVAD candidates for post-LVAD outcomes.

Key Words: biomarkers ■ cell-free nucleic acids ■ epigenomics ■ heart failure ■ liquid biopsy

T he contemporary management of advanced heart failure has evolved with the use of mechanical circulatory support devices. Durable left ventricular assist devices (LVAD) offer longer survival and improved quality of life in patients with refractory symptoms despite maximal medical therapy.[1] Over the past 20 years, survival with durable LVADs has improved, but rates of complications such as infections and hemocompatibility-related adverse events (HRAE) remain high. Consequently, 5-year mortality from these complications and

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WHAT IS NEW?

- This study is one of the first to evaluate nuclear and mitochondrial circulating cell-free DNA (cfDNA) as biomarkers in patients with left ventricular assist device (LVAD).
- Systemic and tissue-specific circulating cfDNA levels significantly reduce with LVAD implantation, demonstrating a dynamic response to a reduction in systemic and tissue-specific injury.
- Pre-LVAD cfDNA levels are associated with adverse post-LVAD events including infections and hemocompatibility-related complications.

WHAT ARE THE CLINICAL IMPLICATIONS?

- cfDNA may serve as a noninvasive biomarker to monitor organ injury and immune activation in patients with LVAD.
- Preimplantation cfDNA levels could be used for advanced therapy risk stratification by helping to identify high-risk patients.

Nonstandard Abbreviations and Acronyms

BNP	B-type natriuretic peptide
cfDNA	cell-free DNA
HRAE	hemocompatibility-related adverse event
IPW	inverse probability weighting
mtcfDNA	mitochondrial cell-free DNA
ncfDNA	nuclear cell-free DNA
NT-proBNP	N-terminal pro-B-type natriuretic peptide

progressive heart failure remains elevated at 50% to 60%.[2]

Patient selection and timing of implantation remain the primary challenges in improving outcomes for patients with advanced heart failure. Clinical tools such as the Interagency Registry for Mechanically Assisted Circulatory Support Profiles are available to determine eligible patients and predict LVAD outcomes. However, these tools rely on subjective assessments and do not incorporate the use of biomarkers, in part because few biomarkers to date have provided prognostic and mechanistic insight into the effects of LVADs. While BNP (B-type natriuretic peptide) and NT-proBNP (N-terminal pro-B-type natriuretic peptide) are biomarkers that can risk-stratify patients with heart failure, their utility as a target for therapeutic response has been limited.[3–6]

In contrast to traditional biomarkers, plasma cell-free DNA (cfDNA) offers a distinct advantage due to its ability to reflect not only cardiac injury but also extracardiac injury. cfDNA is released into the circulation following tissue injury and cell death, capturing a broader spectrum of injury patterns. Importantly, cfDNA fragments retain the epigenetic signatures of their tissue of origin, allowing for the profiling of tissue-specific injury.[7,8] The measurement of cfDNA has been used clinically as a noninvasive marker in oncology surveillance and diagnosis of solid organ transplant rejection, where donor-derived cfDNA detects rejection earlier than existing gold standard diagnostics.[9,10] In patients with heart failure, high levels of cfDNA have been associated with adverse outcomes.[11] However, the tissue injury patterns in the advanced heart failure population, as well as the impact of therapies such as durable LVAD on cfDNA, have not yet been explored.

We hypothesized that durable LVAD implantation would result in decreased evidence of tissue and systemic injury as assessed by cfDNA levels. To test this hypothesis, we analyzed total and tissue-specific cfDNA profiles in 2 independent cohorts. In cohort 1, a multicenter cohort of patients with advanced heart failure on the transplant waitlist, we compared cfDNA levels and the tissue sources in patients with and without LVAD. To further assess the relationship of LVAD and cfDNA profiles, we then assessed cfDNA profiles in cohort 2, a separate group of LVAD candidate patients with paired samples collected before and after LVAD placement, allowing for within-subject comparison.

METHODS

Data Availability

Data are available upon request.

Study Design and Patient Selection

This study was composed of 2 separate prospective cohorts (Figure 1A), each intended for independent retrospective analysis. Cohort 1 included patients with advanced heart failure on the heart transplant waitlist as part of [redacted]. This multicenter prospective cohort study enrolled adult heart transplant waitlist patients from 5 [redacted] heart transplant centers from May 2015 to December 2020, including [redacted]. Patients were enrolled at heart transplantation listing, and blood samples were collected and processed for plasma. We included all adult participants who had plasma collected before transplant. Exclusion criteria included those who had not yet received a heart transplant or those with insufficient plasma for analysis. In addition, demographic and clinical information including pretransplant laboratory test results, inotrope use, and mechanical circulatory support device use was collected. The study was approved by the institutional review boards of all 5 centers and [redacted].

A separate cohort (cohort 2) of 40 patients with advanced heart failure receiving durable LVADs, defined by any surgically implanted LVADs including HeartMate 2, HeartMate 3, and HeartWare, was studied to identify within-subject cfDNA changes after LVAD implantation (Figure 1B). This single-center study cohort from [redacted] prospectively recruited durable LVAD candidates from March 2014 to January 2018
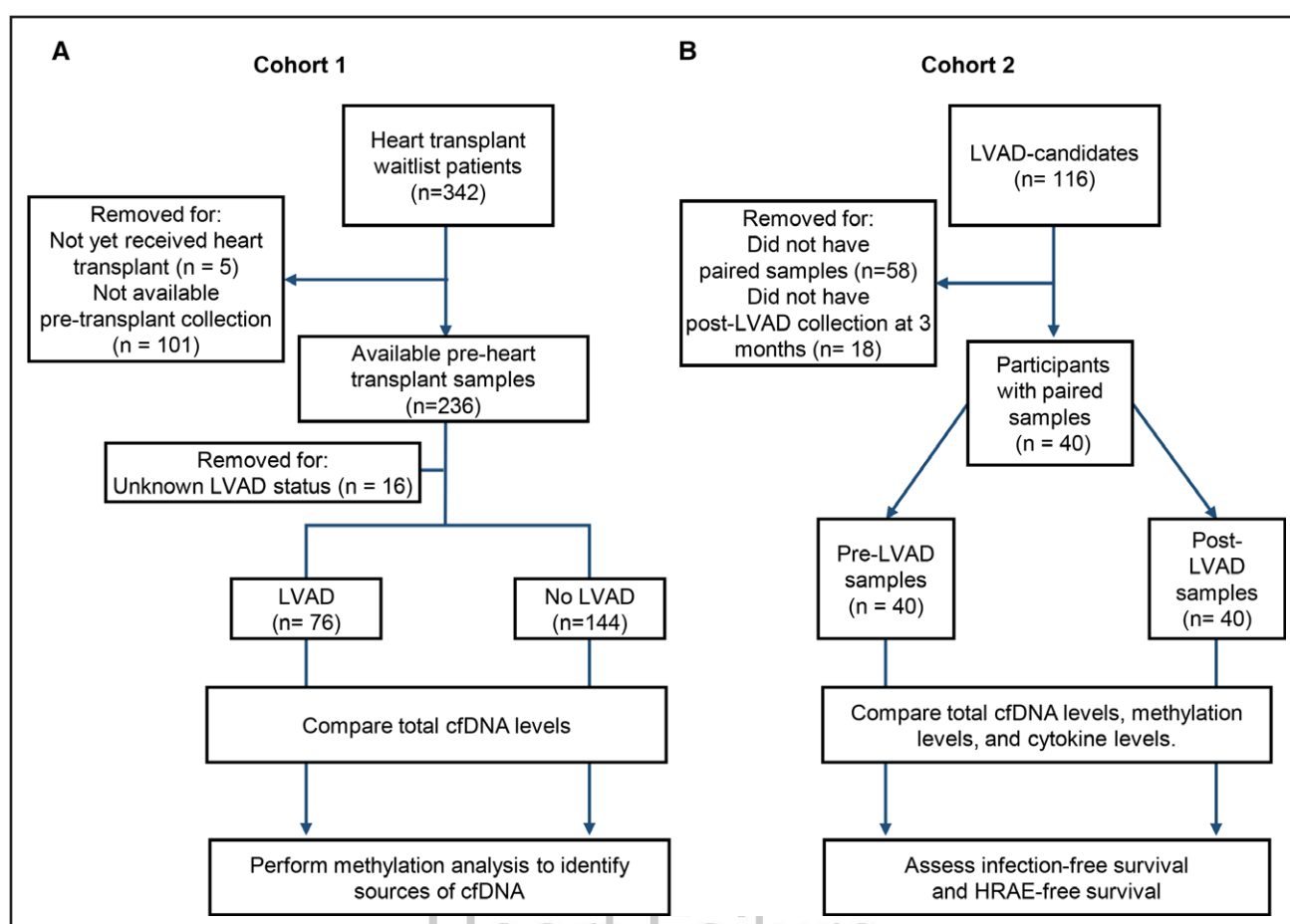
Figure 1. STROBE diagrams.
A, Patient flow diagram for cohort 1, where plasma samples were collected from patients with advanced heart failure awaiting heart transplant.
B, Cohort 2 represented patients with advanced heart failure who were selected for durable left ventricular assist device (LVAD) placement and had paired samples before and after LVAD. A separate group of consecutively enrolled healthy controls (n=48) served as a comparator group (median age, 57 years; 60.5% male) to establish baseline values for cell-free DNA (cfDNA). HRAE indicates hemocompatibility-related adverse event.

and collected paired plasma samples before and after LVAD. The pre-LVAD samples were taken within 2 weeks before LVAD implantation to reflect the patient's status immediately before device placement. The post-LVAD samples were collected during outpatient follow-up after hospital discharge, with the outpatient collection closest to 3 months post-implantation chosen to represent a steady-state period following LVAD placement. This time point was selected to ensure that participants included in the analysis were clinically stable enough to have been discharged from the hospital and followed in an outpatient setting. Exclusion criteria included those without paired samples before and after LVAD and those who did not have a collection close to 3 months after LVAD. We also collected baseline demographic data, health care utilization data of hospital and intensive care unit lengths of stay, and clinical data including laboratory test results and clinical outcomes. The study was approved by the institutional review board. All patients in cohort 1 and cohort 2 provided written informed consent.

Healthy control participants were included as a normal reference cohort. These samples were collected at the time of volunteer blood donation at [redacted]. All healthy control participants provided written informed consent.

All plasma samples underwent cfDNA analysis, which was then compared in those with and without LVAD to understand the effect of device placement on systemic and tissue-specific cfDNA levels.

cfDNA Analysis

The process of cfDNA isolation and quantification has been previously described.[8,12] In short, cfDNA was extracted from plasma and quantified using digital droplet polymerase chain reaction by targeting AP3B1, TERT, AGO1, and RPP30 for nuclear cfDNA (ncfDNA) and ND1 for mitochondrial cfDNA (mtcfDNA). For methylation analysis, the extracted cfDNA underwent bisulfite conversion using the EZ DNA methylation-gold kit (Zymo Research). Library preparation was performed using the Accel-NGS Methyl-Seq DNA Library Kit with Unique Dual Indexing (Swift Biosciences). The libraries were pooled in equimolar concentrations (5 ng per sample) and sequenced in 100-bp paired-end mode using NovaSeq 6000 (Illumina) to achieve a sequencing depth of 140 to 200 million read pairs per sample.

The sequencing reads were mapped to a human reference genome (version hg38) with Bismark (version 0.23.0).

Methylated cytosine bases (CpG) were identified, and the tissue origins of cfDNA were deconvoluted using Bismark (https://github.com/OpenOmics/methyl-seek) and the UXM algorithm as reference (https://github.com/nloyfer/UXM_deconv).[13,14]

Study End Points

The primary analysis compared total and tissue-specific cfDNA levels with and without LVAD. To test the predictive utility of cfDNA as observed in other diseases, we further assessed the association between cfDNA levels and LVAD-associated clinical outcomes. LVAD-related clinical end points were defined as the following: time to important LVAD outcomes including major infection, HRAE (a composite outcome of stroke, device thrombosis, and major bleeding), and mortality. These clinical end points were selected for their established relevance in the LVAD population and were defined based on the Interagency Registry for Mechanically Assisted Circulatory Support Adverse Event Definitions (Table S1).

Statistical Analysis

Data were summarized by median (interquartile range) for continuous variables and frequency (percentage) for categorical variables. To compare patients and healthy controls and compare the unpaired patient groups with or without LVAD in cohort 1, the Wilcoxon rank-sum tests were used to assess the differences in the total and tissue-specific cfDNA levels. Paired t tests were used to compare the paired data between pre-LVAD and post-LVAD samples in cohort 2. To adjust for differences in baseline characteristics and potential confounding factors, a propensity score−based inverse probability weighting (IPW) multivariable regression analysis was performed to compare the cfDNA measures between the patients with and without LVAD.[15–17] The covariates for IPW included age, sex, race, body mass index, transplant center, ischemic versus nonischemic cardiomyopathy, transplant listing status, presence of diabetes, extracorporeal membrane oxygenation use, and inotrope use, and these covariates were also included in the final IPW model. This approach provided a covariate-balanced sample of patients with and without LVAD with the balance of baseline covariates assessed using absolute standardized differences. Heatmaps were used to display the correlation matrix and compare the cfDNA levels between different patient groups. Hierarchical clustering analysis using the Ward method was applied to identify clusters of biomarkers in heatmaps. To assess the association of pre-LVAD cfDNA and clinical outcomes in cohort 2, the event rate and event-free survival probabilities were estimated by the Kaplan-Meier method and compared by log-rank tests between patient groups with different cfDNA levels. Outcomes were censored 2 years post-LVAD because <20% patients were still at risk after 2 years. Random survival forest machine learning method was used to provide variable importance ranking in predicting the outcome for total and tissue-specific cfDNA. Analysis was performed using the R statistical Software, version 4.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

Table 1. Patient Characteristics of Cohort 1

	No LVAD (n=144)	LVAD (n=76)	Overall (n=220)
Cohort 1			
Age, y; median (Q1−Q3)	56 (48−61)	56 (44−61)	56 (47−61)
Sex, n (%)			
Female	37 (25.7)	17 (22.4)	54 (24.5)
Male	107 (74.3)	59 (77.6)	166 (75.5)
Race, n (%)			
White	77 (53.5)	26 (34.2)	103 (46.8)
Black	50 (34.7)	40 (52.6)	90 (40.9)
Other	17 (11.8)	9 (11.8)	26 (11.8)
BMI, kg/m²; median (Q1−Q3)	26.8 (17.2−38.1)	28.8 (18.4−42.0)	27.4 (17.2−42.0)
Heart failure cause, n (%)			
Ischemic cardiomyopathy	41 (28.5)	30 (39.5)	71 (32.3)
Nonischemic cardiomyopathy	103 (71.5)	46 (60.5)	147 (66.8)
Baseline serum sodium, mmol/L; median (Q1−Q3)	138.5 (138.0−140.5)	137.0 (137.0−137.5)	138 (137−139)
Baseline serum Cr, mg/dL; median (Q1−Q3)	1.2 (0.9−1.5)	1.1 (0.9−1.3)	1.2 (0.9−1.5)
Inotrope use, n (%)	55 (38.2)	14 (18.4)	69 (31.4)
IABP use, n (%)	11 (7.6)	0 (0)	11 (5.0)
ECMO use, n (%)	5 (3.5)	6 (7.9)	5 (2.3)
Transplant listing status, n (%)			
1A	29 (20.1)	24 (31.6)	53 (24.1)
1B	45 (31.3)	41 (53.9)	86 (39.1)
2	68 (47.2)	8 (10.5)	76 (34.5)

Transplant listing status was determined based on the United Network for Organ Sharing database using the 3-tier system. BMI indicates body mass index; Cr, creatinine; ECMO, extracorporeal membrane oxygenation; IABP, intra-aortic balloon pump; and LVAD, left ventricular assist device.

RESULTS

Cohort Demographics

Cohort 1 included 236 advanced heart transplant wait-listed patients. Sixteen samples were excluded due to unknown LVAD status within the UNOS database, leaving 220 samples for analysis (Figure 1A). Of this cohort, 76 samples (34.5%) were from patients with a durable LVAD and 144 samples (65.5%) from those who did not have an LVAD. Basic demographics including median age, sex, and body mass index were comparable between those with and without LVAD (Table 1; Table S2). Patients with LVAD had a higher prevalence of ischemic cardiomyopathy and a more severe transplant listing status compared with patients without LVAD.

Cohort 2 consisted of 40 patients with paired samples collected before and after LVAD placement (Figure 1B). Demographics and baseline characteristics are shown in Table 2. Median follow-up time was 2 years after LVAD implantation. Infections occurred in 57.5% (23/40), HRAEs in 25% (10/40), and deaths in 35% (14/40) of patients.

Elevated cfDNA From Cardiac and Noncardiac Sources in Heart Failure

In cohort 1, consisting of patients with advanced heart failure with and without LVAD, ncfDNA was 4× higher (median, 9794 versus 2386 copies/mL; $P<0.001$), and mtcfDNA was 1.5× higher (median, 134707 versus 82054 copies/mL; $P=0.01$) compared with healthy individuals (Figure 2A). The high levels of cfDNA were associated with clinical surrogates of worse disease severity, including BNP (Spearman $\rho=0.39$; $P=0.01$; Figure S1A), as well as use of intra-aortic balloon pumps (median, 39810 versus 10233; $P=0.006$), inotropes (median, 18707 versus 9862 copies/mL; $P<0.001$), and extracorporeal membrane oxygenation (median, 68097 versus 9410 copies/mL; $P=0.01$; Figure S1B). The tissue sources of cfDNA were also distinct in these patients with advanced heart failure compared with healthy individuals. The patients demonstrated significantly elevated cfDNA from innate immune cells (median, 10304 versus 1172 copies/mL; $P<0.001$), adaptive immune cells (median, 86 versus 5 copies/mL; $P=0.007$), and cardiomyocytes (median, 47 versus 11 copies/mL; $P=0.003$) compared with healthy controls. There were also elevated cfDNA from extracardiac sources, including the liver, vascular endothelium, and gastrointestinal cells (Table S3).

Similarly, in cohort 2, patients with advanced heart failure showed elevated cfDNA levels compared with healthy individuals. Specifically, pre-LVAD ncfDNA was 8-fold higher (median, 18980 versus 2386 copies/mL; $P<0.001$), and mtcfDNA was 13-fold higher (median, 1051882 versus 82054 copies/mL; $P<0.001$) than in healthy controls (Figure 2B). In this cohort, the patients with advanced heart failure of cohort 2 demonstrated

higher tissue-specific cfDNA from innate immune cells (median, pre-LVAD 7866 versus post-LVAD 2711 copies/mL; $P<0.001$) and extracardiac sources, including liver, vascular endothelium, gastrointestinal, kidney, lung, and megakaryocyte cells (Table S4).

cfDNA Reduces With LVAD Placement

LVAD implantation was associated with reduced cfDNA levels in both cohorts. In cohort 1, patients with LVADs had significantly lower ncfDNA (median, 11423 versus 7912 copies/mL; $P=0.019$) and mtcfDNA levels (median, 152802 versus 102593 copies/mL; $P=0.014$) levels compared with patients without LVAD (Figure 2A). In propensity score–based IPW multivariable regression analysis, the presence of an LVAD was associated with a 32% reduction in ncfDNA compared with no LVAD (mean ratio, 0.683 [95% CI, 0.511–0.913]; $P=0.01$) after adjusting for baseline characteristics and potential confounding factors (Figure S2). There was no significant

Table 2. Baseline Characteristics of the Participants in Cohort 2

Cohort 2	
Age, y; median (Q1–Q3)	64.5 (56.5–70.5)
Sex, n (%)	
Female	9 (22.5)
Male	31 (77.5)
Race, n (%)	
White	25 (62.5)
Black	12 (30.0)
Other	3 (7.5)
BMI, kg/m²; median (Q1–Q3)	27.6 (24.4–33.4)
Heart failure cause, n (%)	
Ischemic cardiomyopathy	17 (42.5)
Nonischemic cardiomyopathy	23 (57.5)
Baseline serum sodium, mmol/L; median (Q1–Q3)	136.0 (133–137)
Baseline serum Cr, mg/dL; median (Q1–Q3)	1.3 (1.1–1.7)
Inotrope use, n (%)	34 (85.0)
IABP use, n (%)	4 (10)
ECMO use, n (%)	1 (2.5)
Intermacs profile, n (%)	
Profile 1	4 (10.0)
Profile 2	7 (17.5)
Profile 3	25 (62.5)
Profile 4	3 (7.5)
Profile 5	1 (2.5)
Intended goal of LVAD support, n (%)	
Bridge to transplantation	20 (50)
Destination therapy	20 (50)

BMI indicates body mass index; Cr, creatinine; ECMO, extracorporeal membrane oxygenation; IABP, intra-aortic balloon pump; Intermacs, The Interagency Registry for Mechanically Assisted Circulatory Support; and LVAD, left ventricular assist device.
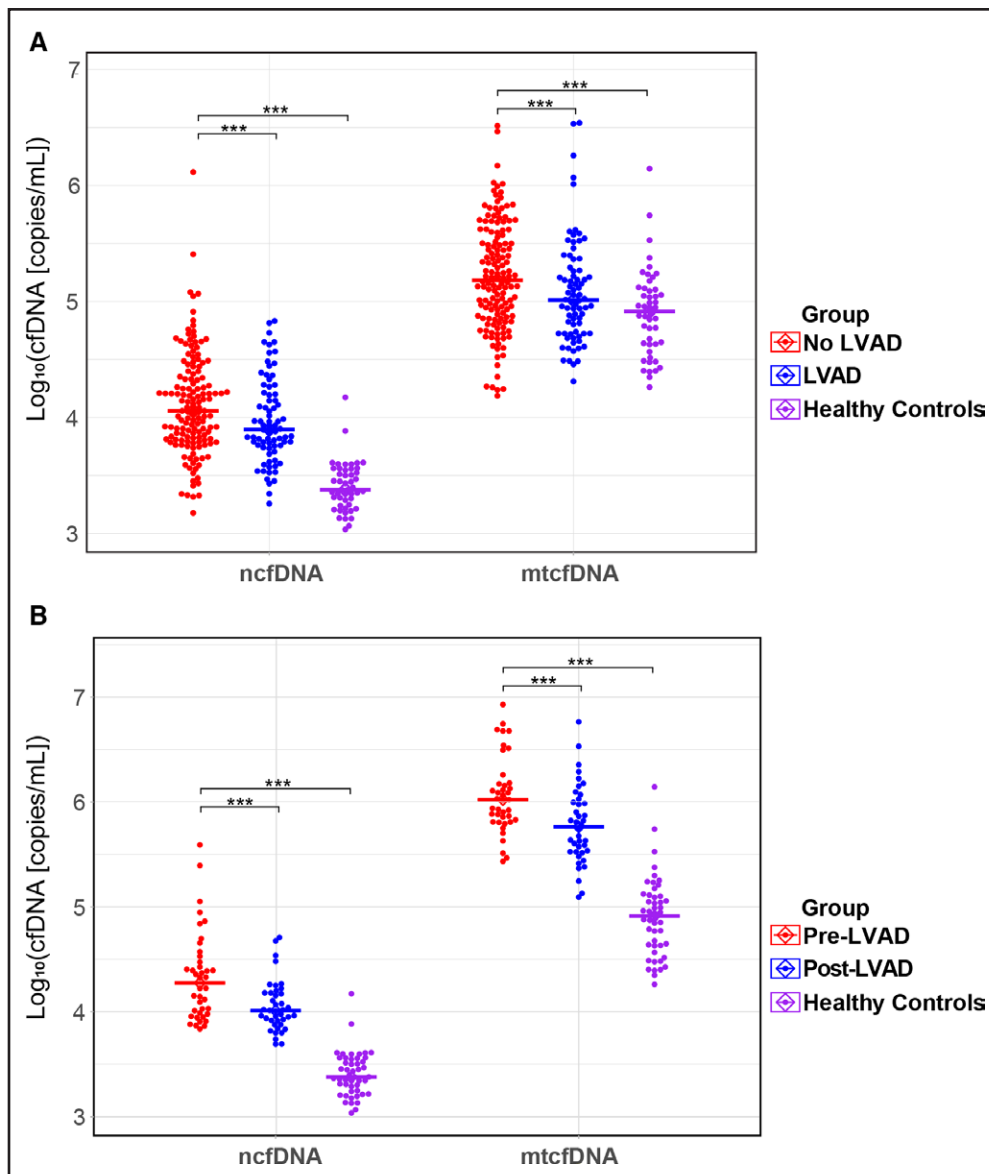
Figure 2. Cell-free DNA (cfDNA) levels are elevated in patients with advanced heart failure and decrease with durable left ventricular assist device (LVAD) placement.

A, Beeswarm plots of nuclear cfDNA (ncfDNA) and mitochondrial cfDNA (mtcfDNA) levels in cohort 1 and healthy controls. Nuclear cfDNA and mtcfDNA are displayed as medians of log10-transformed values in copies/mL. cfDNA levels from cohort 1 were compared with those of healthy individuals by the Wilcoxon rank-sum test. Within cohort 1, participants were divided by the presence of an LVAD before transplant listing, and their cfDNA levels were compared by the Wilcoxon rank-sum test. ***P<0.001. **B**, Beeswarm plots of ncfDNA and mtcfDNA levels in cohort 2 and healthy controls. Nuclear cfDNA and mtcfDNA are displayed as medians of log10-transformed values in copies/mL. The Wilcoxon rank-sum test was used to compare patients before LVAD placement to healthy individuals by the Wilcoxon rank-sum test. Within Cohort 2, serial samples before and after LVAD in each participant were compared with the paired t tests. ***P<0.001.

difference in mtcfDNA levels after adjustment for baseline covariates using IPW analysis (P=0.21). The conventional multivariable adjusted linear models accounted for baseline factors yielded similar results (Table S5).

Similarly, in cohort 2, LVAD placement resulted in approximately a 50% reduction in both ncfDNA (median, 18 980 versus 10 228 copies/mL; P<0.001) and mtcfDNA (median, 1,051,882 versus 579 609 copies/ mL; P=0.002) levels when comparing paired samples before and after LVAD implantation (Figure 2B). Notably,

the post-LVAD cfDNA levels remained greater than those of healthy controls (ncfDNA, P<0.001; mtcfDNA, P<0.001; Table S4).

LVAD Placement Was Associated With Reduction in cfDNA From Innate Immune Inflammatory Cells and Extracardiac Tissues

The tissue-specific cfDNA profiles were distinct among patients with advanced heart failure with and without an

LVAD (Figure S3). In cohort 1, patients with LVAD demonstrated substantial reductions in ncfDNA levels from various tissue types affected in heart failure. Specifically, cfDNA from innate immune cells (median, 4042 versus 10 304 copies/mL; P<0.001), liver (median, 100 versus 317 copies/mL; P=0.008), vascular endothelium (median, 424 versus 791 copies/mL; P=0.004), and megakaryocytes (median, 396 versus 2605 copies/mL; P<0.001) were lower in patients with LVADs compared with patients without LVADs (Table S3).

In cohort 2 using paired data within the same subject, post-LVAD samples demonstrated reductions in cfDNA levels from innate immune (median, 4952 versus 7866 copies/mL; P<0.001), liver (median, 141 versus 584 copies/mL; P<0.001), vascular endothelium (median, 819 versus 1398 copies/mL; P=0.007), and gastrointestinal cells (median, 15 versus 148 copies/mL; P=0.001; Figure 3) compared with pre-LVAD samples. In contrast, ncfDNA from cardiac, kidney, lung, and adaptive immune cells did not significantly change with LVAD placement (Figure 3B; Table S4).

cfDNA Levels Were Associated With Higher Risks of HRAE- and Infection-Free Survivals After LVAD

High pre-LVAD ncfDNA levels were associated with poor post-LVAD outcomes in cohort 2. Patients were stratified by pre-LVAD ncfDNA levels, resulting in median levels of 8857, 21 156, and 49 813 copies/mL for the lowest, middle, and highest cfDNA tertiles. Patients with pre-LVAD cfDNA in the highest tertile demonstrated inferior infection-free survival (HR, 2.94 [95% CI, 1.31–6.56]; P=0.006; Figure 4A; Figure S4A) compared with the rest of the cohort. In univariable analysis, this group also showed worse HRAE-free survival (HR, 3.24 [95% CI, 1.03–10.3]; P=0.034; Figure 4B; Figure S4B). In contrast, mtcfDNA level was not predictive of similar outcomes (infection, P=0.72; HRAE, P=0.07).

To explore the relative association of total ncfDNA and tissue-specific cfDNA on clinical outcomes, we performed an exploratory random forest machine learning analysis. Pre-LVAD total ncfDNA and megakaryocyte-specific cfDNA were the most important tissue types in determining infection-free survival (Figure S5A). In determining HRAE outcomes, pre-LVAD mtcfDNA and adipocyte-specific cfDNA were the most important (Figure S5B). In addition, we calculated the c-statistic for each cfDNA subtype to assess their predictive performance, and the results are presented in Table S6.

DISCUSSION

In this study, we leverage the high sensitivity of cfDNA as a biomarker to profile the spectrum and trends in tissue injury following LVAD placement. In 2 independent cohorts, LVAD placement resulted in a significant reduction in total ncfDNA and mtcfDNA, measures of systemic injury. We observed a significant reduction in tissue-specific cfDNA levels from innate immune inflammatory cells and end organs affected during advanced heart failure. Pre-LVAD cfDNA levels were associated with important post-LVAD outcomes, including infection- and HRAE-free survival after LVAD implant.

These findings align with current paradigms of cellular and immunogenic alterations in patients with advanced heart failure. In our advanced heart failure cohorts, the largest cfDNA contributors were immune cells, adding to a growing body of evidence indicating that excessive inflammation is deeply embedded in heart failure pathobiology.[18] Specifically, activation of innate immune and adaptive immune signaling pathways has been shown to increase myocardial tissue injury and fibrosis in animal models.[19–23] Modulation of such an inflammatory and immune response may improve outcomes and reduce heart failure progression.

In this context of heightened inflammation, LVAD placement reduced levels of innate immune-specific cfDNA, though not completely to the levels of healthy individuals, consistent with existing data supporting inflammatory modulation with LVAD implantation.[24,25] The changes in innate immune signaling have led to the development and validation of clinical scores featuring inflammatory surrogates, such as the neutrophil-to-lymphocyte ratio, which have been shown to predict survival for patients with temporary mechanical circulatory support devices.[26] Such scores, however, notably exclude monocytes and macrophages, which are established as major contributors of inflammatory cytokines resulting in vascular endothelial injury and myocardial fibrosis.[27–29] In fact, evidence that targeting classically activated (M1) and alternatively activated (M2) macrophages attenuates myocardial fibrosis in mouse models is mounting, suggesting that treatments targeting these subsets can influence cardiac remodeling.[30,31] While not directly targeting monocytes and macrophages, LVAD implantation resulted in nearly a 50% decrease in monocyte- and macrophage-specific ncfDNA in our cohorts. Circulating cell counts of monocyte subsets have been demonstrated to diminish with medical treatment of acute decompensated heart failure; however, this has not correlated with circulating cytokine levels, suggesting that cell counts alone may not accurately reflect monocyte function.[32] In contrast, cfDNA may be a more representative marker of monocyte/macrophage turnover and functional activity.

In addition to its effects on immune-active cells, cfDNA analysis corroborates existing evidence of durable LVAD's ability to reduce end-organ injury.[33–35] Because cfDNA is released during necrosis and apoptosis, it serves as an indicator of cell death.[36] Furthermore, given the short half-life of cfDNA, it enables real-time
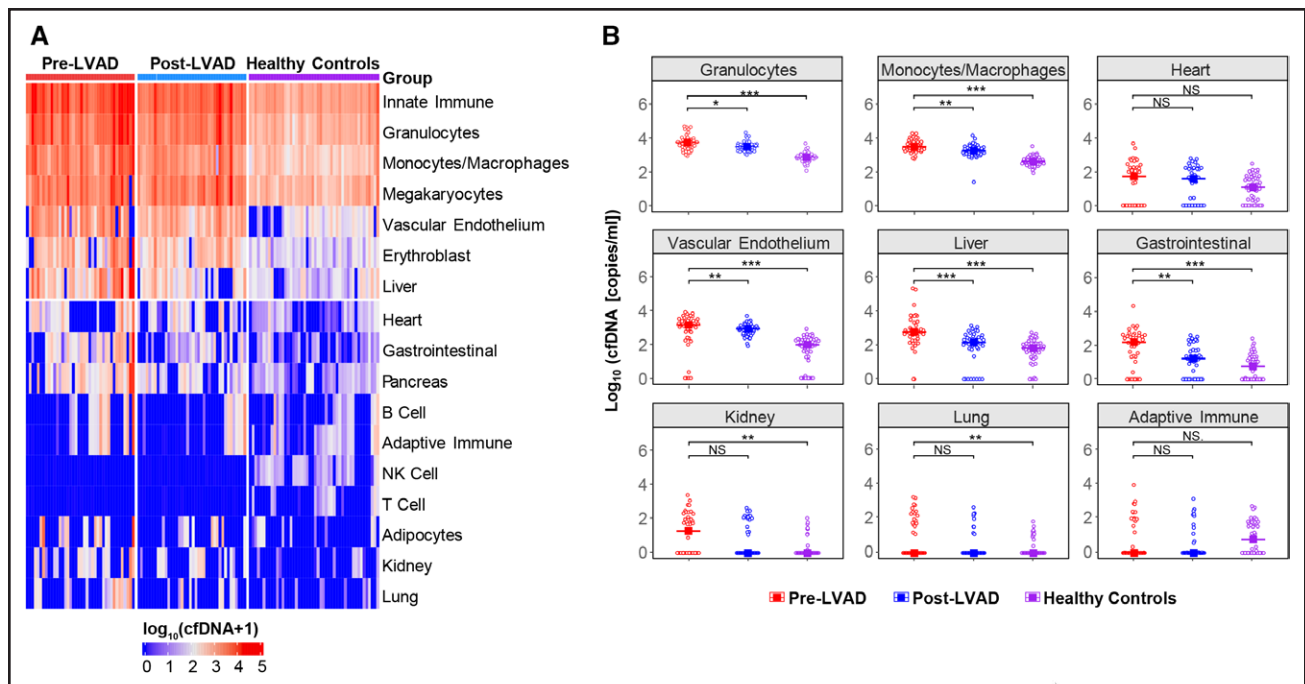
Figure 3. Cell-free DNA (cfDNA) originating from immune and extracardiac organs is reduced after left ventricular assist device (LVAD) implantation.

A, Heatmap of cfDNA levels across various tissue types in cohort 2 and healthy controls. Samples are compared pre-LVAD, post-LVAD, and against healthy individuals. Each row represents tissue sources of cfDNA, and the color gradient represents the log10-transformed cfDNA levels in copies/mL. Unsupervised clustering was used to organize the samples and tissue types, highlighting patterns of similarity. **B**, Beeswarm plots of tissue-specific cfDNA in cohort 2 and healthy controls. cfDNA levels are displayed with a horizontal line indicating the median value of log10-transformed values in copies/mL. Comparisons of tissue-specific cfDNA levels between pre-LVAD participants and healthy controls were conducted using the Wilcoxon rank-sum tests. Paired *t* tests were used to compare paired pre-LVAD and post-LVAD cfDNA values. *$P<0.05$, **$P<0.01$, and ***$P<0.001$.

analysis of tissue injury.[37] In these heart failure cohorts, multiple end organs, including the lung, kidney, and liver, showed evidence of progressive injury. Liver and gastrointestinal-specific cfDNA decreased with LVAD placement, suggesting a reduction in end-organ injury, because tissue-specific cfDNA levels correlate with other markers of end-organ injury.[38] This observation is likely representative of decreased congestion and improved perfusion with device therapy. We also observed significant decreases in vascular endothelial cfDNA after LVAD placement, suggesting reduced vascular endothelial injury. Our vascular endothelial findings conflict with prior evidence supporting worse peripheral arterial endothelial function with continuous flow LVADs.[39,40] A more recent cohort of 11 patients with LVAD demonstrated neutral to beneficial effects in coronary artery endothelial function, suggesting that the endothelial effects from LVADs may not be uniform.[41] The observed reductions in cfDNA from important end organs and immune cells add molecular evidence that LVADs improve clinical stability in patients with advanced heart failure.[42,43]

Notably, we found no significant difference in heart-specific cfDNA with device implantation in contrast to prior studies demonstrating improvements in markers of cardiovascular stress and fibrosis.[44,45] While cardiomyocyte-specific

cfDNA has been previously reported to be higher in patients with heart failure, that cohort does not represent this study's demographic of end-stage heart disease.[46] For this cohort with advanced heart disease, the findings may suggest fewer viable cardiomyocytes capable of recovery. Other patients with end-stage organs, such as lung transplant candidates, have not shown elevated lung-specific cfDNA compared with healthy individuals.[47]

Finally, our findings indicate that high cfDNA levels are associated with adverse clinical outcomes such as HRAEs, infection, and death, suggesting that cfDNA may be a useful predictor of post-LVAD outcomes. This finding is consistent with evidence from various diseases, including heart failure, pulmonary arterial hypertension, advanced lung disease, and COVID-19, where high cfDNA levels have been associated with worse disease severity and increased risk of adverse events.[7,8,11,47] Extending this concept to the heart failure population, our findings reaffirm that cfDNA elevation is suggestive of worse disease pathology and poor outcomes. The clinical implications are substantial, as recent reports of 5-year major bleeding and major infection rates remain high at 34% and 55%, despite significant reductions in the risks of developing HRAEs and death with the use of magnetically levitated LVADs.[1,48] Therefore, assessing cfDNA
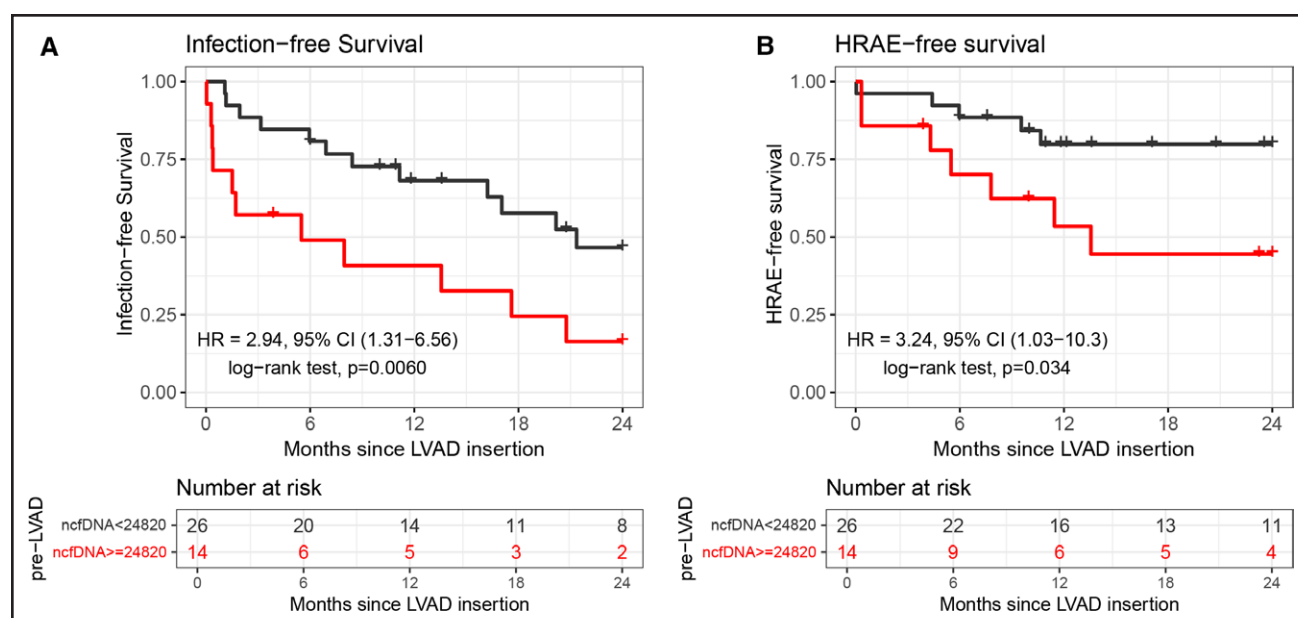
Figure 4. Elevated cell-free DNA (cfDNA) before left ventricular assist device (LVAD) placement is associated with worse infection-free and hemocompatibility-related adverse event (HRAE)–free survival after LVAD implantation.
A, Kaplan-Meier curve for infection-free survival in cohort 2. The log-rank test was used to compare infection-free survival in participants within the highest tertile of pre-LVAD nuclear cfDNA (ncfDNA; >24 820 copies/mL) compared with the rest of the participants. **B**, Kaplan-Meier curve for HRAE-free survival in cohort 2. The log-rank test was used to compare HRAE-free survival in participants within the highest tertile of pre-LVAD ncfDNA (>24 820 copies/mL) compared with the rest of the participants.

levels before LVAD implantation may provide prognostic insights into the risk of developing these complications. Furthermore, methylation analysis can enhance understanding of individual outcomes by providing further insight into the underlying tissue injury pattern and pathophysiology, leading to better targeted therapies.

With pending validation in larger cohorts, cfDNA levels in conjunction with methylation analysis may become effective tools for understanding the therapeutic response to LVADs while simultaneously assessing the risk of specific adverse outcomes. This approach has already proven valuable in precision oncology and transplantation. For example, in transplantation, allograft-derived cfDNA levels increase with acute rejection and reduce with treatment. In oncology, circulating tumor DNA levels have been effective in monitoring response to chemotherapy and surgical excision in various cancers.[49,50] When methylation techniques are added, cardiac cfDNA levels have been successfully used to predict anthracycline-related cardiac dysfunction.[51] Similarly, we hypothesize that the changes in tissue-specific cfDNA may be instrumental in not only understanding the biologic adaptations with LVAD therapy but also in dynamically predicting the risk of individual LVAD-associated adverse outcomes.

Limitations

Although efforts were made to strengthen findings by using 2 separate cohorts, the study's generalizability remains limited by its observational study design and small sample size. Due to the observational design of the study, unmeasured confounders may not have been accounted for. While propensity-weighted analysis of prespecified variables and a secondary cohort with paired serial samples before and after LVAD were added to address confounding in cohort 1, residual confounding may still exist. Within cohort 2, while demographic variables are likely accounted for in paired analysis, hemodynamic and inotropic support, as well as other unmeasured clinical variables, may have been different between the 2 time points. Furthermore, cohort 2 only included participants who survived to 3 months, limiting applicability to those with early post-LVAD complications or mortality. Due to the limited number of subjects and events, multivariable analyses are limited in addressing other potential confounders. Therefore, these findings should be interpreted as exploratory and hypothesis-generating, necessitating follow-up validation studies with larger, multicenter studies with serial collections capable of analyses with multivariable regression.

Furthermore, both cohorts were highly selected such that the participants were transplant- or LVAD-eligible, potentially excluding significant noncardiac comorbidities affecting tissue-specific cfDNA levels. The differences in ncfDNA between the 2 cohorts could potentially reflect differences in heart failure disease categories, disease severity, intent of LVAD therapy, or other unmeasured confounders. Future studies should prioritize a broader, multicenter approach to sample patients with advanced

heart failure, obtaining a more heterogeneous group that includes the many comorbidities found in patients with heart failure.

Finally, we acknowledge that while the methylation deconvolution algorithm includes many tissue types relevant to heart failure pathobiology, the algorithm does not incorporate an exhaustive list of tissue types. Furthermore, the reference library for DNA methylation signatures was developed using normal human cell types and may not fully reflect the complexity of disease states. However, prior studies have demonstrated that cell type–defining signatures remain largely stable across pathological states and can be correlated with organ-specific injury markers, including troponin, AST/ALT, and creatinine.[52–54] While this suggests that our approach provides meaningful biological insights, we acknowledge that epigenetic modifications in disease could impact tissue-specific deconvolution. Future studies are needed to determine if epigenetic changes defining cell identity remain consistent in disease states.[55]

Conclusions

Taken together, our findings further highlight an in-depth inflammatory cells and tissue injury profile following LVAD placement. Our results demonstrate that cfDNA could serve as a noninvasive biomarker to risk-stratify LVAD candidates and to assess the therapeutic benefit of LVAD placement. Future studies should focus on validating these findings in large, multicenter cohorts longitudinally such that we gain mechanistic insight into personalized risk stratification for LVAD complications.

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