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### Permalink

<https://escholarship.org/uc/item/9sb9j1p5>

### Journal

Journal of Cardiac Failure, 24(4)

### ISSN

1071-9164

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### Publication Date

2018-04-01

### DOI

10.1016/j.cardfail.2018.02.001

Peer reviewed



Published in final edited form as:

*J Card Fail.* 2018 April ; 24(4): 255–265. doi:10.1016/j.cardfail.2018.02.001.

## Diastolic Dysfunction in Individuals with Human Immunodeficiency Virus Infection:

### Literature Review, Rationale and Design of the Characterizing Heart function on Anti-Retroviral Therapy (CHART) Study

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## Abstract

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### DISCLOSURES

**Javed Butler:** Research support from the National Institutes of Health, the European Union, and the Patient Centered Outcomes Research Institute, and consultant to Amgen, Astra-Zeneca, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, CVRx, Janssen, Luitpold, Medtronic, Novartis, Relypsa, Roche, Vifor, and ZS Pharma. **Andreas P. Kalogeropoulos:** Research support from the American Heart Association and consultant to Roche Diagnostics. **Kevin J. Anstrom:** None. **Priscilla Y. Hsue:** Research support from the National Institutes of Health and Pfizer; honoraria received from Gilead. **Raymond J. Kim:** Research support from Bayer, Stealth Biotherapeutics, and Astellas. **Rebecca Scherzer:** None. **Sanjiv J. Shah:** Research support from the National Institutes of Health (R01 HL127028 and R01 HL107577) and the American Heart Association, Actelion, AstraZeneca, Bayer, and Novartis; and consultant to Actelion, Amgen, AstraZeneca, Bayer, Boehringer-Ingelheim, Cardiora, Eisai, Gilead, Ironwood, Merck, MyoKardia, Novartis, Sanofi, and United Therapeutics. **Svati H. Shah:** Research support from the National Institutes of Health and the American Heart Association, and holds a patent on an unrelated finding. **Eric J. Velazquez:** Research support from National Institutes of Health, Novartis, Amgen, Pfizer, Alnylam, Philips, GE, Bay Labs; honoraris from Novartis, Amgen, Merck, Pfizer, Expert Exchange, Abiomed. **Adrian F. Hernandez:** Research support from AstraZeneca, Bayer, Luitpold, Glaxo Smith Kline, Merck, Novartis, Portola Pharmaceuticals and Verily; honorarium from Amgen, Bayer, Boehringer-Ingelheim Boston Scientific, Myokardia, Novartis and Sanofi. **Patrice Desvigne-Nickens:** None. **Eugene Braunwald:** For the work under consideration, Dr. Braunwald reports grant support to his institution from Duke University for his role as Chair of the NHLBI Heart Failure Network. For outside the submitted work, Dr. Braunwald reports grant support to his institution from Merck and Company, Astra Zeneca, Novartis, Daiichi Sankyo and Glaxo Smith Kline; personal fees for consultancies with The Medicines Company and Theravance; personal fees for lectures from Medscape and Menarini International; uncompensated consultancies and lectures from Merck and Novartis.

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Antiretroviral therapy (ART) has been associated with a shift in the epidemiology of HIV-associated cardiomyopathy from a phenotype of primarily left ventricular (LV) systolic dysfunction to LV diastolic dysfunction (DD). Patients with HIV receiving ART have higher rates of DD when compared to age-matched controls and develop DD at a younger age. However, little is known about the natural history and pathogenesis of DD in virally suppressed HIV-infected patients. Current evidence suggests that immune processes modulate the risk for cardiac involvement in HIV-infected persons. Ongoing inflammation appears to have myocardial effects and accelerated myocardial fibrosis appears to be a key mediator of HIV-induced DD. The Characterizing Heart Function on Antiretroviral Therapy (CHART) study aims to systematically investigate determinants, mechanisms, and consequences of DD in HIV-infected patients. We will compare ART-treated, virally suppressed HIV-infected individuals with and without DD and HIV-infected individuals with DD in regards to (1) systemic inflammation, myocardial stress, and subclinical myocardial necrosis through circulating biomarkers; (2) immune system activation through cell surface receptors; (3) myocardial fibrosis by cardiac magnetic resonance; (4) markers of fibrosis and remodeling, oxidative stress, and hypercoagulability; (5) left atrial function through echocardiography; (6) proteomic and metabolomic profiles; and (7) phenotype signatures derived from clinical, biomarker, and imaging data.

### Keywords

human immuno-deficiency virus; diastolic dysfunction; heart failure; pathophysiology

## BACKGROUND AND RATIONALE

### Introduction

Antiretroviral therapy (ART) has transformed the clinical profile of human immunodeficiency virus (HIV) from an acute infection with a high mortality risk into a treatable, chronic disease with near-normal life expectancy, especially in high-income countries.<sup>1</sup> By the year 2030, it is anticipated that 73% of HIV-infected individuals will be aged 50 or older and that 78% will have cardiovascular disease.<sup>2</sup> Although cardiac involvement in HIV infection has been long recognized,<sup>3</sup> effective combination ART has been associated with a shift in the epidemiology of HIV cardiomyopathy and heart failure (HF) from a phenotype of primarily left ventricular (LV) systolic dysfunction to LV diastolic dysfunction (DD).<sup>4,5</sup> However, there is little information available on the natural history and pathogenesis of DD and in HIV-infected subjects who are virally suppressed on optimal ART therapy. The growing prevalence of DD in HIV is a concern, given the link between DD, HF with preserved ejection fraction (HFpEF), and mortality.

### Diastolic Dysfunction and HFpEF in HIV-Infected Persons

In older adults without HIV, DD is frequently present, with a prevalence ranging from 15% to over 30% depending on the definition and the population studied.<sup>6-8</sup> DD is associated with increased risk for atrial fibrillation<sup>9</sup> and HF<sup>10</sup>; and portends higher risk for all-cause mortality.<sup>11</sup> Previous studies have shown that the development of DD is preceded by obesity, hypertension, and metabolic syndrome, conditions that are associated with elevated

inflammatory cytokines and endothelial dysfunction.<sup>12</sup> Studies in patients with HIV receiving ART have demonstrated declining rates of LV systolic dysfunction,<sup>3</sup> but higher rates of DD when compared to age-matched controls (Table 1).<sup>13–16</sup> Also, patients with HIV tend to develop DD at a younger age.<sup>5</sup> It is still unclear why HIV-infected patients develop DD more frequently and at a younger age. Although early work suggested that certain ART agents may lead to cardiovascular complications, recent studies suggest minimal effect of ART on DD risk.<sup>16</sup> On the other hand, a central role for inflammation in the development of DD in the general population has been postulated,<sup>12</sup> and there is evidence of persistent immune activation and ongoing inflammation despite ART and viral suppression in HIV-infected patients.<sup>17</sup>

Despite ample evidence of high prevalence of DD in HIV-infected patients, epidemiological data on HFpEF in these patients are scarce. In the large Veterans Aging Cohort Study (VACS), the incidence of HFpEF among HIV-infected veterans ranged from 1.11 cases per 1000 patient-years in the 40–49 age group to 8.19 in those age 70 or older, with an overall increased risk of HFpEF (hazard ratio, 1.21; 95% CI, 1.03–1.41) compared to uninfected veterans.<sup>18</sup> In that study, HIV-infected veterans had increased risk for HF with preserved (< 50%), borderline (40%–49%), and reduced (<40%) left ventricular ejection fraction (LVEF) compared to their uninfected counterparts.<sup>18</sup> Of note, a CD4 count <200 cells/mm<sup>3</sup> was associated with an increased risk for both HF with preserved and reduced LVEF.<sup>18</sup> In a cohort of 5041 HIV-infected patients receiving care at an urban setting,<sup>19</sup> patients with HF (*N*= 216) were older and more likely to be black, hypertensive, and have diabetes. In that study, ~ 40% of HF cases were classified as HFpEF (LVEF ≥ 50%).<sup>19</sup> Peak HIV viral load > 100,000 copies/mL and nadir CD4 count <200 cells/mm<sup>3</sup> were associated with significantly elevated odds of HF.<sup>19</sup> Taken together, these data imply that immune processes modulate risk for HF in HIV-infected persons.

### The Role of Inflammation and Immune Activation

Systemic low-level Inflammation has been proposed as a key mechanism in the development of DD and HFpEF in the general population.<sup>12</sup> Although ART reduces the degree of immune activation and inflammation in HIV-infected persons,<sup>20,21</sup> there is persistent T cell activation<sup>22</sup> and inflammation<sup>23</sup> in these patients. Biomarkers of inflammation remain elevated in the setting of effectively treated and suppressed HIV infection. In a study of 61 HIV-infected patients on ART with undetectable viral load, chronic activated and senescent CD8 cells and apoptotic CD4 and CD8 cells were more frequent and tumor necrosis factor  $\alpha$  levels were higher compared to matched controls after a median of 6 years on ART.<sup>17</sup> In HIV-infected patients, T-cell activation is linked to arterial stiffness,<sup>24</sup> and expansion of intermediate and non-classical monocytes has been shown,<sup>25</sup> which are associated with subclinical atherosclerosis and coronary calcium progression.<sup>25</sup> Elevated levels of soluble CD163, a scavenger receptor on monocytes shed by proteolytic cleavage with pro-inflammatory stimulation, have been associated with coronary plaques<sup>26</sup> and arterial inflammation<sup>27</sup> in HIV-infected patients. Increased arterial stiffness has been linked to development of DD in the general population.<sup>28,29</sup>

There are several proposed mechanisms for this ongoing immune activation and systemic low-level inflammation in HIV-infected patients, including residual HIV replication,<sup>30</sup> gut mucosal injury and microbial translocation,<sup>31</sup> co-infections,<sup>32</sup> and impaired homeostatic drive.<sup>33</sup> Regardless of underlying mechanisms, elevated levels of inflammatory and coagulation biomarkers are strongly predictive of cardiovascular disease and non-AIDS morbidity in HIV-infected patients. Among 9764 HIV-infected patients without history of cardiovascular disease, doubling of baseline interleukin-6 and D-dimer levels was associated with 41% and 45% elevated risk, respectively, for cardiovascular events after a median of 2.6 years.<sup>34</sup>

This ongoing inflammation in HIV-infected patients appears to have myocardial effects, according to insights offered by cardiac magnetic resonance (CMR) studies. In a study of 28 HIV-infected patients on ART with adequate viral suppression (<200 copies/mL), native T1 relaxation times, relative T2 signal intensity ratio, and early gadolinium enhancement ratio, were all elevated compared to control subjects, indicating myocardial inflammation in HIV-infected patients.<sup>35</sup> Another CMR study in 103 HIV-infected persons receiving ART reported similar findings, including subclinical myocardial edema and fibrosis, higher LV mass and lower systolic function, and frequent pericardial effusions,<sup>36</sup> pointing to chronic systemic inflammation involving the myocardium and pericardium.

### **The Role of Cardio-Metabolic Risk Factors**

Cardio-metabolic risk factors such as glucose and lipid metabolism disturbances that induce a pro-inflammatory state and promote DD in the general population are at least equally prevalent in HIV-infected patients. In a meta-analysis of 65 studies comprising over 55,000 HIV-infected patients across 5 continents, the prevalence of metabolic syndrome was 16.7% to 31.3% depending on the definition.<sup>37</sup> In a study of 151 HIV-infected but ART-naive, 150 HIV-infected on ART, and 153 HIV-negative adults in Tanzania, more HIV-infected adults on ART had metabolic syndrome versus HIV-negative controls (21.3% vs 7.8%,  $p=0.008$ ) and elevated lifetime cardiovascular risk (34.7% vs 17.0%,  $p<0.001$ ), which seems to develop 3–4 years after ART initiation.<sup>38</sup> Lipid profiles appear to be altered by both HIV infection and ART.<sup>39</sup> Thus, there is an unmet need to elucidate the role of conventional versus HIV-specific risk factors for the development of DD in HIV-infected individuals, and the underlying biological pathways mediating DD risk.

### **Myocardial Fibrosis as a Potential Mediator of HIV-Induced Diastolic Dysfunction**

Besides standard clinical and subclinical structural and functional derangements, CMR can identify focal and diffuse myocardial fibrosis as well as myocardial inflammation and edema.<sup>40</sup> T1 mapping allows for quantification of the myocardial extracellular volume fraction, a validated index of extracellular matrix expansion.<sup>40,41</sup> In the absence of amyloid or edema, extracellular volume is associated with diffuse interstitial fibrosis, a hallmark of LV remodeling.<sup>40</sup> Myocardial inflammation and interstitial fibrosis, both focal and diffuse, have a higher prevalence in HIV-infected patients (Table 2).<sup>35,36,42,43</sup> In addition, HIV-infected patients have increased myocardial lipid content.<sup>43,44</sup> Importantly, increased myocardial lipid levels and diffuse myocardial fibrosis (as measured by the extracellular volume index on CMR) both correlate with impaired myocardial function in HIV-infected

patients.<sup>43</sup> In a large cohort of adults without known HIV infection,<sup>45</sup> myocardial fibrosis quantified by extracellular volume on CMR was higher among patients with HFpEF and those at risk for HFpEF, suggesting that fibrosis precedes HFpEF. A common finding in CMR studies is that HIV-infected patients have impaired LV systolic function (as measured by strain parameters) despite normal LVEF.<sup>35,36,42,43</sup> Of note, impaired systolic function using echocardiographic strain imaging has been shown in patients with HFpEF in two clinical trials.<sup>46,47</sup> However, it is important to note that although reduced longitudinal strain is a marker of subclinical myocardial dysfunction, it is certainly not specific for HIV-related myocardial dysfunction and does not necessarily signify a progressive condition.

Therefore, accelerated myocardial fibrosis appears to be a key mediator of HIV-induced systolic and diastolic dysfunction. This hypothesis is corroborated by biomarker data in patients with HIV. Soluble ST2, which promotes cardiac fibrosis and is linked to DD<sup>48</sup> and mortality in HFpEF,<sup>49</sup> is associated with cardiac dysfunction and mortality in HIV-infected patients.<sup>50</sup> Galectin-3, which is associated with fibrosis, incident HF, and mortality,<sup>51</sup> is elevated in HIV-infected patients.<sup>52</sup> Growth differentiation factor 15, which is produced by myocytes and endothelial cells and correlates with cardiac mass and fibrosis,<sup>53</sup> predicts all-cause mortality in HIV-infected patients.<sup>50</sup> Disturbances in matrix metalloproteinases and their tissue inhibitors have been reported seen in HIV-infected patients<sup>54</sup>; these changes have been associated with LV hypertrophy and HF.<sup>55</sup> However, the exact mechanisms that accelerate focal and diffuse fibrosis and associated ventricular dysfunction in HIV-infected patients have not been elucidated yet.

### **Biomarkers of Myocardial Strain and Injury in HIV-Infected Patients**

Natriuretic peptides and cardiac troponins are markers of myocardial strain and injury, respectively, and have been associated with risk for HF and subsequent outcomes.<sup>56,57</sup> In a nested case-control study, baseline NT-pro-BNP levels were significantly higher among HIV-infected patients vs. controls who experienced subsequent cardiovascular events.<sup>58</sup> In HIV-infected women, NT-pro-BNP was elevated compared to controls and predicted mortality.<sup>59</sup> In a recent study, N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) was higher and high-sensitivity cardiac troponin I was more frequently detectable in HIV-infected patients despite normal LVEF.<sup>50</sup> In another study of 155 young, asymptomatic HIV-infected patients with LVEF  $\geq$  50% on cardiac computed tomography (except for 2 patients), high-sensitivity cardiac troponin T was detectable in 50%,<sup>52</sup> indicating that subclinical myocyte injury is frequently present even among HIV-infected patients at low risk for cardiovascular disease. Overall however, data are limited in HIV-infected patients, and the potential of these markers to identify those at high risk for DD and HFpEF is unknown.

## **THE CHARACTERIZING HEART FUNCTION ON ANTIRETROVIRAL THERAPY (CHART) STUDY**

### **Objective**

The purpose of the CHART study is to investigate the determinants, mechanisms, and consequences of DD in HIV-infected patients. We will compare ART-treated, virally suppressed HIV-infected individuals with and without DD and HIV- individuals with DD in

regards to (1) persistent systemic inflammation through circulating biomarkers; (2) immune system activation through cell surface receptors; (3) myocardial fibrosis by CMR; (4) circulating biomarkers of fibrosis and remodeling, oxidative stress, and hypercoagulability; (5) left atrial function through echocardiography; (6) myocardial stress and subclinical necrosis through circulating biomarkers; (7) proteomic and metabolic profiles; and (8) clinical, biomarker, and imaging data to derive phenotype signatures.

### Study Design

CHART is a prospective case-control study; the design is summarized in Figure 1.

### Hypothesis

The primary hypothesis is that HIV-infected/DD+ participants will demonstrate a higher degree of systemic inflammation than the HIV-infected/DD- group despite similar ART and viral suppression. We also hypothesize that a higher fibrosis burden will be present in the HIV-infected/DD+ group and that left atrial function will be impaired compared to HIV-infected/DD- and HIV-/DD+ participants.

### Enrolling Centers

The Heart Failure Clinical Research Network (HFN) is a clinical research initiative funded by the National Heart Lung and Blood Institute (NHLBI) with the aim of improving our understanding and management of HF and discover innovative treatment options for HF. The HFN provides a platform for collaborative research by bringing together many centers of excellence across North America. The HFN consists of 9 Regional Clinical Centers and their affiliated sites whose investigators provide scientific leadership in the development of the scientific agenda. The primary goal is to conduct multiple clinical trials to evaluate treatments and strategies to improve management of acute and chronic HF. Ancillary sites can participate in specific HFN protocols. The Coordinating Center at Duke Clinical Research Institute coordinate regulatory, data, and clinical operations. Additional information about the HFN is available at [www.hfnetwork.org](http://www.hfnetwork.org). For the CHART Study, patients will be enrolled at 11 centers (University of Pennsylvania Health System, PA; University Hospitals of Cleveland Medical Center, OH; Duke University Medical Center, NC; Brigham and Women's Hospital, MA; Mayo Clinic, MN; Washington University School of Medicine, MO; Thomas Jefferson University, PA; University of Vermont Medical Center, VT; Tufts University Medical Center, MA; Emory University Hospital, GA).

### Study Populations

We plan to enroll a minimum of 200 ART-treated, virally suppressed HIV-infected subjects with (a) no DD (HIV-infected/DD- cohort; N=100) and (b) with DD (HIV-infected/DD+ cohort; N=100). These groups will be compared to HIV- subjects with DD (HIV-/DD+ cohort) from the Multi-Ethnic Study of Atherosclerosis (MESA) study, an NIH-funded longitudinal cohort.<sup>60</sup> MESA participants were aged 45–84 and free of cardiovascular disease at enrollment (2000–2002). MESA Exam 6 will evaluate 3500+ participants with comprehensive echocardiography and blood samples for biomarkers; this will form the HIV-/DD+ cohort for the present study. We will select 100 age- and sex-matched participants

from MESA without DD based on the CHART criteria for DD outlined below. Importantly, CHART and MESA will have an identical dynamic echocardiography protocol, with a single core laboratory for both studies, and thus we expect these data to be fully comparable. However, MESA participants will not have CMR data for comparison.

### Eligibility Criteria

The enrollment criteria are summarized in Table 3. Although DD may occur in younger HIV-infected persons, most HIV studies of DD have included patients >40 years old.<sup>13–16</sup> Younger patients with HIV have less comorbidity that contributes to DD. Because the main concern in HIV is the effect of DD on long term complications, and in order to elucidate the role of traditional vs. HIV-specific factors, we will enroll middle-aged and older HIV-infected individuals to compare with HIV- subjects with DD.

### Definition of Diastolic Dysfunction

DD will be defined according to the criteria shown in Table 4, which are based on a combination of reduced early diastolic relaxation and evidence of cardiac structural abnormalities that are known to be associated with DD. We chose these criteria instead of the 2016 American Society of Echocardiography (ASE) recommendations for the diagnosis of DD<sup>61</sup> because we sought to identify DD prior to the onset of symptomatic HFpEF; the 2016 ASE diastolic function recommendations are more specific for overt elevation of LV filling pressures.<sup>62</sup> Furthermore, E/e' and PASP may be elevated for reasons other than DD in the setting of HIV, such as a high output state due to hepatitis C-related liver disease (for E/e' or PASP) or HIV-related pulmonary vascular disease (for PASP).

### Research Procedures

**Clinical Data Collection**—Detailed clinical history, physical examination, routine laboratory, medications, and other clinical data will be collected from all participants, as well as blood samples for biomarker determinations (Figure 1). All participants will also undergo an electrocardiogram and complete a Kansas City Cardiomyopathy Questionnaire for quality of life assessment.

**Echocardiography**—Each participant will undergo comprehensive transthoracic echocardiography. Speckle-tracking will be used to evaluate ventricular and atrial mechanics.<sup>63</sup> Passive leg raise will be used to determine the ability of the left atrium to augment its reservoir, conduit, and contractile functions in response to increased preload.<sup>64</sup> The effect of leg raise on standard diastolic function parameters will also be examined. Echocardiograms will be centrally interpreted at the Northwestern University Cardiovascular Imaging Core Laboratory.

**Cardiac Magnetic Resonance**—Each participant will undergo a standard CMR examination, including cine imaging using a steady-state free precession sequence and delayed contrast-enhancement imaging after IV extracellular gadolinium administration using a segmented inversion recovery sequence. Delayed enhancement will allow assessment of the presence, location, and extent of focal myocardial scarring. Myocardial T1



mapping will also be performed at sites capable of obtaining shortened modified Look-Locker inversion recovery<sup>65</sup> images at 1.5 Tesla. Native T1 values and extracellular volume fraction will be assessed. Subjects undergoing T1 mapping will have a hematocrit determination on the day of CMR for calculation of extracellular volume fraction. The Duke Cardiac Magnetic Resonance Core will serve as the core lab.

**Biomarkers**—Multiple domains of biomarkers, including fibrosis and remodeling, arterial inflammation, hyper-coagulability, and oxidative stress, will be evaluated (Table 5). Samples will be stored at  $-80^{\circ}\text{C}$  at the Heart Failure Network core biobank at the University of Vermont.

**Metabolomics and Proteomics Profiling**—For targeted profiling, tandem flow injection mass spectrometry will be used, including 45 acylcarnitine species (mitochondrial metabolism), 15 amino acids, and a set of conventional analytes (lipids, glucose, ketones, non-esterified fatty acids and CRP).<sup>66</sup> For non-targeted profiling, gas chromatography and liquid chromatography will be used.<sup>67,68</sup> Identification of non-targeted metabolite peaks will be performed using a retention-time-locked metabolite library. Peaks will be identified by matching exact masses to a database of >40,000 metabolites (Human Metabolome Database).<sup>69</sup> Proteomic profiling of 1310 proteins will be performed using a DNA aptamer-based platform to target proteins (SomaLogic, Inc), as well as other targeted protein arrays and traditional protein assays. To identify novel proteins or metabolites that differentiate HIV+/DD+ and HIV+/DD- subjects, we will perform targeted analyses of fibrosis and inflammation-related proteins and the full array of metabolomics data. Profiling will be conducted at the Duke Molecular Physiology Institute.

### Sample Size and Power Calculations

The primary comparison for power calculation purposes is the difference in log-transformed interleukin (IL)-6 levels between HIV-infected/DD+ and HIV-infected/DD- groups. Log-transformed IL-6 is expected to have a standard deviation (SD) of approximately 0.75.<sup>70</sup> Sample sizes of 100 subjects in each group will provide 80% and 90% power to detect differences of 0.30 and 0.35, respectively, in a 2-sample *t*-test with a 2-sided type I error of 0.05. These calculations suggest that comparisons of continuous variables between HIV-infected/DD- and HIV-infected/DD+ groups will have 80% and 90% power to detect differences of 0.40 and 0.46 SD, respectively. Comparisons with the HIV-/DD+ cohort will have more power due to the larger number of HIV-/DD+ subjects available in MESA.

### Analytic Plan

Quantitative imaging (CMR and echocardiography) parameters and circulating biomarkers with skewed distributions will be appropriately transformed to normalize distributions. Unadjusted comparisons between groups will be based on *t*-tests (for comparisons that include only the HIV+/DD+ vs. HIV+/DD- groups, e.g. CMR data) and analysis of variance (for HIV+/DD+ vs. HIV+/DD- vs. HIV-/DD- comparisons). For adjusted comparisons, we will use linear regression models with the parameter of interest as the outcome, study group as the main exposure, and clinical characteristics as covariates (including demographics, clinical history items, HIV-related blood work, biochemistry, and medication classes). For

discrete parameters (e.g. presence of fibrosis), we will use chi-squared tests for unadjusted comparisons and logistic regression models for adjusted comparisons following a similar approach as described above.

For proteins and metabolites, multivariable generalized linear regression models, adjusted for potential confounders, after transforming variables appropriately, will be used to test for differences in mean values of analytes between HIV-infected/DD+ and HIV-infected/DD- subjects. Analytes with >25% values as zero (e.g. below the lower limit of quantification) will be analyzed as binary traits. Benjamini and Hochberg false discovery rate will be used for multiple omics data comparisons.<sup>71</sup>

## DISCUSSION

The CHART study seeks to better understand DD, one of the major predisposing risk factors for development of HFpEF and other cardiovascular disease, in HIV-infected patients receiving contemporary ART. Similar to other individuals with chronic inflammatory conditions, HIV infection can serve as a model to better understand the role of inflammation in DD. Furthermore, understanding DD pathogenesis in HIV will allow for identification of treatment targets for patients at risk of HFpEF. The CHART study will determine clinical, imaging and molecular characteristics of this pathogenesis, and enable evaluation of general vs. HIV-specific components.

Our enrollment criteria maximize the ability to detect impaired LV relaxation associated with structural heart disease. Such impairment is indicative of chronically elevated left atrial pressure (left atrial size) or presence of LV hypertrophy. We excluded E/e' ratio and elevated pulmonary artery pressure as indicators of DD because of the possibility of non-DD causes of increases in these values in patients with HIV (e.g., high output state from co-infection; HIV-associated pulmonary hypertension etc.) and also because these parameters are elevated in patients with overt, symptomatic HFpEF. However, it is important to acknowledge that many different mechanisms can contribute to DD such as relaxation abnormalities, changes in myocardial stiffness, elevation of central blood volume, ventricular interaction, reductions in longitudinal shortening, and atrial pathology, among others. Therefore, echocardiographic assessment and classification of DD, no matter how careful, is a necessary simplification of a complex condition for the practical purposes of a clinical study.

Echocardiography in CHART will assess standard DD parameters; provide a comprehensive cardiac assessment; and provide novel metrics of cardiac reserve by combining speckle-tracking with dynamic preload maneuvers. In patients with HFpEF, left atrial dysfunction leads to pulmonary hypertension, reduced exercise capacity, and poor clinical outcomes.<sup>72</sup> Altered response to preload, e.g. with leg raising, can detect diminished left atrial reserve and help identify patients who have an exaggerated rise in LV filling pressure when preload is increased.<sup>64,73</sup> Besides reports of left atrial dilatation,<sup>74</sup> little is known about the role of left atrial function in DD in HIV-infected patients.

There remains a knowledge gap about factors that lead to elevated risk for DD and HFpEF in HIV infection, and other pathways have also been proposed, e.g. mitochondrial toxicity<sup>75</sup>

and endoplasmic reticulum stress.<sup>76</sup> Of note, older ART regimens, such as the nucleoside reverse transcriptase inhibitors zidovudine and stavudine, have been associated with mitochondrial toxicity,<sup>77</sup> but the impact of newer classes of medications, such as integrase inhibitors, on DD and HF in HIV remains unknown. The broad representation of pathways included in the proteomics and metabolomics panels will enable testing of many known pathways and identification of novel mechanisms. High throughput -omics approaches have identified novel markers and mechanisms in general, but have not been specifically applied to investigation of DD and HF pathogenesis in HIV to date.

Despite a growing amount of evidence suggesting that systemic low-level inflammation involving the myocardium and diffuse myocardial fibrosis play a central role in the development of DD in patients with HIV, there are no serial studies to inform us on the rate of progression of these changes in the myocardium and extracellular matrix and importantly, on the clinical significance of these changes. For example, although patients with HIV demonstrate diffuse myocardial fibrosis as indicated by elevated myocardial extracellular volume on CMR, we do not know what threshold of extracellular volume is associated with future development of DD. In addition, the incidence of new-onset HFpEF in patients with HIV and DD has not been reported. The CHART study offers a unique opportunity to study changes in myocardial structure and function over time in HIV-infected patients with or without DD at baseline and their clinical significance. We are currently in the planning stages of a follow-up study in order to perform repeat CMR, echocardiography, and biomarker assessments in CHART participants 2 years after the baseline visit. We are also planning to evaluate for transition from DD- to DD+ phenotype and development of clinical HFpEF in all participants as part of the follow-up study. Finally, an important next step would be to develop interventions to halt the progression of DD in HIV-infected patients.

## CONCLUSION

The CHART study seeks to better understand DD in HIV infected individuals who are receiving ART. DD is one of the major predisposing risk factors for development of HFpEF, and a risk factor that has been linked with other cardiovascular diseases and mortality. The CHART study may assist in better understanding of traditional and non-traditional risk factors of DD pathogenesis in both HIV infected and uninfected individuals, identify the role of generic versus HIV-specific DD factors in HIV-infected patients, and in turn may provide impetus for development of screening, prevention, and treatment options for these patients at risk for developing cardiovascular complications, including HF.

## Acknowledgments

**Funding Sources:** The Heart Failure Clinical Research Network is supported by the NHLBI, National Institutes of Health Funding/Support: U10 HL084904 (awarded to the coordinating center) and U01 HL084861, U10 HL110312, U10 HL110337, U10 HL110342, U10 HL110262, U10 HL110297, U10 HL110302, U10 HL110309, U10 HL110336, and U10 HL110338 (awarded to the regional clinical centers). This work is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, and Blood Institute or the National Institutes of Health.

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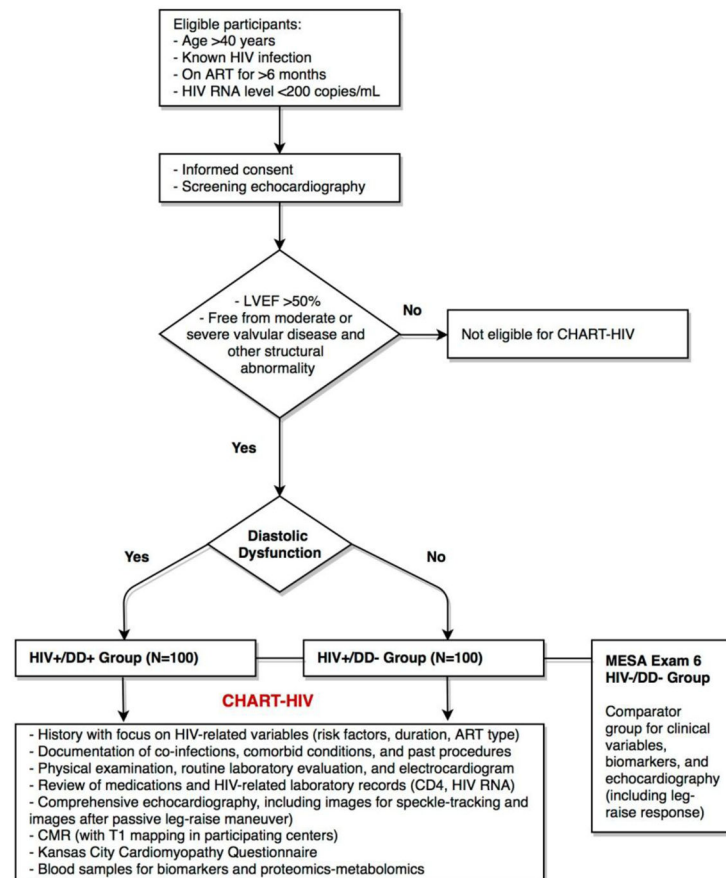
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**Figure 1. Overview of Study Design of the Characterizing Heart function on Anti-Retroviral Therapy (CHART) Study**

ART: antiretroviral therapy; CD4: cluster of differentiation 4; CMR: cardiac magnetic resonance; DD: diastolic dysfunction; HIV: human immunodeficiency virus; LVEF: left ventricular ejection fraction; RNA: ribonucleic acid

**Table 1**  
Diastolic Dysfunction Among HIV Infected Persons on Antiretroviral Therapy in Comparative Studies

Study	Population	Findings
Schuster et al 2008 <sup>13</sup>	30 HIV-infected men (age 42.1 ± 4.7 years, duration of HIV infection 10.4 ± 4.7 years, duration of ART 5.3 ± 2.1 years) and 26 age-matched HIV- controls	HIV-infected patients had similar height-indexed LV mass vs. controls (40.6 ± 9.5 vs. 37.5 ± 9.3 g/m; P > 0.05), but higher prevalence of DD (abnormal relaxation or pseudonormal filling), 64% vs. 12% (P < 0.001). LV systolic function indexes were lower and pulmonary artery pressure was higher in patients with HIV compared with controls.
Hsue et al 2010 <sup>14</sup>	196 HIV-infected adults (median age, 47 years; 85% male; median duration of HIV infection 15 years; 82% on ART) and 52 controls	Median BSA-indexed LV mass was higher in HIV-infected vs. HIV- (77.2 vs. 66.5 g/m <sup>2</sup> ; P < 0.001). LV ejection fraction was similar. Among HIV-infected, 50% had mild DD vs. 29% in HIV- (P = 0.008). HIV-infected patients had an adjusted odds ratio of 2.4 for DD as compared with controls (P = 0.019).
Luo et al 2014 <sup>15</sup>	325 HIV-infected initially ART-naïve patients, repeat evaluation after 48 weeks of ART, 97 age-matched HIV- controls	HIV-infected patients had a higher prevalence of DD vs. controls (16.5% vs 7.2%, P = 0.027) and LV systolic dysfunction (7.3% vs 2.1%, P = .056). DD increased from baseline to week 48 (23.3%; P = 0.056 vs. baseline) in HIV-infected patients.
Fontes- Carvalho et al 2015 <sup>16</sup>	206 HIV-infected persons (88 ART-naïve and 116 on ART, 41.7 ± 9.4 years, 70.4% male) and 30 controls	Prevalence of DD in HIV-infected patients 23% vs. 3.3% in controls (P = 0.01), but not different between ART-naïve (19%) and on-ART (23%) HIV-infected patients. No differences in systolic function.

ART = antiretroviral therapy; BSA = body surface area; DD = diastolic dysfunction; HIV = human immuno-deficiency virus; LV = left ventricular

Table 2

## Myocardial Fibrosis Among HIV Infected Persons in Comparative Studies

Study	Population	Findings
Holloway et al 2013 <sup>42</sup>	90 patients with HIV on ART (median age 43 years), duration of HIV infection 7.4±6.0 years; 39 age-matched controls (median age 40 years) without CV disease	Myocardial fibrosis by LGE, predominantly in the LV basal inferolateral wall, was observed in 76% of patients with HIV vs. 13% of controls (P<0.001); peak myocardial systolic and diastolic longitudinal strain were lower in HIV patient; patients with HIV had 47% higher median myocardial lipid levels
Thiara et al 2015 <sup>43</sup>	95 patients with HIV (age, 49±10 years; 95% on ART for 9±6 years), duration of HIV infection 14±8 years; 30 matched healthy adults (age, 46±8 years) without known CV disease	Myocardial extracellular volume was 0.28±0.04 in patients with HIV vs. 0.26±0.04 in controls (P=0.02) but focal myocardial scarring (by LGE) was similar (8.6% and 7.7%, respectively; P=0.8); mean LV radial strain was decreased in patients with HIV vs. controls (21.7% ± 8.6% vs. 30.5% ± 14.2%; P=0.004)
Luetkens et al 2016 <sup>35</sup>	28 patients with HIV (age, 49±9 years) on ART, duration of HIV infection 9.7±6.9 years; 22 healthy adults (age, 45±16 years) without known CV disease	Myocardial fibrosis by LGE, predominantly in the mid-ventricular and basal inferolateral LV wall, was present in 82.1% of patients with HIV vs. 27.3% of controls (P<0.001); patients with HIV had lower LVEF and global longitudinal (17.7±3.4% vs. 20.2±3.2%, P<0.001) and circumferential (21.2±4.6% versus 24.7±5.1%, P<0.001) strain; parameters indicating inflammation were elevated in patients with HIV
Ntusi et al 2016 <sup>36</sup>	103 patients with HIV without known CV disease (age, 44±10; 13 ART naïve; 29 on protease-inhibitor regimen; 61 on nucleoside reverse-transcription inhibitor regimen); 92 healthy controls (age, 45±10)	Patients with HIV had more myocardial areas with elevated short-tau inversion recovery (indicating myocardial injury) vs. controls, higher average native T1 values (969 vs. 956 ms; P=0.01), more frequent LGE (83% vs. 16%; P<0.001), and 9% lower peak diastolic strain rate (P<0.001); pericardial effusions were more common in patients with HIV

ART= antiretroviral therapy; CV=cardiovascular; HIV= human immunodeficiency virus; LGE = late gadolinium enhancement; LV = left ventricular; LVEF=left ventricular ejection fraction

**Table 3**

Main Inclusion and Exclusion Criteria

	HIV-infected/DD- (N=100)	HIV-infected/DD+(N=100)	HIV-/DD+ (MESA Study)
<b>Inclusion Criteria</b>			
Age	>40 years	>40 years	>40 years
HIV antibody	Positive	Positive	Negative/unknown
ART	>6 months	>6 months	Not applicable
HIV RNA level	<200 copies/mL	<200 copies/mL	Not applicable
LVEF	>50%	>50%	>50%
Diastolic dysfunction*	Absent	Present	Present
<b>Exclusion Criteria</b>			
History of AIDS	Any documented CD4 cell count of less than 200 or AIDS-defining opportunistic infections		
LVEF	Past LVEF <50%		
Valve	Moderate or severe valve stenosis or regurgitation, or past repair or replacement		
Coronary disease	History of percutaneous or surgical revascularization or active angina		
Arrhythmias	Persistent atrial fibrillation		
Blood pressure	>160 mmHg systolic or >100 mmHg diastolic BP		
Comorbidity	Comorbid inflammatory disease (e.g. rheumatoid arthritis or systemic lupus erythematosus)		
Cancer	Active cancer or cancer chemotherapy treatment in the prior year		
Medications	Chronic use of steroid or anti-inflammatory therapy		
CMR eligibility	Contraindication to CMR or gadolinium injection		

AIDS: acquired immune deficiency syndrome; ART= antiretroviral therapy; BP: blood pressure; CD4: cluster of differentiation 4; CMR: cardiac magnetic resonance; HIV= human immuno-deficiency virus; LVEF = left ventricular ejection fraction; RNA: ribonucleic acid \* As defined in Table 3.

**Table 4****Criteria for Diastolic Dysfunction for HIV-Infected Participants**

<b>1</b>	Left ventricular ejection fraction > 50%
<b>2</b>	Evidence of impaired left ventricular relaxation: septal e' < 7 cm/s or lateral e' < 10 cm/s
<b>3</b>	Evidence of chronically elevated left ventricular filling pressure or left ventricular hypertrophy (or concentric remodeling): <ol style="list-style-type: none"> <li><b>a.</b> left atrial volume index &gt; 28 ml/m<sup>2</sup> or</li> <li><b>b.</b> left ventricular hypertrophy (left ventricular mass index &gt; 95/m<sup>2</sup> in women, &gt; 115 g/m<sup>2</sup> in men) or</li> <li><b>c.</b> concentric left ventricular remodeling (relative wall thickness &gt; 0.42)</li> </ol>

**Table 5**

## Study Biomarkers

<b>Inflammation</b>	Interleukin-6, high-sensitivity C-reactive protein
<b>Immune activation</b>	sCD163, sCD14, monocyte expression of CD14 and CD16, T-cell activation and subsets: Th1, Treg, CD4+, CD8+ T cell activation
<b>Coagulation</b>	D-dimer, fibrinogen
<b>Arterial inflammation</b>	Oxidized low-density lipoprotein, lipoprotein-associated phospholipase A2
<b>Fibrosis and remodeling</b>	soluble ST2, GDF-15, C1TP:MMP-1 ratio, Galectin-3, TIMP-1, PIIINP, PINP, PICP
<b>Oxidative stress</b>	Myeloperoxidase
<b>Stress</b>	N-terminal pro-B-type natriuretic peptide
<b>Necrosis</b>	High-sensitivity troponin I

C1TP: carboxyl-terminal telopeptide of collagen type I; GDF: growth differentiation factor; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase; PICP: carboxyl-terminal peptide of procollagen type I; PINP: amino-terminal peptide of procollagen type I; PIIINP: amino-terminal peptide of procollagen type III