Immune Correlates of Diffuse Myocardial Fibrosis and Diastolic Dysfunction Among Aging Women With Human Immunodeficiency Virus

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Abstract

Aged women with Human Immunodeficiency Virus (HIV) experience increased heart failure risk, an adverse outcome strongly associated with myocardial fibrosis (MF). We prospectively evaluated 22 HIV-infected females and 22 non-HIV-infected controls. MF was measured with cardiac magnetic resonance. We also assessed diastolic function and inflammatory CD14+CD16+ monocytes. MF, diastolic dysfunction (DD), and activated monocytes correlated with each other and with indices of systemic monocyte activation. Heart failure outcomes were worse in HIV-infected females. Among HIV-infected females, MF is associated with indices of systemic monocyte activation and increased heart failure risk.

Keywords: HIV; women; myocardial fibrosis; diastolic dysfunction; inflammation.

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Heart failure and associated adverse outcomes threaten the health of aging women with human immunodeficiency virus (WHIV) on antiretroviral therapy (ART). WHIV face increased heart failure risk [1, 2] and worse heart failure outcomes [1] compared with non-HIV-infected women. Moreover, WHIV with heart failure exhibit a predilection toward heart failure with preserved ejection fraction [1]—a heart failure subtype uniquely intransigent to medical therapies. This predilection appears to be more pronounced than that among men with HIV and heart failure [3]. Thus, imperatives exist to characterize pre–heart failure phenotypes among aging WHIV and to identify potential pathogenetic drivers and/or markers of these states. Such efforts may facilitate the development and application of heart failure prevention strategies tailored to this at-risk population.

We explored mechanisms of HIV-attributable heart failure risk among women through a clinical-translational research study synthesizing advanced cardiac and immune phenotypic data. Prospectively recruited, matched groups of women with and without HIV underwent cardiac magnetic resonance imaging (MRI) and detailed assessments of systemic monocyte activation. We hypothesized that WHIV (vs controls) would evidence diffuse myocardial fibrosis and diastolic dysfunction in association with indices of systemic monocyte activation. Our hypothesis was fueled by 3 antecedent observations: First, ART-treated HIV infection has been associated with myocardial structural pathology, including fibrosis and/or steatosis, in all-male or predominantly male cohorts [4–7]. Myocardial fibrosis, in turn, has been linked to systemic monocyte activation and in situ myocardial macrophage infiltration in a pilot autopsy study focused on men with HIV [8]. Finally, HIV infection incites the most pronounced, sustained surge in innate immune hyperreactivity among women [9].

Our work builds on existing cardiac MRI studies on HIV-associated heart failure risk [4–7] in the following ways. To begin, we focused on mechanisms of HIV-attributable heart failure risk specifically among women by comparing WHIV vs those without HIV. A compelling body of literature has shown that mechanisms of HIV-attributable CVD risk among women do not simply parallel those among men [9]. Furthermore, our immune phenotypic techniques focus not only on circulating immune biomarkers that may represent markers of disease; rather, through application of flow cytometry, we also examined cellular/molecular immune changes that we hypothesized may contribute uniquely to heart failure pathogenesis among WHIV. Based on current understandings of how monocytes/macrophages engender myocardial fibrosis [10], we focused our flow cytometric assessments on proportions of circulating monocyte subpopulations and on monocyte subtype expression of cellular receptors implicated in honing to the vasculature and/or transendothelial migration to target tissues. Among WHIV in our cohort, we sought to identify relationships between measures of heightened systemic immune activation and...
surrogate measures of heart failure risk (eg, myocardial fibrosis and diastolic dysfunction).

**METHODS**

**Study Design and Participants**

We prospectively recruited ART-treated WHIV aged 40–75 years without known CVD or diabetes. Non-HIV-infected women were recruited and group-matched to the WHIV based on age and body mass index (BMI). Forty-three participants recruited from the greater Boston area (August 2016–November 2017) provided informed consent to undergo cardiac MRI, blood sampling, and anthropometric assessment. Thirty-four women (20 WHIV and 14 control participants) completed procedures yielding evaluable cardiac MRI data. The Partners Institutional Review Board approved the study, which was registered on ClinicalTrials.gov (NCT02874703; Supplementary Methods).

**Immune Phenotyping**

For WHIV, HIV viral load was determined by ultrasensitive reverse-transcription polymerase chain reaction (Roche Diagnostics/Cobas Ampliprep/Cobas TaqMan HIV-1 test version 2.0). Levels of immune markers were assessed using commercially available enzyme-linked immunosorbent assay kits. Flow cytometry was performed using techniques previously published and data were analyzed using FlowJo software version 8.7.1 (TreeStar; Supplementary Methods).

**Cardiac MRI Acquisition and Analysis**

Participants underwent cardiac MRI on a 3.0-T system (Skyra, Siemens) employing standard protocols for assessment of cardiac structure/function, late gadolinium enhancement imaging for assessment of focal myocardial fibrosis, and T1 imaging with a Look-Locker sequence for assessment of diffuse myocardial fibrosis, the extracellular volume fraction. Diastolic function was quantified by measurement of circumferential diastolic strain rate using myocardial tissue tracking (Supplementary Methods).

**Statistical Analysis**

Normally distributed data are reported as mean ± standard deviation (SD) or median (interquartile range [IQR]). Between-group comparisons were made using the Student t test, Wilcoxon rank-sum test, or χ2 test, and bivariate analyses were performed using a Spearman correlation coefficient or a Pearson correlation coefficient, all as appropriate. JMP Pro software (version 11.0; SAS Institute) was employed for statistical analyses, with P < .05 considered significant.

**RESULTS**

**Participant Characteristics**

Results are reported for the 34 women (20 WHIV and 14 control participants) who completed procedures yielding evaluable cardiac MRI data. By design, both groups of women were similar in age (mean, 52 ± 4 years vs 53 ± 6 years; P = .61) and BMI (mean, 32 ± 7 kg/m² vs 32 ± 7 kg/m²; P = .73) (Table 1). There were no statistically significant differences between groups in race or ethnicity. Among WHIV, the median CD4 count was 773 (IQR, 526–1202) cells/µL and the median viral load was undetectable (Table 1).

**Cardiac Structural and Functional Parameters Among Women With HIV Versus Those Without HIV**

Diffuse myocardial fibrosis was increased among WHIV vs non-HIV-infected women (mean extracellular volume fraction, 0.34 ± 0.06 vs 0.29 ± 0.04; P = .002) (Supplementary Table 1; Figure 1). A single WHIV demonstrated focal myocardial fibrosis. Diastolic function was reduced among WHIV vs non-HIV-infected women (mean circumferential diastolic strain rate, 1.10 ± 0.23 s⁻¹ vs 1.39 ± 0.27 s⁻¹; P = .003) (Supplementary Table 1; Figure 1). Left ventricular mass indexed to body surface area was higher among WHIV (median, 48.4 [IQR, 43.9–56.7] g/m² vs 42.3 [IQR, 40.4–47.2] g/m²; P = .01), whereas the left ventricular ejection fraction was comparable between groups (Supplementary Table 1). Additionally, between-group differences in native T1, a measure of myocardial inflammation, were not significant (Supplementary Table 1).

**Systemic Immune Phenotypes Among Women With HIV Versus Those Without HIV**

Compared with non-HIV-infected women, WHIV demonstrated increased levels of CCL2 (mean, 210 ± 43 pg/mL vs 167 ± 45 pg/mL; P = .009), soluble CD14 (sCD14) (median, 1904 [IQR, 1551–2224] ng/mL vs 1501 [IQR, 1329–1668] ng/mL; P = .008), and soluble CD163 (sCD163) (mean, 1260 ± 293 ng/mL vs 938 ± 308 ng/mL; P = .005) (Supplementary Table 2). WHIV also demonstrated increased density of expression of the cell-surface receptor CD163 on circulating inflammatory CD14⁺CD16⁺ monocytes (mean fluorescence intensity [MFI], 1204.0 ± 380.6 vs 925.3 ± 353.3; P = .04), and an increased density of expression of the cell-surface chemokine receptor CCR2 on circulating classical CD14⁺CD16⁻ monocytes (MFI, 77.1 ± 33.6 vs 37.1 ± 17.7; P = .0001), inflammatory CD14⁺CD16⁻ monocytes (MFI, 83.0 ± 24.2 vs 50.3 ± 18.3; P = .0001), and nonclassical CD14⁺CD16⁻ monocytes (MFI, 38.4 ± 21.7 vs 21.8 ± 14.8; P = .01) (Supplementary Table 2).

**Immune Correlates of Diffuse Myocardial Fibrosis and Diastolic Dysfunction Among WHIV**

Levels of the monocyte activation marker sCD163 correlated with myocardial fibrosis (r = 0.53; P = .02) but not with diastolic dysfunction (Supplementary Table 3). Levels of the chemokine CCL2 and of the monocyte activation marker sCD14 related neither to myocardial fibrosis nor diastolic dysfunction. The density of expression of the cell-surface receptor CD163 on circulating inflammatory CD14⁺CD16⁻ monocytes related neither to myocardial fibrosis nor diastolic dysfunction. Most notably, the density of expression of the cell surface receptor CCR2 on circulating...
inflammatory CD14+CD16+ monocytes correlated directly with myocardial fibrosis (r = 0.48; P = .04) and inversely with diastolic function (r = −0.49; P = .03) (Supplementary Table 3).

DISCUSSION

We employed advanced noninvasive imaging and detailed immunophenotyping techniques to compare myocardial structure and function among aging, asymptomatic women with and without HIV and to identify, among the WHIV, immune correlates of pre–heart failure phenotypes. We determined that, compared with non-HIV-infected women, aging WHIV on stable ART exhibited an increase in diffuse myocardial fibrosis as well as reduced diastolic function. WHIV also demonstrated increased systemic immune activation by select measures (including higher levels of monocyte activation markers such as sCD163), as well as increased density of expression of key cell-surface chemokine receptors on certain subpopulations of circulating monocytes. In our group of studied WHIV, levels of sCD163 correlated with myocardial fibrosis, whereas expression of CCR2 on circulating inflammatory CD14+CD16+ monocytes related directly to myocardial fibrosis and inversely to diastolic function.
Our findings that ART-treated WHIV without known CVD exhibit increased myocardial fibrosis and reduced diastolic function compared with matched non-HIV-infected women may partially explain epidemiologic observations of increased heart failure risk [1, 2] and worse heart failure outcomes [1] among WHIV vs women without HIV. Myocardial fibrosis and/or diastolic dysfunction may be inferred to represent reversible early disease phenotypes, which, untreated, are liable to progress to heart failure and portend death [11]. For example, among diabetic patients, each increase in the extracellular volume of 0.03 has been shown to confer a 50% increased risk in incident heart failure hospitalization and death [11].

Previous work has suggested that not only resident macrophages but also circulating monocytes may play a role in instigating and propagating myocardial inflammation and ensuing myocardial fibrosis [10]. The population of myocardial macrophages is largely regenerated from within; however, in states of systemic immune activation, circulating monocytes may be recruited and retained within the myocardium and there differentiate into macrophages [10]. Macrophages, particularly of the M2 phenotype, secrete inflammatory cytokines that prompt collagen production by adjacent fibroblasts [10]. Cognizant of chronically heightened innate immune reactivity among WHIV [9], we sought to compare indices of systemic immune activation, circulating monocytes may be recruited and retained within the myocardium and there differentiate into macrophages [10]. Macrophages, particularly of the M2 phenotype, secrete inflammatory cytokines that prompt collagen production by adjacent fibroblasts [10].

Cognizant of chronically heightened innate immune reactivity among WHIV [9], we sought to compare indices of systemic immune activation among women with and without HIV and to assess potential systemic immune correlates of myocardial fibrosis and diastolic dysfunction among WHIV. In our studied cohort, ART-treated WHIV (vs non-HIV-infected women) exhibited an increased density of expression of the cell-surface chemokine receptor CCR2 on circulating classical CD14+CD16+ monocytes, inflammatory CD14+CD16+ monocytes, and nonclassical CD14+CD16+ monocytes. Further, among WHIV, CCR2 expression on circulating inflammatory CD14+CD16+ monocytes related directly to myocardial fibrosis and inversely to diastolic function.

The known function of the CCR2 receptor and demonstrated behavior of circulating inflammatory CD14+CD16+ monocytes, taken together, lend biologic plausibility to the notion that circulating inflammatory CD14+CD16+ monocyte CCR2 expression may promote myocardial fibrosis. Specifically, CCR2 expression permits circulatory inflammatory CD14+CD16+ monocytes to hone toward and migrate into target tissues, such as the myocardium. Monocytes thus recruited may then develop into macrophages [10]. Hulsmans et al showed in murine models that monocyte recruitment to the myocardium enhanced the density of macrophages. These macrophages, via cytokine signaling with paracrine and autocrine effects, in turn triggered fibroblast overproduction of collagen and reduced macrophage-mediated fibrinolysis, resulting in myocardial fibrosis and diastolic dysfunction [12]. Of interest, studying human recipients of sex-mismatched heart transplants, Bajpai et al demonstrated that in the myocardium, reparative CCR2-negative macrophages are replenished solely through local proliferation while inflammatory/profibrotic CCR2-positive macrophages are sustained via monocyte recruitment [13]. In addition, in studies of PHIV, Williams et al have shown that CCR2 expression by CD14+CD16+ monocytes primes these cells for transendothelial migration and portends end-organ damage including HIV-associated neurocognitive disorder [14].

A limitation of our hypothesis-generating study includes the cross-sectional study design, precluding determinations on causation. We noted a correlation between circulating inflammatory monocyte expression of the honing receptor CCR2 and myocardial pathology among WHIV. However, the extent to which CCR2 expression on circulating inflammatory monocytes engenders increased density of CCR2

Figure 1. Diffuse myocardial fibrosis and diastolic function among women with human immunodeficiency virus (WHIV) and women without human immunodeficiency virus (HIV). A. Diffuse myocardial fibrosis, as quantified by the cardiac magnetic resonance imaging (MRI) measure of extracellular volume fraction, was increased among WHIV (n = 19, extracellular volume fraction uninterpretable for 1 participant) compared with non-HIV-infected women (n = 14) (P = .002 by t test). B. Diastolic function, as quantified by the cardiac MRI measure of circumferential diastolic strain rate, was reduced among WHIV (n = 20) compared with non-HIV-infected women (n = 14) (P = .003 by t test). In both graphs, data are presented as box plots, where the box represents the median and interquartile range and the whiskers represent the minimum and maximum values.
macrophages in the myocardial structural space and attendant in situ fibrosis can only be ascertained through a longitudinal study in which cardiac biopsy samples are obtained. The small sample size of our study also represents a limitation, impeding our ability to assess whether specific immune indices relate to myocardial fibrosis and diastolic dysfunction among WHIV controlling for other relevant parameters (eg, specific ART exposures) [15]. Moreover, although women with and without HIV in our study exhibited similar age and BMI, between-group differences in other cardiovascular risk factors may have contributed to the observed heightened myocardial pathology among WHIV. Recruitment of study participants from a single geographic region in the northeast United States constitutes another limitation of our study, limiting generalizability. The characterizing HIV-related diastolic dysfunction (CHART) study, a cardiac MRI–based longitudinal study in which cardiac biopsy samples are obtained, will further insights on mechanisms of HIV-associated heart failure. Additional work is needed to compare myocardial structure/function and systemic immune activation among large numbers of aging women with and without HIV in HIV-endemic regions.

Through our study, we noted increased myocardial fibrosis and reduced diastolic function among asymptomatic WHIV vs non-HIV-infected women. This finding may help explain why WHIV have an increased risk of heart failure and worse outcomes compared with women without HIV. Furthermore, we observed that that among WHIV, honing receptor expression on circulating inflammatory monocytes was related to key heart failure precursors (myocardial fibrosis, diastolic dysfunction). This observation intimates new avenues for research on immunomodulatory strategies geared toward heart failure prevention in this at-risk population.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
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Author contributions. M. V. Z. was involved in study concept/design, study recruitment/performance, data acquisition, data analysis/interpretation, and manuscript writing. M. T. was involved in data acquisition, data analysis/interpretation, and manuscript writing. L. A. S. was involved in study recruitment/performance and data analysis/interpretation. A. R. and C. P. M. were involved in study recruitment/performance. J. R., D. C., J. E. H., A. M. N., M. J. S., V. A. T., and T. L. S. were involved in data analysis/interpretation. L. S. S., M. J.-H., M. D. N., and T. H. B. were involved in data acquisition and data analysis/interpretation. T. G. N. was involved in study concept/design, data acquisition, data analysis/interpretation, and manuscript writing. All authors contributed to critical revision of the manuscript.

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