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ORIGINAL RESEARCH

Sex, HIV Status, and Measures of Cardiac Stress and Fibrosis in Uganda

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BACKGROUND: Biomarkers of myocardial stress and fibrosis are elevated in people living with HIV and are associated with cardiac dysfunction. It is unknown whether sex influences these markers of heart failure risk in sub-Saharan Africa, where HIV burden is high and where the vast majority of women with HIV live.

METHODS AND RESULTS: Echocardiograms and 6 plasma biomarkers (suppression of tumorigenicity-2, growth differentiation factor 15, galectin 3, soluble fms-like tyrosine kinase-1, NT-proBNP [N-terminal pro-B-type natriuretic peptide], and cystatin C) were obtained from 100 people living with HIV on antiretroviral therapy and 100 HIV-negative controls in Uganda. All participants were ≥45 years old with ≥1 major cardiovascular risk factor. Multivariable linear and logistic regression models were used to assess associations between biomarkers, echocardiographic variables, HIV status, and sex, and to assess whether sex modified these associations. Overall, mean age was 56 years and 62% were women. Suppression of tumorigenicity-2 was higher in men versus women ($P<0.001$), and growth differentiation factor 15 was higher in people living with HIV versus controls ($P<0.001$). Sex modified the HIV effect on cystatin C and NT-proBNP (both P for interaction <0.025). Women had more diastolic dysfunction than men ($P=0.02$), but there was no evidence of sex-modifying HIV effects on cardiac structure and function. Cardiac biomarkers were more strongly associated with left ventricular mass index in men compared with women.

CONCLUSIONS: There are prominent differences in biomarkers of cardiac fibrosis and stress by sex and HIV status in Uganda. The predictive value of cardiac biomarkers for heart failure in people living with HIV in sub-Saharan Africa should be examined, and novel risk markers for women should be further explored.

Key Words: cardiac biomarkers ■ cardiac fibrosis ■ echocardiography ■ HIV ■ sex

Cardiovascular disease (CVD) is a significant source of morbidity and mortality for people living with HIV (PLWH) globally.[1] In sub-Saharan Africa, chronic HIV infection is prevalent and rates of CVD are rising.[2-4] A modeling study based on Global Burden of Disease data suggests that HIV contributes up to 10% to 15% of population-attributable risk for CVD in sub-Saharan Africa.[4]

The association between HIV and CVD is complex and multifactorial. One important pathway of risk appears to be HIV-induced inflammation and immune activation,[5-7] leading to vascular disease but also systolic and diastolic dysfunction, and eventually clinical heart failure.[8] Women with HIV tend to have higher levels of chronic inflammation and appear to be more susceptible to inflammation-induced comorbidities, such as heart failure and other CVDs,[9] but are often underrepresented in studies conducted in high-income countries, where they make up <25% of the HIV+ population.[10] In addition to markers of generalized inflammation, biomarkers of myocardial stress and fibrosis are also elevated in PLWH compared with controls and are associated with cardiac dysfunction independent of traditional CVD risk factors and HIV-related factors.[5]

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CLINICAL PERSPECTIVE

What Is New?

- Epidemiologic studies suggest that HIV-related heart failure risk may be higher for women compared with men, but little is known about the mechanisms of this risk.
- This study examines sex as an effect modifier of the associations between HIV infection, biomarkers of cardiac stress and fibrosis, and measures of left ventricular structure and function.
- A small number of prior studies from high-income countries have examined sex effects on cardiac biomarkers and echocardiographic measures in people living with HIV; this study examines these sex effects in a sub-Saharan African population, where most women with HIV live.

What Are the Clinical Implications?

- Mechanisms of subclinical cardiac dysfunction may differ between men and women in sub-Saharan Africa.
- The effect of HIV on biomarkers and echocardiographic measures of heart failure risk appears to be different in women compared with men.

Nonstandard Abbreviations and Acronyms

Gal-3	galectin 3
GDF-15	growth differentiation factor 15
PLWH	people living with HIV
sFLT-1	soluble fms-like tyrosine kinase-1
ST-2	suppression of tumorigenicity-2

In the general population, studies have shown sex differences in these cardiac biomarkers and their association with CVD risk.[11-13]

Previous studies have investigated the effect of HIV on cardiac biomarkers and measures of cardiac structure and function, but little is known about how sex influences these associations. Studying the effects of both sex and HIV status is an important step in understanding the pathophysiological characteristics of HIV-related cardiac dysfunction, predicting disease progression, and developing potentially effective therapies. Figure 1 is a conceptual model of our study, which is the first of our knowledge to investigate whether sex modifies the effect of HIV on cardiac biomarkers, the effect of HIV on cardiac structure and function, or the relationship between biomarkers and cardiac structure and function. We additionally aimed to describe sex effects on biomarkers and cardiac structure and function after adjusting for potentially confounding traditional risk factors. More important, to increase the relevance and generalizability of our findings to a global context, we planned our study in sub-Saharan Africa, where the vast majority of women with HIV live.

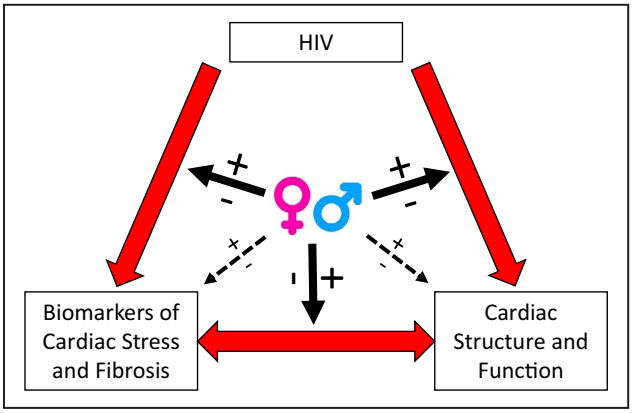


Figure 1. Conceptual model.
The primary aim of our study was to investigate whether sex modifies the HIV effect on biomarkers of cardiac stress and fibrosis or echocardiographic measures of cardiac structure and function (solid arrows). Our secondary aim was to describe sex effects on biomarkers and cardiac structure and function after adjusting for potentially confounding traditional risk factors (dashed arrows).

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Population

The study participants consisted of 100 PLWH and 100 age- and sex-matched HIV-uninfected people ≥45 years of age. The PLWH were recruited from the Joint Clinical Research Center near Kampala, Uganda, and enrolled between April 2015 and May 2017. PLWH were on antiretroviral therapy (ART) for >6 months with a stable regimen for >12 weeks, and had an HIV viral load <400 copies/mL. Controls were recruited from local internal medicine clinics. All participants (PLWH and controls) had ≥1 major CVD risk factor (hypertension, diabetes mellitus, smoking, low high-density lipoproteins, hypercholesterolemia, or family history of early coronary artery disease). Participants were excluded if they had a history of known coronary artery disease, peripheral artery disease, ischemic stroke, active chronic inflammatory condition, use of chemotherapy or immunomodulating agents, or estimated glomerular filtration rate <30 mL/min per 1.73 m^2. The Joint Clinical Research Center Community Advisory Board was consulted

for the design, conduct, and dissemination of the research, which was reviewed and approved by the Institutional Review Boards of University Hospitals Cleveland Medical Center and the Joint Clinical Research Center and by the Uganda National Council for Science and Technology. All subjects signed written informed consent.

Cardiac Biomarkers

The following 6 soluble protein biomarkers were measured in batch from cryopreserved plasma stored at −80°C: NT-proBNP (N-terminal pro-B-type natriuretic peptide), suppression of tumorigenicity-2 (ST-2), galectin 3 (Gal-3), cystatin C, growth differentiation factor 15 (GDF-15), and soluble fms-like tyrosine kinase-1 (sFLT-1). ST-2, Gal-3, sFLT-1, and GDF-15 were measured by ELISA (R&D Systems, Minneapolis, MN). NT-proBNP was measured by electrochemiluminescence (Roche Diagnostics, Indianapolis, IN). Cystatin C was measured by nephelometry (Siemens, Munich, Germany).

NT-proBNP is released under conditions of myocyte stretch and is used to assess hemodynamic myocardial stress.[5,11,14,15] ST-2, a member of the interleukin-1 receptor family, is also upregulated with myocyte stretch and is a marker of cardiac stress[16,17] as it is induced in myocytes in response to mechanical strain.[5] In addition, ST-2 is a marker for adverse cardiac remodeling and tissue fibrosis.[14] Studies have shown ST-2 to be involved in heart failure[16,18] and increased left ventricular (LV) mass[14,19] as well as to be an initiator of cardiac fibrosis[14,16,20,21] and ventricular remodeling.[16,22,23] Gal-3 is a proinflammatory and profibrotic protein expressed in macrophages, epithelial cells, and endothelial cells.[14,16] Cystatin C is a marker of renal dysfunction,[5,15] as it circulates in bodily fluids and is freely filtered across the glomerular membrane.[11,24] Higher cystatin C has also been correlated with greater cardiovascular mortality, heart failure, and increased LV mass, concentricity, and wall thickness.[11,24] GDF-15 is a marker of apoptosis,[5,15] is elevated in cardiac myocytes in response to inflammation, tissue injury, and pressure overload, and helps to regulate cell differentiation and tissue repair.[5] Higher levels of GDF-15 have been linked to mortality and greater number of cardiovascular events independent of traditional biomarkers and other CVD risk factors.[16,17,25-27] sFLT-1 is an endogenous inhibitor of endothelial growth factors, including vascular endothelial growth factor, which provides vasculature with essential survival and maintenance signals,[28] and placental growth factor, which modulates cardiovascular remodeling.[29] sFLT-1 has been shown to be elevated in patients with heart failure and directly associated with ischemic heart disease and all-cause mortality in patients undergoing dialysis.[28-31]

Additional Study Procedures

For both PLWH and controls, demographics and medical history were obtained using standardized questionnaires and clinical chart review. Current and nadir CD4+ count, time since diagnosis, current ART, and total duration of ART were obtained for PLWH participants by chart review. Height, weight, waist, and hip measurements and blood pressure were measured by trained study staff. Participants in the control cohort were confirmed to be HIV uninfected with a rapid HIV test. Blood was drawn after a 12-hour fast for clinical laboratory analyses performed at the Joint Clinical Research Center, including blood cholesterol levels. Ten-year risk of atherosclerotic CVD was calculated using the pooled-cohort equations[32] for White race, because the Black race equations are not validated for a sub-Saharan African population. We additionally chose not to use the Black race equations because of prior work showing a lower than expected rate of coronary disease in this cohort despite a high burden of risk factors.[2]

Echocardiograms were performed by a trained physician-sonographer using a Philips CX50 and a standardized protocol, as reported previously.[33] Briefly, American Society of Echocardiography guidelines[34,35] were used for 2-dimensional echocardiographic measurements of chamber size, LV mass index, LV systolic function, and LV diastolic function. More specifically, the biplane method of disks was used to assess chamber sizes and LV ejection fraction; however, when the LV endocardial border could not be traced, the LV ejection fraction was estimated to the nearest 5% using all possible views. LV mass index was calculated using the cube formula from linear measurements of the interventricular septum, the LV internal diameter, and the posterior wall at end diastole in the 2-dimensional parasternal long-axis view. The mitral E/A ratio, tissue Doppler of the mitral annulus, tricuspid regurgitation velocity, and left atrial volumes were used to assess diastolic function. Images with poor quality, E-A wave fusion, or other missing wave forms were excluded from the relevant outcome measures. A cardiologist (C.L.H.), blinded to HIV status and clinical variables, performed speckle tracking strain measurements using Image Arena, TomTec 2D Cardiac Performance Analysis version 1.1.3.

Statistical Analysis

Baseline participant characteristics were stratified by HIV and sex and summarized as frequency (percentage) for categorical variables and median (interquartile range) for continuous variables. Statistical comparisons between groups were made using *t*-tests and Wilcoxon rank-sum tests for continuous variables and

χ^2 or Fisher exact tests for categorical variables, as appropriate. All biomarkers were nonnormally distributed and were natural log transformed as necessary for regression models described below.

After describing the baseline characteristics, our primary analyses were guided by the conceptual model of our study (Figure 1). We first examined differences in cardiac biomarkers by sex and HIV status. Initial unadjusted comparisons were made between groups using Wilcoxon rank-sum tests. Then, we constructed multivariable linear regression models adjusted for HIV, sex, age, and traditional cardiac risk factors (systolic blood pressure, diabetes mellitus, smoking, total cholesterol, and body mass index). An HIV*sex interaction term was added to assess whether sex modified the HIV effect. Where a significant interaction was observed at a $P<0.05$, we visually plotted the estimates separately for men versus women.

In a similar manner, the 4 echocardiographic outcomes of interest (LV mass, diastolic dysfunction, LV ejection fraction, and LV global longitudinal strain) were described by sex and HIV status. We have previously examined in detail the HIV effect on these 4 key echocardiographic variables of cardiac structure and function.[33] For this analysis, we made additional multivariable-adjusted sex comparisons using regression models. Multivariable linear regression (LV mass, LV ejection fraction, and LV global longitudinal strain) and logistic regression (diastolic dysfunction) models were adjusted for the same variables as above. Effect modification was tested with the addition of an HIV*sex interaction term.

Finally, we assessed relationships between biomarkers and the echocardiographic measures of cardiac structure and function. Although the relationship may be bidirectional, for the purposes of this analysis, the echocardiographic measures were considered as outcome variables. Unadjusted associations were assessed by linear and logistic regression. Again, using similar multivariable-adjusted models, we separately examined biomarker*HIV and biomarker*sex interactions to assess whether HIV status or sex modified the relationship between biomarkers and echocardiographic outcomes. Because of the large number of significant ($P<0.05$) interactions for LV mass index, we then created separate multivariable-adjusted models with z-standardized estimates of the biomarker effect for men and women. We visually plotted these estimates for men versus women to facilitate interpretation of the data. In addition, because of the higher prevalence of hypertension diagnosis among women versus men despite similar systolic blood pressure, we performed a sensitivity analysis of the biomarker*sex interaction in LV mass index models that were adjusted for hypertension diagnosis instead of systolic blood pressure.

SAS version 9.4 was used for statistical analyses. $P<0.05$ was considered statistically significant, and P values were not corrected for multiple comparisons.

RESULTS

Demographic and Clinical Characteristics Stratified by HIV Status and Sex

The demographic and clinical characteristics of the study population are described in Table 1 by sex and HIV status. Compared with controls, PLWH had less diabetes mellitus, lower body mass index, higher waist/hip ratio, higher high-density lipoprotein, and lower 10-year atherosclerotic CVD risk (all $P\leq0.05$). Women had higher body mass index but had lower waist/hip ratio than men ($P<0.0001$). Hypertension was also more prevalent, low-density lipoprotein was higher, and hemoglobin was lower in women compared with men (all $P\leq0.05$). Among PLWH, women and men had similar HIV disease characteristics, except that women had higher current CD4+ count (median, 593 [interquartile range, 504–718] versus 409 [interquartile range, 358–499] cells/mm^3; $P=0.007$).

Cardiac Biomarkers

Table 2 displays cardiac biomarker levels stratified by HIV status and sex. In unadjusted analyses, ST-2 was higher in men versus women ($P<0.0001$) and GDF-15 was higher in PLWH versus controls ($P<0.0001$). After adjustment for traditional risk factors, women had lower ST-2 ($P=0.0003$). In adjusted models, sex modified the effect of HIV status on 2 biomarkers: (1) cystatin C was lower in men with HIV compared with uninfected men, but similar among women with and without HIV (P for interaction=0.002; Figure 2A); and (2) NT-proBNP was similar among men with and without HIV, but higher in women with HIV compared with uninfected women (P for interaction=0.021; Figure 2B).

LV Structure and Function

In a similar manner, sex and HIV effects were examined for each of the 4 echocardiographic outcomes of interest (Table 3). As we have previously reported for this cohort,[33] PLWH had higher LV mass index ($P=0.03$) and a trend toward worse LV global longitudinal strain ($P=0.07$) compared with controls. In models fully adjusted for traditional risk factors, women had 12 mg/m^2 lower LV mass index ($P=0.02$) and borderline statistically higher odds of diastolic dysfunction (adjusted odds ratio, 2.5; 95% CI, 0.86–7.4; $P=0.09$; Table 4). In fully adjusted models, we did not find evidence of sex modifying the effect of HIV on measures of LV structure and function (all P for interaction >0.1).

Table 1. Characteristics of Study Participants Stratified by Sex and HIV Status

Characteristics	Total Cohort (n=200)	Women		Men		*P* Value: HIV vs Control*	*P* Value: Men vs Women*
		Living With HIV (n=62)	Controls (n=62)	Living With HIV (n=38)	Controls (n=38)		
Demographics							
Age, y	55 (51–60)	55 (51–60)	56 (52–60)	53 (50–61)	55 (49–60)	0.47	0.79
Medical history							
Diabetes mellitus	71 (36)	15 (24)	24 (39)	11 (29)	21 (55)	0.005†	0.13†
Hypertension	170 (85)	57 (92)	56 (90)	32 (84)	25 (66)	0.11†	0.002†
Current smoker	8 (4)	1 (2)	2 (3)	3 (8)	2 (5)	>0.999†	0.26†
CVD risk factors and score							
Body mass index, kg/m^2	29 (25–33)	30 (26–34)	32 (28–34)	25 (22–27)	28 (24–30)	0.02	<0.0001
Waist/hip ratio	0.90 (0.85–0.96)	0.88 (0.84–0.92)	0.86 (0.82–0.89)	0.98 (0.93–1.0)	0.93 (0.90–0.97)	0.001	<0.0001
Systolic BP, mm Hg	152 (138–170)	153 (144–168)	152 (136–175)	152 (136–160)	152 (134–174)	0.40	0.44
Total cholesterol, mg/dL	215 (176–246)	221 (176–257)	217 (185–248)	207 (174–240)	196 (169–230)	0.42	0.41
LDL, mg/dL	138 (110–169)	142 (113–173)	141 (112–174)	123 (94–154)	137 (112–168)	0.91	0.04
HDL, mg/dL	55 (45–65)	60 (49–71)	55 (47–60)	56 (46–62)	49 (40–59)	0.01	0.02
10-y ASCVD risk, %	8 (4–12)	5 (3–8)	6 (4–10)	11 (6–17)	12 (8–20)	0.05	<0.0001‡
Other laboratory tests							
Hemoglobin, g/dL	14 (13–15)	14 (13–15)	14 (13–15)	15 (14–16)	15 (15–16)	0.19	<0.0001
Coronary artery calcium score 0	182 (91)	53 (86)	61 (99)	35 (92)	33 (90)	0.36	0.62
HIV characteristics							
Current CD4+ count		593 (504–718)		409 (358–499)			0.007§
Nadir CD4+ count		141 (75–199)		138 (61–216)			0.82‡,§
HIV duration, y		12 (10–12)		12 (10–13)			0.23‡,§
ART duration, y		11 (9–12)		11 (9–13)			0.24‡,§

Data reported as median (interquartile range) or number (percentage). ART indicates antiretroviral therapy; ASCVD, atherosclerotic CVD; BP. blood pressure; CVD, cardiovascular disease; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

**P* value from independent 2-sample *t* test.

†*P* value from χ^2/Fisher exact test.

‡*P* value from Wilcoxon rank-sum test.

§Comparisons between men and women in HIV+ subjects.

Association of Cardiac Biomarkers With Measures of LV Structure and Function

Of the biomarkers measured, NT-proBNP and GDF-15 were most strongly associated with measures of LV structure and function after adjusting for HIV, sex, age, systolic blood pressure, diabetes mellitus, smoking, cholesterol, and body mass index (Table S1). Both NT-proBNP and GDF-15 were positively associated with LV mass index and diastolic dysfunction, and were negatively associated with LV ejection fraction (all $P \leq 0.05$). In addition, ST-2 and cystatin C were significantly associated with LV mass index ($P \leq 0.01$). sFLT-1 and Gal-3 were not significantly associated with any measures of LV structure and function.

Sex-Specific Relationships Between Biomarkers and LV Mass

In models that tested sex as a modifier of the relationship between biomarkers and measures of LV structure and function, the most striking results were for LV mass index, where 4 of 6 biomarkers had statistically significant sex interactions. As shown in Figure 3, the standardized estimate of the relationship between the biomarker and LV mass index was consistently higher for men than women across all biomarkers measured (*P* for interaction <0.05 for cystatin C, NT-proBNP, Gal-3, and sFLT-1). Similar findings were seen when we adjusted for hypertension diagnosis instead of systolic blood pressure (*P* for interaction <0.05 for

Table 2. Cardiac Biomarkers Stratified by Sex and HIV Status

Biomarker	Total Cohort	Women		Men	
		PLWH	Controls	PLWH	Controls
GDF-15, pg/mL	1086 (687–2645)	1779 (1038–4379)	687 (583–906)	2135 (1176–4201)	828 (592–1353)
sFLT-1, pg/mL	104 (90–118)	104 (87–123)	106 (89–122)	97 (88–108)	106 (94–116)
Gal-3, ng/mL	10.4 (8.5–13.4)	11.0 (8.7–13.6)	10.7 (8.9–11.3)	8.9 (7.5–11.5)	10.4 (8.6–13.7)
NT-proBNP, pg/mL	49 (21–91)	58 (32–126)	48 (18–75)	42 (12–83)	39 (22–86)
ST-2, pg/mL	10796 (8369–14174)	8965 (7555–11658)	9961 (7889–12038)	14468 (10927–16165)	12459 (10123–17498)
Cystatin C, µg/mL	0.78 (0.70–0.87)	0.79 (0.71–0.87)	0.79 (0.69–0.87)	0.72 (0.61–0.81)	0.8 (0.73–0.96)

Data reported as median (interquartile range). Gal-3 indicates galectin-3; GDF-15, growth differentiation factor 15; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PLWH, people living with HIV; sFLT-1, soluble fms-like tyrosine kinase-1; and ST-2, suppression of tumorigenicity-2.

cystatin C, Gal-3, and sFLT-1; *P* for interaction=0.180 for NT-proBNP).

For the 3 other echocardiographic measures (diastolic dysfunction, global longitudinal strain, and ejection fraction), the only other statistically significant sex interactions were for Gal-3 and diastolic dysfunction (biomarker*sex interaction *P*=0.02) and sFLT-1 and ejection fraction (biomarker*sex interaction *P*=0.02).

DISCUSSION

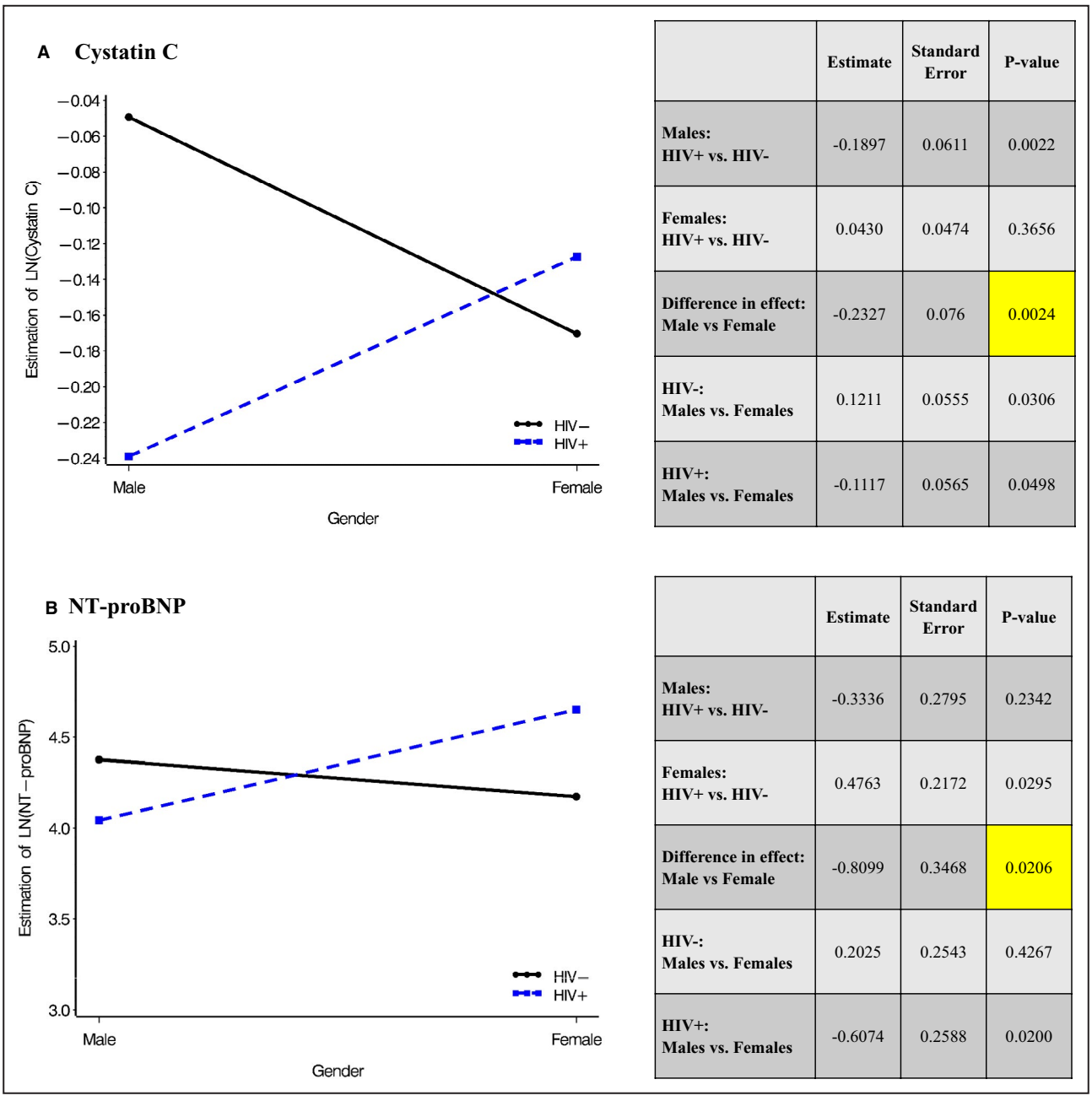
In this innovative study, we examined the influence of sex on HIV-associated heart failure risk markers and subclinical cardiac dysfunction in a sub-Saharan African context. Similar to prior studies from the United States, we describe substantial sex differences in biomarkers of cardiac stress and fibrosis and measures of cardiac structure and function; however, our study additionally focused on sex as a modifier of the HIV effect and the relationship between biomarkers and subclinical cardiac abnormalities. The HIV effects on 2 of the 6 biomarkers were different for women compared with men. Most significantly, the relationship between biomarkers and subclinical alterations in cardiac structure and function, in particular, LV mass index, was stronger in men. Our findings suggest that traditional biomarkers may ultimately prove less effective for predicting outcomes for women with HIV in sub-Saharan Africa, and additional, novel risk markers need to be further explored.

Sex and Subclinical Cardiac Dysfunction in PLWH

CVD remains a significant source of morbidity and mortality in the population with HIV. The ability to identify PLWH who are at increased risk for CVD has significant implications for heart failure prevention strategies and improved outcomes for these patients. Measuring cardiac-specific biomarkers may provide more accurate CVD risk stratification for the population with HIV than traditional risk assessment models because of the inflammation and immune activation associated with HIV.[5] More important, biomarkers differentially associated with LV structure and function in women versus men may open a window into being able to identify "high-risk" individuals with subclinical disease.[36]

In the general population, sex differences in cardiac biomarkers are well described.[14,37] For example, Lau et al studied sex differences in circulating cardiac biomarkers among Framingham Heart Study participants and found that of 71 biomarkers measured, 86% differed significantly between men and women, of which 37 were higher in women and 24 were higher in men.[13]

Despite these known sex differences in cardiac biomarkers, few studies have studied sex differences in cardiac biomarkers among PLWH, primarily because most studies are performed in high-income studies, where men outnumber women with HIV. In an analysis of markers similar to ours, Fitch et al found that high-sensitivity cardiac troponin-T, Gal-3, and ST-2 were significantly higher in participants with HIV versus participants without HIV, and in sex-stratified analysis, the HIV effect seemed to be more pronounced in men compared with women.[14] Although we studied some of the same markers (Gal-3, ST-2, and NT-proBNP), our results did not necessarily reflect a similar trend. For example, we found that men with HIV had lower NT-proBNP than controls but women with HIV had higher NT-proBNP than controls, even after adjustment for potential confounders. This may reflect differences



	Estimate	Standard Error	P-value
Males: HIV+ vs. HIV-	-0.1897	0.0611	0.0022
Females: HIV+ vs. HIV-	0.0430	0.0474	0.3656
Difference in effect: Male vs Female	-0.2327	0.076	0.0024
HIV-: Males vs. Females	0.1211	0.0555	0.0306
HIV+: Males vs. Females	-0.1117	0.0565	0.0498

	Estimate	Standard Error	P-value
Males: HIV+ vs. HIV-	-0.3336	0.2795	0.2342
Females: HIV+ vs. HIV-	0.4763	0.2172	0.0295
Difference in effect: Male vs Female	-0.8099	0.3468	0.0206
HIV-: Males vs. Females	0.2025	0.2543	0.4267
HIV+: Males vs. Females	-0.6074	0.2588	0.0200

Figure 2. Graphical representation of the HIV*sex interaction for cystatin C (A) and NT-proBNP (N-terminal pro-B-type natriuretic peptide) (B).
Highlighted *P* value represents the *P* for interaction in the multivariable linear regression model. All estimates adjusted for age, sex, systolic blood pressure, diabetes mellitus, current smoking status, total cholesterol, and body mass index. LN, natural log.

between US and sub-Saharan African populations, or differences in how we accounted for confounding variables. Although Fitch et al focused on coronary artery disease, we chose to evaluate associations of biomarkers with echocardiographic measures of LV structure and function, because we are particularly interested in the risk of heart failure in women with HIV, which has been well described.[38,39] In sub-Saharan Africa, in particular, it is likely that heart failure may be a more important cardiovascular outcome than atherosclerotic coronary disease, as suggested by our prior study of low rates of detectable coronary calcium in this cohort.[2]

To our knowledge, we are the first to show sex as a modifier of the association between biomarkers and LV mass index, which appears to be elevated in PLWH in African and US cohorts.[33,40] Increases in echocardiographic-derived LV mass index may reflect true myocyte hypertrophy in response to LV pressure overload (ie, chronic hypertension), but may also

Table 3. Measures of LV Structure and Function by Sex and HIV Status

Measure		Women		Men	
	Total Cohort	PLWH	Controls	PLWH	Controls
LV mass index, g/m^2 (n=179)	80 (67 to 97)	77 (67 to 98)	74 (63 to 88)	94 (74 to 110)	74 (64 to 95)
Diastolic dysfunction (yes), % (n=177)	42	55	43	32	30
LV global longitudinal strain, % (n=196)	–16.4 (–18.8 to –14.6)	–16.5 (–18.3 to –15.1)	–18.0 (–19.3 to –14.4)	–15.8 (–17.6 to –13.5)	–16.4 (–18.3 to –15.2)
LV ejection fraction, % (n=194)	67 (60 to 74)	70 (61 to 75)	67 (60 to 74)	65 (59 to 71)	66 (63 to 74)

Data reported as median (interquartile range) for continuous variables or percentage for categorical variables. LV indicates left ventricular; and PLWH, people living with HIV.

represent expansion of intercellular volume, whether from edema or fibrosis, as shown by T1 mapping in cardiac magnetic resonance imaging studies.[41] Of note, increased LV mass index is highly correlated with hypertension, and a high percentage of participants in our study had hypertension. Whether similar HIV effects and sex interactions might be seen in a more metabolically healthy population with less hypertension should be examined in future studies. In our study, biomarkers were less strongly associated with LV mass index in women, suggesting that the biomarkers may also be less strongly associated with downstream clinical heart failure. Future studies such as our cohort and others are also needed to characterize heart failure risk among PLWH in sub-Saharan Africa and should be designed to evaluate sex as a modifier of that risk.

Table 4. Multivariable-Adjusted Effect of Female Sex on Cardiac Biomarkers and Echocardiographic Outcomes

Variable	Adjusted β/OR*	95% CI	*P* Value
Cardiac biomarkers†			
GDF-15	–0.154	(–0.435 to 0.127)	0.2820
sFLT-1	–0.039	(–0.129 to 0.051)	0.3949
Gal-3	–0.020	(–0.171 to 0.132)	0.7963
ST-2	–0.318	(–0.489 to –0.148)	0.0003
LV structure and function			
LV mass index (mg/m^2)	–12.0	(–22.5 to –1.5)	0.0252
Diastolic dysfunction (yes)	OR, 2.5	(0.86 to 7.4)	0.0929
LV global longitudinal strain (%)	–0.81	(–1.97 to 0.36)	0.1749
LV ejection fraction (%)	–1.28	(–5.86 to 3.29)	0.5808

Gal-3 indicates galectin 3; GDF-15, growth differentiation factor 15; LV, left ventricular; OR, odds ratio; sFLT-1, soluble fms-like tyrosine kinase-1; and ST-2, suppression of tumorigenicity-2.

*Estimates for female sex adjusted for HIV status, age, systolic blood pressure, diabetes mellitus, current smoking status, total cholesterol, and body mass index.

†All biomarkers were natural log transformed before analyses. Cystatin C and NT-proBNP (N-terminal pro-B-type natriuretic peptide) are not shown herein because of the presence of a significant HIV*sex interaction, as shown in Figure 2.

HIV Effects on Cardiac Stress and Fibrosis

In addition to knowledge of sex-specific differences, it is important to better understand mechanisms of HIV-associated heart failure risk beyond traditional risk factors in a sub-Saharan African context. With effective ART treatment, the primary phenotype of cardiac dysfunction in PLWH has changed from LV systolic dysfunction to LV diastolic dysfunction.[42] PLWH have higher prevalence of diastolic dysfunction than people without HIV and are afflicted with diastolic dysfunction at a younger age.[42] The exact mechanisms of HIV-associated diastolic dysfunction have yet to be fully elucidated, but evidence suggests that systemic inflammation, myocardial stress, subclinical myocardial necrosis, and myocardial fibrosis and remodeling may play a role.[42] PLWH are more likely than people without HIV to have both focal and diffuse myocardial inflammation and interstitial fibrosis,[41-45] and myocardial fibrosis is associated with impaired myocardial function in PLWH.[45] Myocardial fibrosis has been shown to precede heart failure with preserved ejection fraction in adults without HIV infection.[42,46] Similarly, accelerated myocardial stress and fibrosis may be a major contributing factor to HIV-induced systolic and diastolic dysfunction.[42]

The effect of HIV on biomarkers of inflammation and immune activation and their associations with many markers of CVD risk is well documented, but much less is known about the specific markers of cardiac stress and fibrosis included in our current study. Cardiac biomarker associations with LV structure and function, like those shown in our study, support the hypothesis that myocardial inflammation and fibrosis may help drive subclinical CVD in HIV.[42] Overall, our study found 4 (NT-proBNP, GDF-15, ST-2, and cystatin C) of 6 cardiac biomarkers to be associated with increased LV mass index, more diastolic dysfunction,

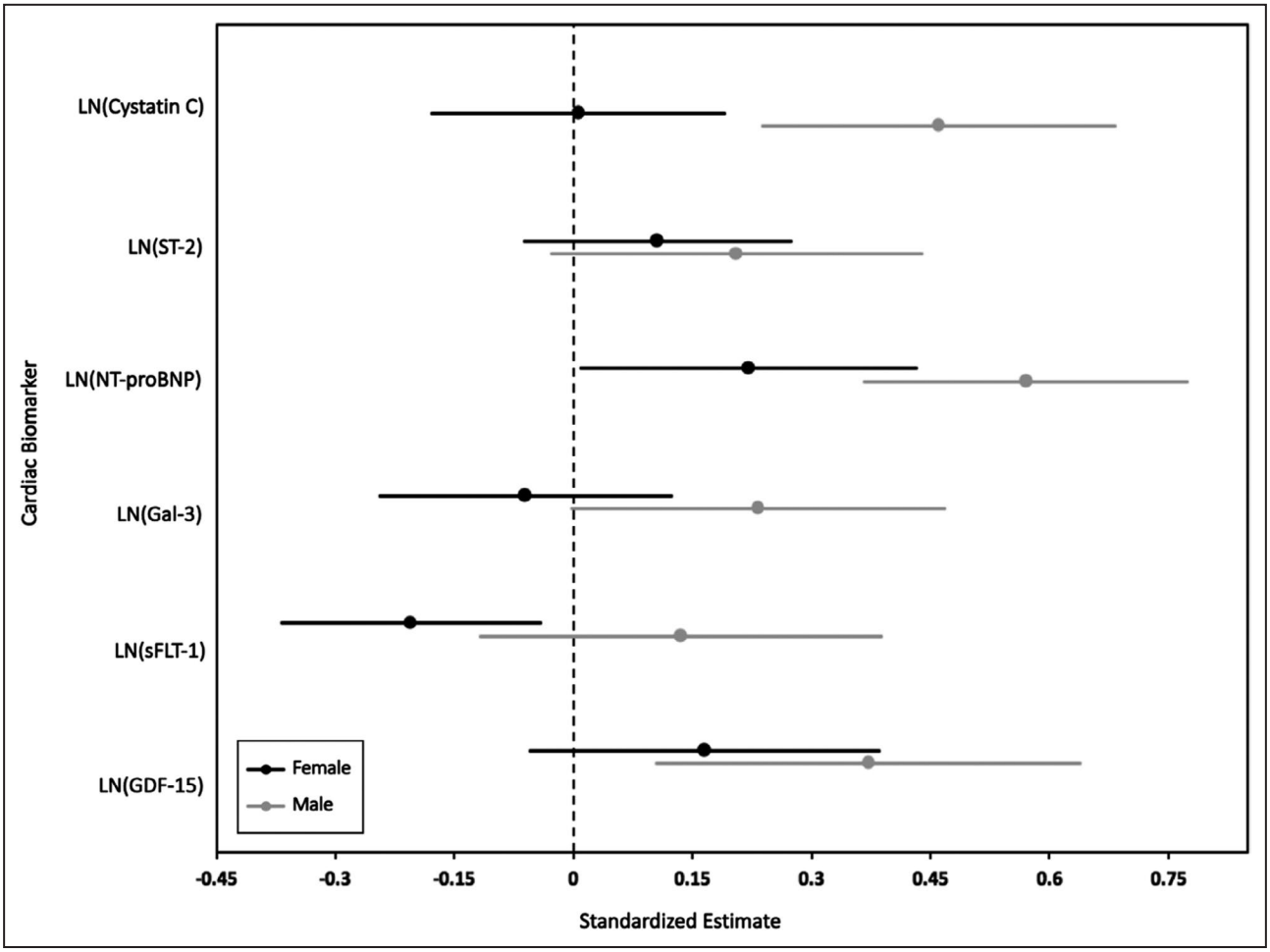


Figure 3. Sex modifies the relationship between cardiac biomarkers and left ventricular (LV) mass index.
The standardized estimate of the association of LV mass index with each of the cardiac biomarkers is plotted separately for men and women. Error bars represent 95% CI. All estimates are adjusted for HIV, age, systolic blood pressure, diabetes mellitus, total cholesterol, and body mass index. Gal-3 indicates galectin 3; GDF-15, growth differentiation factor 15; LN, natural log; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sFLT-1, soluble fms-like tyrosine kinase-1; and ST-2, suppression of tumorigenicity-2.

or both. We found that PLWH had significantly higher levels of GDF-15 compared with controls in a sub-Saharan African cohort that included 60% women, a result that was similarly reported by Secemsky et al in a San Francisco, CA, population.[5] GDF-15 has been shown to be elevated in heart failure and correlate with disease severity in people without HIV[5,47] and is associated with all-cause mortality in PLWH.[5] Soluble ST-2 is an additional biomarker associated with diastolic dysfunction, cardiac fibrosis, and mortality in PLWH. Our study found that ST-2 was significantly associated with LV mass index in a Ugandan population. This result is also corroborated by findings in the Secemsky et al study, which reported that ST-2 was strongly associated with diastolic dysfunction in a US population.[5]

Although the predictive value of cardiac biomarkers requires further investigation, the differential levels of biomarkers related to myocardial stress and fibrosis in PLWH versus controls and in association with measures of LV structure and function reported in our study and others[5,14,15,48] may help to elucidate pathophysiologic mechanisms of CVD in PLWH.

Strengths and Limitations

The sub-Saharan African setting and inclusion of a large proportion of women are significant strengths of our study. Limitations of our study include the cross-sectional design, which permits examination of associations but not establishment of causality. It is possible that observed associations (or, in some cases, lack of association) between biomarkers and outcomes are attributable to residual or unmeasured confounders. Our study was designed to focus on PLWH who are being treated with ART. Because our study did not include a group of untreated PLWH, our results are not generalizable to an untreated population. We additionally cannot assess to what degree associations seen in our study are attributable to

residual effects of the virus versus ART effects and how these effects may differ in women versus men. However, in the era of widespread availability of ART, the more relevant clinical question is whether there are sex differences in cardiovascular risk among ART-treated PLWH. Our population was older, with high rates of diabetes mellitus; thus, our results may not be applicable to younger populations with lower rates of diabetes mellitus. In addition, most participants in our study were hypertensive, with fewer of the male control participants having hypertension. Our results may not be generalizable to populations without hypertension. Finally, we acknowledge that further studies in larger and diverse populations are needed to bolster the external validity of our findings.

CONCLUSIONS

We demonstrate differences in biomarkers of cardiac fibrosis and stress by sex and HIV status in Uganda. In addition, sex may modify the relationships between biomarkers and LV structure and function. Future studies should examine the predictive value of these differentially expressed cardiac biomarkers for cardiovascular events in this population.

ARTICLE INFORMATION

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Supplementary Material

Table S1

REFERENCES

1. Feinstein MJ, Hsue PY, Benjamin LA, Bloomfield GS, Currier JS, Freiberg MS, Grinspoon SK, Levin J, Longenecker CT, Post WS. Characteristics, prevention, and management of cardiovascular disease in people living with HIV: a scientific statement from the American Heart Association. *Circulation*. 2019;140:e98–e124. DOI: 10.1161/CIR.0000000000000695.
2. Alencherry B, Erem G, Mirembe G, Ssinabulya I, Yun C-H, Hung C-L, Siedner MJ, Bittencourt M, Kityo C, McComsey GA, et al. Coronary artery calcium, HIV and inflammation in Uganda compared with the USA. *Open Heart*. 2019;6:e001046. DOI: 10.1136/openhrt-2019-001046.
3. Gersh BJ, Sliwa K, Mayosi BM, Yusuf S. Novel therapeutic concepts: the epidemic of cardiovascular disease in the developing world: global implications. *Eur Heart J*. 2010;31:642–648. DOI: 10.1093/eurheartj/ehq030.
4. Shah ASV, Stelzle D, Lee KK, Beck EJ, Alam S, Clifford S, Longenecker CT, Strachan F, Bagchi S, Whiteley W, et al. Global burden of atherosclerotic cardiovascular disease in people living with HIV. *Circulation*. 2018;138:1100–1112. DOI: 10.1161/CIRCULATIONAHA.117.033369.
5. Secemsky EA, Scherzer R, Nitta E, Wu AH, Lange DC, Deeks SG, Martin JN, Snider J, Ganz P, Hsue PY. Novel biomarkers of cardiac stress, cardiovascular dysfunction, and outcomes in HIV-infected individuals. *JACC Heart Fail*. 2015;3:591–599. DOI: 10.1016/j.jchf.2015.03.007.
6. Stein JH, Hsue PY. Inflammation, immune activation, and CVD risk in individuals with HIV infection. *JAMA*. 2012;308:405–406. DOI: 10.1001/jama.2012.8488.
7. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, Ledergerber B, Lundgren J, et al. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. *PLoS One*. 2012;7:e44454. DOI: 10.1371/journal.pone.0044454.
8. Sinha A, Feinstein M. Epidemiology, pathophysiology, and prevention of heart failure in people with HIV. *Prog Cardiovasc Dis*. 2020;63:134–141. DOI: 10.1016/j.pcad.2020.01.002.
9. Abelman RA, Mugo BM, Zanni MV. Conceptualizing the risks of coronary heart disease and heart failure among people aging with HIV: sex-specific considerations. *Curr Treat Options Cardiovasc Med*. 2019;21:41. DOI: 10.1007/s11936-019-0744-1.
10. Centers for Disease Control and Prevention. HIV and Women: HIV Incidence. https://www.cdc.gov/hiv/group/gender/women/incidence.html. Published 2019. Accessed July 7, 2020.
11. Lew J, Sanghavi M, Ayers CR, McGuire DK, Omland T, Atzler D, Gore MO, Neeland I, Berry JD, Khera A, et al. Sex-based differences in cardiometabolic biomarkers. *Circulation*. 2017;135:544–555. DOI: 10.1161/CIRCULATIONAHA.116.023005.
12. Gijsberts CM, Gohar A, Ellenbroek GHJM, Hoefer IE, de Kleijn DPV, Asselbergs FW, Nathoe HM, Agostoni P, Rittersma SZH, Pasterkamp G, et al. Severity of stable coronary artery disease and its biomarkers differ between men and women undergoing angiography. *Atherosclerosis*. 2015;241:234–240. DOI: 10.1016/j.atherosclerosis.2015.02.002.
13. Lau ES, Paniagua SM, Guseh JS, Bhambhani V, Zanni MV, Courchesne P, Lyass A, Larson MG, Levy D, Ho JE. Sex differences in circulating biomarkers of cardiovascular disease. *J Am Coll Cardiol*. 2019;74:1543–1553. DOI: 10.1016/j.jacc.2019.06.077.
14. Fitch KV, DeFilippi C, Christenson R, Srinivasa S, Lee H, Lo J, Lu MT, Wong K, Petrow E, Sanchez L, et al. Subclinical myocyte injury, fibrosis and strain in relationship to coronary plaque in asymptomatic HIV-infected individuals. *AIDS*. 2016;30:2205–2214. DOI: 10.1097/QAD.0000000000001186.
15. Scherzer R, Shah SJ, Secemsky E, Butler J, Grunfeld C, Shlipak MG, Hsue PY. Association of biomarker clusters with cardiac phenotypes and mortality in patients with HIV infection. *Circ Heart Fail*. 2018;11:e004312. DOI: 10.1161/CIRCHEARTFAILURE.117.004312.
16. Tuegel C, Katz R, Alam M, Bhat Z, Bellovich K, de Boer I, Brosius F, Gadegbeku C, Gipson D, Hawkins J, et al. GDF-15, galectin 3, soluble ST2, and risk of mortality and cardiovascular events in CKD. *Am J Kidney Dis*. 2018;72:519–528. DOI: 10.1053/j.ajkd.2018.03.025.
17. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, Ho JE, Fradley MG, Ghorbani A, Xanthakis V, et al. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation*. 2012;126:1596–1604. DOI: 10.1161/CIRCULATIONAHA.112.129437.
18. Maiolino G, Rossitto G, Pedon L, Cesari M, Frigo AC, Azzolini M, Plebani M, Rossi GP. Galectin-3 predicts long-term cardiovascular death in high-risk patients with coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2015;35:725–732. DOI: 10.1161/ATVBAHA.114.304964.
19. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG, Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident

heart failure in the community. *J Am Coll Cardiol*. 2012;60:1249–1256. DOI: 10.1016/j.jacc.2012.04.053.
20. MacKinnon AC, Liu X, Hadoke PW, Miller MR, Newby DE, Sethi T. Inhibition of galectin-3 reduces atherosclerosis in apolipoprotein e-deficient mice. *Glycobiology*. 2013;23:654–663. DOI: 10.1093/glycob/cwt006.
21. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JPM, Schroen B, André S, Crijns HJGM, Gabius H-J, Maessen J, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation*. 2004;110:3121–3128. DOI: 10.1161/01.CIR.0000147181.65298.4D.
22. Calvier L, Miana M, Reboul P, Cachofeiro V, Martinez-Martinez E, de Boer RA, Poirier F, Lacolley P, Zannad F, Rossignol P, et al. Galectin-3 mediates aldosterone-induced vascular fibrosis. *Arterioscler Thromb Vasc Biol*. 2013;33:67–75. DOI: 10.1161/ATVBAHA.112.300569.
23. Vergaro G, Prud'homme M, Fazal L, Merval R, Passino C, Emdin M, Samuel JL, Cohen Solal A, Delcayre C. Inhibition of galectin-3 pathway prevents isoproterenol-induced left ventricular dysfunction and fibrosis in mice. *Hypertension*. 2016;67:606–612. DOI: 10.1161/HYPERTENSIONAHA.115.06161.
24. Patel PC, Ayers CR, Murphy SA, Peshock R, Khera A, de Lemos JA, Balko JA, Gupta S, Mammen PPA, Drazner MH, et al. Association of cystatin c with left ventricular structure and function: the Dallas Heart Study. *Circ Heart Fail*. 2009;2:98–104. DOI: 10.1161/CIRCHEARTFAILURE.108.807271.
25. Rohatgi A, Patel P, Das SR, Ayers CR, Khera A, Martinez-Rumayor A, Berry JD, McGuire DK, de Lemos JA. Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study. *Clin Chem*. 2012;58:172–182. DOI: 10.1373/clinchem.2011.171926.
26. Kempf T, Horn-Wichmann R, Brabant G, Peter T, Allhoff T, Klein G, Drexler H, Johnston N, Wallentin L, Wollert KC. Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. *Clin Chem*. 2007;53:284–291. DOI: 10.1373/clinchem.2006.076828.
27. Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin Chem*. 2017;63:140–151. DOI: 10.1373/clinchem.2016.255174.
28. Di Marco GS, Kentrup D, Reuter S, Mayer AB, Golle L, Tiemann K, Fobker M, Engelbertz C, Breithardt G, Brand E, et al. Soluble flt-1 links microvascular disease with heart failure in CKD. *Basic Res Cardiol*. 2015;110:30. DOI: 10.1007/s00395-015-0487-4.
29. Gruson D, Hermans MP, Ferracin B, Ahn SA, Rousseau MF. Sflt-1 in heart failure: relation with disease severity and biomarkers. *Scand J Clin Lab Invest*. 2016;76:411–416. DOI: 10.1080/00365513.2016.1190863.
30. Guo Q, Carrero JJ, Yu X, Barany P, Qureshi AR, Eriksson M, Anderstam B, Chmielewski M, Heimburger O, Stenvinkel P, et al. Associations of VEGF and its receptors sVEGFr-1 and -2 with cardiovascular disease and survival in prevalent haemodialysis patients. *Nephrol Dial Transplant*. 2009;24:3468–3473. DOI: 10.1093/ndt/gfp315.
31. Ky B, French B, Ruparel K, Sweitzer NK, Fang JC, Levy WC, Sawyer DB, Cappola TP. The vascular marker soluble fms-like tyrosine kinase 1 is associated with disease severity and adverse outcomes in chronic heart failure. *J Am Coll Cardiol*. 2011;58:386–394. DOI: 10.1016/j.jacc.2011.03.032.
32. Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, Greenland P, Lackland DT, Levy D, O'Donnell CJ, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association task force on practice guidelines. *Circulation*. 2014;129:S49–S73. DOI: 10.1161/01.cir.0000437741.48606.98.
33. Buggey J, Yun L, Hung C-L, Kityo C, Mirembe G, Erem G, Truong T, Ssinabulya I, Tang WHW, Hoit BD, et al. HIV and pericardial fat are associated with abnormal cardiac structure and function among Ugandans. *Heart*. 2020;106:147–153. DOI: 10.1136/heartjnl-2019-315346.
34. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, Flachskampf FA, Gillebert TC, Klein AL, Lancellotti P, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. 2016;29:277–314. DOI: 10.1016/j.echo.2016.01.011.
35. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. 2015;28:e14. DOI: 10.1016/j.echo.2014.10.003.
36. Zanni MV, Awadalla M, Toribio M, Robinson J, Stone LA, Cagliero D, Rokicki A, Mulligan CP, Ho JE, Neilan AM, et al. Immune correlates of diffuse myocardial fibrosis and diastolic dysfunction among aging women with human immunodeficiency virus. *J Infect Dis*. 2020;221:1315–1320.
37. Daniels LB, Maisel AS. Cardiovascular biomarkers and sex: the case for women. *Nat Rev Cardiol*. 2015;12:588–596. DOI: 10.1038/nrcardio.2015.105.
38. Janjua SA, Triant VA, Addison D, Szilveszter B, Regan S, Staziaki PV, Grinspoon SA, Hoffmann U, Zanni MV, Neilan TG. HIV infection and heart failure outcomes in women. *J Am Coll Cardiol*. 2017;69:107–108. DOI: 10.1016/j.jacc.2016.11.013.
39. Womack JA, Chang C-C, So-Armah KA, Alcorn C, Baker JV, Brown ST, Budoff M, Butt AA, Gibert C, Goetz MB, et al. HIV infection and cardiovascular disease in women. *J Am Heart Assoc*. 2014;3:e001035. DOI: 10.1161/JAHA.114.001035.
40. Hsue PY, Hunt PW, Ho JE, Farah HH, Schnell A, Hoh R, Martin JN, Deeks SG, Bolger AF. Impact of HIV infection on diastolic function and left ventricular mass. *Circ Heart Fail*. 2010;3:132–139. DOI: 10.1161/CIRCHEARTFAILURE.109.854943.
41. Ntusi N, O'Dwyer E, Dorrell L, Wainwright E, Piechnik S, Clutton G, Hancock G, Ferreira V, Cox P, Badri M, et al. HIV-1-related cardiovascular disease is associated with chronic inflammation, frequent pericardial effusions, and probable myocardial edema. *Circ Cardiovasc Imaging*. 2016;9:e004430. DOI: 10.1161/CIRCIMAGING.115.004430.
42. Butler J, Kalogeropoulos AP, Anstrom KJ, Hsue PY, Kim RJ, Scherzer R, Shah SJ, Shah SH, Velazquez EJ, Hernandez AF, et al. Diastolic dysfunction in individuals with human immunodeficiency virus infection: literature review, rationale and design of the characterizing heart function on antiretroviral therapy (CHART) study. *J Card Fail*. 2018;24:255–265. DOI: 10.1016/j.cardfail.2018.02.001.
43. Luetkens JA, Doerner J, Schwarze-Zander C, Wasmuth JC, Boesecke C, Sprinkart AM, Schmeel FC, Homsi R, Gieseke J, Schild HH, et al. Cardiac magnetic resonance reveals signs of subclinical myocardial inflammation in asymptomatic HIV-infected patients. *Circ Cardiovasc Imaging*. 2016;9:e004091. DOI: 10.1161/CIRCIMAGING.115.004091.
44. Holloway CJ, Ntusi N, Suttie J, Mahmod M, Wainwright E, Clutton G, Hancock G, Beak P, Tajar A, Piechnik SK, et al. Comprehensive cardiac magnetic resonance imaging and spectroscopy reveal a high burden of myocardial disease in HIV patients. *Circulation*. 2013;128:814–822. DOI: 10.1161/CIRCULATIONAHA.113.001719.
45. Thiara DK, Liu CY, Raman F, Mangat S, Purdy JB, Duarte HA, Schmidt N, Hur J, Sibley CT, Bluemke DA, et al. Abnormal myocardial function is related to myocardial steatosis and diffuse myocardial fibrosis in HIV-infected adults. *J Infect Dis*. 2015;212:1544–1551. DOI: 10.1093/infdis/jiv274.
46. Schelbert EB, Fridman Y, Wong TC, Abu Daya H, Piehler KM, Kadakkal A, Miller CA, Ugander M, Maanja M, Kellman P, et al. Temporal relation between myocardial fibrosis and heart failure with preserved ejection fraction: association with baseline disease severity and subsequent outcome. *JAMA Cardiol*. 2017;2:995–1006. DOI: 10.1001/jamacardio.2017.2511.
47. Kempf T, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W, Ponikowski P, Filippatos GS, Rozentryt P, Drexler H, et al. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol*. 2007;50:1054–1060. DOI: 10.1016/j.jacc.2007.04.091.
48. Hanna DB, Lin J, Post WS, Hodis HN, Xue X, Anastos K, Cohen MH, Gange SJ, Haberlen SA, Heath SL, et al. Association of macrophage inflammation biomarkers with progression of subclinical carotid artery atherosclerosis in HIV-infected women and men. *J Infect Dis*. 2017;215:1352–1361. DOI: 10.1093/infdis/jix082.

SUPPLEMENTAL MATERIAL

Table S1. Relationship between cardiac biomarkers and measures of LV structure and function.

	LV Mass Index	Diastolic Dysfunction (Yes)	Global Longitudinal Strain	LV Ejection Fraction
LN (GDF-15)				
β or OR	8.47	1.90	-0.47	-2.43
95% CI	(3.33, 13.61)	(1.07, 3.36)	(-1.06, 0.13)	(-4.80, -0.05)
p-value	0.0014	0.03	0.13	0.05
LN (sFLT-1)				
β or OR	-7.24	0.36	1.24	-0.46
95% CI	(23.77, 9.29)	(0.06, 2.16)	(-0.61, 3.09)	(-7.83, 6.90)
p-value	0.39	0.26	0.19	0.90
LN (Gal-3)				
β or OR	7.56	0.52	-0.59	1.16
95% CI	(-2.04, 17.16)	(0.18, 1.47)	(-1.70, 0.53)	(-3.21, 5.53)
p-value	0.12	0.22	0.30	0.60
LN (NT-proBNP)				
β or OR	8.80	1.61	-0.20	-2.49
95% CI	(6.13, 11.47)	(1.31, 2.29)	(-0.53, 0.14)	(-3.77, -1.22)
p-value	<.0001	0.0086	0.25	0.0002
LN (ST-2)				
β or OR	11.17	1.54	0.001	-1.22
95% CI	(2.57, 19.77)	(0.62, 3.82)	(-0.99, 0.99)	(-5.13, 2.68)
p-value	0.01	0.36	0.999	0.54
LN (Cystatin C)				
β or OR	21.59	1.72	0.36	-0.98
95% CI	(8.91, 34.27)	(0.42, 7.06)	(-1.16, 1.88)	(-6.90, 4.95)
p-value	0.001	0.45	0.64	0.75

Multivariable linear regression models adjusted for HIV, sex, age, systolic blood pressure, diabetes, smoking, cholesterol, and body mass index. LN, natural log; OR, odds ratio; GDF-15, growth differentiation factor; sFLT-1, soluble fms-like tyrosine kinase-1; Gal-3, galectin-3; NT-proBNP, N-terminal pro-B-type natriuretic peptide; ST-2, suppression of tumourigenicity-2.