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Inflammation, Immune Activation, Immunosenescence, and Hormonal Biomarkers in the Frailty-Related Phenotype of Men with or at Risk for HIV

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Conflicts of Interest:

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Abstract

Background

The extent to which inflammation, immune activation/immunosenescence, and hormonal abnormalities are driven by HIV or frailty is not clear.

Methods

HIV-infected frail men (n=155) were matched to non-frail, HIV-infected (n=141) and HIV-uninfected (n=150) men by age, calendar year, and antiretroviral therapy (ART) use (HIV only). Frailty was defined by ≥3 frailty-related phenotype criteria (weight loss, exhaustion, low activity, slowness) at ≥2 visits, or at 1 visit with ≥1 criteria at ≥2 visits. IL-6, hs-CRP, sTNFR-1 and 2, DHEA-S, free testosterone, HOMA-IR, IGF-1, and %CD4+CD28-, %CD8+CD28-, %CD4+CD38+DR+, %CD8+CD38+DR+ T-cells were measured. Log-linear regressions were adjusted for *a priori* selected covariates to determine differences by frailty and HIV status.

Results

In multivariate analyses adjusted for covariates, among HIV-infected men, frailty was associated with higher IL-6 and hs-CRP and lower free testosterone and DHEA. In contrast, HIV infection but not frailty was associated with significantly greater immune senescence (% CD4+28- or CD8+28- T-cells) and immune activation (% CD4+CD38+HLA-DR+ and CD8+CD38+HLA-DR+).

Conclusions

Frailty among HIV-infected men was associated with increased inflammation and lower hormone levels, independent of co-morbid conditions. Interventions targeting these pathways should be evaluated to determine the impact on prevention or reversal of frailty among HIV-infected men.

Introduction

Frailty is a syndrome characterized by an increased vulnerability to stressors in the face of limited physiologic reserve [1]. A frailty phenotype was defined by Fried et al. as impairment in at least three of five domains: physical slowness, fatigue, low activity, weakness, and physical shrinking [2]. Other authors have used a model of an accumulation of deficits to define frailty, or have modified the original Fried phenotype using a combination of objective and subjective measures of health to fit the population or available data [3]. Regardless of the specific definition, frailty has been associated with increased healthcare costs and utilization, poor outcomes following surgical procedures, increased need for skilled nursing care among other outcomes, and ultimately, an increased risk of death [4].

A similarity between the syndrome of frailty and AIDS was recognized early in the HIV era [5]. The occurrence of a frailty-related phenotype was later reported in the Multicenter AIDS Cohort Study (MACS), where the greatest proportion of the frailty-related phenotype was among persons with low CD4+ T lymphocyte counts (CD4) or those not receiving antiretroviral therapy (ART) [6, 7]. A subsequent MACS study demonstrated that the frailty-related phenotype was associated with lower AIDS-free and overall survival, even among persons who achieved HIV-1 virologic suppression [8]. A frailty index developed from self-reported data in the Veterans Aging Cohort Study (VACS) was likewise predictive of hospitalization and mortality [9]. These phenotypes are different than the original Fried frailty definition in that they include subjective measures only and lack extensive validation; however, their association with mortality suggests that both are valuable measures of vulnerability to stressors.

Both frailty and HIV infection are associated with heightened inflammation, hormonal abnormalities, and impairments in the immune system [10, 11]. The extent to which these factors are driven by HIV or frailty, particularly among virologically suppressed HIV-infected individuals, is less clear. A better understanding of the underlying mechanisms that drive frailty can inform the most appropriate and efficient pathways to target for prevention and treatment of frailty. The goal of this study was to describe differences in levels of inflammatory, immune, and hormonal markers by frailty status among persons with HIV infection (i.e., comparing HIV-infected men with and without frailty) and by HIV serostatus without the influence of frailty (i.e., comparing non-frail HIV-infected and HIV-uninfected men). We hypothesized that both frailty and HIV infection would be associated with abnormalities across all three domains.

Methods

Study population

The MACS is a prospective study of the natural and treated history of HIV infection among men who have sex with men in the United States with sites in Baltimore, MD-Washington, DC; Chicago, IL; Pittsburgh, PA; and Los Angeles, CA. Enrollment of 6972 participants occurred during three time periods: 1984-1985, 1987-1990, and 2001-2003. At each semi-annual study visit, physical exams were performed, questionnaires administered, and blood was drawn for laboratory testing and storage. Self-reported use of antiretroviral medications was summarized at each visit to define prior and current use of ART. Informed consent was obtained from each participant, and approval was provided by each local institutional review board.

Selection of cases and controls, by exposure status

This analysis employed a matched study design of HIV-infected men without AIDS (defined as no history of an AIDS-defining illness[12]) and HIV-uninfected men in the MACS. The exposure of interest was frailty, defined as follows. First, the frailty-related phenotype was summarized by four self-reported subjective measures [6] describing weight loss, exhaustion, low activity levels and slowness. Weight loss was defined as self-reported unintentional weight loss of 10 pounds or more since the previous visit. Other criteria were derived from the Short-Form (SF)-36: exhaustion was defined as answering yes to the question: "During the past 4 weeks, as a result of your physical health, have you had difficulty performing your work or other activities (for example, it took extra effort)?" Low activity was defined as feeling "limited a lot" in response to: "Does your health now limit you in vigorous activities, such as running, lifting heavy objects, participating in strenuous sports?" Slowness was defined as feeling "limited a lot" in response to: "Does your health now limit you in walking several blocks?" HIV-infected men were considered frail if they had either: a) two or more visits meeting at least 3 frailty-related phenotype criteria or b) one visit meeting ≥3 criteria and two subsequent visits meeting 1-2 criteria. The index visit (i.e., study entry) for each case was the first frail visit. HIV-infected and -uninfected controls with no history of meeting any frailty-related phenotype criteria were matched to each case by age within 3 years and calendar year of visit. Among HIV-infected participants, controls were additionally matched at the index visit by the reported use of HIV therapy, categorized as highly-active ART (HAART), non-HAART therapy, or no ART. The definition of HAART was guided by the Department of Health and Human Services guidelines [13].

Health status and clinical characteristics

Confounders considered for inclusion were taken from data collected prior to the index visit and included: smoking status (never, former or current), hepatitis C virus infection (detectable hepatitis C RNA in serum), depressive symptoms (Center for Epidemiologic Studies Depression score of > 16)[14], diabetes, dyslipidemia, and hypertension. HIV-related variables included nadir CD4 (prior to index visit), CD4 at the index visit, detectable plasma HIV-1 RNA (viral load) at the index visit (based on the limit of detection of the method in use at that time), ART type (no therapy, non-HAART, or HAART), and ART exposure [15]. CD4 cell counts were measured with standardized flow cytometry [16, 17]. 77% had viral load determined by the Roche ultra-sensitive 2nd generation assay (limit of detection < 50 copies/ml); the remainder were tested by the Roche Cobas TaqMan (limit of detection < 20 copies/ml). Testosterone therapy use was self-reported and available for HIV-infected men only.

Laboratory analyses for outcome variables

Plasma, serum, and peripheral blood mononuclear cells (PBMC) markers were assessed at the index visit for biomarkers selected within three outcome domains (inflammation, hormone, and immune activation/senescence). Inflammation biomarkers were measured from stored serum samples and included: high-sensitivity C reactive protein (hs-CRP), interleukin (IL)-6, and the soluble receptors for TNF-alpha (sTNFRI and sTNFRII). Markers were measured in duplicate using commercially available enzyme-labeled immunosorbent sandwich assays (ALPCO Diagnostics, Windham, NH) and values were averaged for analysis [18]. Insulin concentrations were measured using radioimmunoassay (Linco Research, St. Charles, MO). Dehydroepiandrosterone sulfate (DHEA-S), free testosterone, and insulin-like growth factor 1 (IGF-1) were measured from cryopreserved samples by enzyme immunoassay for DHEA-S

(DRG International Inc, Springfield, NJ), immunoenzymometric assay for IGF-1 (Immunodiagnostics Systems Inc, Fountain Hills, AZ), liquid chromatographic-tandem mass spectrometry (LC-MS/MS) for total serum testosterone (all measured in Bhasin Laboratory, Boston University, Boston, MA). Sex hormone binding globulin was measured using radioimmunoassay and free testosterone was then calculated from total testosterone and sex hormone binding globulin measurement using the Vermeulen equation [19]. Fasting glucose and insulin have been measured at each semiannual MACS visit since 1999, processed at a central laboratory (Heinz Laboratory, Pittsburgh, PA), and were used for calculation of the homeostatic model assessment of insulin resistance (HOMA-IR) [20].

For measurement of markers of immune activation and senescence by flow cytometry, frozen PBMCs were thawed and stained with anti-CD3 APC-H7, anti-CD8 Pacific Blue, anti-CD38 PE, anti-HLA-DR FITC, and anti-CD28 APC monoclonal antibodies (BD Biosciences, San Jose, CA) and LIVE/DEAD Fixable Aqua Dead Cell Stain (Life Technologies, Carlsbad, CA) for 20 minutes at room temperature. The PBMC were then washed twice with FACS buffer (1% BSA in PBS), resuspended in 500 µL FACS Lysing Solution (BD Biosciences), and analyzed on a FACS Canto II flow cytometer (BD, San Jose, CA) using FACSDiva software (BD San Jose, CA). Data from at least 10,000 viable $CD8^+$ cells were collected per tube. The percentages of cells expressing CD38, HLA-DR, and CD28 among $CD8^+$ and CD8- T cells (the latter reflecting primarily, but not entirely, $CD4^+$ T cells) were determined using fluorescence-minus-one (FMO) controls.

Statistical analyses

Fisher's exact test and the non-parametric Kruskal-Wallis test were used for univariate comparisons of clinical characteristics and outcomes for categorical and continuous variables,

respectively. Linear regression models were used to describe differences by exposure group. To account for leftward skewness of the biomarker distributions, these values were log transformed. Two models were fit for each biomarker: one model was restricted to HIV-infected men with the exposure being frailty; the other model was restricted to non-frail men with the exposure being HIV infection. Models were of the form: log(biomarker) = A_0 + A_1 x Exposure + A_z x Z + e, where *e* represents residuals that were normally distributed with mean 0 and variance sigma-squared and *Z* represents a vector of confounders. The difference was described in terms of percent and calculated as [exp(A_1)-1] x 100. Confounders identified *a priori* were age, race (black vs. non-black), body mass index (BMI) in the log scale, obesity (BMI ≥ 30 kg/m^2), smoking status, hepatitis C infection, diabetes, dyslipidemia, hypertension and depressive symptoms for all models. For the models in which the effect of frailty was assessed among HIV-infected men, additional HIV-related variables included low CD4 nadir (< 350 cells/μL), low current CD4 cell count (< 350 cells/μL), detectable HIV-1 viral load, and type of ART (no ART, non-HAART, or HAART). An alpha level of 0.05 was considered significant.

To maximize the use of available data, minimize the effect of outlying values, and reduce the amount of missing data, individual variables were summarized from observations collected temporally close to the index visit. Specifically, the summary of 3 visits within 1 year prior to and including the index visit was used. If 3 visits were not available in this time interval, the first visit no more than 6 months after the index visit was used to fill in the missing data. For continuous variables, the mean level from up to 3 visits prior to and including the visit were used. For categorical variables, the presence of each condition was defined by at least two occurrences in the three visits prior to and including the index visit.

Results

Of 4005 potentially eligible participants seen between 1994 and 2010, a total of 155 HIV-infected men met our frailty criteria: 101 (65%) had ≥ 2 visits with ≥3 frailty-related phenotype criteria; 54 (35%) had 1 visit with ≥3 frailty-related phenotype criteria and ≥2 visits with 1-2 criteria. HIV-infected frail men were matched to 141 non-frail HIV-infected and 150 non-frail HIV-uninfected men. The median ages were between 47 and 49 years, and the median year of the index visits was 2006 (Table 1).

Characteristics of the study population are detailed in Table 1. Groups were significantly different in many characteristics, for example: frail HIV-infected men had a much higher prevalence of depressive symptoms by CES-D (54.8%) compared to non-frail HIV-infected and -uninfected men (5.0% vs. 4.7%, respectively, Table 1). Among HIV-infected men, frail men had a similar proportion of low nadir CD4 cell count, but a higher proportion of current CD4 cell count <350 cells/μL and detectable HIV-1 viral load compared to HIV-infected non-frail men. Similar proportions of frail and non-frail HIV-infected participants were on HAART (74.8% vs. 72.3%, respectively). Frail men were more likely to report testosterone therapy use (13.8% vs. 5.2%), although 16% (n=25) and 18% (n=26) of frail and non-frail HIV-infected were missing self-reported testosterone use (data not collected for HIV-uninfected men).

Differences in markers of inflammation, immune activation and senescence, and hormonal dysfunction between HIV-uninfected men, HIV-infected non-frail, and HIV-infected frail men are shown in Table 2 (unadjusted). Levels of inflammatory markers (IL-6, hsCRP, sTNFR1, sTNFR2) were highest among frail, HIV-infected men and lowest among non-frail, HIV-uninfected men. Markers of immune activation (%CD38+HLA-DR+ expression on CD4+ or

CD8+ T-cells) and senescence (% of CD28- CD4+ or CD8+ T-cells) were similar among HIV-infected frail and HIV-infected, non-frail men, and were significantly lower among HIV-uninfected men (all p <0.001). Frail, HIV-infected men had significantly lower levels of DHEA-S (p<0.001), free testosterone (p=0.045), and IGF-1 (p<0.001), and a trend towards worsening insulin resistance (HOMA-IR; p=0.057) compared to non-frail, HIV-infected men. In contrast, only insulin resistance was worse by HIV status, with significantly greater HOMA-IR (p<0.001) among the non-frail, HIV-infected vs -uninfected men.

Adjusted differences between the groups in markers of inflammation, immune activation and senescence, and hormone biomarkers are shown in Figures 1-3, respectively. Among HIV-infected men, frailty was associated with 52% higher IL-6 (p<0.001) and 69% higher hs-CRP concentrations (p<0.001; Figure 1a). Only sTNFR2 was significantly greater among HIV-infected versus uninfected, non-frail men (22%, p<0.001; Figure 1b). Cellular markers of immune activation or senescence did not differ significantly by frailty status among HIV-infected men (Figure 2a). In contrast, HIV-infected men had significantly higher percentages of T-cells expressing CD38+HLA-DR+ and CD28- compared to HIV-uninfected men (Figure 2b). Among HIV-infected men, the presence of frailty was significantly associated with lower free testosterone (17%, p= 0.020) and lower DHEA-S (18%, p= 0.035), with a trend towards worsened insulin resistance (20%; p=0.051; Figure 3a). In contrast, among non-frail men, HIV infection was significantly associated with greater insulin resistance (26%, p= 0.003), but not with DHEA-S, free testosterone or IGF-1 levels (Figure 3b).

To further investigate the potential impact of detectable compared to undetectable HIV-1 RNA, a sensitivity analysis was performed, restricting the analysis to HIV-uninfected men, and HIV-

infected frail (N=76) and non-frail (N=86) men with an undetectable viral load (Supplemental Table 1). Univariate comparisons were similar to Table 2 and did not change significance level, with the exception that the percentage of CD4+ with CD28- expression was significantly lower among frail vs non-frail HIV-infected men. In the adjusted comparison (Supplemental Table 2), similar to the overall findings (Figures 1a-c), the HIV-infected frail men had higher hsCRP (p= 0.005), lower free testosterone (p= 0.011) and higher insulin resistance (p= 0.010) compared to HIV-infected non-frail men. The difference in IL-6 was attenuated and no longer significant (p=0.147) and frail HIV-infected men had significantly lower %CD4+28- T-cells (p= 0.025). Among HIV-infected versus HIV-uninfected men, the difference in sTNFR2 was attenuated but remained significant (p=0.049); differences in markers of immune senescence persisted (all p<0.001).

Discussion

The degree to which multisystem regulation in older, HIV-infected men is altered by HIV infection versus the presence of frailty has not previously been described. Here, by analyzing both HIV status and frailty status together, we have shown that IL-6 and hs-CRP were associated with frailty among HIV-infected men. In addition, we have shown for the first time that lower DHEA-S and lower testosterone were also associated with frailty in those men, consistent with the concept of multisystem dysregulation. In contrast, HIV serostatus but not frailty was associated with cellular immune activation and immunosenescence.

Even with effective ART, inflammatory markers remain elevated among HIV-infected compared to HIV-uninfected controls [21]. While pronounced differences by HIV serostatus were expected, the adjusted differences were driven more by frailty for IL-6 and hs-CRP. Overall, the

association between inflammation and frailty is consistent with several prior studies in HIV-infected populations, irrespective of the frailty definition, age range, or HIV risk factors of the population studied. We previously found significantly greater IL-6 but not CRP among HIV-infected frail versus non-frail adults [22], perhaps due to differences in liver function or other downstream inflammatory signaling between the populations. In a separate analysis of men in the MACS [23], HIV-infected men who were frail by the Fried criteria had 50% greater CRP concentrations than HIV-infected non-frail men. In the ALIVE cohort of HIV-infected and -uninfected injection drug users, both IL-6 and sTNFR1 levels increased with increasing frailty, and this relationship was slightly stronger among the HIV-infected participants [24]. In contrast, in the VACS, inflammation was associated with a higher score on the VACS Index, an index of HIV-related variables and other comorbid conditions, but not with a subjective frailty index [25]. Additionally, in the AGEhiv Cohort, frailty was not associated with markers of inflammation or monocyte activation [26]. Differences in HIV disease severity, frailty definitions, comorbidities and coinfections, and other unrecognized confounders may account for discrepancies between studies, but as a whole, these studies add to the growing literature on the relationship between frailty and the chronic inflammation of HIV infection.

It is well recognized that markers of T-cell senescence and T-cell activation are higher among HIV-infected [27] compared to HIV-uninfected persons, but the degree to which these elevations are attributable to frailty or to HIV is not clear. The lack of association of these cellular markers with frailty in our population differed findings from a prior study [22], which found a strong association between frailty and immune activation (%CD38+HLA-DR+ expression on CD8+ T-cells) [22]. In the prior study, the median % of CD8+CD38+HLA-DR+ was 15%, all participants were ART-treated, and 96% virologically suppressed [22]. In contrast, in the current study the percentage of CD38+HLA-DR+ on CD8+ cells were much higher for HIV-infected men, even

when restricted to those with virologic suppression (Supplemental Tables). The reasons for these differences are unclear, but could be partially explained by different characterizations of frailty.

Insulin resistance or diabetes, low DHEA-S, and low testosterone have been associated with frailty and components of frailty including fatigue, weakness, and low energy, in multiple studies of HIV-uninfected cohorts [10, 28-33]. In HIV-infected populations, frailty (by varying definitions) has been previously associated with diabetes [34, 35], low testosterone [36], and low IGF-1 [37], each in separate cohorts. Although low levels of DHEA-S were seen in asymptomatic HIV disease prior to HAART [38] and have been associated with progression to AIDS [39], no prior studies in HIV-infected populations had shown an association with frailty. Whether treatments to replace or normalize these hormones might result in improvement in HIV-infected adults, remains to be clearly established in either HIV-infected or uninfected populations. Data on the effects of DHEA supplementation on physical function in the general elderly population are inconclusive [40], with some studies [41] but not all [42], demonstrating improved strength and function. Furthermore, the benefit of testosterone replacement therapy on components of frailty remains controversial [43].

Several limitations of our study should be mentioned. First, we utilized the frailty-related phenotype to establish a diagnosis of frailty. The frailty-related phenotype has been utilized in several prior studies within the MACS Cohort, as grip strength and gait speed were not added to the MACS until 2007. The phenotype is associated with increased mortality and poor outcomes [8], but relies on subjective report and therefore may reflect a greater predominance of depressive symptoms, subjective fatigue, and self-image than the Fried frailty phenotype.

Second, the median year of assessment was 2006, so some of our data preceded the HAART era and were subject to a lack of viral suppression. Further, relatively few observations were from the modern ART era of the potent integrase-strand transfer inhibitors. Matching by calendar year and ART-use, with further adjustment for virologic suppression, minimized the ART differences while maximizing the available data across the diverse HIV treatment periods. The MACS includes only men who have sex with men, and the present study included predominantly middle-aged Caucasian men; thus generalizability to women, much older populations, or populations with greater racial/ethnic diversity or differing HIV risk factors may be limited. Furthermore, no HIV-uninfected frail control group was included. Differences in body fat and muscle mass beyond BMI may have influenced markers of inflammation, activation, and hormone dysfunction, but image-based body composition measures were not available for most participants. Similarly, additional measures of immune senescence such as CD57 or proliferation assays may have provided a better assessment of the true senescence status. Lastly, the cross-sectional nature of the data does not allow inferences to be made regarding causality.

Prior studies suggest a causal role of inflammation in muscle mass decline, muscle turnover, and weight loss, contributing to components of frailty [44-47]. In the present study, associations persisted in multivariate models adjusted for comorbid conditions, suggesting that the frailty-associated inflammation and hormonal dysregulation were not merely a result of greater comorbid disease burden among frail participants. Thus, our findings emphasize the potential importance of inflammation (IL-6 and hs-CRP) and hormonal dysregulation in frailty among HIV-infected adults. However, considering the cross-sectional design and the small, but significant difference, our findings cannot provide a basis for recommending testosterone or DHEA-S replacement or anti-inflammatory therapy as a treatment for frailty. Indeed, few

interventions outside of exercise have been shown to effectively reverse the trajectory of frailty [48]. Results from ongoing studies on the anti-inflammatory roles of statins, ACE-inhibitors/angiotensin-receptor blockade, metformin, or probiotics may support a role for these therapies in the future [49]. For now, interventions should focus on limiting ongoing exposures to inflammation through lifestyle factors such as maintenance of a healthy weight and diet, physical activity, and adequate sleep; optimizing comorbidity management; and preservation of gonadal function (i.e., early initiation of ART, avoidance of alcohol, marijuana, and opiates). Ultimately, early and long-lasting interventions that impact multiple pathways will likely prove most effective in slowing or preventing frailty, particularly in older HIV-infected adults.

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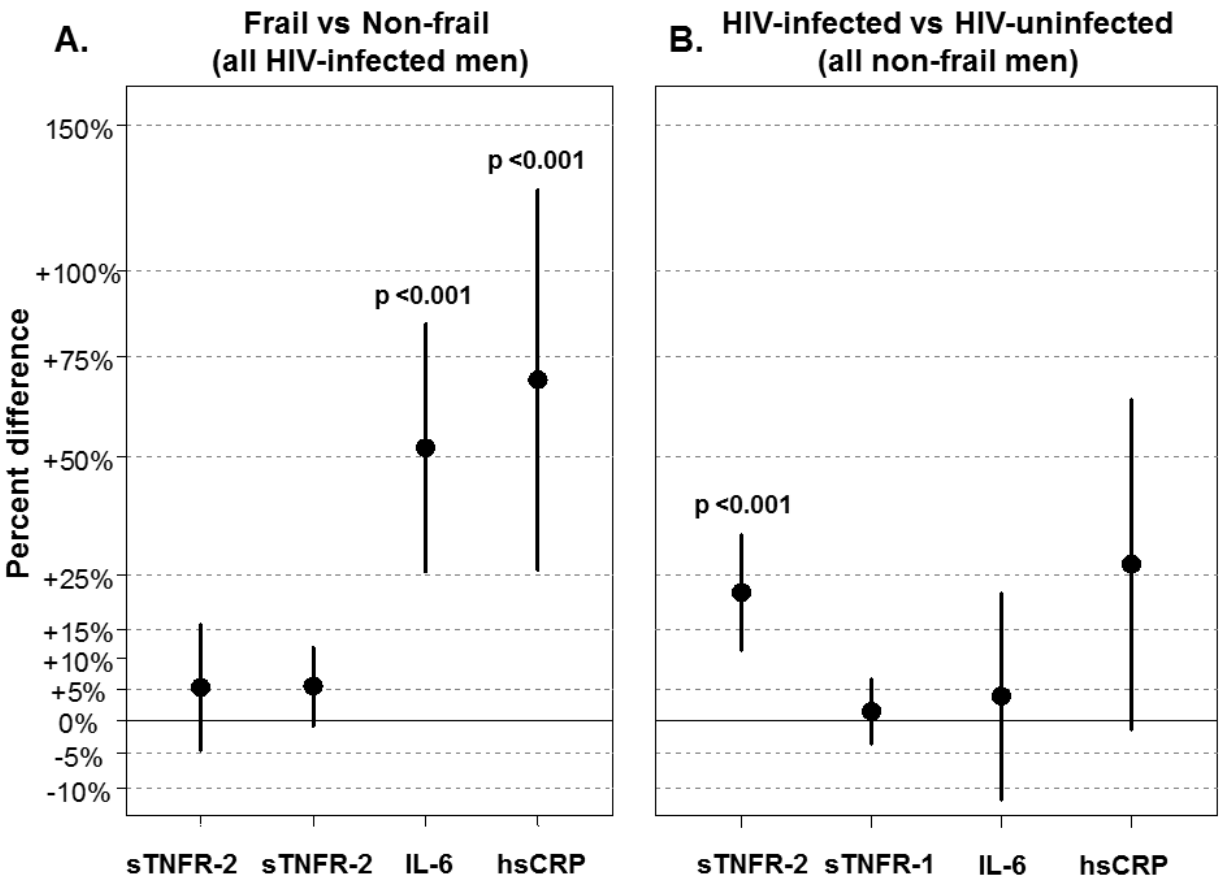
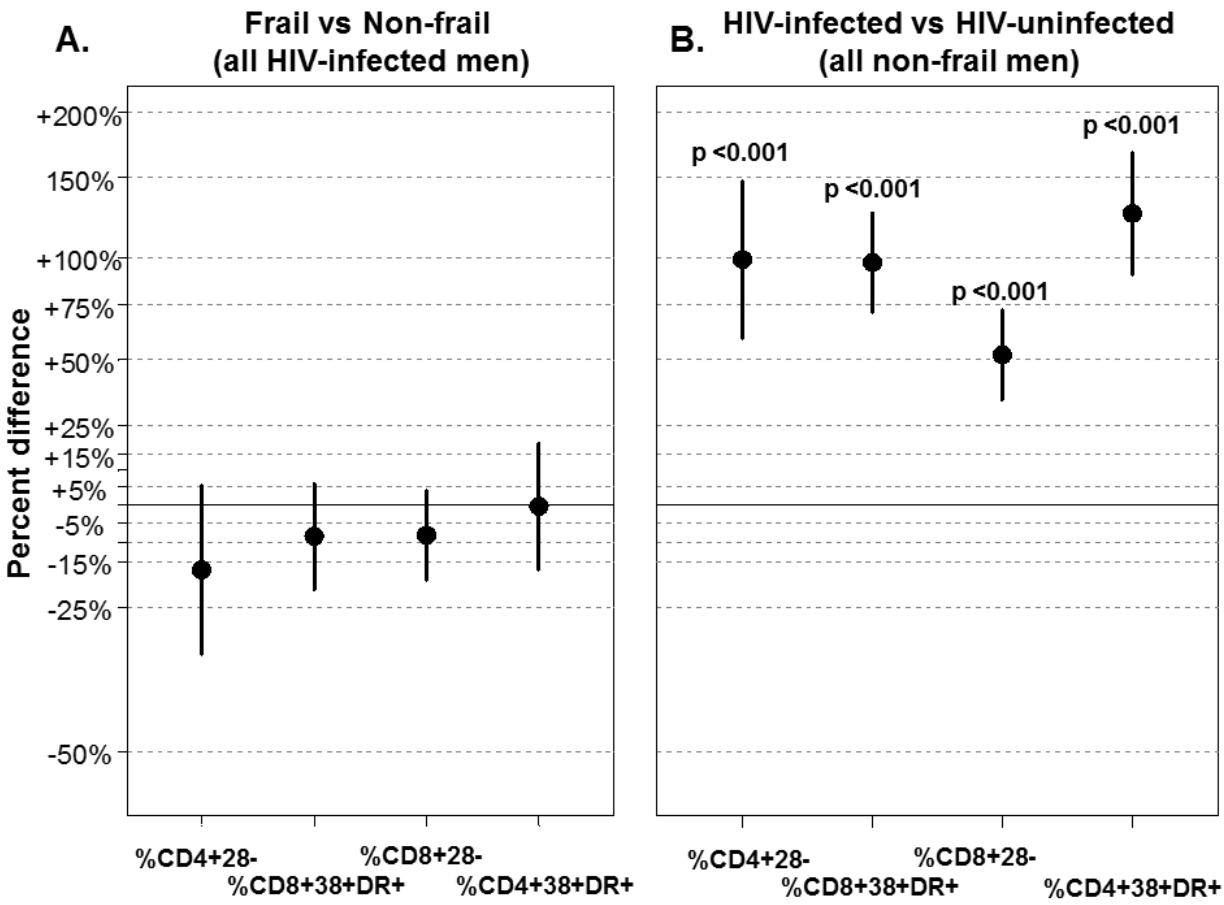
Figure Legends:

Figure 1. Adjusted differences in inflammatory marker levels by frailty status among HIV-infected men (Panel 1a), and by HIV serostatus among non-frail men (Panel 1b), represented by point estimates (dots) and 95% confidence intervals (vertical lines). The study design was matched by age, calendar year of visit, and ART use (among HIV-infected men); models were additionally adjusted for age, black race, BMI in the log scale, obesity, smoking status (never, former or current smoker), HCV infection, diabetes, dyslipidemia, hypertension and presence of depressive symptoms (CES-D > 16). Among HIV-infected men (Panel 1a), models were further adjusted for nadir CD4 cell count < 350 cells/μL (yes or no), current CD4 < 350 cells/μL (yes or no), current detectable HIV-1 RNA (yes or no), and type of antiretroviral therapy (none, non-HAART or HAART).

Figure 2. Adjusted differences in markers of immune activation and senescence by frailty status among HIV-infected men (Panel 2a) and by HIV serostatus among non-frail men (Panel 2b), represented by point estimates (dots) and 95% confidence intervals (vertical lines; see Figure 1 legend for covariate adjustment).

Figure 3. Adjusted differences in markers of hormonal regulation by frailty status among HIV-infected men (Panel 3a) and by HIV serostatus among non-frail men (Panel 3b), represented by point estimates (dots) and 95% confidence intervals (vertical lines; see Figure 1 legend for covariate adjustment).





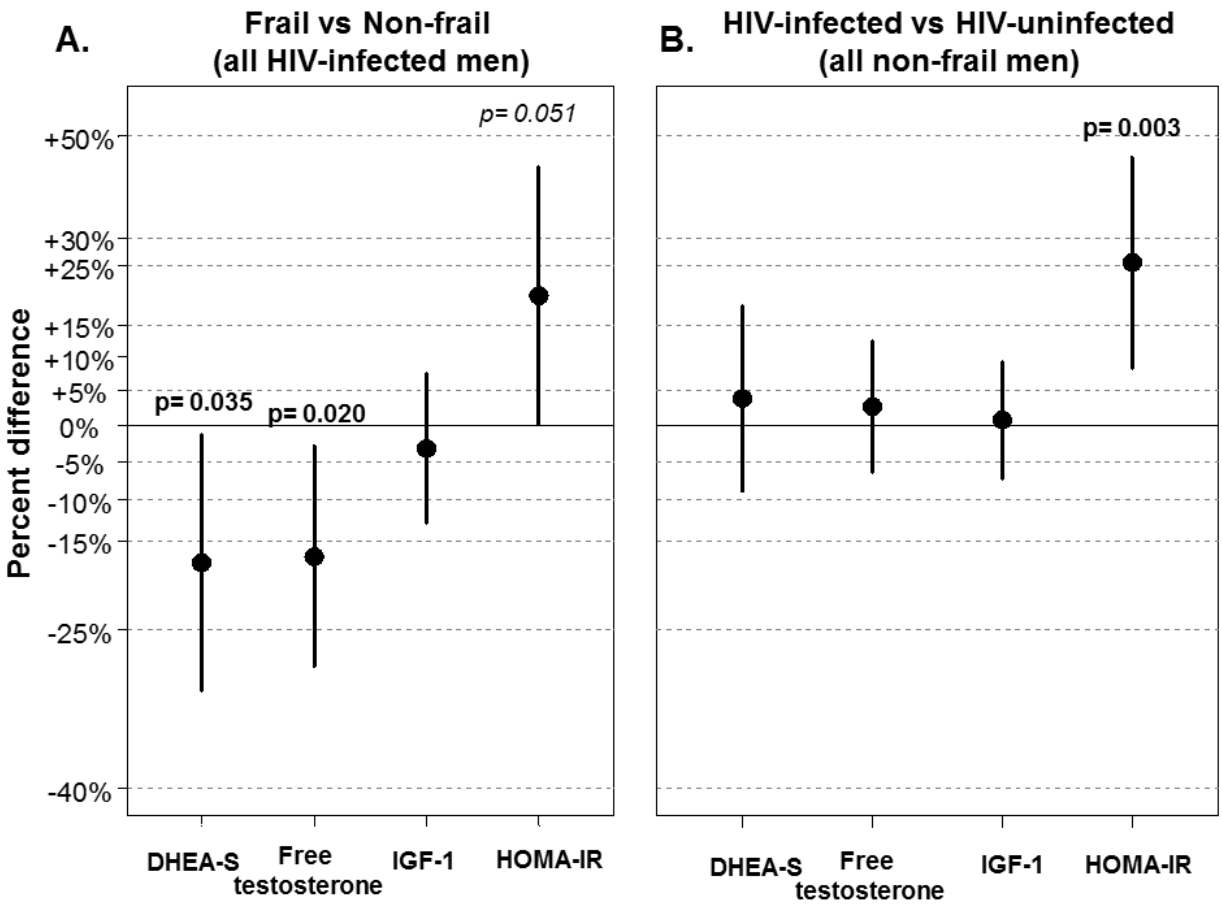


Table 1. Demographic and clinical characteristics of the study population at the index visit.

Variable	*HIV-uninfected* *N= 150 (%)*	*Non-frail HIV-infected* *N= 141 (%)*	*Frail HIV-infected* *N= 155 (%)*	*P-value*
Age (years)	48.45 [41.53, 53.06]	47.67 [40.38, 52.99]	49.11 [41.41, 53.88]	0.71 †
Date	2006 [2002, 2008]	2006 [2001, 2008]	2006 [2001, 2008]	0.76 †
Black race	25 (16.7)	40 (28.4)	51 (32.9)	0.003 ‡
Hispanic	8 (5.3)	14 (9.9)	17 (11)	0.18 ‡
Metabolic health				
Body mass index, kg/m^2	25 [23.53, 27.17]	25.65 [23.62, 27]	23.9 [21.8, 27.5]	0.024 †
Obese	16 (10.7)	5 (3.7)	23 (14.9)	0.004 ‡
Fasting glucose > 100 mg/dL	37 (30.8)	45 (41.7)	65 (54.2)	0.001 ‡
Diabetes	7 (4.7)	22 (15.6)	41 (26.5)	<.001 ‡
Dyslipidemia	94 (75.2)	106 (89.8)	114 (84.4)	0.010 ‡
Metabolic syndrome	34 (22.7)	57 (40.4)	75 (48.4)	<.001 ‡
Cardiovascular and renal health				
Hypertension	26 (17.3)	40 (28.4)	65 (41.9)	<.001 ‡
Estimated glomerular filtration (by CKD-EPI), ml/min|1.73m^2	84.03 [74.57, 98.65]	97.19 [82.89, 107.66]	93.82 [78.29, 106.45]	<.001 †
Behavioral and psychological health				
Never smoker	45 (30)	40 (28.4)	26 (17.1)	<.001 ‡
Former smoker	85 (56.7)	71 (50.4)	56 (36.8)	
Current smoker	20 (13.3)	30 (21.3)	70 (46.1)	

	HIV-uninfected	*Non-frail HIV-infected*	*Frail HIV-infected*	
Variable	*N= 150 (%)*	*N= 141 (%)*	*N= 155 (%)*	*P-value*
≥ 14 alcoholic drinks per week	18 (12)	11 (7.8)	6 (3.9)	0.031 ‡
Illicit drug use	95 (63.3)	76 (53.9)	94 (61.8)	0.214 ‡
Hepatitis C infection	2 (1.3)	6 (4.3)	27 (17.5)	<.001 ‡
Depressive symptoms (CES-D > 16)	7 (4.7)	7 (5.0)	85 (54.8)	<.001 ‡
HIV-related health and therapy				
Testosterone therapy	NA*	6 (5.2)	18 (13.8)	0.030 ‡
Incident AIDS	0 (0)	13 (9.2)	30 (19.4)	<.001‡
Nadir CD4	NA	287 [184, 400] (N= 141)	272 [164, 438] (N= 154)	0.783 †
Nadir CD4 < 200	NA	44 (31.2)	54 (34.8)	0.538 ‡
Nadir CD4 < 350 cells//µL	NA	95 (67.4)	96 (61.9)	0.33 ‡
Current CD4 (cells/µL)	NA	559 [400.67, 693.67]	476 [290.67, 635.67]	0.012 †
Current CD4 < 350 cells/µL	NA	23 (16.3)	49 (31.6)	0.003 ‡
Detectable viral load	NA	58 (41.4)	78 (50.3)	0.130 ‡
Non-HAART	NA	24 (17)	27 (17.4)	0.098 ‡
HAART	NA	102 (72.3)	116 (74.8)	
No ART	NA	15 (10.6)	12 (7.7)	
Any NRTI exposure	NA	127 (90.1)	142 (93.4)	0.394‡
Cumulative NRTI exposure, year	NA	5.97 [1.84, 10.3]	5.08 [2.12, 9.33]	0.515†
Any NNRTI exposure	NA	75 (53.2)	97 (63.8)	0.075‡

Variable	*HIV-uninfected* *N= 150 (%)*	*Non-frail HIV-infected* *N= 141 (%)*	*Frail HIV-infected* *N= 155 (%)*	*P-value*
Cumulative NNRTI exposure, years	NA	0.13 [0, 2.78]	1.2 [0, 3.73]	0.056†
Any INSTI exposure	NA	6 (4.3)	8 (5.3)	0.788‡
Cumulative INSTI exposure, years	NA	0 [0, 0]	0 [0, 0]	0.692†
Any entry inhibitor exposure	NA	0 (0)	1 (0.7)	1.000‡
Any d4T exposure	NA	70 (49.6)	89 (58.6)	0.129‡
Cumulative d4T exposure, years	NA	0 [0, 3.43]	0.59 [0, 2.94]	0.737†
Any ZDV exposure	NA	96 (68.1)	108 (71.1)	0.612‡
Cumulative ZDV exposure, years	NA	2.26 [0, 5.3]	1.39 [0, 4.35]	0.437†
Any indinavir exposure	NA	38 (27)	45 (29.6)	0.697‡
Cumulative indinavir exposure, years	NA	0 [0, 0.14]	0 [0, 0.68]	0.783†
Any nelfinavir exposure	NA	26 (18.4)	37 (24.3)	0.256‡
Cumulative nelfinavir exposure, years	NA	0 [0, 0]	0 [0, 0]	0.332†

Medians [IQR] and % (n). P-values are based on Kruskal-Wallis non-parametric rank sum for continuous variables (†) and Fisher's exact test for categorical variables (‡). NA*, not available; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CES-D, Center for Epidemiologic Studies-Depression.NRTI, nucleoside/tide analogue reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; INSTI, integrase strand transferase inhibitor; d4t, stavudine; ZDV, zidovudine Illicit drug use included crack/cocaine use, heroin/opioids, amphetamines, speedballs or injection drug use.Body mass index (BMI) was calculated as kg/m^2 and obesity defined as ≥30 kg/m^2; diabetes (hemoglobin A1c ≥ 6.5%, fasting glucose >126 mg/dL or diagnosis of diabetes with use of medications); dyslipidemia (fasting total cholesterol ≥200 mg/dL, LDL ≥130 mg/dL, HDL <40 mg/dL, triglycerides ≥150 mg/dL or use of lipid lowering medications with self-reported/clinical diagnosis of dyslipidemia); metabolic syndrome was defined by the presence of at least three of the following: waist circumference ≥102 cm, fasting triglycerides ≥150 mg/dL, fasting glucose ≥100mg/dL, on diabetic medications with a history of diabetes or self-report, HDL cholesterol <40 mg/dL, SBP ≥ 130 mmHg or DBP ≥ 85 or on hypertensive medications with a self-reported or clinical history of hypertension; hypertension was defined by SBP ≥140 or DBP ≥90 mmHg, and history of hypertension or diagnosis of hypertension with use of antihypertensive medications.

Table 2. Univariate comparison biomarkers of inflammation, T-cell activation and senescence, and hormonal regulation by HIV-serostatus and frailty status.

Variable	*HIV-uninfected N= 150*	*Non-frail HIV-infected N= 141*	*Frail HIV-infected N= 155*	*P-value for HIV-infected, frail vs. non-frail*	*P-value for HIV-infected non-frail to HIV-uninfected men*
Inflammation					
IL-6, pg/ml	1.2 [0.8, 1.6]	1.4 [1.0, 2.0]	2.3 [1.6, 3.5]	<.001	0.002
hsCRP, ng/L	0.8 [0.4, 1.8]	1.4 [0.6, 2.4]	2.2 [1.0, 5.2]	<.001	0.001
sTNFR1, pg/ml	1182 [1039, 1300]	1203 [1061, 1335]	1357 [1152, 1618]	<.001	0.21
sTNFR2, pg/ml	2505 [2040, 2992]	3313 [2518, 4247]	4203 [3028, 6334]	<.001	<.001
T-cell activation and senescence					
%CD8+ CD38+HLA-DR+	14.4 [10.1, 20.7]	33.8 [20.1, 49.3]	37.5 [23.1, 53.7]	0.26	<.001
%CD4+ CD38+HLA-DR+	5.8 [3.9, 8.35]	14.0 [7.8, 21.4]	15.5 [8.4, 29.1]	0.076	<.001
%CD8+28-	40.3 [25.5, 54.8]	57.7 [47.0, 69.4]	55.2 [40.7, 67.5]	0.18	<.001
%CD4+28-	11.5 [5.6, 23.5]	19.9 [13.0, 34.4]	17.4 [9.1, 34.3]	0.22	<.001
Hormonal regulation					
DHEA-S, ng/mL	1.2 [0.8, 1.6]	1.2 [0.8, 1.8]	0.8 [0.4, 1.4]	<.001	0.46
Free testosterone*, pg/mL	85 [71, 106]	86 [70, 106]	70 [48, 99]	0.045	0.74

Variable	*HIV-uninfected N= 150*	*Non-frail HIV-infected N= 141*	*Frail HIV-infected N= 155*	*P-value for HIV-infected, frail vs. non-frail*	*P-value for HIV-infected non-frail to HIV-uninfected men*
IGF-1, ug/L	118 [96, 138]	116 [92, 144]	107 [77, 138]	<.001	0.92
HOMA-IR	2.25 [1.64, 3.01]	2.93 [2.17, 4.89]	3.43 [2.24, 6.8]	0.057	<.001

Median [IQR] (N available data). P-values are based on the Kruskal-Wallis non-parametric rank sum test. IL-6, interleukin-6; hs-CRP, high-sensitivity C-reactive protein; sTNFR-1 and 2, soluble tumor necrosis factor receptors-1 and 2; DHEA-S, dehydroepiandrosterone sulfate; IGF-1, insulin-like growth factor-1; HOMA-IR, homeostatic model assessment –insulin resistance; * includes men on testosterone replacement therapy