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Association of Functional Impairment with Inflammation and Immune Activation in HIV-1-Infected Adults on Effective Antiretroviral Therapy

Kristine M Erlandson[1,2], Amanda A Allshouse[3], Catherine M Jankowski[2], Eric J Lee[1], Kevin M Rufner[4], Brent E Palmer[5], Cara C Wilson[1], Samantha MaWhinney[3], Wendy M Kohrt[2], Thomas B Campbell[1]

[1]University of Colorado Denver, Department of Medicine, Division of Infectious Diseases

[2]University of Colorado Denver, Department of Medicine, Division of Geriatric Medicine

[3]University of Colorado Denver, Department of Biostatistics and Informatics

[4]University of Colorado Denver, Department of Medicine, Division of Gastroenterology and Hepatology

[5]University of Colorado Denver, Department of Medicine, Division of Allergy and Clinical Immunology

Corresponding Author Contact Information: Kristine Mace Erlandson, MD, University of Colorado Anschutz Medical Campus, Department of Medicine, Division of Infectious Diseases, 12700 E. 19th Avenue, Mail Stop B168; Aurora, CO 80045, Fax: 303-724-4926; Telephone: 303-724-4941, Kristine.Erlandson@ucdenver.edu

ABSTRACT:

Background: The relationships of inflammation, immune activation and immune senescence markers with functional impairment in aging HIV-1-infected persons are unknown.

Methods: HIV-1-infected persons, aged 45 to 65 years, with plasma HIV-1 RNA <48 copies/mL on antiretroviral therapy underwent standardized functional testing. In a nested case-control analysis, low-function cases were matched (1:1.5) by age, gender and HIV-1 diagnosis date to high-function controls. Markers of inflammation, T-cell activation, microbial translocation, immune senescence and immune recovery estimated functional status in conditional logistic regression. Primary analyses were adjusted for CD4 count, smoking, and hepatitis.

Results: 31 low-function cases were compared to 49 high-function controls. After adjusting, lower proportions of CD4+ T-cells and higher proportion of CD8+ T-cells, higher CD38/HLA-DR expression on CD8+ T-cells, and higher interleukin-6 were associated with a significantly greater odds of low-function (all OR ≥ 1.1; $P \leq 0.03$). Other inflammatory, senescence and microbial translocation markers were not significantly different (all $P \geq 0.11$) between low and high-function groups.

Conclusion: Functional impairment during successful antiretroviral therapy was associated with higher CD8+ T cell activation and interleukin-6 levels. Interventions to decrease immune activation and inflammation should be evaluated for effects on physical function and frailty.

BACKGROUND

A cornerstone of "successful aging" is the ability to maintain functional independence [1,2]. A key and often modifiable, element of functional independence is the preservation of physical function. Physical function impairments in older adults increase the risk of morbidity and mortality. Among those aging without HIV-1 infection, declines in physical function and development of frailty are strongly associated with inflammation and immune system dysfunction, including immune activation, senescence and altered T-lymphocyte subsets [3-8].

Aging with human immunodeficiency virus (HIV)-1 infection is associated with early decline in physical function and development of frailty [9]. Early development of frailty or a frailty-like syndrome has been demonstrated in several HIV cohorts, although findings have been most apparent in those not taking antiretroviral therapy or with the lowest CD4+ T-cell counts [10-14]. Performance-based measures targeted at the upper end of the functional spectrum among middle-aged and older adults with HIV-1 infection have identified greater than expected impairments in walking speed, balance, ability to rise from a chair and peak exercise capacity [15,16] .

Given that immune activation and inflammation have been associated with impaired physical function and frailty in older non-HIV-infected persons, and HIV-1 infection is associated with chronic immune activation and inflammation despite otherwise successful antiretroviral therapy [17], we hypothesized that functional impairment in HIV-1 infection would be associated with markers of immune activation and inflammation. Because immune senescence is associated with frailty in non-HIV-infected elderly patients, and immune senescence and microbial translocation have

been associated with chronic HIV-1 infection, we further hypothesized that markers of immune senescence and microbial translocation would also be associated with functional impairment.

METHODS

Study Population

All individuals receiving care for HIV-1 infection in the Infectious Diseases Group Practice at the University of Colorado were evaluated for participation. Individuals meeting the following criteria were eligible: 1) 45 to 65 years of age; 2) able to consent and participate in study procedures; and 3) taking a minimum of two antiretroviral drugs for at least six months with one undetectable plasma HIV-1 RNA (<48 copies/mL) and no plasma HIV-1 RNA >200 copies/mL in the prior six months. Approval was obtained by the Colorado Multiple Institutional Review Board and informed consent was obtained from all participants.

Clinical Assessments

All participants completed a standardized interview and available medical records were reviewed. Self-reported time since HIV diagnosis, nadir CD4 lymphocyte count, and HIV treatment history were confirmed by medical records when available. The presence or absence of the following comorbidities were determined by medical records: seizure disorder, dementia, stroke, neuropathy, psychiatric disease, arthritis, osteopenia or osteoporosis (prior stress fracture or T-score < -1 on bone densitometry scan), diabetes, kidney disease (calculated creatinine clearance <30 mL/min by

Cockcroft-Gault), malignancy (excluding non-melanoma skin cancer), solid organ transplant, lung disease, hypertension, cardiovascular disease, viral hepatitis (presence of hepatitis B virus surface antibody or hepatitis C virus antibody), and chronic liver disease. Medications were determined by medical record review and self-report. Laboratory values were the most recent values available in the medical record. The Veterans Aging Cohort Study Index was calculated using the following parameters as previously described: CD4 count, viral load, age, aspartate aminotransferase, alanine aminotransferase, platelets, hemoglobin, hepatitis C, and estimated glomerular filtration rate [18,19]. Of a possible 164 points, higher values indicate greater mortality risk, and scores of ≤ 34 are associated with the lowest mortality [18,19].

Functional Assessments

A frailty phenotype was assessed as previously described by Fried, et al [20]. Shrinking was defined as unintentional weight loss of ≥ 10 pounds, or decrease of 5% of body weight in the last year (self-reported and verified by records when available). Exhaustion was defined by three to four times per week of feeling “everything I do is an effort” or “sometimes I just cannot get going” [11,12,14,20]. Low activity was defined as self-report of being “limited a lot” in vigorous physical activities from the Short-Form (SF)-36 ® [11,12,14]. Weakness was assessed by the average of three dominant hand grip measurements using a single Lafayette dynamometer, applying previously defined gender and body mass index (BMI) cutoffs [20]. Slowness was defined by 4.5-m walk time: men ≤173 cm and women ≤159 cm in height requiring ≥7 seconds or men >173 cm and women >159 cm requiring ≥6 seconds met a criterion [20]. One point was given

for each abnormality. Three to five points were considered low function, one or two points moderate function, and zero points high function [20].

The Short Physical Performance Battery assessed tandem stand, walking speed, and sit-stand test time, with zero points indicating inability to complete a task and four points indicating performance within the expected range [21]. Tandem stand was measured by ability to stand heel-to-toe for ten seconds, walking speed by the faster of two 4-m walks at usual pace, and sit-stand test time by five repetitions of sit-to-stand without use of the arms [21]. A score of less than 9 is highly predictive for subsequent disability and was considered low function, 9-11 points moderate function, and 12 points (no deficits) high function [21,22].

400-m walk time was measured on a set walking course by asking the participant to walk as quickly as possible to complete the distance [23,24].

We defined high-function as ability to complete a 400-m walk and no deficits on either the frailty phenotype or the Short Physical Performance Battery. Low-function was defined as a score of 3 or more on the frailty phenotype or a score of less than 9 on the Short Physical Performance Battery with at least 1 deficit on the opposing test. Moderate function was defined as at least 1 deficit on the frailty phenotype or Short Physical Performance Battery but not meeting the definition of low-function.

Flow Cytometric Assays

For measurement of HLA-DR and CD38 expression, whole blood was stained with fluorescently labeled anti-CD3/CD4/CD38/HLA-DR or anti-CD3/CD8/CD38/HLA-DR monoclonal antibodies (BD Biosciences, San Jose, CA). Cells were incubated for

30 minutes at room temperature, red blood cells (RBCs) were lysed, and remaining cells fixed by the addition of 450 µL of FACS Lysing solution (BD Biosciences) before they were analyzed.

For CD28 expression, RBCs were lysed before staining by adding 4 mL of PharmLyse (BD Biosciences, San Jose, CA) to 1 mL of whole blood. The blood was incubated for 5 minutes at room temperature and centrifuged at 300 x g for 5 minutes to pellet the white blood cells. The cells were washed with FACS buffer (2% human serum in PBS) and the pellet was resuspended in 100 µLs of FACS buffer. The staining cocktail containing fluorescently labeled anti-CD3, -CD4, -CD8 and -CD28 mAbs was added and the cells were incubated at 4^{o}C for 20 minutes. Cells were washed once with FACS buffer, resuspended in 100 µL of fixation buffer (1% paraformaldehyde) and stored at 4^{o}C until analysis. A fluorescence minus one (FMO) control which did not contain CD28 was included.

All flow cytometric assays used a FACSCalibur flow cytometer (BD Immunocytometry Systems, San Jose, CA). The senescence panel was acquired using Cell Quest Pro software (BD Immunocytometry Systems, San Jose, CA) and 100,000 events were collected. The percentage of CD28 expression on CD4 and CD8 T-cells was determined using the FMO stained cells to set the positive gate. CD38 and HLA-DR expression was analyzed using setting (voltage and compensation) determined by FACSComp using CaliBRITE beads (BD Biosciences, San Jose, CA). Samples were analyzed using the HLA-DR/CD38 Multiset Algorithm which automatically gates and determines the percentage of HLA-DR+/CD38+ CD4 and CD8 T-cells.

Quantitative Polymerase Chain Reaction Assays

Leukocyte telomere length was measured in cryopreserved whole blood. Genomic leukocyte DNA was isolated using Perfect Pure DNA Blood kit from 5 PRIME (Fischer Scientific, Pittsburgh, PA). Quantitative real-time amplification of the telomere sequence was performed by Cawthon's method [25], with modifications as described by O'Callaghan [26] to obtain absolute telomere length. Each sample was analyzed in quadruplet and the interclass correlation was 0.89 (95% CI 0.86, 0.91).

16s rDNA was isolated from cryopreserved EDTA plasma using a modified DNAeasy extraction (Qiagen Inc, Valencia, CA), lysophilized, then quantified utilizing real-time PCR by methods previously described [27]. Samples were analyzed in duplicate; the coefficient of variation was 18.3%. Variance of pooled controls had a mean cycle threshold of 41.2 (95% CI 39.4, 43.0); 39.4 was set as the zero cut-off, and all values were normalized to 39.4 cycles.

Measurement of Soluble Molecules in Plasma and Serum

Commercially available enzyme-linked immunosorbent assays (ELISA) were used to measure serum IL-6 and TNF-alpha (R&D Systems, Minneapolis, MN), plasma soluble CD14 (sCD14; R&D Systems Inc, Minneapolis, MN), lipopolysaccharide binding protein (LBP; Hycult, Plymouth Meeting, PA), endotoxin core IgM antibodies (EndoCAb; Hycult, Plymouth Meeting, PA) and intestinal fatty acid binding protein (iFABP; R&D Systems Inc, Minneapolis, MN). Highly sensitive C-reactive protein (hs-CRP) was measured by immunoturbidimetrics (Beckman Coulter, Brea, CA). Plasma lipopolysaccharide (LPS) concentration was run in triplicate and measured with the

Endotoxin Detection Kit (Lonza, Walkersville, Maryland) after dilution of plasma 1:10 in endotoxin-free water (Hyclone, Logan, Utah) and incubation at 80°C for 15 minutes. All assays followed the instructions specified by the manufacturer. The coefficient of variation was 1.2% for sCD14 and 17.6% for LPS.

Study Design and Data Analysis

A nested case-control study included all persons identified as low-function as cases. Each case was matched to a high-function control. If more than one high-function control was identified, cases were matched to two controls until the target sample size of 80 subjects was reached. Matching was by rank order of age within two years, gender, and time since HIV diagnosis (prior to 1996 or after 1996). Thirteen cases were matched to one control and 18 cases were matched to two controls. All persons included as cases and controls were asked to return for a second visit for collection of blood samples. If participants had an infection or other acute illness, blood samples were not collected until a minimum of 14 days after symptom resolution. Persons who had taken oral corticosteroids within the prior four weeks were excluded from the case-control analysis. Blood samples were not collected on individuals who did not participate in the case-control analysis.

Odds Ratios (OR) and 95% confidence intervals (CI) are presented from conditional logistic regression for primary analysis where conditional odds of low function was estimated for each measure, with adjustment for most recent CD4+ T-cell count, viral hepatitis (positive hepatitis B virus surface antibody and/or hepatitis C virus antibody) and smoking. Mean differences and 95% CI from mixed model comparisons

are reported for secondary analyses, where continuous measures are summarized as mean and 95% CI from mixed model with matching identifier treated as a cluster and adjustment for most recent CD4+ T-cell count, viral hepatitis and smoking. Skewed measures were log transformed (IL-6, hs-CRP, 16s rDNA) prior to analysis. Categorical measures are presented as frequency and percent. The monotonic relationship between CD4+ and CD8+ T-cell activation and CD4+ T-cell count was assessed with the Spearman correlation coefficient. Analyses were performed in SAS v9.2 (Cary, NC). Data were collected and managed with Research Electronic Data Capture (REDCap) hosted at the University of Colorado [28].

RESULTS

Study Population

Between February and November, 2010, 542 HIV-1-infected persons met eligibility requirements and were asked to participate: 171 either did not respond to correspondence or were not interested; 2 died prior to obtaining consent; 369 consented and 359 completed functional testing. 33 (9%) were identified as low-function, 186 (52%) as moderate and 140 (39%) as high-function. One low-function person moved from the area and the other could not be contacted. 31 low-function cases were matched to 49 high-function controls and underwent laboratory evaluations at the second study visit. Overall, participants were 85% male and 74% Caucasian with a median age of 50.8 years (interquartile range 47.7-55.7 years), median CD4+ lymphocyte count of 551 cells/μL (interquartile range 361-768 cells/μL), and HIV-1 RNA below limits of detection in 95% of subjects [29].

The low- and high-function groups were similar by baseline demographic characteristics with the exception that low-function cases were more likely to be current smokers (Table 1). Low-function cases had lower nadir CD4+ T-cell count, a greater number of comorbid conditions, higher Veterans Aging Cohort Study Index Scores and were prescribed a greater number of non-antiretroviral medications (Table 1). Three subjects had detectable plasma HIV-1 RNA on routine laboratory monitoring after the screening visit (1 low-function case had 491 copies/mL, 2 high-function controls had 78 and 1670 copies/mL). One low-function case had a prior liver transplant and was receiving cyclosporine and sirolimus at the time of evaluation. Although total CD4+ T-cell count was not significantly different between low-function and high-function groups (Table 1), lower CD4%, higher CD8%, and lower CD4/8 ratio were associated with greater odds of low-function status (Table 2). Significant differences were detected between low- and high-function groups in CD4+ T-cell percentage (Figure 1A), CD8+ T-cell percentage (Figure 1B), and CD4/CD8 ratio (Figure 1C).

Markers of Immune Activation and Inflammation

To determine variability in CD38/HLA-DR expression on CD8+ T-cells, 20 subjects had a repeat blood draw two to five days after the initial blood draw. Among these 20 subjects, interclass correlation was 0.88 (95% CI 0.73, 0.95).

CD8+ but not CD4+ T-cell expression of CD38/HLA-DR was associated with a greater odds of being low-function, and remained significant after adjusting for CD4+ T-cell count, smoking, and hepatitis B or C (Table 2). Low-function persons had higher percentages of CD8+ and CD4+ T-cells co-expressing CD38 and HLA-DR compared to

high-function persons (Figures 1D and 1E). In multivariate analyses adjusting for CD4+ T-cell count, smoking, and hepatitis, CD38/HLA-DR on CD8+ T-cells remained significantly higher among low-function persons (adjusted difference = 4.8, 95% CI 1.4, 8.3; adjusted *P* = 0.02*)* but significant differences in CD4+ T-cell activation were no longer detected (adjusted-difference = 2.7, 95% CI -0.09, 5.4; adjusted *P* = 0.07). Among the three subjects with detectable plasma HIV-1 RNA (Table 1), CD8+ T-cell expression of CD38/HLA-DR increased with increasing plasma HIV-1 RNA concentration (7% in a control with 78 copies/mL, 17% in a case with 491 copies/mL, and 25% in a control with 1670 copies/mL). CD8+ T-cell expression of CD38/HLA-DR remained higher in low-function cases (17.6 ± 1.4%) than high-function controls (12.6 ± 1.2%) when the three subjects with detectable plasma HIV-1 RNA were excluded (*P* = 0.003). Among all subjects, higher CD4+ and CD8+ T-cell activation were moderately inversely correlated with CD4+ T-cell count (r = -0.41, *P* < 0.001 and r = -0.38, *P* = 0.002, respectively).

Higher plasma sCD14 was associated with greater odds of being low-function, however differences were not robust after adjustment (Table 2). Similarly, plasma sCD14 concentration was higher among low- compared to high-function persons in univariate (Figure 1F) analysis but was not significant after multivariate analysis (adjusted difference= 0.3; 95% CI 0.5, 6.0; adjusted *P* = 0.09).

Although each log increase in IL-6 increased the odds of low-function classification by 1.2 (95% CI 1.02, 1.51, p=0.03), TNF-alpha and hs-CRP were not associated with a greater odds of low-function (Table 2). In mixed model regression, plasma IL-6 and TNF-alpha concentrations were significantly higher among low- than

high-function persons in univariate analyses (Figure 2A and 2B). IL-6 but not TNF-alpha concentration remained significantly higher in the adjusted model (IL-6 $\log_{10}$ unadjusted difference = 0.36, 95% CI -0.54, -0.18, $\log_{10}$ adjusted difference = 0.31, 95% CI -0.50, -0.12, adjusted *P* = 0.002 and TNF-alpha adjusted difference = 0.5, 95% CI -0.02, 1.0; adjusted *P* = 0.06). There was a trend towards higher hs-CRP concentration in low-function cases (Figure 2C).

Markers of Microbial Translocation

Markers of microbial translocation were not significantly associated with a greater odds of being low-function (Table 2) and in secondary analyses were not significantly different between low- and high-function groups in unadjusted or adjusted analyses: 16S rDNA, LPS, LBP, i-FABP, or endoCAB (Figures 3A-E).

Markers of Immunosenescence

Lack of CD28 expression on CD4+ or CD8+ T-cells was not associated with greater odds of low-function (Table 2) and was not significantly different between functional groups (Figure 4A and 4B). Quadruplicate measurements of telomere length had an interclass correlation of 0.89 (95% CI 0.86, 0.91). Mean leukocyte telomere length was not associated with greater odds of low-function (Table 2) and was not significantly different between functional groups (Figure 4C). Among all subjects, there was no significant correlation between age and percentage of CD8+ T-cells without CD28 expression (r = 0.21, *P* = 0.06) or telomere length (r = 0.03, *P* = 0.71).

DISCUSSION

Functional status incorporates the concomitant processes of aging, inflammation, HIV-1 infection, comorbid diseases and lifestyle. Using a case-control design, we demonstrated significant independent associations of functional impairment with IL-6 and CD8+ T-cell activation in patients on effective combination antiretroviral therapy. Our findings add to a growing body of evidence for the association of chronic immune activation and inflammation to the development of non-AIDS morbidity and mortality [30-32]. To our knowledge, this is the first evidence linking physical function and frailty to immune activation and inflammation among persons with HIV-infection.

Similar to prior studies of frailty and disability among HIV-uninfected elderly, we detected an association between high IL-6 and low function and a trend towards higher hs-CRP and TNF-alpha, suggesting an overall inflammatory milieu among functionally impaired persons. Moreover, increased CD8+ T-cell activation was also associated with functional impairment among HIV-1 infected persons. Higher IL-6 is closely linked to frailty, disability and mortality among HIV-uninfected elderly [3,7], while CD8 T-cell activation is associated with disease progression and poor immune recovery in HIV-1 infected persons [17]. However, the role of CD8+ T-cell activation in physical function impairment is largely unexplored in HIV- infected or -uninfected populations [8].

We also detected higher monocyte activation, as measured by sCD14, although the differences were not statistically significant after adjusting for smoking status. Monocyte activation is associated with frailty and disability in HIV-uninfected persons [8,33] and with mortality among HIV-infected persons [36]. Given that sCD14 is released from monocytes upon LPS stimulation, we expected to find evidence of

microbial translocation among low-functioning persons. Contrary to our hypothesis, differences in microbial translocation markers were not detected. Given that all subjects were treated with effective antiretroviral therapy, we suspect immune reconstitution from antiretroviral therapy attenuated any potential differences between low- and high-functioning individuals. Furthermore, variability in the assays and transient fluctuation in microbial translocation products may have impacted our ability to detect differences.

In HIV-uninfected persons, chronic inflammation, antigen stimulation, and age lead to immune remodeling manifest by decline in CD4+ T-lymphocytes, increase in CD8+ T-lymphocytes, and expansion of CD28(-)T-lymphocytes with shortened telomeres [3]. These changes have been associated with frailty and disability in HIV-uninfected elderly [3-5,34,35]. Although the median number of CD4 lymphocytes for our subjects was greater than 500 cells/μL and current CD4 T cell count was similar between cases and controls, low-functioning cases had lower nadir CD4 T cell count and lower percent of CD4+ T cells. Thus, our findings suggest that lower nadir CD4 count prior to initiation of antiretroviral therapy and continued perturbations in CD4+ T cell proportions during antiretroviral therapy place HIV-infected patients at risk for functional impairment through chronic immune activation and inflammation.

Although HIV-uninfected elderly with low-functional status have increased markers of immune senescence, we did not detect significant differences in CD8+ CD28(-) T-cells, CD4+CD28(-) T-cells, or telomere length between low- and high-functioning groups. Although telomere length does appear to shorten with time and with several disease processes, considerable inter-individual variability is due to an array of genetic, environmental, and lifestyle factors, which complicates between-group

comparisons [36]. Telomere length from our cohort (mean 64.8 kb/diploid genome, SD 22) was similar to an uninfected cohort 15 years older (mean 69.5 kb/diploid genome, SD 37) [26] suggesting an increase in cell turnover unrelated to functional capacity.

The strengths of our study were the careful matching of low- and high-functioning participants to control for potential effects of age, gender, and duration of HIV infection on inflammatory and activation markers. A relatively narrow age range provided focus on the relationships between inflammation and physical impairment in early aging, rather than age-related changes in the elderly. Limitations to our study include the cross-sectional and observational design. Longitudinal studies are needed to investigate whether increased activation and inflammation are associated with a decline in functional capacity, or whether interventions to decrease inflammation and immune activation improve physical function. We did not control for differences in comorbid conditions or medications and larger studies are needed to study the effects of specific comorbidities or medications (including statins) on physical function, immune activation and inflammation.

Our study was the first comparison of markers of immune activation, immune senescence, and microbial translocation in HIV-infected persons with phenotypes defined by functional capacity. We identified a distinct clinical phenotype based on physical function and frailty assessment that is associated with markers of inflammation and immune activation that predict poor prognosis in HIV-1 infected persons. A measurement of physical function can be easy, fast and inexpensive. Future interventions targeted at decreasing immune activation and inflammation in HIV-1

infected persons should assess the clinical impact of therapy through measures of physical function.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Conflict of Interest: The authors declare no conflicts of interest.

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REFERENCES

1. Rowe JW, Kahn RL. Successful aging. Gerontologist **1997**;37:433-40.
2. Hawton A, Green C, Dickens AP, et al. The impact of social isolation on the health status and health-related quality of life of older people. Qual Life Res **2011**;20:57-67.
3. Yao X, Li H, Leng SX. Inflammation and immune system alterations in frailty. Clinics in geriatric medicine **2011**;27:79-87.
4. Moro-Garcia MA, Alonso-Arias R, Lopez-Vazquez A, et al. Relationship between functional ability in older people, immune system status, and intensity of response to CMV. Age (Dordr) **2011**.
5. Risques RA, Arbeev KG, Yashin AI, et al. Leukocyte telomere length is associated with disability in older u.s. Population. J Am Geriatr Soc **2010**;58:1289-98.
6. De Fanis U, Wang GC, Fedarko NS, Walston JD, Casolaro V, Leng SX. T-lymphocytes expressing CC chemokine receptor-5 are increased in frail older adults. J Am Geriatr Soc **2008**;56:904-8.
7. Reuben DB, Cheh AI, Harris TB, et al. Peripheral blood markers of inflammation predict mortality and functional decline in high-functioning community-dwelling older persons. J Am Geriatr Soc **2002**;50:638-44.
8. Fahey JL, Schnelle JF, Boscardin J, et al. Distinct categories of immunologic changes in frail elderly. Mech Ageing Dev **2000**;115:1-20.
9. Deeks SG. Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. Top HIV Med **2009**;17:118-23.
10. Terzian AS, Holman S, Nathwani N, et al. Factors associated with preclinical disability and frailty among HIV-infected and HIV-uninfected women in the era of cART. J Womens Health (Larchmt) **2009**;18:1965-74.

11. Desquilbet L, Jacobson LP, Fried LP, et al. HIV-1 infection is associated with an earlier occurrence of a phenotype related to frailty. J Gerontol A Biol Sci Med Sci **2007**;62:1279-86.

12. Desquilbet L, Margolick JB, Fried LP, et al. Relationship between a frailty-related phenotype and progressive deterioration of the immune system in HIV-infected men. J Acquir Immune Defic Syndr **2009**;50:299-306.

13. Oursler KK, Goulet JL, Crystal S, et al. Association of age and comorbidity with physical function in HIV-infected and uninfected patients: results from the Veterans Aging Cohort Study. AIDS Patient Care STDS **2011**;25:13-20.

14. Onen NF, Agbebi A, Shacham E, Stamm KE, Onen AR, Overton ET. Frailty among HIV-infected persons in an urban outpatient care setting. J Infect **2009**;59:346-52.

15. Oursler KK, Sorkin JD, Smith BA, Katzel LI. Reduced aerobic capacity and physical functioning in older HIV-infected men. AIDS Res Hum Retroviruses **2006**;22:1113-21.

16. Richert L, Dehail P, Mercie P, et al. High frequency of poor locomotor performance in HIV-infected patients. Aids **2011**;25:797-805.

17. Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. The Journal of pathology **2008**;214:231-41.

18. Justice AC, McGinnis KA, Skanderson M, et al. Towards a combined prognostic index for survival in HIV infection: the role of 'non-HIV' biomarkers. HIV Med **2010**;11:143-51.

19. Tate JJ, AC. Change in a prognostic index for survival in HIV infection after on year on cART by level of adherence. Presented at: 48th Annual IDSA Meeting; 2010; Vancouver.

20. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci **2001**;56:M146-56.

21. Guralnik JM, Simonsick EM, Ferrucci L, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol **1994**;49:M85-94.

22. Chiarantini D, Volpato S, Sioulis F, et al. Lower extremity performance measures predict long-term prognosis in older patients hospitalized for heart failure. Journal of cardiac failure **2010**;16:390-5.

23. Vestergaard S, Patel KV, Bandinelli S, Ferrucci L, Guralnik JM. Characteristics of 400-meter walk test performance and subsequent mortality in older adults. Rejuvenation Res **2009**;12:177-84.

24. Simonsick EM, Montgomery PS, Newman AB, Bauer DC, Harris T. Measuring fitness in healthy older adults: the Health ABC Long Distance Corridor Walk. J Am Geriatr Soc **2001**;49:1544-8.

25. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res **2002**;30:e47.

26. O'Callaghan N, Dhillon V, Thomas P, Fenech M. A quantitative real-time PCR method for absolute telomere length. BioTechniques **2008**;44:807-9.

27. Jiang W, Lederman MM, Hunt P, et al. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. J Infect Dis **2009**;199:1177-85.

28. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. Journal of biomedical informatics **2009**;42:377-81.

29. Erlandson KM, Allshouse AA, Jankowski CM, et al. Comparison of Functional Status Instruments in HIV-Infected Adults on Effective Antiretroviral Therapy. HIV Clin Trials **2012**;13:324-34.

30. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS medicine **2008**;5:e203.

31. Kalayjian RC, Machekano RN, Rizk N, et al. Pretreatment levels of soluble cellular receptors and interleukin-6 are associated with HIV disease progression in subjects treated with highly active antiretroviral therapy. J Infect Dis **2010**;201:1796-805.

32. Kaplan RC, Sinclair E, Landay AL, et al. T cell activation predicts carotid artery stiffness among HIV-infected women. Atherosclerosis **2011**;217:207-13.

33. Leng SX, Tian X, Matteini A, et al. IL-6-independent association of elevated serum neopterin levels with prevalent frailty in community-dwelling older adults. Age Ageing **2011**;40:475-81.

34. Semba RD, Margolick JB, Leng S, Walston J, Ricks MO, Fried LP. T cell subsets and mortality in older community-dwelling women. Exp Gerontol **2005**;40:81-7.

35. Woo J, Tang NL, Suen E, Leung JC, Leung PC. Telomeres and frailty. Mech Ageing Dev **2008**;129:642-8.

36. Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? A review. J Gerontol A Biol Sci Med Sci **2011**;66:202-13.

Figure Legend

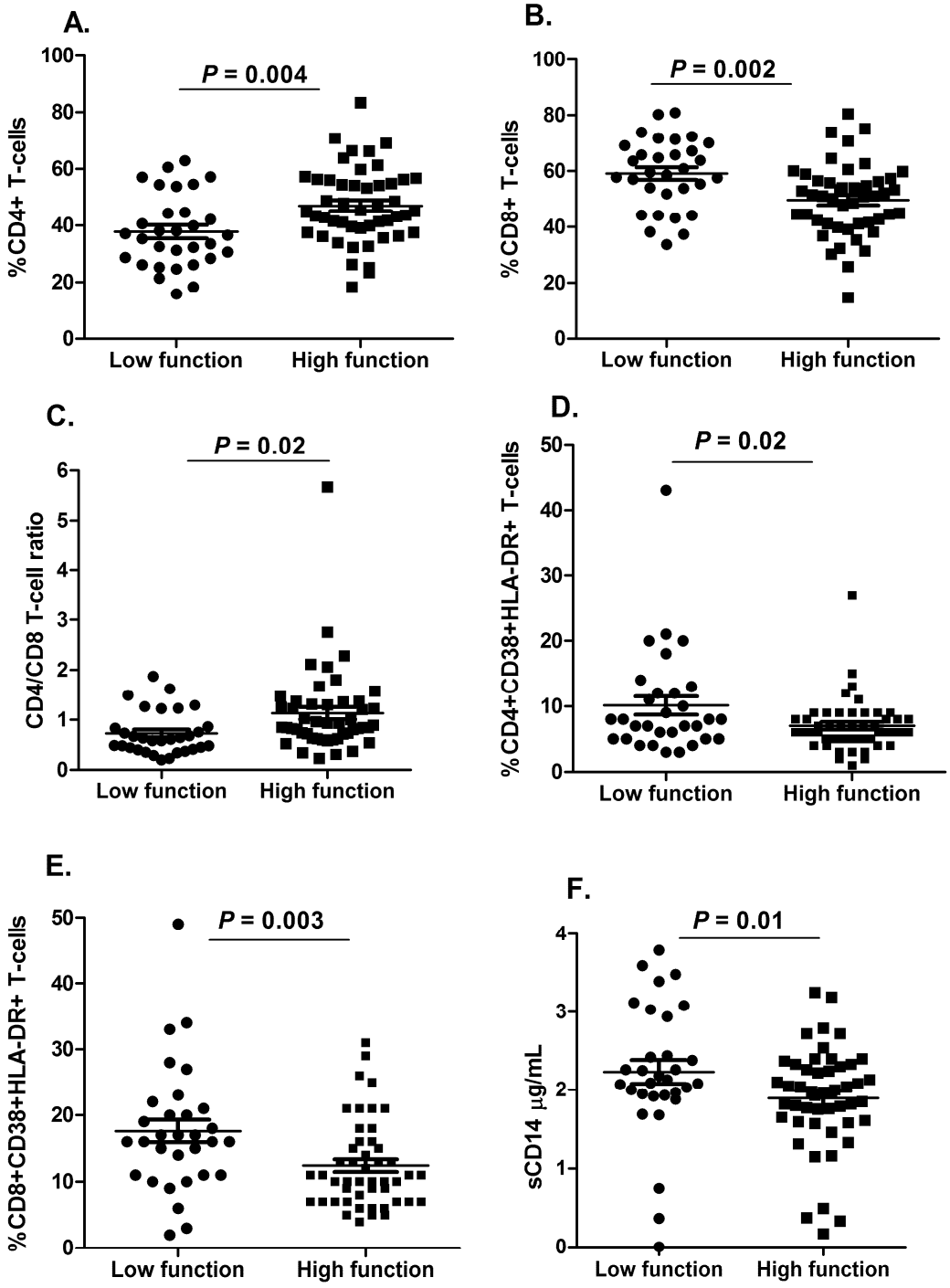
Figure 1. Comparison of T-lymphocyte subsets and immune activation (CD38 and HLA-DR expression on CD4+ and CD8+ T-cells, soluble [s] CD14) between low and high function groups. Mean and standard error (SE) represented by bars and error bars, *P* value from unadjusted comparison of means in a mixed-effects model to account for clustering from matched design. *A.* % CD4+ T-cells; *B*.% CD8+ T-cells; *C.* Ratio CD4+: CD8+ T-cells *D*. % CD38/HLA-DR on CD4+ T-cells; *E*. % CD38/HLA-DR on CD8+ T-cells *F*. Plasma sCD14.

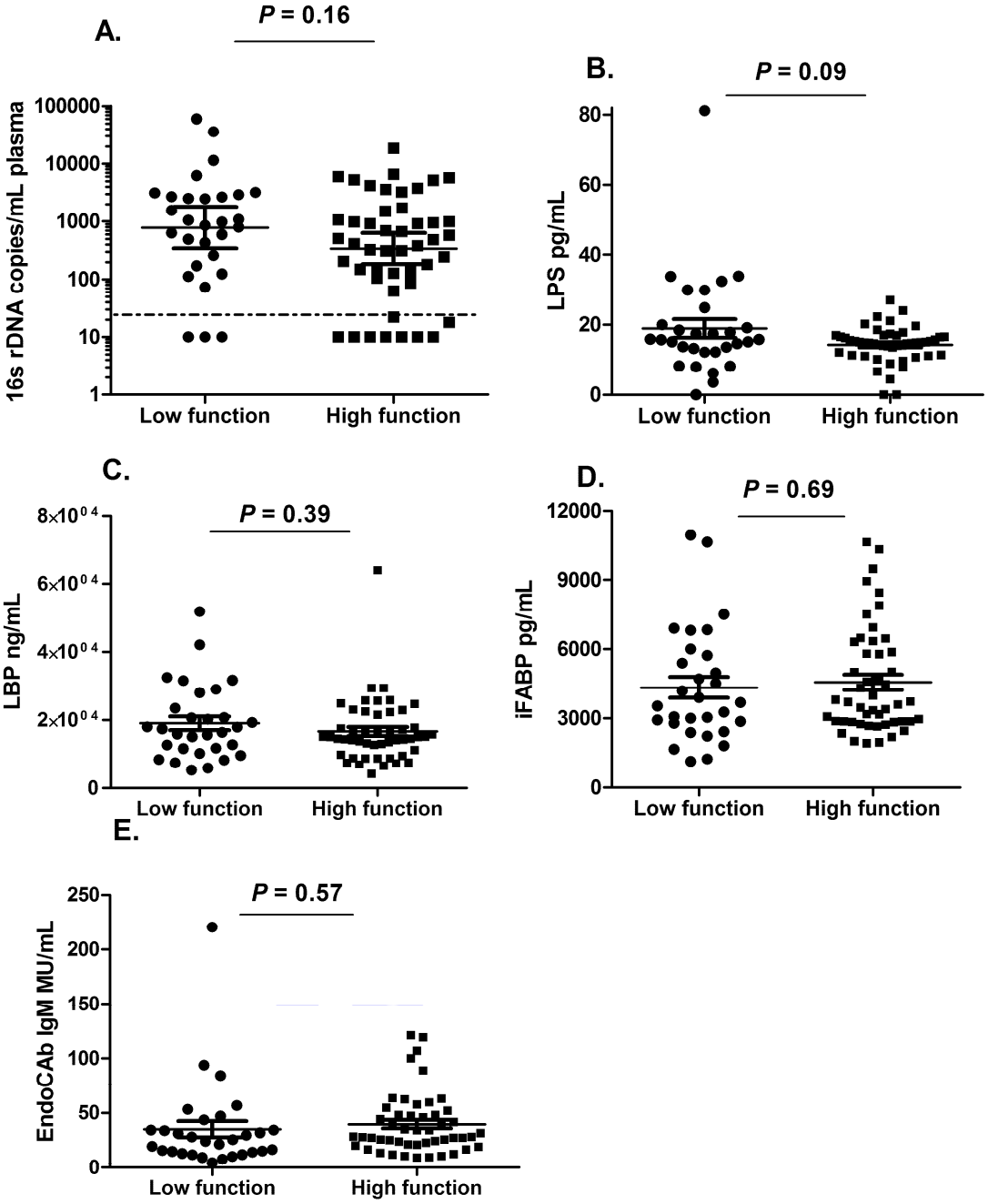
Figure 2. Comparison of inflammatory markers between low and high function persons. Geometric mean and 95% confidence interval (interleukin [IL]-6 and highly sensitive C-reactive protein [hs-CRP]) or mean and standard error (tumor necrosis factor [TNF]-alpha) represented by bars and error bars, *P* value from unadjusted comparison of means in a mixed-effects model to account for clustering from matched design. Comparisons for IL-6 and hs-CRP are log-transformed. *A*. serum IL-6; *B.* serum TNF-alpha; and *C*. serum hs-CRP.

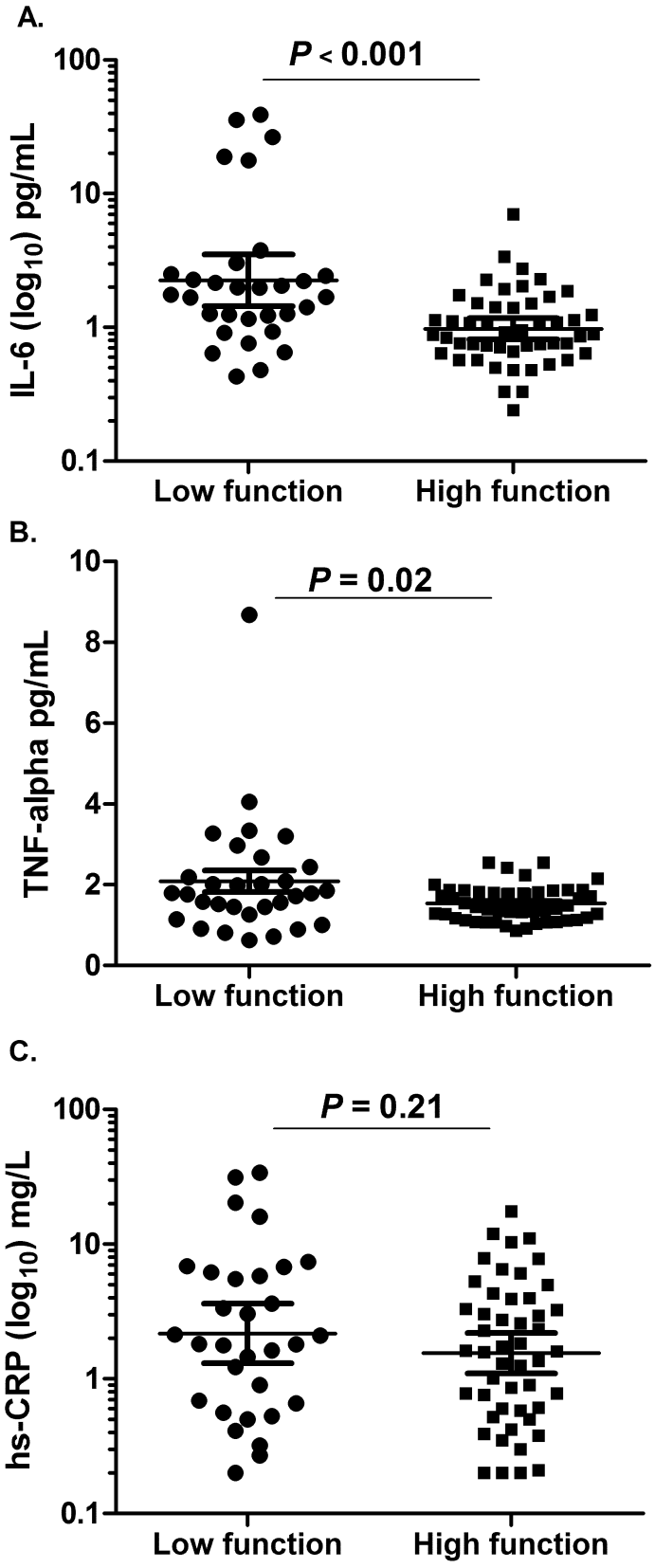
Figure 3. Comparison of microbial translocation markers between low and high function persons. Mean and standard error (SE) or geometric mean and 95% confidence interval (16S rDNA) are represented by bars and error bars, *P* value from unadjusted comparison of means in a mixed-effects model to account for clustering from matched design. Comparison for 16s rDNA is log-transformed. *A.*16S rDNA; dashed line represents assay limit of detection. *B*. plasma lipopolysaccharide (LPS); *C.*

plasma LPS binding protein (LBP); *D*. plasma intestinal fatty acid binding protein (iFABP); and *E*. plasma endotoxin core IgM antibodies (EndoCAb).

Figure 4. Comparison of immunosenescence markers between low and high function persons. Mean and standard error represented by bars and error bars, *P* value from unadjusted comparison of means in a mixed-effects model to account for clustering from matched design. *A*. Absence of CD28 on CD4+ T-cells; *B*. Absence of CD28 on CD8+ T-cells; *C*. Telomere length







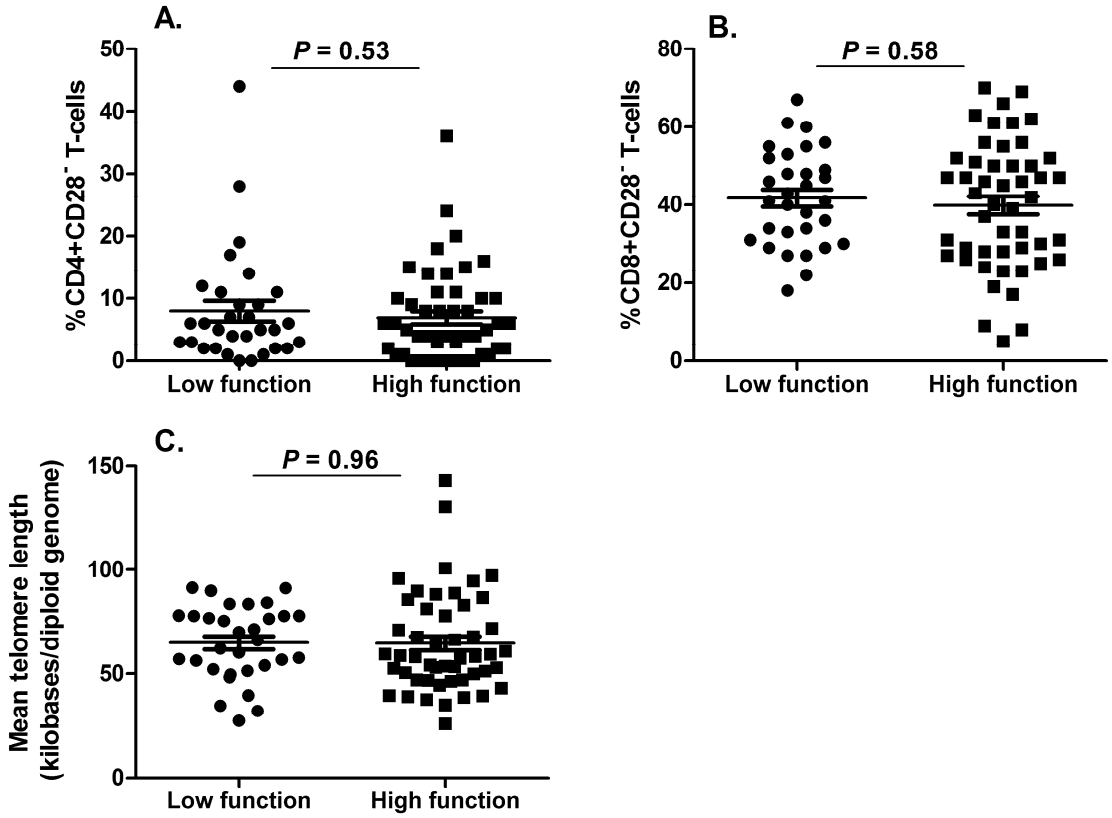


Table 1. Odds ratio associated with low function by demographic and clinical characteristics.

Characteristic	Low function N=31 (%)	High function N=49 (%)	Odds Ratio (95% CI)	*P* value
Demographics				
Age (mean, SE)	52.9 (0.8)	52.7 (0.8)	1.1 (0.8, 1.4)	0.59
Female	8 (26)	10 (20)	--	--
Hispanic/Latino	7 (23)	6 (12)	2.3 (0.6, 8.5)	0.20
Non-white	8 (26)	12 (25)	1.0 (0.3, 3.0)	1.0
Current smoker	15 (48)	10(20)	4.3 (1.4, 13.4)	0.01
HIV Risk:				
Man who has sex with men	18 (58)	34 (69)	0.5 (0.1, 1.9)	0.32
Intravenous drug use	4 (13)	6 (12)	0.9 (0.2, 4.2)	0.90
Heterosexual	9 (29)	12 (25)	1.2 (0.3, 4.2)	0.83
Other	2 (7)	2 (4)	1.4 (0.2, 10.3)	0.73
HIV and Comorbidities				
Years since HIV diagnosis (mean, SE)	15.1 (1.4)	15.4 (1.4)	0.9 (0.8, 1.2)	0.56
Years continuous ART (mean, SE)	8.5 (1.1)	9.3 (1.0)	1.0 (0.9, 1.1)	0.48
Nadir CD4+ T-cells/μL (mean, SE)	110 (27)	178 (22)	0.8 (0.6, 1.0)[a]	0.05

Most recent CD4+ T-cells/μL (mean, SE)	549 (49)	639 (40)	0.9 (0.8, 1.1)[b]	0.19
Detectable HIV-1 RNA (≥ 48 copies/mL)	1 (3)	2 (4)	1.0 (0.09, 11.0)	1.0
Number of comorbidities	3.6 (0.3)	2.3 (0.2)	1.6 (1.2, 2.2)	0.006
Hepatitis B or C	14 (45)	12 (25)	2.4 (0.9, 6.5)	0.10
Body mass index (kg/m^2, mean, SE)	27.1 (1.7)	25.8 (1.3)	1.0 (0.8, 1.3)	0.77
Veterans Aging Cohort Study Index Score	25.9 (2.3)	15.3 (2.1)	2.2 (1.3, 3.9)	0.005
Medications				
Protease inhibitor	17 (55)	22 (45)	1.6 (0.6, 2.9)	0.31
NNRTI	15 (48)	23 (47)	1.1 (0.4, 2.6)	0.88
Integrase inhibitor	5 (16)	12 (24)	0.5 (0.2, 2.0)	0.35
Number of non-antiretroviral medications[c]	6.0 (0.5)	4.2 (0.4)	1.2 (1.0, 1.5)	0.04
HMG-CoA reductase inhibitor class	6 (19)	15 (31)	0.4 (0.1, 1.5)	0.19
Acyclovir or valacyclovir	6 (19)	11 (23)	0.9 (0.3, 2.7)	0.85
Nonsteroidal anti-inflammatory	4 (13)	6 (12)	1.2 (0.3, 4.1)	0.83

Abbreviations: SE, standard error; CI, confidence interval; VACS, Veterans Aging Cohort Study; ART, antiretroviral therapy; NNRTI, Non-nucleotide reverse transcriptase inhibitor; HMG-CoA, Hydroxymethylglutaryl Coenzyme A

a. Per increase of 50 cells/μL
b. Per increase of 100 cells/μL
c. Most common non-ART medications in descending order: antidepressant, benzodiazepine, opiate, HMG-CoA reductase inhibitors (“statins”), angiotensin-converting enzyme inhibitor/angiotensin receptor blocker

Table 2. Odds of low functional status associated with exposure to activation, inflammation, microbial translocation, or senescence markers.

Exposure variable	Low function (N=31)	High function (N=49)	Unadjusted Odds Ratio (95% CI)	Unadjusted P value	Adjusted[a] Odds Ratio (95% CI)	Adjusted[a] P value
%CD4	37.8 (33.1, 42.5)	46.9 (43.1, 50.8)	0.9 (0.90, 0.98)	0.006	0.9 (0.89, 0.99)	0.01
%CD8	59.1 (54.5, 63.7)	49.6 (45.9, 53.3)	1.1 (1.02, 1.12)	0.005	1.1 (1.01, 1.13)	0.01
CD4/CD8	0.7 (0.5, 1.0)	1.1 (0.2, 1.4)	0.8 (0.71, 0.95)	0.009	0.8 (0.69, 0.97)	0.02
Immune Activation						
%CD38/HLA-DR/CD4	10.1 (8.0, 12.2)	7.0 (5.3, 8.7)	1.4 (0.98, 2.08)	0.06	1.4 (0.91, 2.24)	0.12
%CD38/HLA-DR/CD8	17.6 (14.8, 20.5)	12.5 (10.1, 15.0)	1.1 (1.02, 1.19)	0.01	1.1 (1.02, 1.25)	0.02
sCD14 (µg/mL)	2.3 (2.1, 2.6)	1.9 (1.7, 2.1)	1.1 (1.01, 1.21)	0.02	1.1 (0.98, 1.17)	0.14
Inflammation						
IL-6 (pg/mL)	2.2 (1.6, 3.1)	1.0 (0.8, 1.3)	1.3 (1.05, 1.50)[b]	0.01	1.2 (1.02, 1.51)[b]	0.03
TNF-alpha (pg/mL)	2.1 (1.7, 2.4)	1.5 (1.3, 1.8)	1.4 (0.96, 2.07)[c]	0.079	1.5 (0.91, 2.34)[c]	0.11
hs-CRP (µg/mL)	2.2 (1.4, 3.5)	1.6 (1.1, 2.3)	1.1 (0.97, 1.17)[b]	0.22	1.1 (0.96, 1.19)[b]	0.25
Microbial translocation						
16s rDNA (copies/mL)	616 (221, 1717)	242 (108, 542)	1.0 (0.98, 1.06)[b]	0.33	1.0 (0.97, 1.07)[b]	0.48
LPS (pg/mL)	18.3 (14.8, 21.9)	14.3 (11.5, 17.2)	1.0 (0.98, 1.11)	0.18	1.0 (0.96, 1.13)	0.32
LBP (ng/mL)	1.84 (1.48, 2.21) $x10^4$	1.66 (1.35, 1.97) $x10^4$	1.0 (0.95, 1.15)[d]	0.37	1.0 (0.90, 1.12)[d]	0.95

i-FABP (pg/mL)	434 (347, 521)	456 (388, 525)	1.0 (0.95, 1.03)[e]	0.68	1.0 (0.91, 1.02)[e]	0.19
endoCAB (MMU/mL)	35.2 (22.9, 47.4)	39.64 (30.0, 49.3)	1.0 (0.98, 1.01)	0.52	1.0 (0.98, 1.01)	0.51
Immune senescence						
CD28(-)CD4	8.0 (5.1, 10.9)	6.9 (4.5, 9.2)	1.0 (0.97, 1.08)	0.44	1.0 (0.96, 1.12)	0.34
CD28(-)CD8	41.8 (36.4, 47.2)	39.9 (35.6, 44.2)	1.0 (0.98, 1.04)	0.54	1.0 (0.98, 1.07)	0.23
Telomere length	64.9 (57.2, 72.0)	64.7 (58.4, 70.9)	1.0 (0.98, 1.02)	0.95	1.0 (0.99, 1.04)	0.28

Data presented as mean ± SE for variables with normal distribution, geometric mean with 95% confidence interval for skewed variables (IL-6, hs-CRP, and 16s r-DNA. Odds ratios represent an increase of 1 unit unless otherwise indicated. CI, confidence interval; MMU/mL, median units of IgM/mL

a. Adjusted for most recent CD4 count, tobacco use, and hepatitis B or C
b. Per 0.1 log change
c. Per 0.5 pg/mL change
d. Per 2000 ng/mL change
e. Per 200 pg/mL change