T-Cell Immune Dysregulation and Mortality in Women With Human Immunodeficiency Virus

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Background. Dysregulation of adaptive immunity is a hallmark of human immunodeficiency virus (HIV) infection that persists on antiretroviral therapy (ART). Few long-term prospective studies have related adaptive immunity impairments to mortality in HIV, particularly in women.

Methods. Among 606 women with HIV in the Women's Interagency HIV Study, peripheral blood mononuclear cells collected from 2002 to 2005 underwent multiparameter flow cytometry. Underlying cause of death was ascertained from the National Death Index up to 2018. We examined associations of $CD4^+$ and $CD8^+$ T-cell activation (%$CD38^+$HLA-DR^+), senescence (%$CD57^+CD28^-$), exhaustion (%PD-1^+), and nonactivation/normal function (%$CD57^-CD28^+$) with natural-cause, HIV-related, and non-HIV-related mortality.

Results. At baseline, median participant age was 41, and 67% were on ART. Among 100 deaths during a median of 13.3 years follow-up, 90 were natural-cause (53 non-HIV-related, 37 HIV-related). Higher activation and exhaustion of $CD4^+$ T cells were associated with risk of natural-cause and non-HIV-related mortality, adjusting for age, demographic, behavioral, HIV-related, and cardiometabolic factors at baseline. Additional adjustment for time-varying viral load and $CD4^+$ T-cell count did not attenuate these associations. $CD8^+$ T-cell markers were not associated with any outcomes adjusting for baseline factors.

Conclusions. Persistent $CD4^+$ T-cell activation and exhaustion may contribute to excess long-term mortality risk in women with HIV, independent of HIV disease progression.

Keywords: HIV; T-cell; immune activation; mortality.

Activation of the innate and adaptive immune system is a hallmark of human immunodeficiency virus (HIV) infection that persists despite suppressive antiretroviral therapy (ART) [1, 2]. On the adaptive immunity side, T-cell activation proceeds with 3 signals: antigen recognition by the T-cell receptor, receptor co-stimulation, and an inhibitory termination signal [3]. These signals tightly regulate an effective immune response to acute infection, in which the immune response is terminated after pathogen clearance. In HIV infection, where the immune response cannot clear the antigen, persistent T-cell activation drives excessive proliferation and differentiation and ultimately, senescence and exhaustion [3, 4]. T-cell senescence and exhaustion are 2 states of dysfunction that both involve poor effector functions (eg, cytotoxic capacity and cytokine secretion) [5]. Senescence is characterized by high differentiation and low proliferative activity, often measured by loss of CD28 and gain of CD57 surface expression [6], whereas exhaustion is characterized by sustained expression of inhibitory receptors, such as programmed death-1 (PD-1) [7]. Hypothesized causes of persistent immune dysregulation in treated HIV infection include ongoing low-level viral replication, microbial translocation, coinfection with other viruses, and lymphoid fibrosis [1].

Persistent immune dysregulation may contribute to the higher risk of non-HIV-related conditions (eg, cardiovascular disease [CVD], neurocognitive decline, cancer) and mortality observed in people with HIV compared to people without HIV [8–12]. While consistently strong evidence points to the role of innate immune activation (eg,

Received 4 June 2021; editorial decision 23 August 2021; accepted 25 August 2021; published online August 27, 2021.

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Presented in part: 28th Conference on Retroviruses and Opportunistic Infections, Virtual, 6–10 March 2021.

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https://doi.org/10.1093/infdis/jiab433

monocyte activation, inflammation) in morbidity and mortality in treated HIV, the association of adaptive immune markers with these outcomes is not clear [13]. In resource-limited settings, among participants initiating ART, T-cell activation has been associated with HIV clinical progression and mortality independent of $CD4^+$ T-cell counts [14, 15]; however, the association of T-cell activation with mortality in ART-treated resource-rich settings (eg, the United States [US]) was not significant independent of $CD4^+$ T-cell counts [16, 17]. The CD4/CD8 cell ratio, a surrogate for insufficient adaptive immune recovery during HIV infection, was associated with non-AIDS-related mortality in a small case-control study [18], but not in a large prospective cohort collaboration study [19]. T-cell senescence and exhaustion have not been associated with mortality in HIV [13, 16, 17].

To our knowledge, there are no large, prospective studies with long-term follow-up examining the associations of dysregulated T-cell states, such as T-cell activation, senescence, and exhaustion, with mortality in people with HIV. Moreover, previous studies predominantly among male populations [16, 17] did not consider generalizability to women. Here, we describe the relationships of T-cell activation (indicated by cell surface marker $CD38^+HLA\text{-}DR^+$), senescence ($CD57^+CD28^-$), and exhaustion ($PD\text{-}1^+$), as well as nonactivated normal T-cell function ($CD57^-CD28^+$) [20], with HIV-related and non-HIV-related mortality in 606 women with HIV enrolled in the Women's Interagency HIV Study (WIHS), with a median 13.3 years of follow-up.

MATERIALS AND METHODS

Study Population

The WIHS was a multicenter cohort of women with and without HIV in the US (part of the Multicenter AIDS Cohort Study/WIHS Combined Cohort Study as of 2020) that collected clinical, demographic, and behavioral data semiannually through interviews, physical examinations, and laboratory tests, previously described in detail [21]. Participants provided written informed consent and were compensated for participation.

Women in the current analysis participated in a vascular disease substudy beginning in 2004 at the original 6 WIHS sites (Bronx, New York; Brooklyn, New York; Chicago, Illinois; San Francisco, California; Los Angeles, California; Washington, D.C.) [22, 23]. All WIHS participants were eligible for participation in the vascular substudy at the time of recruitment. The vascular substudy featured high-resolution B-mode carotid artery ultrasound at a baseline visit (2004–2006; wave 1) and follow-up visits occurring every 2–3 years through 2013 (waves 2–4). Among women who attended the wave 2 visit, previously frozen peripheral blood mononuclear cell (PBMC) samples from the time roughly corresponding to the wave 1 visit were selected for immune phenotyping (sample collection dates ranged from 2002 to 2005). Both women with and without HIV were included in immune phenotyping (n = 612 with HIV; n = 223 HIV-seronegative), but only women with HIV were included in the mortality analysis due to the small number of deaths (n = 17) in women without HIV during follow-up. We excluded 3 women with prevalent self-reported coronary heart disease and 3 women missing cause of death, leaving 606 women with HIV included in the current mortality analysis.

Laboratory Methods

Immune phenotyping was performed using multiparameteric flow cytometry. Cryopreserved PBMCs were thawed and rested overnight at 37°C in an incubator supplied with 5% carbon dioxide. PBMCs were stained first with LIVE/DEAD Fixable Aqua stain (Molecular Probes, Inc), followed by cell surface staining with fluorochrome-conjugated monoclonal antibodies to CD3, CD4, CD8, CD57, CD28, PD-1, HLA-DR, and CD38. After staining, cells were washed and fixed in 2% formaldehyde. Flow cytometry acquisition was performed on a BD LSRII (BD Biosciences) and data analyzed using FlowJo (Tree Star, Ashland, Oregon). Analyses of markers of interest ($CD38^+HLA\text{-}DR^+$, $CD57^+CD28^-$, $PD\text{-}1^+$, $CD57^-CD28^+$) were performed after stringent gating on singlet live (Aqua-) $CD3^+CD4^+$ or $CD3^+CD8^+$ T cells.

Study Measures

The predictor variables were $CD4^+$ and $CD8^+$ T-cell surface markers of activation (%$CD38^+HLA\text{-}DR^+$), senescence (%$CD57^+CD28^-$), exhaustion (%$PD\text{-}1^+$), and nonactivated normal function (%$CD57^-CD28^+$). To facilitate comparison between predictors in statistical analyses, these variables were *z* score standardized.

Mortality and underlying cause of death were ascertained from the National Death Index (NDI, National Center for Health Statistics, Hyattsville, Maryland). NDI data were available through 31 December 2017 for the Bronx, Washington, D.C., San Francisco, and Chicago sites, through 31 December 2018 for the Brooklyn site, and through 31 December 2014 for the Los Angeles site. Natural-cause mortality was defined as death from any cause except external causes including accidents, intentional self-harm, or assault (*International Classification of Diseases, Tenth Revision* [*ICD-10*] codes V01–Y89), or use of psychoactive substances (*ICD-10* codes F11–F16, F18–F19). HIV-related death was defined as death that includes an *ICD-10* code for HIV disease as the underlying cause (*ICD-10* codes B20–B24). Non-HIV-related death was defined as any natural-cause death that did not include an *ICD-10* code for HIV disease. We also performed an exploratory analysis of CVD-related mortality, regardless of underlying cause of death. CVD-related mortality was defined as a natural-cause death from any "major cardiovascular diseases" (*ICD-10* codes I00–I78 [24]) in the multiple causes of death, excepting death from "cardiac arrest, unspecified." This definition was used because the number of underlying-cause CVD deaths was too small (n = 13).

Statistical Analysis

We used cause-specific hazard models [25] to estimate the associations of T-cell biomarkers with natural-cause mortality, HIV-related mortality, non-HIV-related mortality, and CVD-related mortality. Thus, participants who experienced a competing event for the respective outcome during follow-up were censored at the time of competing event in Cox proportional hazards models. Left truncation was employed to account for immortal time between PBMC sample collection and the start of person-time at risk (2–3 years later). We developed nested models to serially adjust for sets of a priori potential confounding variables, corresponding to the baseline (ie, sample collection) visit: age, demographic factors (study site, race/ethnicity, income, education), behavioral factors (crack or cocaine use, injection drug history, viral hepatitis history, alcohol use, smoking), HIV-related factors ($CD4^+$ T-cell count, HIV RNA load, ART, AIDS), and cardiometabolic factors (body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, antihypertension medication, lipid-lowering medication, diabetes). We checked whether further adjustment for C-reactive protein (CRP; marker of inflammation), the CD4/CD8 ratio (marker of adaptive immune health), or cytomegalovirus (CMV) and Epstein-Barr virus (EBV) immunoglobulin G (IgG) levels (markers of coinfection) [26], altered our results. Finally, we examined time-dependent models adjusting for time-varying HIV RNA load and $CD4^+$ T-cell count, to explore confounding by HIV disease progression during follow-up.

RESULTS

Participant Characteristics

At baseline, the median age of women with HIV was 41 years (interquartile range [IQR], 35–47 years). The majority of participants were black (58%) or Hispanic (29%), low-income (<$30 000/year; 84%), premenopausal (79%), on ART (67%), had detectable HIV RNA loads (59%), and had $CD4^+$ T-cell counts <500 cells/μL (58%) (Table 1). Among those on ART, 43% had detectable viral load and 58% had low CD4 cell counts. Women without HIV, used for comparison of T-cell biomarkers, were similar to women with HIV regarding demographic characteristics (Table 1).

Women with HIV were followed for a median of 13.3 years (IQR, 10.9–14.1 years) from the time of sample collection, or 10.5 years (IQR, 7.8–11.0 years) from the start of time at risk (ie, accounting for immortal time) (Supplementary Table 1). Among 100 deaths that occurred during follow-up, 90 were due to natural underlying causes (53 non-HIV-related, 37 HIV-related) and 10 to external causes. Additionally, 27 deaths were CVD-related when considering multiple causes of death. Median time from sample collection to death was 8.4 years for natural-cause, 9.2 years for non-HIV-related, and 6.3 years for HIV-related death (Supplementary Table 1). Among those who died, median time from the last WIHS clinic visit to death was 0.36 years (IQR, 0.18–0.54 years). At that last visit, 60% of those who died had detectable HIV RNA load, and 13% had AIDS. AIDS at the last visit and classification of HIV-related death based on the death certificate were in slight agreement ($\kappa = 0.16$ [95% confidence interval {CI}, –.01 to .32]; $P = .07$).

T-Cell Biomarker Characteristics

To confirm suspected T-cell dysregulation in women with HIV, we compared T-cell biomarkers between women with and without HIV. As expected, women with HIV differed significantly from women without HIV in most T-cell biomarkers, with the exception of $CD4^+$ T-cell senescence (Figure 1A). Specifically, women with HIV had higher frequencies of $CD4^+$ and $CD8^+$ T cells expressing activation ($CD38^+HLA\text{-}DR^+$) and exhaustion ($PD\text{-}1^+$) markers, higher frequencies of $CD8^+$ T cells expressing senescence markers ($CD57^+CD28^-$), and lower frequencies of $CD4^+$ and $CD8^+$ T cells expressing markers of nonactivation/normal function ($CD57^-CD28^+$), than women without HIV. In general, women with controlled HIV (undetectable HIV RNA load and $CD4^+$ T-cell count ≥500 cells/μL) had better T-cell biomarker profiles than women with uncontrolled HIV; both groups differed from women without HIV for most biomarkers (Figure 1B).

T-cell biomarkers were correlated, with strong positive correlations between immune markers of the same class on $CD4^+$ and $CD8^+$ T cells, as well as weaker positive correlations among markers of activation, senescence, and exhaustion (Figure 2). Naturally, markers of $CD4^+$ and $CD8^+$ nonactivation/normal function had strong inverse correlations with T-cell senescence markers of the same cell type, and also tended to inversely correlate with activation and exhaustion markers (Figure 2).

T-Cell Biomarkers and Mortality

We used cumulative mortality curves based on the Kaplan–Meier method to describe the unadjusted relationship of T-cell biomarkers, dichotomized at the median, with mortality (Figure 3). Women with higher $CD4^+$ T-cell activation and exhaustion, and lower nonactivation/normal function, appeared to have greater natural-cause mortality during follow-up; these patterns were generally consistent for non-HIV-related and HIV-related mortality, though not always reaching statistical significance. Women with higher $CD4^+$ T-cell senescence had greater HIV-related mortality only. Women with lower $CD8^+$ T-cell nonactivation/normal function appeared to have greater natural-cause mortality, whereas $CD8^+$ T-cell activation, senescence, and exhaustion appeared to be unrelated to natural-cause mortality (Figure 3).

In multivariate-adjusted, cause-specific Cox proportional hazards models, $CD4^+$ T-cell activation and exhaustion were significantly associated with higher risk of natural-cause

Table 1. Characteristics of the Study Participants at Baseline

Characteristic	HIV Positive (n = 606)	HIV Negative (n = 223)
Age, y, median (IQR)	41 (35–47)	40 (34–46)
Race/ethnicity		
Black	353 (58)	152 (68)
Hispanic	178 (29)	55 (25)
White	57 (9)	11 (5)
Other	18 (3)	5 (2)
Income		
<$30 000/year	509 (84)	187 (84)
>$30 000/year	97 (16)	36 (16)
Education		
Did not complete high school	256 (42)	82 (37)
Completed high school	179 (30)	75 (34)
College	159 (26)	64 (29)
More than college	12 (2)	2 (1)
Smoking		
Never	198 (33)	53 (24)
Former	137 (23)	46 (21)
Current	271 (45)	124 (56)
Injection drug use history	172 (29)	53 (24)
Alcohol use		
Abstainer	317 (52)	89 (40)
Light (1–3 drinks/wk)	215 (36)	84 (38)
Moderate (4–13 drinks/wk)	59 (10)	37 (17)
Heavy (>13 drinks/wk)	15 (3)	13 (6)
Menopause or hysterectomy	129 (21)	37 (17)
Diabetes	55 (9)	19 (9)
Hypertension	168 (28)	53 (24)
History of AIDS event	223 (37)	…
On ART	405 (67)	…
ART regimen		
None	201 (33)	…
PI-based[a]	197 (33)	…
NNRTI-based[b]	124 (20)	…
NRTI-based[c]	44 (7)	…
Other[d]	40 (7)	…
Detectable HIV RNA load (>80 copies/mL)	355 (59)	…
$CD4^+$ count <500 T-cells/μL	352 (58)	…
$CD4^+$ count, T-cells/μL, median (IQR)	436 (287–649)	…

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

[a]At least 1 PI and 1 NRTI.

[b]At least 1 NNRTI and 1 NRTI.

[c]Three or more NRTIs.

[d]"Other" includes women on both PI and NNRTI, no NRTI, or <3 NRTIs with no PI/NNRTI.

mortality, adjusting for age and demographic, behavioral, HIV-related, and cardiometabolic risk factors at baseline, as well as time-varying HIV RNA load and $CD4^+$ T-cell count (Figure 4; Supplementary Table 2). Each standard deviation increase in $CD4^+$ T-cell activation or exhaustion was associated with a 56% (95% CI, 14%–113%) and 40% (95% CI, 5%–86%) increased risk, respectively, of natural-cause mortality at any time during follow-up. A significant inverse association of $CD4^+$ T-cell nonactivation/normal function with natural-cause mortality was slightly attenuated upon adjustment for time-varying viral load and $CD4^+$ T-cell count, while markers of $CD4^+$ senescence and $CD8^+$ activation, senescence, exhaustion, and nonactivation/normal function were not associated with natural-cause mortality after adjustment for baseline HIV-related and/or cardiometabolic risk factors (Figure 4; Supplementary Table 2). We did not observe significant effect modification by HIV control (all *P* values for interaction > .16), though sample size was more limited for women with controlled HIV (n = 139) (Supplementary Table 3).

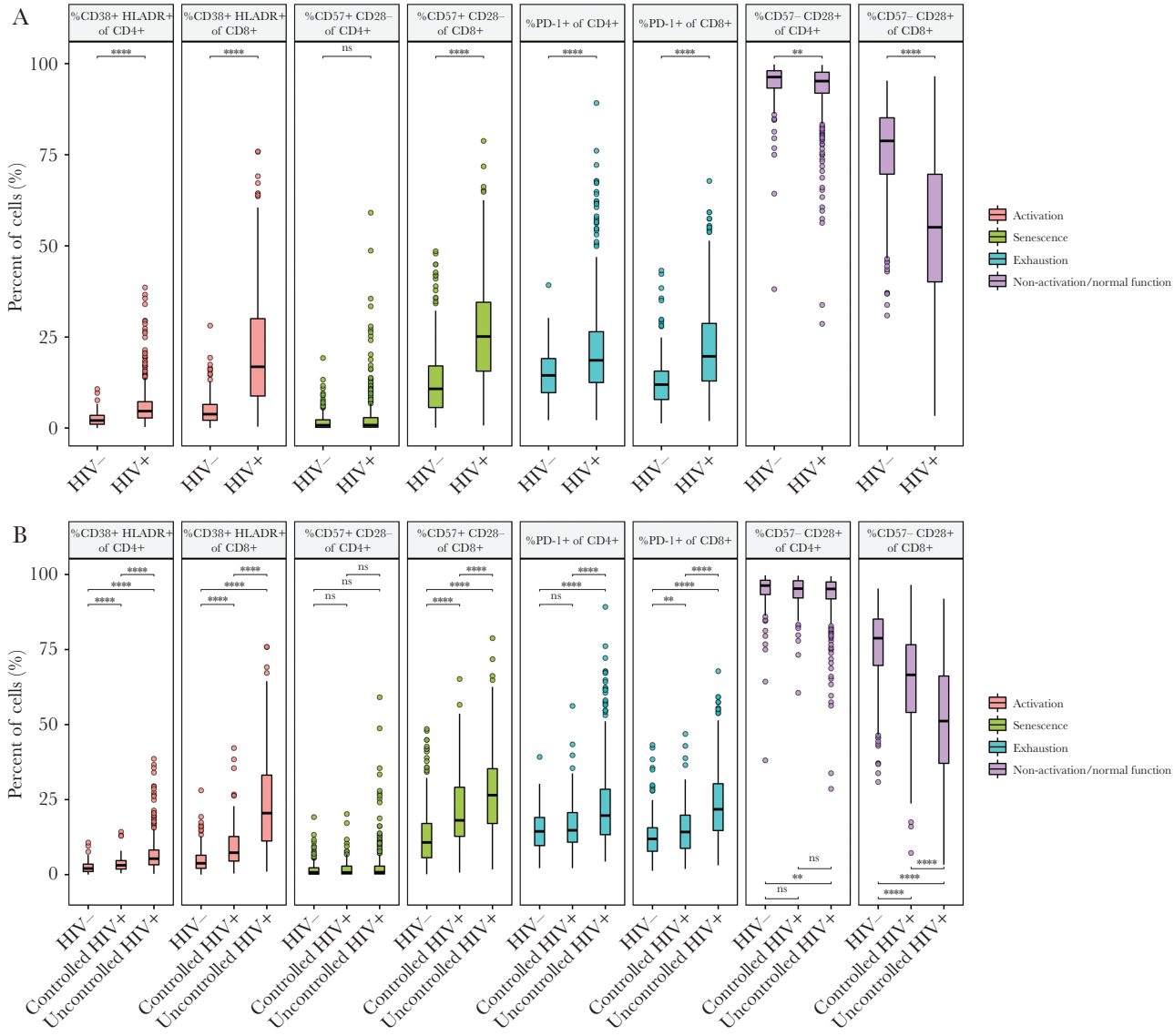

Figure 1. Distribution of T-cell biomarkers in women with or without human immunodeficiency virus (HIV). Boxplots of T-cell biomarkers by HIV serostatus (*A*) and HIV serostatus and HIV control (*B*). Among women with HIV, controlled HIV was defined as undetectable viral load and CD4 T-cell count ≥500 cells/μL, while uncontrolled HIV was defined as detectable viral load or CD4 T-cell count <500 cells/μL. HIV^-, n = 223; HIV^+, n = 606; controlled HIV, n = 139; uncontrolled HIV, n = 467. Asterisks indicate significance in Wilcoxon rank-sum tests: $^{**}P < .01$; $^{****}P < .0001$; ns = not significant ($P > .05$).

We next examined cause-specific models for HIV-related and non-HIV-related mortality. T-cell markers were generally not associated with HIV-related mortality independent of baseline HIV-related and/or cardiometabolic risk factors, with the exception of $CD4^+$ T-cell nonactivation/normal function, which was marginally inversely associated with HIV-related mortality (Figure 4; Supplementary Table 2). This latter association was further attenuated after adjustment for time-varying HIV RNA load and $CD4^+$ T-cell count. For non-HIV-related mortality, $CD4^+$ T-cell activation and exhaustion were significantly associated with higher risk of the outcome, independent of age and demographic, behavioral, HIV-related, and cardiometabolic risk factors at baseline, as well as time-varying HIV RNA load and $CD4^+$ T-cell count (Figure 4; Supplementary Table 2). Adjustment for baseline CRP and CD4/CD8 ratio did not materially impact any model estimates, nor did adjustment for CMV and EBV IgG levels (Supplementary Table 2).

Finally, we explored the associations of T-cell markers with CVD-related mortality. $CD4^+$ T-cell senescence was marginally ($P = .06$) associated with CVD-related mortality, independent of age and demographic, behavioral, and HIV-related risk factors at baseline; the relationship was somewhat attenuated with adjustment for baseline cardiometabolic risk factors (Supplementary Table 4). Other T-cell markers were not associated with CVD-related mortality (Supplementary Table 4).

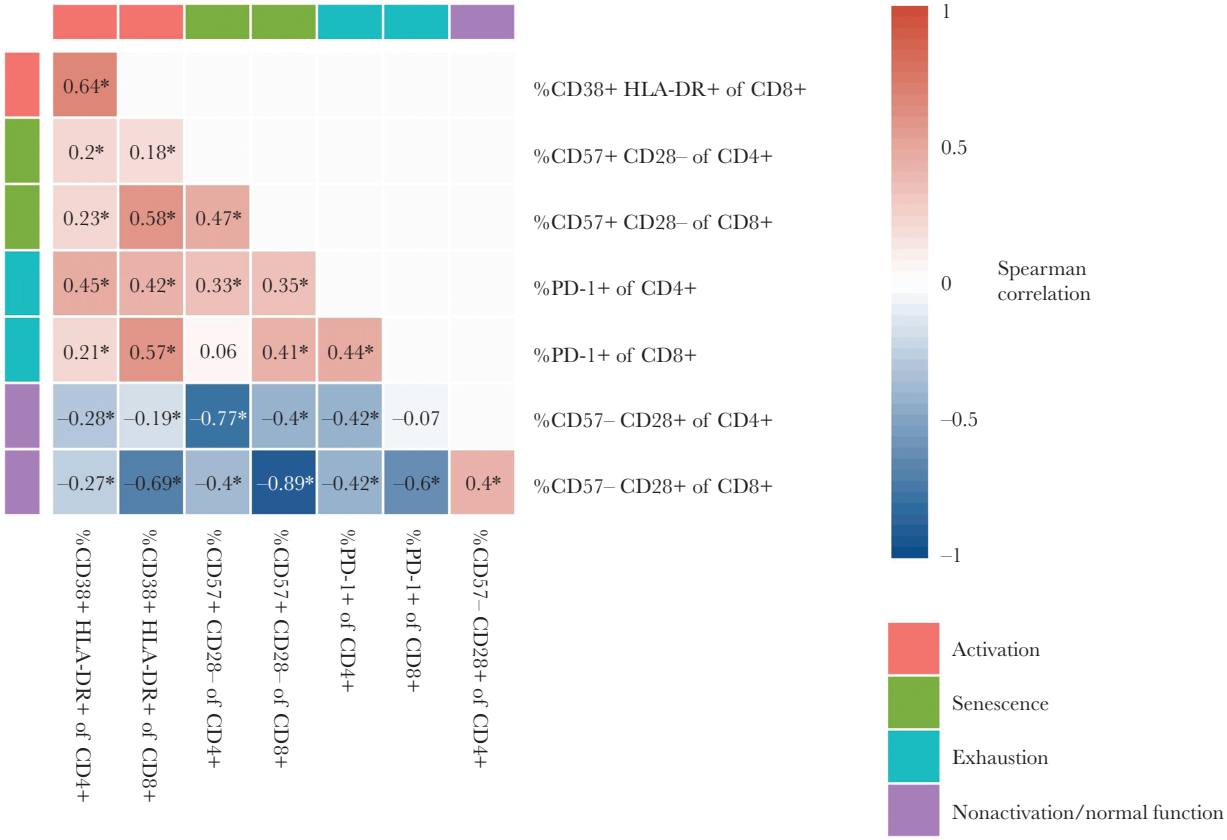

Figure 2. Spearman correlations among T-cell biomarkers in women with human immunodeficiency virus. Correlations (calculated among n = 606 women) are shown on the plot, with * indicating $P < .05$.

DISCUSSION

In this prospective cohort study of women with HIV in the US, $CD4^+$ T-cell activation and exhaustion were significantly associated with increased risk of natural-cause mortality, specifically non-HIV-related mortality, independent of cardiometabolic and HIV-related risk factors. Persistent $CD4^+$ T-cell activation and exhaustion may contribute to excess long-term mortality risk in women with HIV.

It has long been known that T-cell activation, especially of $CD8^+$ T cells, is associated with progression of HIV disease [27], even on ART treatment [2, 15]. $CD8^+$ T-cell activation on ART [15] and $CD4^+$ T-cell activation pre-ART [14] have been associated with all-cause mortality, independent of $CD4^+$ T-cell counts, in resource-limited settings, where infectious complications predominate in cause of death. However, the relationship of T-cell activation with non-HIV-related morbidity and mortality in people with HIV is not well understood. Previous work by our group and others in the WIHS has shown that, independent of HIV RNA load and $CD4^+$ T-cell count, $CD4^+$ T-cell activation was associated with carotid artery stiffness [28, 29], while $CD8^+$ T-cell activation was associated with carotid artery lesions [30]. Unlike our findings, $CD4^+$ and $CD8^+$ T-cell activation was not independently associated with risk of non-AIDS-defining events in the AIDS Clinical Trials Group (ACTG) [17, 31, 32], nor with natural-cause mortality in the Longitudinal Study of the Ocular Complications of AIDS (LSOCA) [16]. Similar to T-cell activation, T-cell senescence and exhaustion markers were not associated with non-AIDS events [17, 32] or natural-cause mortality [16] in previous studies.

Although these previous analyses in ACTG and LSOCA, both resource-rich settings, are not in agreement with our results, our study here differs in several ways. First, our study features a prospective design and uses survival analysis methods, rather than a nested case-control design, thus avoiding imperfect selection of controls and improving precision [33]. Our study also has a longer follow-up time (median, 13.3 years) than the ACTG (median, 3 years) and LSOCA (<1 year) analyses, allowing for greater capture of non-HIV-related deaths, which appeared to drive our findings. Last, our study population comprised only women, whereas the other studies comprised >80% men. While there is still much to learn regarding sex differences in HIV pathogenesis and clinical outcomes, emerging research suggests that premenopausal women may be protected from HIV viral replication by estrogen and/or progesterone [34, 35], and that the effect of HIV on CVD risk may be greater in women than in

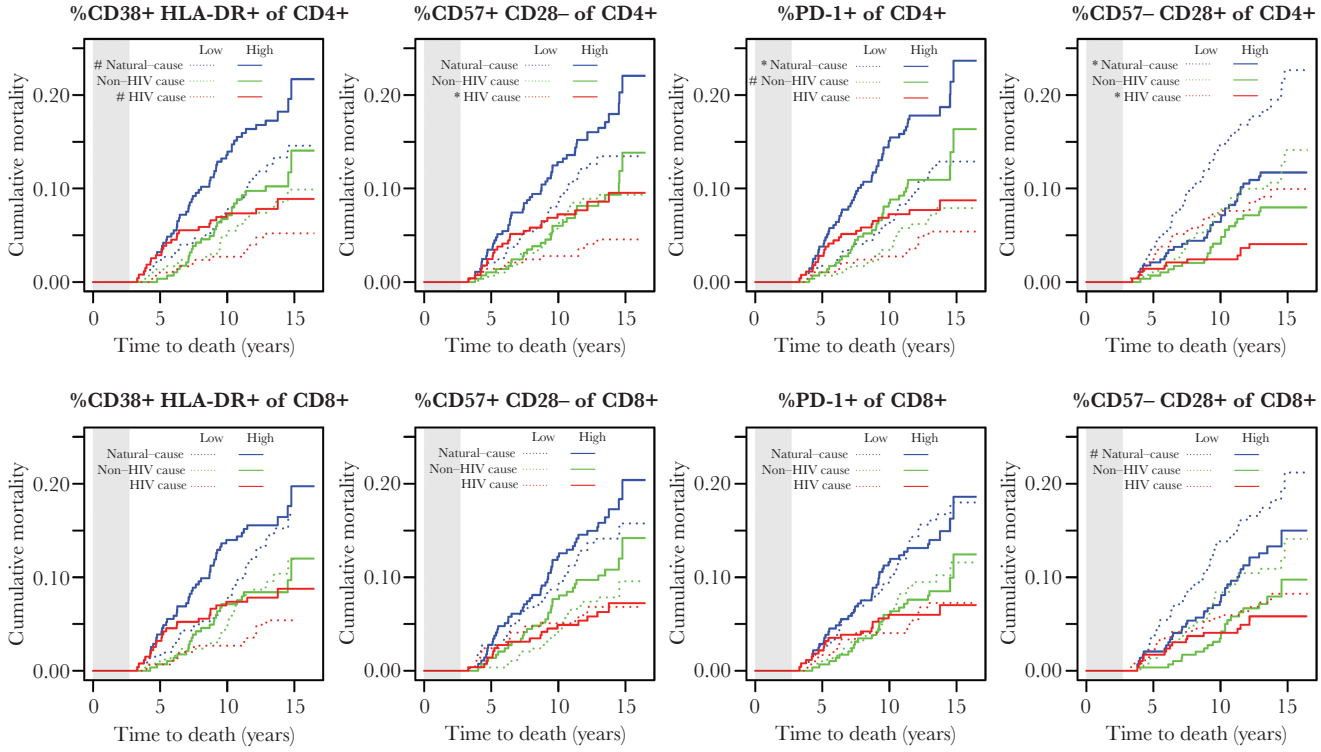

Figure 3. Cumulative mortality curves for binary T-cell biomarkers in women with human immunodeficiency virus (HIV). Unadjusted curves are shown for natural-cause, non-HIV-related, and HIV-related mortality among 606 women (90, 53, and 37 deaths, respectively). Each T-cell biomarker was dichotomized at the median. Shaded area represents the median time to the start of time at risk (2.7 years), accounting for the immortal time after sample collection. Log-rank tests were used to evaluate difference in survival for above vs below the median of a given biomarker. $^{*}P < .05$; $^{\#}P < .10$.

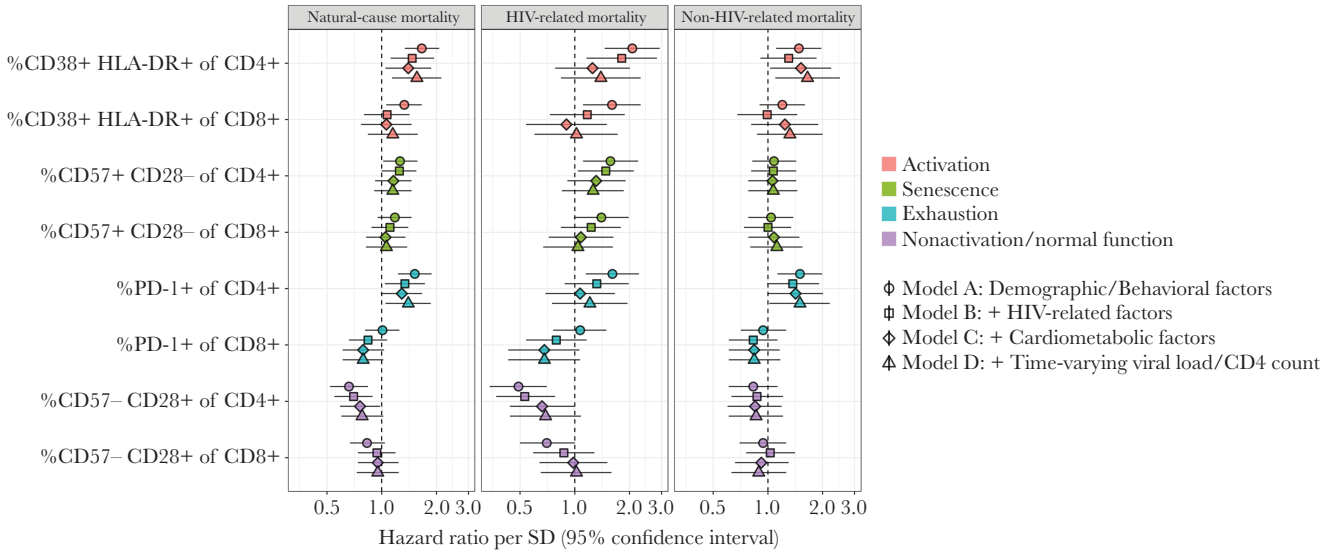

Figure 4. Multivariate-adjusted models of T-cell biomarkers and mortality in women with human immunodeficiency virus (HIV). Cause-specific Cox proportional hazards regression was used to assess the associations of $CD4^{+}$ and $CD8^{+}$ T-cell activation (%$CD38^{+}HLA\text{-}DR^{+}$), senescence (%$CD57^{+}CD28^{-}$), exhaustion (%$PD\text{-}1^{+}$), and nonactivation/normal function (%$CD57^{-}CD28^{+}$) with natural-cause mortality, HIV-related mortality, and non-HIV-related mortality. Immune markers were *z* score standardized; plot shows hazard ratios (95% confidence interval) for 1 standard deviation (SD) increase in the immune markers. Model A adjusts for age, study site, race/ethnicity, income, education, crack or cocaine use, injection drug history, viral hepatitis history, alcohol use, and smoking. Model B adjusts for all model A covariates and $CD4^{+}$ T-cell count, HIV RNA load, antiretroviral therapy, and AIDS. Model C adjusts for all model B covariates and body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, antihypertension medication, lipid-lowering medication, and diabetes. Model D is a time-dependent model, adjusting for the time-fixed covariates in model C as well as time-varying $CD4^{+}$ T-cell count and HIV RNA load.

men [9, 36, 37]. Future studies including both women and men with longer follow-up can uncover whether the associations we have observed of $CD4^+$ T-cell activation and exhaustion with non-HIV-related mortality are specific to women.

Persistent T-cell activation in chronic HIV infection leads to elevated levels of T-cell exhaustion, commonly measured by the PD-1 checkpoint inhibitor. The mechanistic role of T cells expressing PD-1 in HIV pathogenesis has garnered much interest, given the potential opportunity for use of anti–PD-1 immunotherapies in HIV treatment [38]. An accumulation of observational and in vitro studies have demonstrated that $CD4^+$ T cells expressing PD-1 are an important source of the latent HIV reservoir, contributing to HIV persistence on ART [3]. However, small case studies and phase 1 trials of PD-1 blockade have yielded mixed results regarding its ability to reverse HIV latency [38], and other trials are still ongoing [39]. Our observational study is the first to report a significant association of $CD4^+$ T-cell exhaustion with non-HIV-related mortality independent of baseline HIV-related and cardiometabolic factors; this finding could be related to PD-1–expressing $CD4^+$ T cells harboring latent HIV infection, or other mechanisms of T-cell dysfunction such as poor effector function [7].

T-cell senescence, on the other hand, was not significantly related to natural-cause, non-HIV-related, or HIV-related mortality in our study independent of baseline HIV-related and cardiometabolic factors. However, we did observe a marginally significant association of $CD4^+$ T-cell senescence with CVD-related mortality, defined as deaths with any CVD-related cause in the multiple causes of death (ie, regardless of underlying cause). This result is somewhat consistent with previous findings from our group of a relationship of $CD4^+$ T-cell senescence with carotid artery stiffness [29], though not with carotid artery lesions [30]. Since only half of CVD-related deaths in our study had CVD as the underlying cause, future studies with larger sample sizes will be necessary to more confidently determine the association of T-cell biomarkers with underlying CVD-cause mortality.

We also observed that nonactivation/normal function of $CD4^+$ T cells, indicated by $CD57^-CD28^+$ markers, was inversely associated with natural-cause mortality, and particularly HIV-related mortality. T-cell co-stimulatory molecules (eg, CD28) are necessary for optimal T-cell activation, proliferation, and survival [40]—antigenic activation of T cells via the T-cell receptor without co-stimulatory signaling leads to a state of T-cell anergy [3]. This is in line with a protective role of $CD28^+$, nonactivated, normally functioning $CD4^+$ T cells in HIV disease progression, as observed here.

Interestingly, all of our significant findings pertained to $CD4^+$ rather than $CD8^+$ T cells. This was unexpected, given the hypothesized role of $CD8^+$ T-cell activation in HIV depletion of $CD4^+$ T cells [2]. We found that $CD8^+$ T-cell activation was significantly associated with increased risk of HIV-related mortality in unadjusted analyses; however, this association was attenuated upon adjustment for HIV-related factors (including $CD4^+$ T-cell count), suggesting that the effects of $CD8^+$ T-cell activation are mediated through $CD4^+$ T-cell depletion, consistent with other studies in resource-rich settings [2, 16]. Our observed associations of $CD4^+$ T-cell activation and exhaustion with non-HIV-related mortality were not attenuated upon adjustment for baseline or time-varying $CD4^+$ T-cell count, suggesting that $CD4^+$ T-cell dysregulation pathways may operate independently of HIV disease progression to influence mortality.

Strengths of our study include the relatively large sample size, prospective design, long-term follow-up, and population of women with HIV who have been understudied in comparison to men. Additionally, the extensive data collected by the WIHS allowed us to adjust for many potential sources of confounding, including demographic, behavioral, HIV-related, and cardiometabolic factors, as well as biomarkers of inflammation (CRP), adaptive immune health (CD4/CD8 ratio), and coinfections (CMV and EBV antibody levels). Our study was limited by the single time-point of T-cell marker measures, and the insufficient number of deaths to further categorize into more specific underlying cause of death outcomes (eg, CVD, cancer). We lacked comprehensive data on additional biomarkers of inflammation, such as inflammatory cytokines, to better tease out effects of adaptive and innate immune dysregulation on mortality. Additionally, we relied on death certificate information to determine cause of death. While death certificates are the standard for reporting population-based mortality, there are well-known limitations in accuracy [41].

In summary, we observed that activation and exhaustion of $CD4^+$ T cells measured at 1 point in time were significantly associated with future risk of non-HIV-related mortality in women with HIV, independent of HIV disease progression. This result is in contrast with previous studies in predominantly male populations with HIV, in which null associations were observed for T-cell dysregulation markers and mortality [16, 17]. Future research should explore whether sex-specific mechanisms are involved in the relationship of $CD4^+$ T-cell immune dysregulation to non-HIV-related mortality. Meanwhile, ongoing studies in the area of immunotherapy (PD-1 and other checkpoint blockades) will reveal whether such treatment can improve health outcomes in people with HIV.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. The authors gratefully acknowledge the contributions of the study participants and dedication of the staff at the Multicenter AIDS Cohort Study/Women's Interagency HIV Study (WIHS) Combined Cohort Study (MWCCS) sites.

Disclaimer. The contents of this publication are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH).

Financial support. D. B. H., C. A. M., and R. C. K. were supported by the National Heart, Lung, and Blood Institute (grant number K01HL137557 to D. B. H.; grant number K23HL152903 to C. A. M.; grant number R01HL148094 to R. C. K.). K. G. M. was supported by the National Center for Advancing Translational Sciences (award number KL2TR001432). Data in this manuscript were collected by the WIHS, now the MWCCS. MWCCS (principal investigators): Atlanta clinical research site (CRS) (Ighovwerha Ofotokun, Anandi Sheth, and Gina Wingood), U01-HL146241; Baltimore CRS (Todd Brown and Joseph Margolick), U01-HL146201; Bronx CRS (Kathryn Anastos and Anjali Sharma), U01-HL146204; Brooklyn CRS (Deborah Gustafson and Tracey Wilson), U01-HL146202; Data Analysis and Coordination Center (Gypsyamber D'Souza, Stephen Gange, and Elizabeth Golub), U01-HL146193; Chicago–Cook County CRS (Mardge Cohen and Audrey French), U01-HL146245; Chicago-Northwestern CRS (Steven Wolinsky), U01-HL146240; Northern California CRS (Bradley Aouizerat, Jennifer Price, and Phyllis Tien), U01-HL146242; Los Angeles CRS (Roger Detels and Matthew Mimiaga), U01-HL146333; Metropolitan Washington CRS (Seble Kassaye and Daniel Merenstein), U01-HL146205; Miami CRS (Maria Alcaide, Margaret Fischl, and Deborah Jones), U01-HL146203; Pittsburgh CRS (Jeremy Martinson and Charles Rinaldo), U01-HL146208; University of Alabama at Birmingham (UAB)–MS CRS (Mirjam-Colette Kempf, Jodie Dionne-Odom, and Deborah Konkle-Parker), U01-HL146192; and University of North Carolina (UNC) CRS (Adaora Adimora), U01-HL146194. The MWCCS is funded primarily by the National Heart, Lung, and Blood Institute, with additional co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institute on Aging, National Institute of Dental and Craniofacial Research, National Institute of Allergy and Infectious Diseases, National Institute of Neurological Disorders and Stroke, National Institute of Mental Health, National Institute on Drug Abuse, National Institute of Nursing Research, National Cancer Institute, National Institute on Alcohol Abuse and Alcoholism, National Institute on Deafness and Other Communication Disorders, National Institute of Diabetes and Digestive and Kidney Diseases, and National Institute on Minority Health and Health Disparities, and in coordination and alignment with the research priorities of the NIH, Office of AIDS Research. MWCCS data collection is also supported by UL1-TR000004 (University of California, San Francisco Clinical and Translational Science Awards), UL1-TR003098 (Johns Hopkins University Institute for Clinical and Translational Research), UL1-TR001881 (University of California, Los Angeles Clinical and Translational Science Institute), P30-AI-050409 (Atlanta Center for AIDS Research [CFAR]), P30-AI-073961 (Miami CFAR), P30-AI-050410 (UNC CFAR), P30-AI-027767 (UAB CFAR), and P30-MH-116867 (Miami Center for HIV and Research in Mental Health).

Potential conflicts of interest. A. A. A. has received personal funds for consulting from Merck, ViiV, and Gilead; her institution has received funds from Merck and Gilead for her research. A. S. has received grant funding from Gilead Sciences, Inc. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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