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Association between short leukocyte telomere length and HIV infection in a cohort study; no evidence of a relationship with antiretroviral therapy

DeAnna L. Zanet, Anona Thorne, Joel Singer, Evelyn J. Maan, Beheroze Sattha, Armelle Le Campion, Hugo Soudeyns, Neora Pick, Melanie Murray, Deborah M Money, Hélène C.F. Côté, for the CIHR Emerging Team grant on HIV therapy and Aging: CARMA

From the Department of Pathology and Laboratory Medicine, University of British Columbia (D.L.Z., B.S., H.C.F.C), the CIHR Canadian HIV Trials Network (A.T., J.S.), the Department of Obstetrics and Gynecology (D.M.M., E.J.M.), the Department of Medicine (N.P., M.M.), Vancouver, British Columbia, Canada, and the Centre Hospitalier Universitaire Sainte-Justine and Department of Microbiology, Infectiology & Immunology, University of Montreal (A.LC., H.S.), Montreal, Quebec, Canada

Address reprint requests to Dr. Côté at the Department of Pathology and Laboratory Medicine, University of British Columbia, G227-2211 Wesbrook Mall, Vancouver, B.C., V6T 2B5, Canada or at helene.cote@ubc.ca

Alternate corresponding author: DeAnna.Zanet@gmail.com

Summary: HIV infection is independently associated with shorter leukocyte telomere length (LTL) but HIV disease and treatment factors are not, except having a peak viral load >100,000 copies/ml. Smoking and HCV infection also affect LTL in HIV-uninfected and HIV-infected individuals, respectively.

Abstract

Background. HIV-infected individuals appear to age faster than the general population, possibly related to HIV infection, antiretroviral therapy, and/or social/environmental factors. We evaluated leukocyte telomere length (LTL), a marker of cellular aging, in HIV-infected and uninfected adults.

Methods. Clinical data and blood were collected from CARMA cohort study participants. Variables important univariately were multivariate model candidates.

Results. Of the 229 HIV-infected and 166 HIV-uninfected participants, 76% were women, and 71% were current/previous smokers. In a multivariate model of all participants, older age ($p<0.001$), HIV infection ($p=0.04$), active HCV infection ($p=0.02$), and smoking ($p<0.003$) were associated with shorter LTL. An interaction was detected, whereby smoking was associated with shorter LTL in HIV-uninfected subjects only. Among those, age and smoking ($p\leq0.01$) were related to shorter LTL. In two HIV-infected individual models, age ($p\leq0.002$) and either active HCV infection ($p=0.05$), or peak HIV viral load ≥100,000 copies/ml ($p=0.04$) were associated with shorter LTL while other HIV disease or treatment parameters were unrelated.

Conclusions. Our results suggest that acquisition of HIV and viral load are primarily responsible for the association between HIV+ status and shorter LTL. The lack of association between LTL and time since HIV diagnosis, antiretroviral treatment, or degree of immune suppression would implicate HIV infection-related factors rather than disease progression or treatment. Smoking effects on LTL appear masked by HIV, while HCV infection may accelerate LTL shortening, particularly in co-infected individuals. The effect of early therapeutic intervention on LTL in HIV and HCV infections should be evaluated.

Introduction

The success of combination antiretroviral therapy (cART) has increased survival for persons living with human immunodeficiency virus (HIV) but age-associated diseases, including cardiovascular disease, diabetes, osteoporosis, and some cancers appear more prevalent in HIV-infected persons than in the general population [1-3]. This cohort study aims to investigate whether shorter leukocyte telomere length (LTL), a marker of cellular aging, might underlie this apparent accelerated aging.

Telomerase is responsible for the replication of telomeres which protect the ends of chromosomes [4]. While telomeres shorten with each cell division in most cells, telomerase activity contributes to maintenance of telomere length (TL) in germ cells, embryonic stem cells, tissue-specific adult stem cells, placenta, and activated immune cells [5]. TL is therefore considered a marker of cellular replicative history and/or remaining replication capability, and having short telomeres has been linked to many age-associated diseases [6]. HIV infection can lead to chronic immune activation, inflammation and oxidative stress [7]. In addition, HIV proteins can down-modulate expression of telomerase activity [8, 9]. HIV cART may also contribute to premature telomere shortening [10] through inhibition of the reverse transcriptase activity of human telomerase by nucleoside reverse transcriptase inhibitors (NRTIs) [11-13], or induction of oxidative stress by several antiretroviral agents [14, 15]. All these factors may affect telomeres and cellular aging and short telomeres have been reported in small studies ($n \leq 30$ per group) on lymphocyte subsets from HIV-infected individuals compared to those from uninfected persons [16-18]. Recently, one larger South African cohort study reported shorter LTL in HIV-infected individuals compared to non-infected controls, suggestive of accelerated biological aging in the HIV population [19]. Taken together, the combination of HIV infection and cART could contribute to premature aging in persons living with HIV through decreased telomerase activity and/or accelerated telomere shortening. We sought to investigate LTL in this population and the factors associated with shorter LTL.

METHODS

Study design and population

Study participants were HIV-infected and uninfected adults (>19 years) enrolled in the prospective CARMA cohort in Vancouver, Canada, between December 2008 and July 2011. All participants provided written informed consent. The study was approved by the University of British Columbia Clinical Research Ethics Board (H08-02018 and H09-02867). Blood samples, as well as relevant demographic, clinical, and behavioral data were collected. Whole blood relative LTL was measured by qPCR as previously described [20]. Hepatitis C virus (HCV) RNA and high sensitivity C-reactive protein (CRP) testing was done on plasma collected on the day of the visit. More details appear in the supplementary text.

Statistical analyses

Chi-square, Student's t-tests, and Wilcoxon rank sum tests were used to compare the groups' demographic and clinical characteristics at baseline. Univariate linear regression models were used to explore the following explanatory variables in relation to LTL: age, sex, HIV status (infected vs. uninfected), HCV infection status (active vs. cleared vs. never), household income (<$15,000/year vs. ≥$15,000/year), race (Black, Aboriginal, South Asian vs. White), smoking status (current vs. previous vs. never), smoking exposure (pack-years), alcohol (drink-years), illicit drug use ever (yes vs. no), body mass index, and CRP (>3 and ≥1 to ≤3 µg/ml vs. <1 µg/ml) , controlling for age as appropriate. Univariately important variables ($p<0.15$) were used as candidates in the development of a multivariate model. Collinearity between these candidates was determined by examination of contingency tables (Table S8) and multivariate models were then run including the strongly collinear

variables in turn. Variables which were highly non-significant in all models were eliminated going forward. Combinations of remaining variables were then tested, with variables retained in the model as long as the multivariate p-value was <0.1. Interactions between variables of interest were examined and were included in the models as needed. Finally, effect size estimate (β) confidence intervals for statistically non-significant variables were examined in an effort to determine whether we may have missed potentially important explanatory variables. Because paternal and maternal ages at birth were not initially collected and were unavailable for 43% of the participants, they were omitted during model development. Hepatitis B virus (HBV) infection was omitted because rare in our study population.

In a secondary analysis to examine the effect of HIV-related variables, we repeated this analysis twice, first including all HIV-infected participants, and then the more homogeneous subgroup of those on cART with undetectable HIV plasma viral load (pVL). HIV-related explanatory variables examined included time since HIV diagnosis, current and nadir CD4 count, current and peak HIV pVL (highest HIV pVL ever recorded dichotomized at 100,000 copies/mL), current cART status, lifetime duration of cART, type of cART at visit, number of cART regimens and NRTIs, number of cART interruptions >1 week, and percentage of time on cART since HIV diagnosis.

To further support our findings, we conducted a sensitivity analysis including only participants for whom biological father's age at birth was available. We built two multivariate models: one following the same "rules" as for the all-participants model with respect to variable inclusion, and a second with paternal age also included as a candidate variable. The descriptive statistics and other sensitivity analysis results are presented in the Supplement. Statistical analyses were performed using SAS software v9.3 (SAS Institute).

RESULTS

Characteristics of the study participants

For the 229 HIV-infected participants, median age was 40 years (range 20-76), while for the 166 HIV-uninfected participants, it was 38 years (range 20-73). The majority (76%) were women. There was no significant difference between the two groups with respect to age, sex, income, education, and plasma CRP level, and both groups showed similar rates of current smoking and illicit drug use. However, there was a lower proportion of Blacks in the HIV uninfected group and a higher proportion reporting alcohol consumption, while HIV-infected participants had younger parents (Table 1). The median lifetime cART duration was 4 years (range 0-20), 8% were cART-naive, and 39% had received four or more different cART regimens (Table 2).

Univariate regression analyses in all participants

Younger age, Black or South Asian race (*vs.* White), older parental ages at birth, and having never been infected with HIV, HBV or HCV were significantly associated with longer LTL among all participants (Figure 1, Table S1). While no association with sex was seen overall, among White participants, women had longer LTL than men (β=0.26, p=0.02). Low income, smoking (both current status and pack-years), illicit drug use ever, and alcohol drink-years were all associated with shorter LTL while higher CRP level was not.

Interactions and collinearity between variables

Repeating the univariate analyses in each group (Figure 1, Table S1) revealed a number of potential interactions: smoking status, illicit drug use and income <$15,000/year were associated with shorter LTL in HIV-uninfected individuals but not in persons living with HIV. Similarly, HIV infection and income <$15,000/year were only associated with shorter LTL in those who never smoked (data not shown). Finally, HIV infection status was associated with shorter LTL for younger subjects only. Because smoking, race, income, illicit drug

use, and HCV infection showed significant collinearity (see contingency tables in Supplement, Table S8) these variables were considered iteratively for the multivariate model.

HIV-specific explanatory variables

Univariately, having a peak HIV pVL ≥100,000 copies/mL was the only significant HIV-specific LTL predictor, and this remained true after adjusting for age (Figure 2, Table S2). Neither the time since HIV diagnosis, current HIV pVL, current CD4 count, CD4 nadir, nor length or type of cART exposure showed any association with LTL, before or after adjusting for age or HIV duration, as appropriate. The same was true among the subgroup of HIV-infected participants on cART with an undetectable HIV pVL (n= 126) except that the paternal age association was lost.

Predictors of shorter leukocyte telomere length

The final multivariate model (R^2 =0.25) showed that older age, smoking status, HIV infection, and active HCV infection were all independently significantly associated with shorter LTL (Table 3), even after taking into account the interaction between HIV and smoking described above. The magnitude of the effect size estimate (β) suggested the largest effect on LTL was in association with current smoking. HIV infection and active HCV infection effect size estimates were comparable to 24 (95% confidence interval [CI] 13-35) and 9 (1-17) years of aging respectively, although the former may be partly confounded by an age*HIV status interaction. Among all HIV-infected participants, older age and active HCV infection were significantly associated with shorter LTL, the latter's effect estimate comparable to over a decade of aging. An alternate model substituting a categorical variable indicating peak HIV pVL ≥100,000 copies/mL in place of HCV status appeared to predict LTL equally well (Table 3). Among HIV-uninfected participants, only age and smoking were independently associated with shorter LTL (Table 3). Examining β confidence intervals for statistically non-significant variables indicated a possible role for having a detectable HIV pVL at visit but our sample did not enable us to draw firm conclusions with respect to this variable (see Supplement text and Tables).

We performed a secondary analysis on a more homogeneous subgroup consisting of HIV-infected individuals on cART having an undetectable HIV pVL at the time of visit (Tables 1 and 2). The results were similar to those for all HIV-infected individuals (Tables S1, S2 and Table 3), except that active HCV was no longer associated with LTL. Finally, a sensitivity analysis among subjects with paternal age at birth data showed similar results as the final multivariate model (see Supplement Tables S3 to S7).

DISCUSSION

In this cohort of 395 HIV-infected and uninfected adults, after accounting for other important variables, we found that HIV infection and smoking (current more than previous) were strongly associated with shorter LTL. Our data further suggested that HCV infection also negatively affects LTL, particularly in HIV-infected individuals in whom peak HIV viremia also predicts shorter LTL.

HIV

The effect size estimate (β) on LTL for HIV infection is similar to that seen for over a decade of aging, or with current smoking. There was a lack of association between LTL and most HIV-specific factors except peak HIV viremia. Neither time since HIV diagnosis, or progression of HIV disease as measured by current or nadir CD4 count were related to LTL. Furthermore, despite in vitro and ex vivo data showing telomerase inhibition by NRTI [11-13, 21], we saw no evidence of a relationship between LTL and cART exposure, including cART duration, percentage of time on cART since HIV diagnosis, current treatment status, current type of cART, or number of cART interruptions. These results suggest that chronic inflammation (as reflected by CRP), and/or telomerase inhibition by cART do not adequately explain the association seen between HIV and shorter LTL. The fact that few study participants (22/163) were currently treated with the NRTIs showing highest telomerase inhibition in vitro, namely stavudine, zidovudine or didanosine [12], may explain the lack of

association between cART and LTL. Taken together, our observations would suggest instead that acquiring the HIV infection itself may exert a negative effect on LTL that neither significantly worsens nor improves with time or with cART, but that is rather modulated by HIV products or by the immune response toward HIV that take place early on following infection.

The mechanism behind shorter LTL in HIV-infected individuals remains unknown. A plausible explanation consistent with our findings could involve an early and at least partially irreversible loss of telomere in hematopoietic stem cells, the precursors of leukocytes, especially around the time of primary infection and establishment of HIV, possibly affecting the length of all blood cells' telomeres thereafter. HIV infection and HIV Tat have indeed been shown to down-modulate telomerase expression and activity in various blood cell subtypes [9, 22, 23].

Incidentally, shorter LTL, a predictor of cardiovascular disease particularly in younger individuals [24-26], could contribute to the reported association between HIV and increased cardiovascular risk [27, 28]. Endothelial progenitor cells are involved in neovascularisation and vascular tissue injury repair. Since these and leukocytes are both derived from hematopoietic stem cells, shorter LTL has been proposed to also reflect endothelial cell TL [29]. Furthermore, replenishment of leukocytes migrating during inflammation may also trigger increased replication of hematopoietic stem cells, hence shorten their telomeres (reviewed in [29]). Although we found no association between current CRP level and LTL at time of visit, it is possible that HIV-induced inflammation and endothelial activation [30], particularly at times of high viremia, could affect hematopoietic stem cells TL, further reducing LTL and endothelial progenitor cell function. Future studies in various blood cell subsets will be required to explore this hypothesis.

Alternatively, our data are also consistent with the possibility that individuals with short LTL are at higher risk of acquiring HIV. An intriguing preliminary study in healthy individuals showed increased resistance to experimentally induced infection by the common cold virus in participants with longer LTL [31]. Either suggested mechanism would also be consistent with reports of premature cardiovascular aging [28, 32] in this

population, irrespective of current HIV viremia, CD4 count or exposure to cART, and independent of traditional cardiac risk factors.

While other mechanisms such as inhibition of telomerase activity by NRTIs as well as HIV or cART-induced oxidative stress and inflammation may also contribute to telomere shortening in persons living with HIV, our data suggest that these are less important than HIV infection *per se* since HIV disease, cART-related variables and CRP showed no association with LTL. Nevertheless, the low R^2 of the multivariate model for the HIV-infected group suggests that important but as yet unidentified factors are at play in this population.

Hepatitis C virus infection

HCV infection was also associated with shorter LTL in this cohort, in agreement with other studies [33, 34]. This relationship may be linked to decreased lymphocyte telomerase expression or activity [34] and/or to chronic immune activation and inflammation that are reported increased in individuals with chronic HCV. Although we likely lacked power to distinguish between active and cleared HCV, our univariate results suggested a stronger effect in individuals with circulating HCV RNA. Given this, it would be of interest to investigate whether HCV therapy and HCV clearance would positively affect LTL.

Smoking

Our findings are in agreement with previous reports of shorter age-adjusted LTL in current smokers compared to non-smokers [35]. Indeed, smoking is a well-established risk factor in many disease states, including cardiovascular disease, and is especially prevalent in the HIV population [36]. Though some studies have observed an association between pack-years and LTL, we did not.

Both smoking and HIV infection elicit similar pathophysiological changes known to be linked with cardiovascular disease, including systemic oxidative stress, endothelial cell dysfunction, and reduced repair

capacity of endothelial progenitor cells. Taken together, although our results highlight the importance of smoking cessation interventions, other factors, identified or not, appear more strongly related to LTL shortening in HIV-infected individuals.

Other important factors

In general agreement with existing telomere literature, younger paternal age at birth, higher BMI, alcohol use, illicit drug use, and low income were all univariately associated with shorter LTL within the whole cohort. Of these, only paternal age emerged in the multivariate sensitivity analysis. Overall, sex was not associated with LTL, in apparent contrast to other studies reporting longer LTL in women compared to men [37]. While the high prevalence of substance use and HCV infection in our cohort may confound sex-related effects on LTL, it is noteworthy that previous cohort studies included primarily white individuals. Indeed, we also observed longer LTL in women when restricting our analysis to white participants. The univariate association observed between LTL and low income is consistent with reports of a positive relationship between LTL and socio-economic measures [38]. In our cohort, low income was correlated with lower education, substance use (smoking, alcohol, illicit drugs), and Aboriginal ethnicity, and likely behaved as a surrogate variable for a number of other factors reportedly linked to LTL, including poor lifestyle habits, poor nutrition, and lower healthcare access.

Strengths and limitations

A strength of this study is the fact that the HIV-infected and uninfected participants shared very similar socio-demographic characteristics, reducing the confounding effect of factors that differ between the HIV population and the general population. A further strength was its larger size compared to other studies of telomeres in HIV. Limitations include the incomplete data on parental ages, and the fact that a small number of HIV-uninfected participants may be unaware of their HCV infection. The best measure available to assess the effect of HIV duration was the HIV diagnosis date, but while highly correlated with HIV acquisition date (Figure S1), it is not equivalent to date of infection. A number of explanatory variables showed markedly different associations with

LTL according to HIV status or other variables and were therefore modified by them. Although we accounted for important interactions, it is possible that unidentified ones exist.

Cytomegalovirus (CMV) infection has been associated with shorter LTL and immunosenescence [39, 40] but CMV serology data were not available for this study. While our two groups are expected to have similarly high CMV infection rates, HIV-infected individuals may have higher CMV reactivation rates, possibly confounding our results. Cardiovascular risk could not be estimated due to the unavailability of complete lipid data and family history. Additionally, a number of factors such as exercise, psychological stress, life trauma, mental health, and marital status that have been linked to LTL were not evaluated in our study participants.

In conclusion, after controlling for important variables, HIV infection was independently associated with shorter LTL but cART was not. The HIV effect on LTL, which is consistent with the accelerated biological aging seen in HIV-infected individuals, was modified by smoking. Our results further suggest that the HIV effect is related to immune control of HIV viremia. Finally, HCV infection is also associated with shorter LTL in HIV-infected individuals, while in uninfected ones, smoking is more important.

Further research is needed to determine the clinical significance of shorter LTL and potential usefulness of measuring LTL in HIV-infected individuals as a marker of disease risk, particularly cardiovascular risk [3]. Investigating whether cART and HCV therapy early in infection would influence LTL dynamics, cardiovascular health and mortality is also needed.

NOTES

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Table 1. Participant characteristics at study visit[a].

	All Participants (n = 395)	Participants HIV uninfected (n = 166)	Participants HIV infected (n = 229)	P value[b] HIV Infected *vs.* uninfected	Participants HIV infected on cART with undetectable viral load (n = 126)	Participants HIV infected with detectable viral load (n = 88)
Age, median (IQR) [range], y	40 (32-47) [20-76]	38 (31-49) [20-73]	40 (33-49) [20-76]	.40	42 (34-47) [20-76]	37 (31-44) [20-60]
Female	299 (76)	118 (71)	181 (79)	.11	97 (77)	75 (85)
Race						
Aboriginal/First Nations	117 (30)	53 (32)	64 (28)		29 (23)	29 (33)
Black	39 (10)	1 (1)	38 (17)		21 (17)	14 (16)
South Asian	25 (6)	8 (5)	17 (7)	<.01	10 (8)	7 (8)
White	180 (45)	87 (52)	93 (41)		53 (42)	33 (37)
Other/Missing	34 (9)	17 (10)	17 (7)		13 (10)	5 (6)
Maternal age at birth, median (IQR) [range], y (n=210)	25 (21-30) [14-52]	27 (24-31) [15-40]	24 (20-29) [14-52]	<.01	25 (20-29) [14-52]	23 (20-28) [14-46]
Paternal age at birth, median (IQR) [range], y (n=226)	29 (24-35) [15-72]	32 (26-36) [17-48]	27 (23-34) [15-72]	<.01	28 (23-33) [15-72]	26 (22-34) [16-61]
Income <$15,000/y (n=369)	194 (49)	89 (54)	105 (46)	.64	62 (49)	44 (50)
High school graduate (n=366)	249 (63)	115 (69)	134 (58)	.48	78 (62)	46 (52)
HBV diagnosis ever (n=392)	25 (6)	4 (2)	21 (9)	<.01	10 (8)	9 (10)
HCV diagnosis ever (n=394)	136 (34)	48 (29)	88 (38)	.05	41 (32)	41 (46)

HCV infection status						
Active HCV infection	92 (23)	33 (20)	59 (26)	.17	27 (21)	26 (30)
Cleared HCV infection	42 (11)	15 (9)	27 (12)		13 (10)	14 (16)
BMI, median (IQR) [range] (n=374)	25 (22-29) [14-53]	24 (21-28) [14-53]	25 (22-29) [16-46]	.06	26 (22-29) [17-46]	25 (22-29) [16-40]
Smoking status (n= 391)						
Current smokers	195 (49)	86 (52)	109 (48)		44 (35)	55 (63)
Previous smokers	84 (21)	29 (17)	55 (24)	.24	37 (29)	17 (19)
Never smokers	112 (28)	51 (31)	61 (27)		42 (33)	16 (18)
Lifetime smoking — Pack-years, median (IQR) [range] (n=367)	3 (0-14) [0-160]	2 (0-13) [0-57]	4 (0-15) [0-160]	.31	3 (0-16) [0-89]	5 (0-15) [0-160]
Current drug users (daily-weekly) [range] (n=393)	153 (39)	68 (41)	85 (38)	.50	33 (26)	45 (51)
Lifetime ever illicit drug users (daily-weekly) (n=385)	223 (56)	94 (56)	129 (56)	.65	60 (48)	58 (66)
Ex-drug user (n=385)	70 (18)	26 (16)	44 (19)	.20	27 (21)	13 (15)
Current alcohol users (n=389)	227 (57)	110 (66)	117 (51)	<.01	60 (48)	50 (57)
Drink-years, median (IQR) [range] (n=332)	7 (0-42) [0-922]	7 (1-41) [0-922]	6 (0-43) [0-396]	.05	2 (0-43) [0-300]	8 (0-31) [0-396]
Ex-alcohol user (n=389)	107 (27)	47 (28)	60 (26)	.76	32 (25)	23 (26)
CRP[c] — (µg/ml), median (IQR) [range] (n=389)	1.4 (0.5-3.1) [0.5-40.1]	1.3 (0.5-3.3) [0.5-40.1]	1.4 (0.5-3.0) [0.5-40.1]	.77	1.2 (0.5-3.2) [0.5-40.1]	1.7 (0.6-2.9) [0.5-40.1]
CRP <1 µg/ml	162 (41)	73 (44)	89 (39)		55 (44)	27 (31)

CRP ≥1 to ≤3 µg/ml	128 (32)	48 (29)	80 (35)		36 (29)	39 (44)
CRP >3 µg/ml	99 (25)	43 (26)	56 (24)		32 (25)	21 (24)
Leukocyte telomere length, median (IQR) [range]	2.9 (2.6-3.4) [1.4-5.9]	3.0 (2.6-3.5) [1.7-5.0]	2.9 (2.6-3.3) [1.4-5.9]	0.03	2.9 (2.6-3.3) [1.4-5.8]	2.8 (2.5-3.2) [1.9-5.8]

Abbreviations: IQR, interquartile range; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; BMI, body mass index; CRP, C-reactive protein

[a]Data are presented as n (% of total) unless otherwise indicated. Number of subjects is included beside the variables for which data are not complete. Race was given by self-report. See Supplementary appendix for definitions of pack- and drink-years

[b] Characteristics were compared between groups using Chi-square, t or Wilcoxon rank sum test, as appropriate

[c] Samples below the lower limit of the assay (0.6 µg/ml, n=135) were assigned the value of 0.5 µg/ml while those above the higher limit of the assay(40 µg/ml, n=3) were assigned the value of 40.1 µg/ml. A total of 12 participants (4 HIV-infected and 8 HIV-uninfected) had CRP levels >20 µg/ml indicative of possible acute infection.

Table 2. HIV-specific characteristics for HIV-infected participants.

	All participants HIV infected[a] (n = 229)	Participants HIV infected on cART with undetectable viral load (n = 126)	Participants HIV infected with detectable viral load (n =88)
Mode of acquisition			
Heterosexual	112 (49)	56 (44)	40 (45)
Homosexual	7 (3)	5 (4)	1 (1)
Intravenous illicit drug use	41 (18)	17 (13)	20 (23)
Perinatal	3 (1)	1 (1)	2 (2)
Blood Products	7 (3)	6 (5)	1 (1)
Multiple Modes	31 (14)	26 (21)	18 (20)
Unknown or Not Disclosed	28 (12)	15 (12)	6 (7)
Time since HIV diagnosis, median (IQR) [range], y (n=225)	9 (6-13) [0-23]	10 (6-14) [1-22]	8 (4-12) [0-23]
CD4 nadir, median (IQR) [range], cells/mm^3 (n=228)	190 (90-270) [0-1110]	70 (90-240) [0-731]	200 (88-322) [0-1110]
CD4 count at study visit, median (IQR) [range], cells/mm^3	450 (295-630) [20-1570]	480 (340-630) [110-1150]	370 (218-580) [20-1570]
On cART at study visit	163 (71)	126 (100)	30 (34)
Peak HIV pVL ≥100,000, copies/mL (n=216)[b]	110 (48)	68 (54)	39 (44)
Time between HIV diagnosis and peak HIV pVL, median (IQR) [range], y (n=212)	3 (1-7) [0-19]	3 (1-7) [0-17]	4 (1-7) [0-19]
Undetectable HIV pVL at study visit (n=222)	134 (59)	126 (100)	0 (0)
Log HIV pVL, median (IQR) [range], copies/mL (n=222)	1.6 (1.6-2.7) [1.6-5.8]	---	3.4 (2.4-4.2) [1.6-5.8]
cART regimen at visit			
PI only	110 (48)	80 (63)	26 (30)
NNRTI only	40 (17)	36 (28)	2 (3)
PI and NNRTI	3 (1)	3 (2)	0 (0)
NRTI-sparing	2 (1)	2 (2)	0 (0)
Other	10 (4)	8 (6)	2 (3)

Lifetime cART duration, median (IQR), y	4 (1-8)	6 (2-10)	1 (0-6)
Number of different cART regimens (n=228)			
0	18 (8)	0 (0)	14 (16)
1	50 (22)	26 (20)	19 (22)
2	44 (19)	23 (18)	19 (22)
3	27 (12)	19 (15)	8 (9)
≥4	89 (39)	59 (47)	27 (31)
Number of different NRTI (n=228)			
0	18 (8)	0 (0)	14 (16)
1	1 (0)	0 (0)	1 (1)
2	71 (31)	39 (31)	26 (30)
3	28 (12)	16 (13)	12 (14)
≥4	110 (48)	72 (57)	34 (39)
Number of cART interruptions > 1 week (n=210)			
0	61 (27)	54 (42)	4 (4)
1	63 (28)	32 (25)	26 (30)
2	37 (16)	19 (15)	18 (20)
3	17 (7)	10 (8)	6 (7)
≥4	32 (14)	12 (10)	19 (22)
Percentage of time on cART since HIV diagnosis, median (IQR) [range]	42 (14-68) [0-100]	53 (31-83) [2-100]	20 (4-48) [0-96]

Abbreviations: IQR, interquartile range; cART, combination antiretroviral therapy; pVL, plasma viral load; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor

[a]Data are presented as n (% of total) unless otherwise indicated. Number of subjects is included beside the variables for which data are not complete.

[b] This represents the highest HIV pVL on record. Participants with missing data either initiated cART prior to HIV pVL testing (11/229), or no HIV pVL data were available (2/229), usually because treatment was initiated elsewhere.

Table 3. Multivariate analyses of the association between various factors and leukocyte telomere length in all participants and separated by HIV status.

	All Participants (R^2 = 0.25) (n = 388)		Participants HIV uninfected (R^2=0.40) (n = 166)		Participants HIV infected				Participants HIV infected with suppressed viral load (R^2 = 0.091) (n= 115)	
					Model 1 (R^2 = 0. 096) (n = 226)		Model 2 (R^2 = 0.074) (n = 216)			
Predictors	β value (95% CI)	P value	β value (95% CI)	P value	β value (95% CI)	P value	β value (95% CI)	P value	β value (95% CI)	P value
HIV status (infected *vs.* uninfected)	-0.50 (-0.73 to -0.27)	.04	---	---	---	---	---	---	---	---
Age (per 10 y)	-0.21 (-0.27 to -0.15)	<.001	-0.27 (-0.35 to -0.18)	<.001	-0.17 (-0.24 to -0.09)	<.001	-0.13 (-0.21 to -0.05)	.002	-0.14 (-0.24 to -0.03)	<.001
HCV infection status	---	.04	---	---	---	.08	---	---	---	---
Active HCV infection (*vs*. never)	-0.19 (-0.35 to -0.03)	.02	---	---	-0.19 (-0.38 to 0.0004)	.05	---	---	---	---
Cleared HCV infection (*vs*. never)	-0.16 (-0.37 to 0.04)	.12	---	---	-0.21 (-0.47 to 0.05)	.12	---	---	---	---
Smoking status	---	.003	---	<.001	---	---	---	---	---	---
Current smoking (*vs*. never)	-0.55 (-0.77 to -0.32)	<.001	-0.57 (-0.78 to -0.36)	<.001	---	---	---	---	---	---
Previous smoking (*vs*. never)	-0.40 (-0.69 to -0.12)	.005	-0.35 (-0.62 to -0.07)	.01	---	---	---	---	---	---
HIV status*smoking status interaction	---	<.001	---	---	---	---	---	---	---	---
Peak HIV pVL ≥100,000 copies/ml (*vs*. <100,000 copies/ml)	---	---	---	---	---	---	-0.17 (-0.34 to -0.006)	.04	-0.24 (-0.46 to -0.02)	.04

Other variables that were considered during modeling include: race, HBV infection ever, BMI, income, pack-years, drink-years, and illicit drug use. These models did not include paternal or maternal age.
Abbreviations: HCV, Hepatitis C Virus; HBV, Hepatitis B virus; BMI, body mass index; pVL, HIV plasma viral load

Figure Legends

Figure 1. Univariate analyses of the association between possible predictors and leukocyte telomere length. β values and 95% confidence intervals are shown for possible predictors sorted according to their association with LTL: associations with shorter LTL show negative β values. Variables in italics have been adjusted for age at visit as appropriate. Analyses were separated by group: all subjects (light grey triangle), HIV uninfected subjects (dark grey circle) and HIV infected subjects (black square). P values shown are p<0.001 (**), p<0.05 (*) and p<0.15 (†); all variables with p<0.15 (†) were considered in the multivariate modeling.

HCV, hepatitis C virus; HBV, hepatitis B virus; BMI, body mass index

Figure 2. Univariate analyses of the association between HIV-related factors and leukocyte telomere length. β values and 95% confidence intervals are shown for possible predictors sorted according to their association with LTL: associations with shorter LTL show negative β values. Variables in italics have been adjusted for time since HIV diagnosis or age at visit as appropriate. P values shown are p<0.05 (*) and p<0.15 (†); all variables with p<0.15 (†) were considered in the multivariate modeling.

pVL, plasma viral load; cART, combination antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor