### Title

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# TELOMERE LENGTH IS ASSOCIATED WITH HIV INFECTION, METHAMPHETAMINE USE, INFLAMMATION, AND COMORBID DISEASE RISK

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## Abstract

**Background:** HIV infection and methamphetamine dependence (METH) are each associated with inflammation and premature aging, but their impact on biological aging is difficult to measure. Here we examined the impact of HIV and METH on leukocyte telomere lengths (LTL), and the correlations between LTL and other aging biomarkers.

**Methods:** The study was a cross-sectional analysis of 161 individuals categorized by HIV and methamphetamine (METH) dependence status into four groups: HIV−METH− (n=50), HIV −METH+ (n=29), HIV+METH− (n=40), and HIV+METH+ (n=42). We analyzed the relationships of leukocyte telomere length (telomere to single copy gene [T/S] ratio) with demographic and

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JEI provided analyses for the manuscript.

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RH and IG critically edited the manuscript and provided the data used for the analysis.

Author Disclosures

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clinical data as well as a panel of biomarkers of inflammation and endothelial activation measured in blood and cerebrospinal fluid (CSF).

**Results:** HIV and METH were independently associated with shorter T/S ratio, even after adjusting for demographics and leukocyte count ($R^2$=0·59, p<0·0001). Higher plasma C-reactive protein (p=0·0036) and CSF VCAM-1 (p=0·0080) were also associated with shorter T/S ratio. A shorter T/S ratio was associated with higher risk for cardiovascular disease (p<0·0001) and stroke (p<0·0001), worse motor functioning (p=0·037) and processing speed (p=0·023), more depressive symptoms (p=0·013), and higher CSF neurofilament-light (p=0·003).

**Conclusions:** HIV and METH dependence were each associated with shorter telomeres. After adjusting for demographics, HIV, and METH, T/S ratio remained associated with aging-related outcomes including neurocognitive impairment, neurodegeneration, risks of cardiovascular disease and stroke. While not establishing causality, this study supports using the T/S ratio as a biomarker for estimating the impact of HIV and comorbidities on long-term health.

### Keywords

HIV; Methamphetamine; Telomeres; Aging; Cardiovascular

## 1. Introduction

With the remarkable achievements in antiretroviral therapy, life expectancy of people living with HIV (PWH) is approaching that of the general population.(Gueler et al., 2017) However, some studies suggest that PWH are developing “age associated” comorbidities, such as cardiovascular disease and neurocognitive decline, at younger ages compared to uninfected adults. This premature aging is thought to be due to a combination of HIV-associated chronic inflammation, viral effects from persistent low-level HIV replication, antiretroviral therapy (ART)-related toxicities on aging-related biologic pathways(Nasi et al., 2017), and higher prevalence of negative social determinants of health.

Across the United States, the prevalence of addictive drug use is higher among PWH than the general population. In San Diego, as in many other parts of the country, methamphetamine is one of the more commonly used addictive drugs, particularly among men who have sex with men (MSM). Methamphetamine has multiple adverse effects on human physiology. In the brain, methamphetamine dysregulates serotonin and dopamine release; activates glutamate receptors and astrocytes leading to increased production and release of pro-inflammatory cytokines (e.g., interleukin-6, tumor necrosis factor-α); and increases blood-brain barrier permeability also leading to central nervous system (CNS) inflammation.(Loftis et al., 2011; Yamamoto and Raudensky, 2008) Systemic immune activation is also seen with methamphetamine use (e.g., elevated soluble CD14, C-reactive protein). (Carrico et al., 2018) The end result of these processes in the CNS is neurodegeneration, a process that also occurs with physiologic aging.(Good and Radcliffe, 2011; Nakama et al., 2011) The proinflammatory state and the catecholamine surges induced by methamphetamine also adversely affect the vascular system resulting in cardiomyopathies, myocardial infarctions and strokes, outcomes that increase in frequency with age.(Kaye et al., 2007; Paratz et al., 2016) In addition to the vascular and central

nervous systems, methamphetamine may also increase the frequency of many other adverse age related outcomes. (Jones and Rayner, 2015; Tomita et al., 2014)

While extrinsic factors (e.g. extent of methamphetamine use, medical adherence) may also influence the risk of age-related comorbidities, these impacts can be challenging to accurately quantify. Genetic and epigenetic changes can be used to estimate “biologic” age. (Horvath, 2013) These changes can predict cardiovascular, (Sharifi-Sanjani et al., 2017) renal, (Mazidi et al., 2017b) and neurocognitive (Liu et al., 2018) age-related outcomes. Telomeres are the protective DNA complexes at the termini of eukaryotic chromosomes, and shortening of these DNA segments are associated with maladaptive physiologic changes. (Blackburn et al., 2015) Telomere attrition and reductions in telomerase activity occurs with aging in humans, and shortened telomeres are associated with aging-related outcomes. (Fali et al., 2019) Here we examined the impact of HIV infection and methamphetamine use on leukocyte telomere length, hypothesizing that they would negatively impact this measure of biologic aging.

## 2. Methods

### 2.1 Study Cohort

This study was performed in the observational cohort of the Translational Methamphetamine AIDS Research Center (TMARC), which is a multi-center project that focuses on understanding the combined effects of HIV and methamphetamine dependence on brain structure and function. The research performed by TMARC is approved by the University of California San Diego Human Research Protections Program and local IRBs at the sites. Clinical, demographic, and laboratory assessments were obtained from 170 TMARC participants who were categorized by HIV and methamphetamine (METH) dependence status into one of four groups: HIV−/METH− (n = 50), HIV−/METH+ (n = 29), HIV+/METH− (n = 40), and HIV+/METH+ (n = 42). All participants provided written informed consent, including host genetic analysis. HIV infection was confirmed by the MedMira Rapid HIV Antibody Test (MedMira, Halifax, Canada). The presence and history of METH use disorders were determined via the DSM-V criteria for METH abuse and dependence as assessed by the Composite International Diagnostic Interview Version 2·1 [CIDI]. (Organization, 1997) Participants who met criteria for both lifetime METH dependence and either METH abuse or dependence within the past 18 months were included (as defined in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [DSM-V]). (Association, 2013) Exclusion criteria included history of psychosis (e.g., schizophrenia), significant medical (e.g., hepatitis C infection) or neurological (e.g., head injury with loss of consciousness for more than 30 minutes, seizure disorder, stroke) conditions known to affect cognitive functions (determined by self-report and medical records when available). To minimize the confounding effects of acute drug use (intoxication and withdrawal), participants who tested positive for addictive drugs other than METH or cannabis were also excluded. Finally, all HIV+ participants were on suppressive ART with HIV RNA ≤ 200 copies/m.

**Clinical and Laboratory Measures—**Laboratories obtained at the baseline visit included serum electrolytes, fasting glucose, creatinine, total protein, albumin, and fasting lipid profile as well as blood leukocyte, erythrocyte, and platelet number. Glucose, protein, leukocytes, and erythrocytes were also measured in cerebrospinal fluid (CSF) in the subgroup who successfully underwent lumbar puncture (n = 91, 19 HIV−METH−, 19 HIV −METH+, 29 HIV+METH−, 24 HIV+METH+). In both plasma and CSF, an additional panel of biomarkers of inflammation and endothelial activation were measured by immunoassay: C-reactive protein, soluble CD14, soluble tumor necrosis factor receptor (sTNFR)-II, interleukin (IL)-6, CXCL10, monocyte chemoattractant protein (MCP-1)/ CCL2, vascular endothelial growth factor (VEGF), soluble urokinase-like plasminogen activator receptor (suPAR), soluble intercellular adhesion molecule (sICAM)-1, and soluble vascular cell adhesion molecule (sVCAM)-1. Most soluble biomarkers were measured by multiplex assays from Meso Scale Discovery (C-reactive protein, sTNFR-II, IL-6, CXCL10, CCL2, VEGF, suPAR, sICAM-1, and sVCAM-1). Soluble CD14 was measured by traditional immunoassay (R&D Systems). Neuron axonal injury was assessed by measuring neurofilament-light (NFL) in CSF, also by immunoassay (Tecan). In PWH, CD4+ T-cells were counted by flow cytometry and HIV RNA was measured by real-time PCR (Roche Amplicor, lower limit of quantification 40 copies/mL). The antiretroviral regimen and its duration were also recorded for PWH.

## 2.2 Functional and Risk Status Measures

Baseline functional status was measured using the Karnofsky Performance Scale Index and the Fried Frailty Index. (Fried et al., 2001) Vascular disease risk was calculated using the Framingham Cardiovascular Disease (CVD) Risk Score and the Framingham Stroke Risk Score.

## 2.3 Neuropsychological assessments

At the baseline visit, TMARC's standardized neuropsychological testing battery assessed seven cognitive domains commonly impacted by HIV and METH: motor functioning, executive functioning, processing speed, learning, recall, working memory/attention, and verbal fluency. Raw test scores were transformed into demographically-corrected T-scores after adjusting for effects of age, education, sex, and ethnicity. T-scores were averaged within each domain to form domain-specific T-scores and averaged across the entire battery to yield a Global T-Score, as previously described. (Blackstone et al., 2012)

## 2.4 Measuring Leukocyte Telomere Length

Leukocyte telomere length (LTL) was measured at baseline from frozen whole blood stored at −80°C using qPCR, as previously reported, (Cawthon, 2002) and was expressed as the ratio of telomere signal vs. a single copy gene signal, T/S. Assays were performed twice, each in triplicate wells. When the two values differed by more than 7%, a third run was done. The average of the two closest runs were used in the final analysis. The inter-assay coefficient of variation for this study was 2·3%.

### 2.5 Estimating Biological Age

We estimated biological age by first developing a model to predict chronologic age using LTL, adjusting for demographic characteristics (age, sex, race/ethnicity, and education) and leukocyte count or to these variables along with others identified in Table 2 (e.g., BMI). We performed these multivariable regressions stratified by the four groups defined by HIV and METH (HIV−METH−, HIV−METH+, HIV+METH−, HIV+METH+). Biological age was then estimated by inserting the adjusted T/S ratio that resulted from the multivariable regression of each risk group (HIV−METH+, HIV+METH−, HIV+METH+) into the regression equation for the reference group (HIV−METH−) and solving the equation for age.

### 2.6 Statistical Analysis

We examined the impact of HIV serostatus and METH dependence on leukocyte telomere to single copy gene ratio [T/S], adjusting all analyses for age, sex, education, race/ethnicity, and leukocyte count (factors that may affect T/S). (Diez Roux et al., 2009; Gardner et al., 2014; Mazidi et al., 2017a; Needham et al., 2013) To determine whether the independent effects of HIV and METH on T/S were attenuated by clinical laboratory factors, multivariable regression was performed using the Akaike Information Criterion and backward selection. Candidate clinical laboratory factors qualified for inclusion as covariates if the p value for their association with T/S ratio, adjusting for demographic factors and white blood cell count, was ≤ 0·15. We then examined the association of T/S with biomarkers of inflammation and endothelial activation, as well as aging disease-related measures (e.g. Framingham risk scores, Fried Frailty Phenotype) using multivariable regression. Most analyses were again adjusted for age, sex, education, race/ethnicity, and leukocyte count based on published findings on telomere length.(Diez Roux et al., 2009; Gardner et al., 2014; Mazidi et al., 2017a; Needham et al., 2013) The exceptions were disease-related measures that included demographic data in their calculation. Specifically, Framingham risk scores include age and sex and neuropsychological testing T-scores account for age, sex, race/ethnicity, and education. For these variables, these demographic factors were not included in initial analyses but were added as a final step to assess if they influenced findings. The false discovery rate (FDR) approach was applied in the primary analyses of the correlates of T/S ratio to account for type I error. Finally, mediation analyses were performed to see if T/S ratio mediated the impact of demographic variables on biomarkers of inflammation and aging disease-related measures using the 3-step approach outlined by Baron and Kenny (1986).

## 3. Results

### 3.1 Participant Characteristics and Correlates of Telomere Length

Table 1 summarizes the characteristics of the 161 participants. The four groups defined by HIV and METH (i.e., HIV−METH−, HIV−METH+, HIV+METH−, HIV+METH+) differed in multiple characteristics, including age, sex, education, Framingham Stroke Risk, Frailty Phenotype, Beck Depression Inventory, and T/S Ratio. The two HIV groups were generally similar in demographic and disease characteristics, with the exception of HIV+METH+ individuals having shorter estimated duration of HIV infection (p=0·04) and higher CD4+ T-

cell count at the time of assessment than non-users (p=0·01). METH groups were younger (p=0·005); more likely to be men (<0·001); had fewer years of education (<0·001); more likely to be Hispanic (p=0·003); less likely to be black (p=0·037); and had more depressive symptoms than non-users (<0·001), but still only had predominantly mild depression. Although race and ethnicity do impact telomere length, this effect is similar for blacks and Hispanics, (Diez Roux et al., 2009). Therefore we adjusted for race by the presence or absence of European ancestry.

HIV groups also had shorter unadjusted T/S ratios than uninfected groups (p=0·003). To further investigate these differences, subsequent analyses were adjusted for the influence of age, sex, education, race/ethnicity, leukocyte count. Table 2 summarizes the analyses of individual variables and multivariable models of either HIV and METH alone (with adjusting covariates) or with other covariates selected by the Akaike Information Criterion. In the initial multivariable model that included demographic variables, leukocyte count, HIV, and METH, both HIV and METH were statistically significantly associated with shorter telomere length. We then fixed HIV status and METH status in the model and tested other covariates for statistical significance. The best multivariable model included HIV and METH, as well as body mass index (p=0·013), serum globulins (p=0·0024), and serum creatinine (p=0·027) (model $R^2$=0·59, p<0·0001). An interaction term between HIV and METH was not statistically significant (p=0·92). The direction of the associations with telomere length were all in the hypothesized direction (i.e., shorter telomeres were associated with HIV, METH, and higher BMI, globulins, and creatinine). Supplemental Figure 1 displays the relationships between age and T/S ratio stratified by HIV and METH group either unadjusted (Supplemental Figure 1a), adjusted for demographics and leukocyte count (Supplemental Figure 1b), or fully adjusted for other covariates in the best multivariable model in Table 2 (Supplemental Figure 1c). These additional variables were included in the final model as they could reflect other pathophysiologic processes that impact telomere length.

Figure 1 summarizes additional analyses of the influence of HIV and METH on estimated biological age relative to the reference group (HIV−METH−). Figure 1a reinforces the finding from Table 1 that within this cohort, the METH users had younger chronological age than non-users and is shown here to provide a ready comparison to the following figures. The resulting estimated biological age was subtracted from chronological age to yield the data in Figures 1b and 1c. Each risk condition (HIV, METH) resulted in incremental increases in estimated biological age, with a median increase in biological age of 22·3 years (IQR 16·6–27·0 years) in the HIV+METH+ group in the fully adjusted model.

Additional analyses were performed in the HIV groups. These analyses added AIDS diagnosis, nadir and current CD4+ T-cell count, current CD8+ T-cell count, estimated duration of infection, and duration of ART exposure to the list of potential covariates. We did not consider different ART regimens as many participants had prior exposure to multiple types of ART. All p values were > 0·15, except for current CD8+ T-cell count (p=0·106). In models that adjusted for demographic variables and leukocyte count, the best multivariable model identified that shorter T/S ratios were associated with METH dependence (p=0·024),

larger BMI (p=0·023), higher serum creatinine (p=0·018), and higher CD8+ T-cell count (p=0·056) (model $R^2$=0·55, p<0·0001).

### 3.2 Relationship of HIV infection, METH use, and T/S Ratio with Disease-Associated Biomarkers

Aging, HIV, and METH are each associated with multiple biological processes, including inflammation and endothelial activation. In the preceding analysis, we noted that higher serum globulins, which include immunoglobulins and may be an indicator of inflammation, were associated with shorter T/S ratio. To further evaluate if T/S ratio, an indicator of biological aging, was associated with other disease-related biomarkers, we compared it to a panel of biomarkers reflecting inflammation and endothelial activation (as well as neuronal injury in the following section). In analyses of individual biomarkers adjusting for demographic covariates and leukocyte count, a shorter T/S ratio was associated with higher CRP (plasma Figure 2a] and CSF), CXCL10 (CSF), and sVCAM-1 (CSF, Figure 2b) (Supplemental Table 1). Multivariable analyses that also included HIV and METH identified that only plasma CRP and CSF sVCAM-1 were associated with T/S ratio (model $R^2$=0·62, p<0·0001). Multivariable models were then constructed to evaluate if T/S ratio mediated the association of other variables (age, sex, race/ethnicity, education, HIV, METH) on these markers of disease and inflammation. T/S ratio was found to at least partially mediate the relationships between age and CCL2 (effect size[ES]: 0·31, p<0·001 to ES: 0·12, p=0·2) and between age and CSF sVCAM-1 (ES: 0·38, p<0·001 to ES: 0·14, p=0·2), and HIV on CCL2 (ES: 0·23, p=0·006 to ES: 0·12, p=0·12).

### 3.3 Impact on Aging-Related Outcomes

A lower T/S ratio was associated with higher Framingham CVD (Figure 2c) and Stroke (Figure 2d) risk scores (all p values <0·001) and frailty status (p = 0·021, Figure 2i) in unadjusted analyses (Table 3). A lower T/S ratio was also associated with higher CSF NFL levels (p<0·001, Figure 2e), a biomarker of neurodegeneration. After adjustment for demographic characteristics, leukocyte count, HIV, and METH, T/S ratios remained associated with both Framingham scores and NFL, but not frailty status. We also compared T/S ratio to neurocognitive performance and Beck Depression Inventory values, identifying associations between shorter T/S ratios and worse processing speed (Figure 2f), worse motor performance (Figure 2g), and more depressive symptoms (Figure 2h). To determine if the T/S ratio mediated the impact of these variables on physiologic aging outcomes, we constructed several multivariate models. T/S ratio also at least partially mediated the impact of sex on the Framingham CVD score (ES: −0·25, p=0·003 to ES: −0·078, p=0·3), and HIV status on the Framingham Stroke Risk score (ES: 0·16, p=0·06 to ES: 0·041, p=0·6), respectively.

## 4. Discussion

This cross-sectional study demonstrates that HIV infection and METH dependence independently and adversely affect the leukocyte T/S ratio, a biomarker associated with aging-related outcomes. This measure of aging is associated with clinical (e.g. increased mortality with malignancy) (Callahan et al., 2017), cardiovascular disease(Haycock et al.,

2014) and functional outcomes (e.g. frailty) (Haapanen et al., 2018) in the general population. These associations remained statistically significant even after adjusting for the demographic factors that can influence telomere length. (Gardner et al., 2014; Mazidi et al., 2017a; Needham et al., 2013) The findings suggest that the T/S ratio may provide a quantitative measure of the impact of HIV infection, METH dependence, and other comorbidities on long-term health.

The causes of death among PWH have changed with the evolution of ART. Currently, among virally suppressed PWH in the developed world, non-AIDS defining cancers, liver disease, and cardiovascular disease are the most common causes of death.(Farahani et al., 2017) Cardiovascular disease and cancer may also be the major causes of morbidity among persons with METH dependence. (Herbeck et al., 2015) Among PWH, chronic persistent inflammation may mediate the impact of HIV on the increased mortality observed in this population. Similar to other published work, we found evidence that chronic inflammation may also be mediating the impact of HIV infection and METH dependence on telomere length. (Weng, 2008) Here we observed that elevated globulins in the blood were associated with shorter telomere lengths, even after adjusting for HIV infection and METH dependence. Similarly, plasma CRP, another measure of the inflammatory state, and CSF sVCAM-1, an indicator of endothelial activation and a downstream product of inflammation, were also independently associated with shorter telomere length.

Importantly, we also found that the leukocyte T/S ratio remained associated with aging disease-related outcomes, such as indicators of cardiovascular and central nervous system disease, after adjusting for demographic factors, leukocyte count, HIV serostatus, and METH dependence. Moreover, our findings suggest an association between telomere length and poorer neurocognitive performance, which is consistent with research in healthy adults and in other clinical populations. (Yaffe et al., 2011) Our findings were specific to neurocognitive domains of processing speed and motor skills, two domains that decline independently with age and can subsequently contribute to poorer performance on higher level cognitive tasks. (Ebaid et al., 2017) Mechanisms underlying this association could relate to the aforementioned factors (e.g., cardiovascular disease), as well as other neurovascular and/or inflammatory mechanisms. Therefore, as a biomarker, the T/S ratio may be a useful as a summary predictor of age-related comorbidity and may help identify individuals at risk for neurocognitive decline. Such a marker could also be used to identify PWH who might benefit from services to support medical care, social engagement, and independent living.

While the described relationships are all associations given the cross-sectional study design, and do not imply causality, the transitive relationships between HIV and methamphetamine use T/S ratio, and T/S ratio with clinical, physiologic and functional outcomes suggest an important link between HIV (or its treatment), methamphetamine use and premature aging. Limitations of this study include an imbalance between groups by size (HIV−METH+ group was smaller), age, BMI and sex, and the potential for unmeasured confounders such as host genetics, diet, etc… These differences limit our ability to fully adjust for these factors. T/S ratios also vary by cell type, and shifts in cell populations could also impact T/S ratios. Also, other measures of biologic aging, such as DNA methylation, have similarly shown age

acceleration in PWH, although the magnitudes may differ than what was seen in this study. (Gross et al., 2016) Further work is needed to understand the basic mechanisms driving these pathologies so that appropriate interventions can be designed.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Highlights

- Leukocyte telomere length (LTL) is associated with aging-related outcomes
- HIV and METH dependence independently and adversely affect LTL
- LTL may estimate the risk of aging related comorbidities in PWH +/− METH dependance
- LTL may also help identify individuals at risk for neurocognitive decline

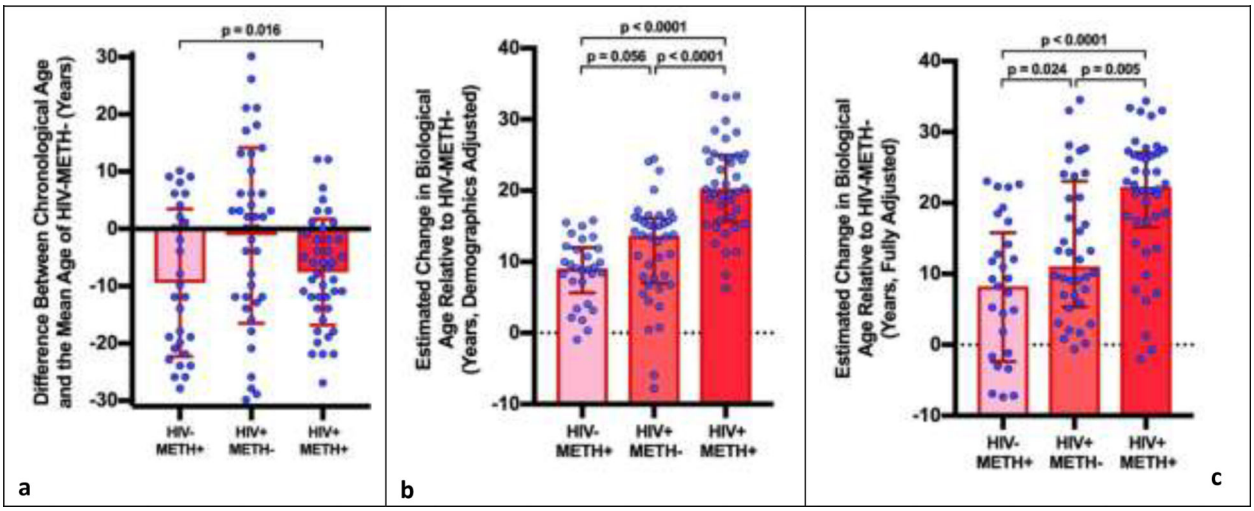


**Figure 1. Chronological and Estimated Biological Age by Group.**

The chronological age of METH users in the cohort is younger than the HIV−METH− group but their biological age is older in fully adjusted models, indicating an adverse effect of METH on biological aging. HIV was independently associated with older estimated biological age. a) Difference between the chronological age of members of the three risk groups and the mean age of the HIV−METH− group. b) Estimated biological age relative to the HIV−METH− group adjusted for demographic covariates (chronological age, sex, race/ethnicity, education) and leukocyte count. c) Estimated biological age relative to the HIV−METH− group fully adjusted for all covariates in the best multivariable model (adds BMI, Globulins, and Creatinine to the prior model).

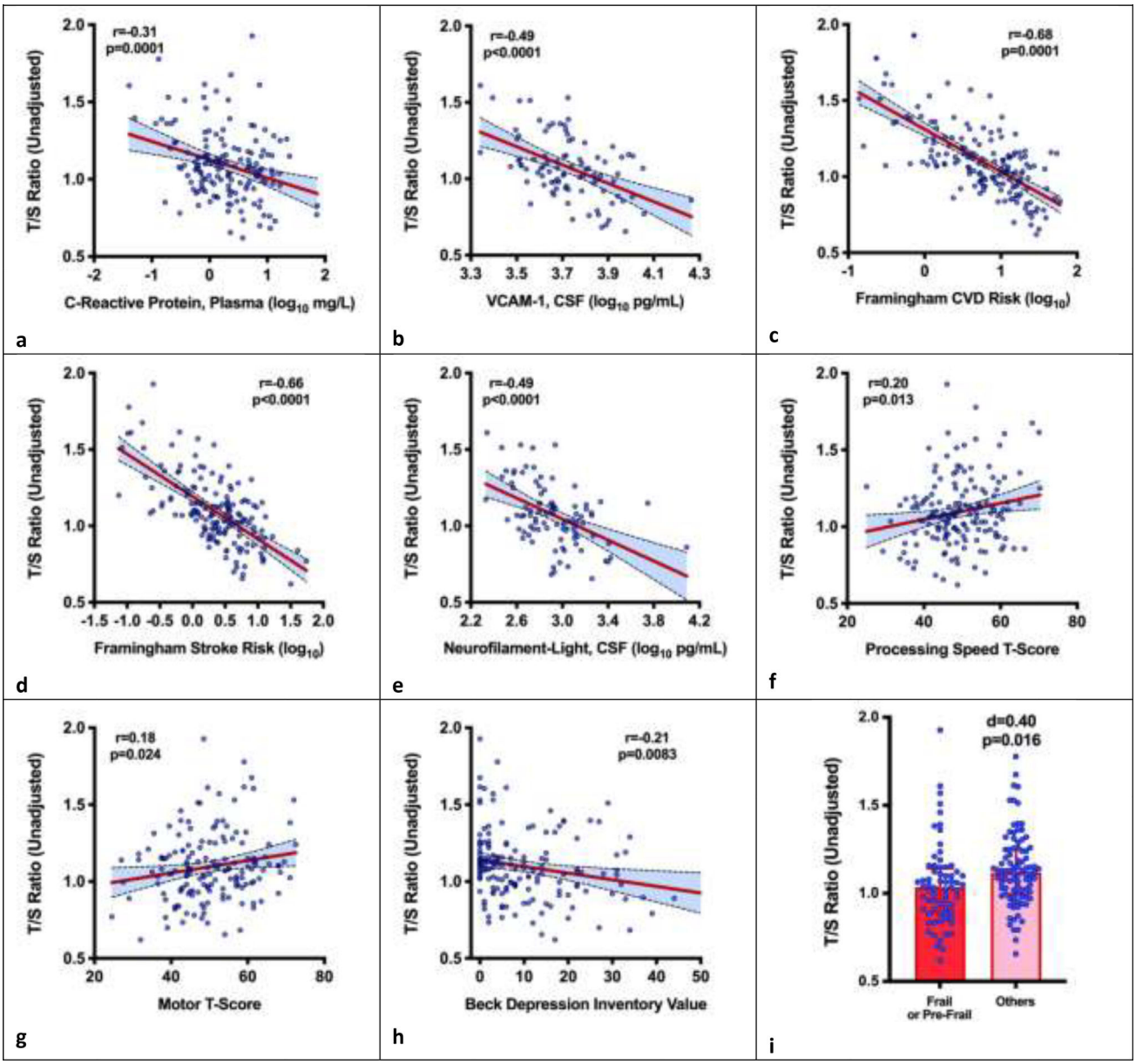


**Figure 2. Correlation of Telomere Length with Biomarkers and Disease-Related Composite Variables.**

T/S Ratio correlated with plasma CRP, CSF sVCAM-1, and CSF NFL as well as Framingham CVD Risk, Framingham Stroke Risk, Beck Depression Inventory, Processing Speed, Motor Functioning, and Frailty/Pre-Frailty.

**Table 1.**

Participant Characteristics.

| | All | HIV-METH− | HIV-METH+ | HIV+METH− | HIV+METH+ | |
|---|---|---|---|---|---|---|
| **Sample Size** | 161 | 50 | 29 | 40 | 42 | **p value** |
| **Age (Years)**[1] | 45 [34·5–55] | 54 [35·5–61·2] | 37 [27·5–52] | 50 [37–58] | 41·5 [35–47·2] | **0·005** |
| **Sex, Women**[2] | 45 (27·9%) | 27 (54%) | 12 (41·4%) | 5 (12·5%) | 1 (2·4%) | **<0·001** |
| **Race/Ancestry (European)**[2] | 87 (54·0%) | 27 (54·0%) | 14 (48·3%) | 25 (62·5%) | 21 (50·0%) | 0·61 |
| **Education (Years)**[1] | 14 [12–16] | 15 [14–16] | 12 [10·5–15] | 1 [12·2–16] | 13·5 [12–14·2] | **<0·001** |
| **Cotinine Level**[1] | 2 [1–6] | 1 [0–2] | 3 [1–6] | 2 [0–5] | 4 [1–6] | **0·02** |
| **Leukocyte Count (/mm$^3$)**[1] | 6·2 [5·1–7·4] | 5·7 [5·0–7·2] | 7·0 [5·4–7·8] | 6·6 [5·2–7·7] | 6·1 [4·9–6·7] | 0·53 |
| **Est. Duration of HIV (Years)**[1] | 10·7 [3·9–17·0] | - | - | 13·8 [3·7–21·9] | 7·7 [4·0–13·7] | **0·04** |
| **AIDS Diagnosis**[2] | 33 (39·3%) | - | - | 20 (50·0%) | 13 (32·5%) | 0·07 |
| **ART Use**[2] | 82 (100%) | - | - | 40 (100%) | 42 (100%) | 1·00 |
| **Duration of ART (Years)**[1] | 4·9 [1·8–11·5] | - | - | 4·9 [1·1–12·7] | 5·0 [2·2–10·6] | 0·99 |
| **HIV RNA, Plasma (≤ 200 cp/mL)**[2] | 82 (100%) | - | - | 40 (100·0%) | 42 (100·0%) | 1·00 |
| **CD4+ T-cell Count, Nadir (/mm$^3$)**[1] | 292·5 [159·8–457] | - | - | 278 [127·2–470·2] | 296·5 [179–462·5] | 0·50 |
| **CD4+ T-cell Count, Current (/mm$^3$)**[1] | 682 [547–884] | - | - | 701 [548–937·8] | 635 [446·2–819·2] | **0·01** |
| **Framingham CVD Risk Score**[1] | 7·2 [2·4–15·4] | 7·2 [1·8–16·5] | 3·8 [1·2–13·5] | 12·1 [3·4–18·2] | 6·3 [3·8–11·8] | 0·29 |
| **Framingham Stroke Risk Score**[1] | 2·5 [1·1–5·1] | 2·6 [0·8–6·4] | 1·3 [0·8–3·8] | 3·4 [1·6–70] | 2·3 [1·7–3·7] | **0·006** |
| **Body Mass Index (BMI)** | 26·9 [23·8–31·6] | 26·7 [22·8–32·4] | 31·1 [25·6–36·0] | 25·8 [24·2–30·1] | 26·6 [23·4–29·8] | **0·008** |
| **Pre-Frail or Frail**[2] | 68 (42·5%) | 12 (24·0%) | 13 (44·8%) | 26 (65·0%) | 17 (40·5%) | **0·001** |
| **Global T-Score** | 48·1 [43·9–51·7] | 49·4 [43·2–55·2] | 47·4 [44·1–50·3] | 47·1 [43·0–51·2] | 48·2 [45·2–49·9] | 0·33 |
| **Beck Depression Inventory**[1] | 6 [2–15·8] | 1·5 [0–6] | 13 [4–18·5] | 10·5 [3–15·8] | 14 [4·5–22·5] | **<0·001** |
| **T/S Ratio**[1] | 1·08 [0·96–1·20] | 1·12 [1·02–1·31] | 1·15 [0·99–1·28] | 1·03 [0·92–1·15] | 1·04 [0·94–1·10] | **0·003** |

Notes: HIV (Human Immunodeficiency Virus), AIDS (Acquired Immunodeficiency Syndrome), ART (Antiretroviral Therapy), CVD (Cardiovascular Disease), T/S (Telomere to Single Copy Gene Ratio).

[1]Median [Interquartile Range]

[2]Number (percent). Statistical comparisons were made using the Mann-Whitney (2 groups) or Kruskal-Wallis (4 groups) tests.

**Table 2.**

**Demographic, Clinical and Laboratory Measures Associated with Leukocyte T/S Ratio in All Participants.**

All multivariable models adjust for age (p value in best model: < 0·0001), sex (0·0015), education (0·0178), race/ethnicity (0·0675), and leukocyte count (0·0936). Individual variables with p values ≤ 0·15 are shown. Individual variables that had p values > 0·15 included Triglycerides, Total Cholesterol, Hemoglobin, Glucose, Alanine Transaminase, Aspartate Transaminase, Total Bilirubin, FIB-4, and Cotinine.

| | Individual Variables with Adjustment for Demographics and Leukocytes | | Additional Adjustment for HIV & METH | | Best Multivariable Model With Other Variables | | |
|---|---|---|---|---|---|---|---|
| | β | P value | β | P value | β | P value | FDR P value |
| **HIV Seropositive** | −0·043 | **0·0027** | −0·040 | **0·004** | −0·034 | 0·0136 | **0·0166** |
| **METH Dependence** | −0·039 | **0·0058** | −0·037 | **0·0097** | −0·037 | 0·0063 | **0·0115** |
| **Body Mass Index** | −0·007 | **0·017** | | | −0·004 | 0·0127 | **0·0166** |
| **Serum Globulins** | −0·092 | **0·0019** | | | −0·092 | 0·0024 | **0·009** |
| **Erythrocyte Diameter Width** | −0.037 | **0·005** | | | | | |
| **Est. Glomerular Filtration Rate** | 0·002 | **0·029** | | | | | |
| **Creatinine** | −0·137 | **0·032** | | | −0·132 | 0·027 | **0·029** |
| **Alkaline Phosphatase** | −0·001 | **0·036** | | | | | |
| **Total Protein** | −0·050 | **0·066** | | | | | |
| **HDL Cholesterol** | 0·001 | **0·087** | | | | | |
| **Albumin** | 0·066 | **0·130** | | | | | |

Notes: HDL (High Density Lipoprotein), FDR (False Discovery Rate)

## Table 3.
## Relationship of T/S Ratio and Disease-Related Variables.

Multivariate analysis found T/S Ratio was associated with Framingham CVD Risk, Framingham Stroke Risk, Pre-Frailty, Executive Functioning, and Motor Functioning. Analyses of T/S Ratio were either unadjusted or adjusted for demographics, leukocyte count, HIV and METH. The calculation of Framingham CVD Risk and Framingham Stroke Risk include age and sex so these were not included in initial demographic adjustments for these variables. Calculation of neurocognitive performance T-scores includes adjustments based on age, sex, ethnicity, and education so these were also not included in initial adjustments.

| | **Unadjusted** | | **Plus Age, Sex, Ethnicity, Education, and Leukocyte Count** | | **Plus HIV and METH** | | | |
|---|---|---|---|---|---|---|---|---|
| | **β** | **p value** | **β** | **p value** | **β** | **p value** | **β** | **p value** |
| **Dependent Variables That Do Not Include Demographic Data in Their Calculation** | | | | | | | | |
| **Pre-Frailty or Frailty** | −1·935 | **0·016** | −0·915 | 0·411 | −0·655 | 0·578 | - | - |
| **Karnofsky Rating** | 11·70 | **<0·0001** | 10·24 | **0·0126** | 8·48 | **0·047** | - | - |
| **Beck Depression Inventory** | −10·26 | **0·0083** | −20·38 | **0·0001** | −13·16 | **0·013** | - | - |
| **Neurofilament Light, CSF** | −0·690 | **<0·0001** | −0·476 | **0·007** | −0·546 | **0·003** | - | - |
| **Dependent Variables That Include Age and Sex in Their Calculation** | | | | | | | | |
| | **Unadjusted** | | **Plus Ethnicity, Education, and Leukocyte Count** | | **Plus HIV and METH** | | **Plus Age and Sex** | |
| **Framingham CVD Risk** | −1·745 | **<0·0001** | −1·617 | **<0·0001** | −1·625 | **<0·0001** | −0·400 | **0·0056** |
| **Framingham Stroke Risk** | −1·568 | **<0·0001** | −1·502 | **<0·0001** | −1·501 | **<0·0001** | −0·345 | **0·0045** |
| **Dependent Variables That Adjust for Age, Sex, Race/Ethnicity, and Education in Their Calculation** | | | | | | | | |
| | **Unadjusted** | | **Plus Leukocyte Count** | | **Plus HIV and METH** | | **Plus Age, Sex, Ethnicity, and Education** | |
| **Global Performance** | 3·893 | **0·081** | 3·224 | 0·152 | 2·354 | 0·319 | 4·047 | 0·220 |
| **Processing Speed** | 7·341 | **0·013** | 6·831 | **0·023** | 5·472 | **0·082** | 7·331 | **0·097** |
| **Motor Functioning** | 8·002 | **0·024** | 7·494 | **0·037** | 6·900 | **0·068** | 11·044 | **0·038** |
| **Working Memory** | 4·362 | 0·163 | 3·850 | 0·226 | 2·566 | 0·442 | 8·320 | **0·069** |
| **Executive Functioning** | 2·348 | 0·449 | 1·003 | 0·747 | −0·304 | 0·926 | 6·923 | 0·125 |
| **Delayed Recall** | 2·260 | 0·494 | 1·623 | 0·629 | 2·807 | 0·426 | −3·462 | 0·474 |
| **Learning** | 1·407 | 0·649 | 0·145 | 0·963 | 0·422 | 0·897 | −2·101 | 0·642 |
| **Verbal Functioning** | 0·081 | 0·980 | 0·052 | 0·990 | −1·573 | 0·647 | −1·464 | 0·757 |

Notes: CSF (Cerebrospinal Fluid), CVD (Cardiovascular Disease),