Associations between plasma biomarkers and changes in cognitive function over two years in people with and without HIV

Short title: Associations biomarkers and cognition in HIV

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Abstract:

Objective: Chronic inflammation may be associated with cognitive disorders in people with HIV (PWH) on antiretroviral treatment (ART). We examine associations between cognitive function (CF) and plasma biomarkers measured in PWH and demographically-similar people without HIV in the POPPY study.

Design: Prospective longitudinal cohort study.

Methods: At baseline and 2-year follow-up, participants completed a cognitive test battery. Global T-scores were derived by averaging domain T-scores. We used linear regression to explore associations between changes in Global T-scores and log-transformed plasma biomarkers of neuronal injury, systemic inflammation and innate immune activation. We explored whether effects of biomarkers differed by HIV status.

Results: 349 participants were included (73% PWH, median [interquartile range, IQR] age 54 [50-60] years, 85% male, 95% white). Among PWH, 98% were on ART, 93% had HIV-RNA ≤50 copies/mL and median [IQR] CD4+ count was 627 [490, 792] cells/mm^3. Mean (standard deviation (SD)) baseline Global T-score was 47.7 (5.9) which increased to 48.9 (5.5) after a median [IQR] follow-up of 26 [24, 29] months. Lower average increases in Global T-scores were seen in those with higher MIP-1α concentrations (parameter estimate: -0.27 [95% CI: -0.51, -0.03] /10% increase) and sCD14 (-0.17 [-0.30, -0.03]), though only MIP-1α (-0.46 [-0.58, -0.10]) remained significant after adjustment. There was no evidence that the associations differed by HIV status.

Conclusion:

Higher MIP-1α and sCD14 showed small associations with lower average increases in Global T-scores, with no differences by HIV status or inflammatory clusters, highlighting the multifactorial influences on cognitive trajectories in people ageing with and without HIV.

Key words: cognition; HIV; Antiretroviral Therapy, Highly Active; inflammation; Immunity, Innate

Introduction

Emerging evidence demonstrates that individuals on effective antiretroviral treatment (ART) continue to exhibit an elevated risk of developing non-AIDS and non-infectious complications compared to age-matched control populations [1–4]. This phenomenon is hypothesised to be partly driven by persistent immune dysfunction, chronic immune activation, and systemic inflammation despite virological suppression in individuals receiving ART [5–7], as evidenced by persistently elevated biomarkers of immune activation and inflammation, compared to populations without HIV [8,9].

HIV-associated dementia is now rarely seen in people taking ART, however milder forms of cognitive disorders continue to be reported in people with HIV on virologically suppressive modern ART [10,11]. The mechanisms underlying the milder forms of cognitive impairment remain unclear. Dysregulation of innate immune responses and chronic inflammation associated with persistently elevated type I and type II interferons, ongoing low-level viral replication, vascular injury, metabolic abnormalities, glial cell dysfunction, decreased white matter integrity and the adverse effects of antiretroviral agents may contribute to the pathogenesis [12–16]. Ongoing progressive neuronal loss has been observed in individuals with HIV on virologically suppressive ART [17], suggesting that neuronal injury may result not only directly from the virus itself, but also from chronic immune activation and inflammation [18]. Furthermore, neurotoxicity may result directly from viral proteins or from certain drugs capable of crossing the blood-brain barrier [19].

While several biomarkers of immune activation (e.g. CCL2, IL-6, soluble CD14 (sCD14), neopterin, kynurenine, quinolinic acid) were associated with more severe forms of cognitive impairment in the pre-ART era [20–25], evidence regarding specific biomarkers that can reliably predict and monitor cognitive function in people on virologically suppressive ART remains limited.

Here, we examine the associations between biomarkers of systemic inflammation and innate immune activation and changes in cognitive function among people with HIV and demographically similar people without HIV.

Methods

Study Design and Participants

The Pharmacokinetic and clinical Observations in PeoPle over FiftY (POPPY) study is a prospective cohort study of people with and without HIV in the United Kingdom (UK) and Ireland. Three sub-cohorts of participants were recruited from eight HIV clinics: (1) 699 people with HIV aged >50 years who were of white or black African race, and acquired HIV through sexual routes; (2) 374 people with HIV aged <50 years, who were frequency-matched to the older group of people with HIV on gender, race, sexual orientation and clinic; and (3) 304 HIV-negative controls aged >50 years, who were frequency-matched to the older group of people with HIV on age, gender, race, sexual orientation and location (in or out of London). The baseline visit took place between April 2013–January 2016 and the wave 3 POPPY follow-up visit took place between May 2015–February 2018. Further detail on the POPPY study has been described previously [26].

This present analysis uses a subset of POPPY study participants who were part of the POPPY-Sleep sub-study (participants attended one study visit between March 2017 and July 2018) [27], who had valid data on 31 plasma biomarkers analysed using two platforms that are immunoassays based on Enzyme Linked Immunosorbent Assay (ELISA) as part of this sub-study, and who had an assessment of cognitive function at both the baseline and wave 3 POPPY study visits [28]. The inflammatory pathways and plasma biomarkers measured were as follows: (1) Coagulation (sCD40L, D-dimer, sP-Selectin), (2) Neuronal injury (NFL, S100B), (3) Endothelial function (sICAM-1, E-Selectin, sVCAM-1, vWF), (4) Systemic inflammation (hsCRP, IL-2, IL-6, TNF-α, IFN-γ, IL-1β, TNF RI,

TNF RII), (5) Innate immune activation (sCD14, MCP-1, sCD163, MIP-1α, IL-10), (6) Immune regulation (IL-1RA, PDL-1, IL-4), (7) Microbial translocation (IL-18, I-FABP, IL-12, LBP), and (8) Atherosclerosis (Lp-PLA2, MPO) [28].

The derivation and characteristics of the inflammatory plasma biomarker clusters have been previously described [28]. In summary, the clusters were derived by conducting principal components analysis (PCA) on the correlation matrix of 31 log-transformed plasma biomarker concentrations followed by agglomerative hierarchical cluster analysis [28]. Three distinct clusters were identified: (1) Gut/immune activation; (2) Neurovascular; and (3) Reference.

Cognitive Function/Performance Outcomes

Cognitive function was assessed at baseline and wave 3 for the main POPPY study using the Cogstate battery [29]. The battery covered six cognitive domains commonly affected by HIV-associated cognitive impairment: visual learning, psychomotor function, visual attention, executive function, verbal learning, and working memory/attention. T-scores (mean: 50, standard deviation (SD): 10) were derived, and a Global T-score of overall cognitive function was obtained by averaging the domain T-scores [29,30].

The primary outcome of interest was the change in Global T-scores between the two POPPY study visits, with a positive change reflecting an improvement in Global T-scores at the second study visit compared to the first, and a negative change a deterioration.

Primary and Secondary Exposures: Plasma Biomarkers and Inflammatory Plasma Biomarker Clusters

For the primary analyses, we examined the associations between the change in Global T-scores between the two POPPY study visits and specific plasma biomarkers selected a priori based on our hypothesis that they would predict changes in cognitive function: NFL, S100B, sCD-14, IL-10, MCP-1, sCD163, MIP-1α, IL-2, IL-6 and TNF-α. For the secondary analyses, we examined associations between the change in Global T-scores between the two POPPY study visits by inflammatory plasma biomarker clusters.

Statistical Analyses

Prior to analyses, we excluded participants without data on the primary outcome (change in Global T-score) or who were identified as influential observations based on data exploration to ensure robustness of statistical analyses.

Participant characteristics

We summarised participant characteristics using medians (interquartile ranges (IQRs)) and counts (proportions) for continuous and binary-categorical characteristics, respectively. We summarised HIV-specific clinical characteristics measured at both the POPPY baseline visit and at or near the POPPY-Sleep Sub-study visit when the plasma biomarkers were measured. We compared general

baseline characteristics measured at the POPPY baseline visit between people with and without HIV using Wilcoxon rank-sum tests for continuous variables, and Chi-squared/Fisher's exact tests for categorical variables, as appropriate. We also compared general baseline characteristics and HIV-specific clinical characteristics between the inflammatory plasma biomarker clusters using Kruskal-Wallis tests for continuous variables, and Chi-squared/Fisher's exact tests for categorical variables, as appropriate.

Linear regression analyses

Linear regression was used to estimate associations between the change in Global T-score and both HIV status and the specific subset of plasma biomarkers hypothesised to be associated with changes in cognitive function. Each of the plasma biomarkers was (natural) log-transformed and included as an independent variable in unadjusted and adjusted linear regression models separately to estimate the association of each plasma biomarker with the change in Global T-score. In adjusted analyses, we adjusted for important confounders identified *a priori*: age, sex, race, education, and HIV status (for plasma biomarker analyses). We used likelihood ratio tests (LRTs) to first explore whether there were significant differences in changes in Global T-scores by HIV status before and after adjustment for important confounders. Subsequently, we used LRTs to explore whether there were significant associations between changes in Global T-scores and the specific subset of plasma biomarkers. We also explored whether any associations between changes in Global T-scores and the specific subset of plasma biomarkers varied by HIV status using LRTs testing if an interaction term between the log-transformed plasma biomarker and HIV status was statistically significant, after adjustment for important confounders. Average predicted Global T-score changes, and their 95% confidence intervals were generated from the final models across the observed range of biomarker concentrations that were statistically significant, holding other covariates constant at representative values.

All analyses were performed using R version 4.1.0, with two-sided p-values <0.05 considered to be statistically significant.

Results

Participant Characteristics

Of the 465 participants who had reliable plasma biomarker measurements and were eligible for inclusion in analyses, 350 participants had complete data on Global T-scores collected at both the baseline and wave 3 visits. One participant was identified to have a particularly extreme change in Global T-scores and was excluded due to potentially inaccurate cognitive testing, further supported by their identification as an influential observation with a high degree of influence on regression estimates, resulting in a final sample of 349 participants.

Of participants in the sample, 254 (72.8%) were people with HIV, the median (IQR) age was 54 (50, 60) years and most were white (94.6%), male (85.1%), and men who have sex with men (MSM) (77.7%). Among those with HIV, most (249 (98.0%)) were on ART, 235 (93.3%) had HIV-RNA <50 copies/mL, and the median (IQR) CD4+ cell count was 627 (490, 792) cells/mm^3 at the POPPY

baseline visit (**Table 1**). Few changes in these HIV-specific measures were observed at the POPPY-Sleep Sub-study visit (**Table 1**). The 115 participants excluded because of missing data on change scores were more likely to be female, non-white, to have acquired HIV through sex between men and women, not consume alcohol currently and to have a BMI >30 kg/m^2 (Supplementary Table A1, Supplemental Digital Content, http://links.lww.com/QAD/D762). Compared to those included with HIV, those excluded with HIV had a lower median CD4+ T-cell count at both the POPPY baseline and Sleep Sub-study visits, shorter median time since HIV diagnosis and lower proportion with a history of dideoxynucleoside (d-drugs) use (Supplementary Table A1, Supplemental Digital Content, http://links.lww.com/QAD/D762).

Among those included in the present analyses, people with and without HIV differed on several characteristics at baseline. People with HIV were younger than those without HIV (median age of 53 vs. 58 years, p<0.001) as expected given the study design, were less likely to currently consume alcohol (82.7% vs. 93.7%, p=0.009), and were more likely to be male (90.6% vs. 69.5%, p<0.001), MSM (84.3% vs. 58.9%, p<0.001), to have a history of recreational drug use in the 6 months prior to baseline (26.8% vs. 15.8%, p=0.03) and to have ever injected drugs (10.2% vs. 2.1%, p=0.01) (**Table 1**).

People with HIV had higher median concentrations of sCD14 (1744.0 vs. 1516.9 x10^3 picograms per millilitre (pg/mL), p<0.001), sCD163 (679.3 vs. 474.8 x10^3 pg/mL, p<0.001) and MCP-1 (157.8 vs. 140.9 pg/mL, p=0.002). People with HIV were more likely to be in the 'Neurovascular' cluster (45.7% vs. 34.7%) and less likely to be in the 'Gut/immune activation' cluster (7.9% vs. 15.8%) compared to those without HIV (global p-value 0.04) (**Table 2**).

Global T-score changes: POPPY Baseline and wave 3 study visits

The median (IQR) time between the baseline and wave 3 cognitive assessments was 26 (24, 29) months. Overall, Global T-scores increased from a mean of 47.7 (SD: 5.9) at baseline to 48.9 (5.4) at wave 3 (mean change 1.2 [95% CI 0.7, 1.7]) (**Table 3**); 133/349 (38.1%) participants experienced a decline in the Global T-Score between the two visits, but for most (111/133, 83.5% or 31.8% of the total group) this decline was <5 (a change that would be deemed to be clinically significant). The mean change in Global T-scores was 1.1 (0.6, 1.7) in people with HIV and 1.3 (0.5, 2.1) in people without HIV (p=0.72 for difference between the two groups), with 98/254 (38.6%) and 35/95 (36.8%) of the two groups having a decline in Global T-scores between visits, respectively.

Observed changes in the Global T-scores were stable across quintiles of the plasma biomarker concentrations overall but were slightly more variable by HIV status (Supplementary Figures A1 and A2, Supplemental Digital Content, http://links.lww.com/QAD/D762). The estimated associations between plasma biomarkers and the change in Global T-scores between the two POPPY study visits are shown in Table 4. Higher concentrations of MIP-1α (-0.27 (-0.51, -0.03)) and sCD14 (-0.17 (-0.30, -0.03)) were associated with lower average increases in Global T-scores, though only the association with MIP-1α (-0.34 (-0.58, -0.10)) remained significant after adjustment. Plots of the average predicted change in Global T-score by values of MIP-1a and sCD14, overall and adjusted for demographic factors, are shown in Supplementary Figure A3, Supplemental Digital Content,

http://links.lww.com/QAD/D762. There was no evidence that the biomarker associations varied by HIV status (Table 4).

Secondary Analyses: Inflammatory Plasma Biomarker Clusters

Of the 349 participants included, 165 (47.3%) were in the 'Reference' cluster, 35 (10.0%) in the 'Gut/immune activation' cluster and the remaining 149 (42.7%) in the 'Neurovascular' cluster (**Table 2**). Consistent with previous findings[28], there were statistically significant between-cluster differences for several baseline characteristics, with those in the 'Neurovascular' cluster being more likely to be living with HIV or obesity, those in the 'Gut/Immune activation' cluster having a higher median age and systolic blood pressure, and those in the 'Reference' cluster being more likely to have a university education **(**Supplementary Table A2, Supplemental Digital Content, http://links.lww.com/QAD/D762**)**.

While a slight increase was observed in the Global T-scores between the POPPY baseline and wave 3 visits as indicated by the positive mean change scores (95% CI) of within-individual change (1.2 (-0.5, 3.0), 1.0 (0.2, 1.7), 1.4 (0.8, 2.0) for 'Gut/immune activation', 'Neurovascular' and 'Reference' clusters, respectively), there were no statistically significant between-cluster differences before or after adjustment for important confounders (Table 5). These data were consistently compatible with models without an interaction term between the inflammatory plasma biomarker cluster and HIV status variables relative to models with this interaction term, suggesting no evidence that between-cluster differences varied across people with and without HIV.

Discussion

Over a 2-year period, we observed a mild mean increase in Global T-scores in our cohort of people with and without HIV. While we did not find clear associations between the change in Global T-scores and most biomarkers, we found that higher concentrations of MIP-1α and sCD14 were associated with lower average increases in Global T-scores, consistent with literature suggesting a link between chemokine dysregulation and cognitive impairment. Chemokines such as MIP-1α have been implicated in neuroinflammation and blood–brain barrier dysfunction, and elevated concentrations have been observed both in people with HIV on ART [31,32] and individuals without HIV with cognitive impairment [33]. However, after adjustment for key demographic covariates, we found that only the MIP-1α association remained statistically significant, and there was no evidence that these relationships differed by HIV status. This further suggests that the impact of specific inflammatory pathways on cognitive function is likely multifactorial and relevant to brain health in all, rather than being unique to HIV. These findings contribute to the existing evidence base on the associations between biomarkers of systemic inflammation and innate immune activation with changes in cognitive function [34,35].

Beyond the specific biomarker associations with changes in cognitive function, our findings demonstrated a distinct inflammatory profile in participants with HIV, who, despite being virologically suppressed on effective ART, exhibited significantly higher concentrations of certain inflammatory plasma biomarkers, particularly sCD14, sCD163, and MCP-1, suggesting ongoing immune activation.

These results are consistent with previous reports indicating that people with HIV on virologically suppressive ART continue to exhibit elevated biomarkers of systemic inflammation and monocyte activation including sCD14 and sCD163 compared to cohorts without HIV [7,36,37]. Elevated concentrations of sCD14 and sCD163 have also been independently associated with cognitive impairment in people with HIV, underscoring their clinical relevance [38,39].

Building on the observations of distinct HIV-associated inflammatory profiles, participants with HIV were more likely to be classified in the 'Neurovascular' inflammatory cluster and less likely in the 'Gut/immune activation' cluster. This finding may indicate distinctive inflammatory phenotypes in people with HIV, potentially shaped by chronic viral infection, ART exposure, immune dysregulation, and other comorbidities and conditions associated with inflammation [40–42] that disproportionately impact people with HIV.

In contrast to studies that have identified associations between immune activation profiles and cognitive trajectories in people with HIV [43], in our study we observed that the mean Global T-score change was broadly similar, with no statistically significant observed differences between the inflammatory clusters, both before or after adjustment, and differences did not vary by HIV status. Several factors likely contribute to these divergent findings. Participants in POPPY were generally healthier, older, predominantly white men with well-controlled HIV and few advanced comorbidities, whereas the Women's Interagency HIV Study (WIHS) cohort as presented in the publication by Rubin et al [43] included only women, greater racial diversity, and more individuals exposed to structural disadvantage, all of which may amplify inflammation–cognition relationships. Furthermore, the WIHS analysis captured inflammatory signatures closer to ART initiation and evaluated cognition over a longer follow-up, whereas POPPY assessed immune markers and cognitive change within a relatively stable, virologically suppressed population over a shorter period. It is also notable that participants excluded from our analysis, who were younger, more often female or from non-White ethnic groups, and had a higher comorbidity burden, more closely resembled the WIHS cohort, where stronger inflammation–cognition associations have previously been reported. Overall, these differences suggest that cognitive trajectories in ageing populations, with or without HIV, are influenced by a complex interplay of demographic, biological, psychosocial, and structural factors, and that inflammation-related cognitive risk is not specific to HIV alone.

Our data suggests that while distinctive inflammatory profiles exist (as represented by the inflammatory clusters in this instance), these inflammatory patterns were not strongly predictive of short-term cognitive function change in our cohort of people with well-controlled HIV and preserved immune function. The absence of associations at the cluster level, despite modest biomarker-specific effects, may reflect dilution of MIP-1α and sCD14 signals when combined with other markers, potentially obscuring associations within broader inflammatory clusters.

The pattern of modest improvements alongside subtle cognitive fluctuations in our cohort, particularly among people with HIV, aligns with findings from other longitudinal studies in cohorts of older people with HIV who are virologically suppressed on ART and without advanced immunosuppression [44]. Whilst a proportion of participants did experience a decline in Global T-scores, these changes were generally small, did not exceed the threshold typically considered clinically significant in most, and

were similar regardless of HIV status, suggesting that they were largely a reflection of random variation. The comparable changes in Global T-score between people with and without HIV suggest that HIV status alone may not fully explain individual differences in cognitive trajectories, a conclusion echoed by studies in both populations that have highlighted the multifactorial role of comorbidities, substance use, and psychosocial factors in shaping cognitive outcomes [45,46].

Our study has several strengths, including a well-characterised, longitudinally followed cohort, the inclusion of people with HIV and demographically similar people without HIV, and detailed immune phenotyping using inflammatory biomarker clustering. However, some limitations should be acknowledged. First, the observational design of our study limits our ability to draw causal inferences. Second, the cohort was predominantly white, male, and MSM, which limits the generalisability of our findings to other key populations affected by HIV, such as women and people of non-white ethnicities. Inflammation-cognition relationships may differ in women and people of non-white ethnicities due to biological differences in immune responses, sex-specific hormonal influences, and differential exposure to social and structural determinants of health[47,48]. Additionally, individuals with different HIV acquisition routes may experience distinct comorbidity profiles or patterns of immune activation, which could influence cognitive outcomes[49]. These factors highlight the need for studies in more diverse populations to fully understand the interplay between inflammation and cognitive function in people living with HIV. In addition, individuals with HIV differed from those not living with HIV in several sociodemographic and behavioural characteristics. Individuals with HIV were younger and more likely to be white, male, MSM, have a history of recreational or injecting drug use and were less likely to consume alcohol, reflective of the demographic profile of the overall POPPY study cohort [26]. These differences may reflect broader social and behavioural patterns in the epidemiology of people with HIV. While certain factors such as comorbidities and psychosocial variables may influence biomarker changes, these factors themselves are often affected by HIV and adjusting for them as potential confounders might inadvertently obscure meaningful associations between HIV, biomarkers, and cognitive function. For this reason, these variables were not included as covariates in our analyses, and this should be borne in mind when interpreting the results. Finally, while statistically significant, the absolute changes in Global T-scores over the 2-year period were small and may not reflect clinically meaningful relevance.

Future research should aim to validate these findings in larger populations of people with HIV and demographically similar people without HIV, and to replicate these findings in other representative populations, including women, older adults, and ethnically diverse populations. Integrating more detailed cognitive testing with longitudinal neuroimaging data and advanced immune profiling may uncover the biological mechanisms linking chronic inflammation and cognition outcomes. Understanding these pathways in both people with and without HIV and integrating inflammatory biomarker profiles into predictive models of cognitive decline could offer insights into identifying at-risk individuals and developing targeted interventions.

Conclusions

In conclusion, while our findings underscore persistent immune activation in people on virologically suppressive ART and suggest modest associations between certain inflammatory biomarkers and cognitive function over a 2-year period, we also highlight the intricacy of these relationships. The apparent lack of interaction between HIV status and inflammatory clusters on changes in cognitive function further supports the view that other non-HIV factors likely also play important roles in determining cognitive trajectories over time. Our results highlight the complexity of factors that may influence inflammation-related cognitive impairment in the context of people on ART with virological suppression, confirming that further research into the interplay between chronic inflammation and cognitive outcomes is warranted, particularly in people ageing with HIV.

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Table 1. Summary characteristics of participants included, overall and by people with and without HIV

Median [IQR] or n (%)	Overall* N=349	People without HIV N=95	People with HIV N=254	p-value
(Socio-)Demographics				
Age, years	54 (50, 60)	58 (54, 61)	53 (47, 59)	<0.001
Male	297 (85.1%)	66 (69.5%)	231 (90.9%)	<0.001

White	330 (94.6%)	91 (95.8%)	239 (94.1%)	0.53
Educational attainment - university degree or above	172 (50.3%)	47 (50.0%)	125 (50.4%)	0.95
Anthropometric Measurements				
BMI ≥30 kg/m^2	53 (15.3%)	16 (17.2%)	37 (14.6%)	0.55
Systolic Blood Pressure (mmHg)	127 (117, 138)	130 (118, 144)	126 (117, 137)	0.10
Diastolic Blood Pressure (mmHg)	79 (72, 85)	78 (72, 84)	79 (72, 86)	0.85
Lifestyle Factors				
MSM sexuality/route of HIV acquisition	271 (77.7%)	56 (58.9%)	215 (84.6%)	<0.001
Current alcohol use**	299 (85.7%)	89 (93.7%)	210 (82.7%)	0.009
History of recreational drug use in past 6 months	83 (23.8%)	15 (15.8%)	68 (26.8%)	0.03
Ever injected drugs	28 (8.0%)	2 (2.1%)	26 (10.2%)	0.01
HIV-specific characteristics				
Measured at POPPY Baseline Visit				
HIV-RNA <50 copies/mL		--	235 (93.3%)	--
CD4+ T-Cell Count (cells/mm^3)		--	627 (490, 792)	--
Nadir CD4+ T-Cell Count (cells/mm^3)		--	199 (100, 320)	--
On any form of ART***		--	249 (98.0%)	--
History of dideoxynucleoside (d-drugs) use****		--	92 (36.2%)	--
History of any AIDS event		--	68 (26.8%)	--
Measured at or Near POPPY-Sleep Sub-study Visit				
HIV-RNA <50 copies/mL		--	238 (94.4%)	--
CD4+ T-Cell Count (cells/mm^3)		--	660 (526, 864)	--
Nadir CD4+ T-Cell Count (cells/mm^3)		--	190 (99, 306)	--
On any form of ART***		--	246 (96.9%)	--

		--	162 (63.8%)	--
On Protease Inhibitors		--	162 (63.8%)	--
On Non-Nucleoside Reverse Transcriptase Inhibitors		--	196 (77.2%)	--
On Integrase Inhibitors		--	50 (19.7%)	--
Cumulative exposure to any form of ART, years***		--	10.8 (5.0, 16.9)	--
Years since HIV Diagnosis		--	18.8 (11.3, 24.9)	--

*The following variables have missing data (number of participants missing data reported in parentheses): Educational attainment (7), BMI (3), baseline HIV-RNA <50 copies/mL (2), baseline CD4+ Cell Count (7), baseline Nadir CD4+ Cell Count (13), POPPY-Sleep Sub-study visit HIV-RNA <50 copies/mL (2), POPPY-Sleep Sub-study visit CD4+ Cell Count (1), POPPY-Sleep Sub-study visit Nadir CD4+ Cell Count (1), Years since HIV Diagnosis (at POPPY-Sleep Sub-study visit) (2).

**Current alcohol use is defined as any current alcohol use versus no current alcohol use

***Note: Participants may have used more than one form of ART treatment.

****History of dideoxynucleoside (d-drugs) use includes previous use of didanosine (ddI), zalcitabine (ddC) and/or stavudine (d4T)

Abbreviations: IQR = interquartile range; BMI = body mass index; MSM = men who have sex with men; ART = antiretroviral therapy

Table 2. Plasma biomarker concentrations (units in picograms per millilitre (pg/mL) unless otherwise stated), overall and by people with and without HIV

Plasma biomarker/Inflammatory plasma biomarker cluster	Overall (N=349)	People without HIV (N=95)	People with HIV (N=254)	p-value
Neuronal Injury				
NFL	57.5 (41.4, 77.9)	63.3 (45.4, 77.0)	55.7 (40.4, 77.9)	0.24
S100B	623.3 (514.6, 781.3)	666.3 (538.4, 790.3)	611.9 (506.9, 765.7)	0.11
Systemic Inflammation				
IL-2	1.0 (0.6, 1.8)	0.9 (0.5, 2.7)	1.0 (0.6, 1.6)	0.49
IL-6	2.1 (1.5, 3.1)	2.1 (1.6, 3.3)	2.2 (1.5, 3.1)	0.88
TNF-α	5.1 (4.2, 6.6)	5.3 (4.0, 7.7)	5.0 (4.2, 6.4)	0.41

Innate Immune Activation				
sCD14 (x10^3 pg/mL)	1622.1 (1335.0, 2036.3)	1516.9 (1239.4, 1,825.0)	1744.0 (1398.3, 2117.5)	<0.001
sCD163 (x10^3 pg/mL)	640.6 (407.8, 977.3)	474.8 (342.1, 804.9)	679.3 (430.7, 1012.2)	<0.001
IL-10	0.7 (0.5, 1.0)	0.8 (0.6, 1.2)	0.7 (0.5, 1.0)	0.16
MCP-1	152.7 (127.9, 194.1)	140.9 (119.6, 174.0)	157.8 (130.7, 202.9)	0.002
MIP-1α	524.8 (454.6, 597.0)	542.2 (454.7, 617.3)	524.1 (454.8, 592.4)	0.63
Inflammatory Biomarker Cluster				
'Reference'	165 (47.3%)	47 (49.5%)	118 (46.5%)	0.04
'Gut/immune activation'	35 (10.0%)	15 (15.8%)	20 (7.9%)	
'Neurovascular'	149 (42.7%)	33 (34.7%)	116 (45.7%)	

Table 3. Summary of Global T-Score measures assessed at baseline and wave 3 of the POPPY Study, overall and by people with and without HIV

Outcome	Observed Mean (Standard Deviation)			Estimated Mean Difference (95% Confidence Interval (CI)) (reference: people without HIV)			
				Unadjusted		Adjusted*	
	Overall N=349	People without HIV N=95	People with HIV N=254	Estimate (95% CI)	LRT p-value	Estimate (95% CI)	LRT p-value
Global T-Score – POPPY Study baseline visit	47.7 (5.9)	49.3 (4.8)	47.1 (6.1)	-2.2 (-3.6, -0.8)	0.002	-1.5 (-2.8, -0.1)	0.04
Global T-Score – POPPY Study wave 3 visit	48.9 (5.4)	50.7 (4.4)	48.3 (5.6)	-2.4 (-3.6, -1.1)	<0.001	-1.9 (-3.1, -0.6)	0.004
Change in Global T-Score –	1.2 (4.4)	1.3 (3.9)	1.1 (4.6)	-0.2 (-1.2, 0.8)	0.72	-0.4 (-1.5, 0.7)	0.48

(wave 3 – baseline)						

Table 4. Results of unadjusted and adjusted analyses exploring associations between Global T-score measures and specific plasma biomarkers

Outcome	Plasma Biomarker/ Index	Unadjusted		Adjusted (age, sex, race and education and HIV status)		
		Estimate (95% CI)*	LRT p-value	Estimate (95% CI)*	LRT p-value	LRT p-value (HIV Status:Plasma Biomarker interaction)**
Change in Global T-Score – (wave 3 – baseline)	S100B	0.02 (-0.10, 0.15)	0.71	0.02 (-0.11, 0.15)	0.78	0.28
	NFL	-0.03 (-0.11, 0.05)	0.46	-0.05 (-0.15, 0.04)	0.25	0.87
	IL-6	-0.03 (-0.11, 0.04)	0.37	-0.03 (-0.11, 0.05)	0.40	0.13
	IL-2	-0.04 (-0.08, 0.01)	0.09	-0.03 (-0.07, 0.02)	0.20	0.04
	TNF-α	-0.01 (-0.10, 0.09)	0.85	0.02 (-0.07, 0.12)	0.61	0.27
	sCD163	0.03 (-0.04, 0.10)	0.44	0.03 (-0.04, 0.10)	0.46	0.55
	MIP-1α	-0.27 (-0.51, -0.03)	0.02	-0.34 (-0.58, -0.10)	0.01	0.21

		Unadjusted estimate (95% CI)	p-value	Adjusted estimate (95% CI)	p-value	LRT p-value
	MCP-1	-0.08 (-0.20, 0.04)	0.18	-0.09 (-0.21, 0.04)	0.17	0.56
	IL-10	0.01 (-0.07, 0.09)	0.86	0.03 (-0.05, 0.10)	0.53	0.92
	sCD14	-0.17 (-0.30, -0.03)	0.02	-0.13 (-0.27, 0.01)	0.06	0.34

Abbreviation: LRT: Likelihood Ratio Test

*Note, interpret the coefficients for the biomarkers as the mean change in the Global T-score measure/outcome for every 10% increase in the biomarker concentration, for example, where a positive coefficient indicates "improved" cognitive function and a negative coefficient indicates "reduced" cognitive function for change scores. **Testing for the addition of an interaction term between HIV status and the biomarker/index, while the adjusted estimate (95% CI) and p-value are from a model without an interaction term. [refer to **Table A3** for interaction effects for people with and without HIV]

Table 5. Summary of regression analyses of Global T-Score measures assessed at baseline and wave 3 of the POPPY Study by inflammatory biomarker cluster

Observed Mean (Standard Deviation)				Estimated Mean Difference (95% Confidence Interval)						
				Unadjusted (Reference Cluster: 'Reference')			Adjusted (Reference Cluster: 'Reference')*			
Overall N=349	'Reference' N=165	'Gut/immune activation' N=35	'Neurovascular' N=149	'Gut/immune activation' N=35	'Neurovascular' N=149	LRT p-value	'Gut/immune activation' N=35	'Neurovascular' N=149	LRT p-value	LRT p-value - interaction HIV status-cluster**

Change in Global T-Score (wave 3 – baseline)	1.2 (4.4)	1.4 (4.1)	1.2 (5.0)	1.0 (4.5)	-0.2 (-1.8, 1.4)	-0.4 (-1.4, 0.5)	0.67	0.4 (-1.3, 2)	-0.6 (-1.6, 0.4)	0.33	0.96

Abbreviation: LRT: Likelihood Ratio Test

*adjusted for HIV status, age, sex, education, and race.

**Testing for the addition of an interaction term between HIV status and the inflammatory biomarker cluster variable, while the "LRT p-value adjusted" is from a model without an interaction term.