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Low-Level HIV RNA in Cerebrospinal Fluid and Neurocognitive Performance: A Longitudinal Cohort Study

Albert M. Anderson[1], Bin Tang[2], Florin Vaida[2], Daniel Mcclernon[3], Reena Deutsch[2], Mariana Cherner[2], Debra Cookson[2], Melanie Crescini[2], Igor Grant[2], Ronald J. Ellis[2,4], Scott L. Letendre[2,5]

[1]Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine

[2]Departments of Psychiatry

[3]bioMONTR Labs, Research Triangle Park, North Carolina

[4]Neurosciences

[5]Medicine, University of California, San Diego

Corresponding Author: Albert M. Anderson, Emory University School of Medicine, 341 Ponce de Leon Avenue, Atlanta, GA 30308, Phone: 404-616-3147, Fax: 404-616-9702, aande2@emory.edu

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Running Head:

Low-Level CSF HIV RNA and cognition

Abstract

Background: Cognitive complications persist in persons with HIV (PWH) during suppressive antiretroviral therapy (ART). Low levels of HIV during ART could contribute to these complications. In this study, we measured cerebrospinal fluid (CSF) HIV using a single copy assay (SCA) to investigate a possible relationship between low level HIV and cognition.

Design/Methods: SCA data were analyzed from three consecutive paired CSF-plasma specimens collected over a mean of 456 days from 96 participants on suppressive ART. Using mixed models, the presence of CSF HIV by SCA as a risk factor for worse neurocognitive performance was examined.

Results: At baseline on the SCA, 45.8% of participants had detectable plasma HIV RNA (median 8 copies/milliliter (ml), interquartile range (IQR) = 3-17 among detectable values), and 17.7% had detectable CSF HIV RNA (median CSF concentration= 3 copies/ml, interquartile range= 2-13 among detectable values). The frequency of CSF HIV RNA detection declined over time in CSF (p=0.018) with a trend toward decline in plasma (p=0.064). Detectable CSF HIV RNA during the study was associated with worse performance in the domains of recall (p=0.014) and motor (p=0.040), and a trend with worse overall global performance (p=0.078). Integrase inhibitor use, while very infrequent in this cohort, was associated with better performance in two domains.

Conclusions: Low-level CSF HIV RNA declines with time but is associated with worse cognitive performance in two domains. Additional research is needed to better understand the relationship between HIV RNA persistence during long-term ART and central nervous system complications in PWH.

Keywords: HIV; Cerebrospinal Fluid; Cognitive Disorders; Antiretroviral Therapy

Introduction

End-organ complications continue to occur more commonly among persons with HIV (PWH) compared to the general population despite antiretroviral therapy (ART).[1] These include central nervous system (CNS) complications such as HIV-associated neurocognitive disorder (HAND) and mental health complications such as depression.[2] Even in the setting of undetectable HIV RNA in blood and minimal neuropsychological (NP) comorbidities, the prevalence of neurocognitive impairment approaches 40% in some cohorts of PWH on ART.[3] HAND is associated with adverse quality of life outcomes and has been linked to premature mortality.[4-7] These may occur because inflammation and neuroglial damage can persist in the CNS despite systemic viral suppression.[8,9]

The pathogenesis of the HAND phenotype remains incompletely understood, though there is significant evidence of an inflammatory component.[10] One hypothesis is that HAND persists, in part, because of continued low-level production of HIV in the CNS. Low level HIV measured by single copy assay (SCA) has been found in the blood of significant proportions of PWH despite ART.[11,12] This has been linked to chronic inflammation in some studies of PWH, while in others the association with systemic inflammation has not been significant.[13-15] In terms of change over time, studies of ART intensification have not reduced low level HIV in blood, at least during relatively short-term follow-

up.[16,17] Observational studies with longer follow-up, however, have suggested that low-level HIV in blood continues to slowly decay over time during ART.[18,19]

Based on a smaller body of evidence, low levels of HIV are also detectable in the brain and cerebrospinal fluid (CSF) of PWH during ART.[20-22] This raises the possibility that the CNS is a reservoir site for HIV and is supported by studies showing viral genetic compartmentalization in the CNS, some of which suggest that this compartmentalization contributes to HAND.[23,24] Recent evidence indicates that HIV DNA can be detected from CSF in 48% of PWH on suppressive ART and that the presence of HIV DNA is associated with worse neuropsychological performance.[25] This also supports the hypothesis that the CNS is an HIV reservoir site that has the potential for adverse clinical outcomes. Little is known about the dynamics of low-level HIV in the CNS during ART over time. In a prior analysis, our group found that low-level HIV RNA in CSF was commonly present among virologically suppressed PWH.[26] However, the longitudinal component of this previous analysis involved a relatively small sample size with only two time points. Other work on low level CSF HIV RNA over time has also been limited by small sample size.[27] In the current study, we aimed to determine the frequency of low-level HIV RNA in CSF and plasma at three time points in a group of participants on ART with plasma and CSF HIV RNA $\leq$ 50 copies/milliliter (ml) on standard testing. We also examined whether the presence of persistently detectable low-level HIV RNA in CSF was associated with worse neuropsychological performance.

Methods

This study included participants from three NIH-funded longitudinal projects that enrolled chronically infected adult PWH on ART and included comprehensive, standardized neurobehavioral assessments.[3,28,29] All of these studies were based at the same site (University of California at San Diego). Specimens and data were included in the current analysis if participants a) used the same ART

regimen at three consecutive assessments, b) had HIV RNA ≤50 copies/ml in CSF and plasma at all three assessments, and c) had a sufficient volume of CSF and plasma in storage at -80^{o}C from all assessments to perform the SCA. At all assessments, CSF and blood were collected within one hour of each other and were processed and placed in storage within two hours. Participants who had severe neuropsychiatric comorbid conditions that could confound attribution of NCI to HIV were excluded. These included: history of traumatic brain injury with loss of consciousness > 30 minutes; stroke history with residual sequelae, uncontrolled active seizure disorder, psychotic disorders such as schizophrenia, and current substance use disorder.

Neurocognitive performance was assessed using a comprehensive and standardized battery of tests, which has been described in detail elsewhere.[3,30] All three studies included the following tests:

Processing speed: WAIS-III Digit Symbol, WAIS-III Symbol Search, Trailmaking A

Learning: HVLT-Revised, BVMT-Revised

Recall: HVLT-Revised Delay, BVMT-Revised Delay:

Executive Function: Wisconsin Card Sorting-64, Trail Making B

Attention/Working Memory: PASAT-50

For the second test in the attention/working memory domain, WAIS III Spatial span was used in the two most recent studies, while WAIS III letter number sequencing was used in the earliest study. Lastly, the two most recent studies also included Stroop color naming (DKEFS version) as an additional processing speed test and Stroop Color-Word (DKEFS version) as an additional executive function test. Scores from these tests when available were also incorporated into their respective domain scores. Demographically uncorrected scaled scores were converted to T scores (Mean= 50; Standard Deviation = 10) that corrected for the effects of age, education, sex, and race/ethnicity on neurocognition.[31-33] For the longitudinal neuropsychological assessments, neurocognitive score adjustment for practice effects was made by using median practice effect data from previous work.[34] Participants also completed two

separate questionnaires on activities of daily living: Lawton Brody Questionnaire and Patient's Assessment of Own Functioning Inventory (PAOFI). To meet criteria for functional impairment as per Frascati criteria, participants had to have three or more complaints on the PAOFI and two or more declines on the Lawton Brody. Depression symptoms were also assessed using the Beck Depression Inventory (BDI)-II score at baseline.

Neurocognitive testing, blood draws, and CSF sampling were all performed on the same day at the three consecutive study visits. Adherence was measured using the AIDS Clinical Trials Group (ACTG) adherence questionnaire, specifically with the question of "what percentage of your prescribed ART doses have you taken in the last four days?". Distribution and effectiveness of ART drugs in the CNS was estimated by the CNS Penetration Effectiveness Score (CPE) method.[35] Routine clinical assays, such as blood CD4+ T-cell count and CSF total protein, were measured in the Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory at the University of California, San Diego Medical Center. HIV-1 RNA was measured in CSF and plasma by real time polymerase chain reaction with a lower limit of quantification of 50 copies/mL (Abbott Diagnostics, Des Plaines, Illinois, USA). Low-level HIV-1 RNA was measured in CSF and plasma with a single copy assay (SCA, bioMONTR Labs Research Triangle Park, NC, USA) that has been validated and used in other CSF studies.[36] This method is based on a proprietary protocol which is used in conjunction with a commercial HIV-1 RNA easyQ reagent kit (bioMerieux Inc, Lyon, France). Briefly, a specimen of up to 2 ml of human CSF is added to lysis buffer containing guanidine thiocyanate. HIV-1 RNA is extracted in combination with the easyMAG platform (bioMerieux, Inc). Eluates containing HIV-1 RNA are aliquoted into 0.5 mL reaction tubes and amplified using 3 enzymes: T7 RNA polymerase, avian myeloblastosis virus reverse transcriptase, and RNase H. Molecular beacons targeting the pol/gag region of HIV-1 RNA are used for amplification and detection by isothermal reactions at 41^{o}C. HIV-1 RNA level is quantified in conjunction with the NucliSENS easyQ HIV-1 v2.0 Director software and a proprietary algorithm

developed by bioMONTR Labs. The dynamic range of this HIV-1 assay is 1–5,000,000 copies/mL. Detectable was therefore at least 1 copy/mL and the assay is quantifiable to 3 copies/mL.

Statistical approach:

Demographics, medical history, and ART characteristics were compared between participants with undetectable (< 1 copy/mL) and detectable (≥1 copy/mL) HIV RNA in either plasma or CSF at first assessment. The statistical comparisons used Student's t-test for continuous variables, and Fisher's exact test for binary and categorical variables. Similarly, global and domain neuropsychological performance at baseline were compared between participants with undetectable and detectable HIV RNA in plasma or CSF using Student's t-tests. Comparison between BDI-II scores was performed with the Wilcoxon rank sum test. Appropriate transformations (e.g. square root for CD4) were applied when indicated to improve symmetry and normality of distributions.

A mixed-effects logistic regression with subject-specific random intercepts was used to model the HIV RNA detectability over time in plasma and CSF separately. Separate models were fitted with time as either a continuous or categorical variable, where the detectable HIV RNA and time are outcome and predictor respectively. Model performance was assessed using the Akaike information criterion (AIC) and the likelihood ratio test. Wald 95% confidence intervals and p-values were reported for the time effects. The association between detectable HIV RNA in plasma and CSF was examined with a longitudinal logistic regression using generalized estimating equations (GEE), with detectable HIV RNA status at each visit in plasma as predictor, and in CSF as outcome. In order to determine the associations of HIV RNA in CSF with CPE, CSF leukocyte count, and plasma HIV RNA, each was regressed on $\log_{10}$(CSF HIV RNA) in linear mixed-effects models, controlling for time. CSF leukocyte count was highly skewed, and $\log_{10}$ transformed prior to analysis.

We examined whether detectable CSF HIV RNA (binary variable) predicts global and domain-specific T-scores using the longitudinal records, using linear mixed-effect models with subject-specific

random intercepts, adjusted for potential confounders, including current CD4+ T-cell count, nadir CD4+ T-cell count, duration of ART (current regimen and all regimens), current use of ART drug classes (e.g., integrase inhibitors (IIs)), number of ART drugs previously failed, and time since baseline. The potential confounders with p-value more than 0.2 were removed from the model sequentially, using backward model selection. Additional analyses examining the role of plasma HIV RNA (detectable/undetectable) as an additional predictor gave identical results as those reported here. Statistical analyses were implemented using R version 3.5.1, 2018. Alpha was set at 0.05.

Results

Ninety-six participants with three consecutive visits were analyzed. Table 1 summarizes the comparisons of demographic and disease characteristics at the first assessment based on detectable HIV RNA on the SCA in either plasma or CSF. For participants with detectable plasma HIV RNA (n=44), the median concentration was 8 copies/milliliter (ml), interquartile range (IQR) = 3-17. Participants with detectable plasma HIV had a lower current CD4+ T-cell count (median 498 cells/µl versus 626 cells/µl, p=0.020). Non-nucleoside reverse transcriptase inhibitor (NNRTI) users tended to be less likely to have detectable HIV RNA in plasma (p=0.06). Participants with detectable plasma HIV RNA also tended to be younger (mean 45.1 years versus 48.4 years, p=0.055), and to be on their current ART regimen for a shorter length of time (mean 1.87 years versus 2.79 years, p= 0.083). For participants with detectable CSF HIV RNA (n=17), the median concentration was 3 copies/ml (interquartile range [IQR] = 2-13). There were no statistically significant differences between participants based on detectable CSF HIV RNA at the first assessment, though the group with detectable CSF HIV RNA tended to have a shorter total length of time on ART (mean 6.4 years versus 9.1 years, p= 0.077)**.** For the 288 total visits, adherence was 100% for 265 visits (92%). There was no significant difference in 100% adherence between plasma detectable visits and plasma undetectable visits (89.2% versus 93.8%, p=0.18). There

was also no difference in 100% adherence between CSF detectable visits and CSF undetectable visits (90.9% versus 92.2%, p=0.74). In terms of HAND status, 92 of 96 participants had functional assessment results at baseline. 40 of 92 (43.5%) met criteria for HAND. Of this 40, 31 (77.5%) had ANI, five (12.5%) had MND, and four (10%) had HAD. Neurocognitive performance at the first assessment was not associated with detectable HIV RNA in plasma or CSF in unadjusted analyses, except for a trend association between detectable HIV RNA in CSF and worse motor performance (p=0.064). Depression symptoms were not significantly different (p=0.56) between those with detectable CSF HIV RNA (median BDI-II= 11, IQR 2-15) compared to those with undetectable CSF HIV RNA (median BDI-II= 9, IQR 4-19). Depression symptoms were also not significantly different (p=0.9) between those with detectable plasma HIV RNA (median BDI-II= 10, IQR 3-18) compared to those with undetectable plasma HIV RNA (median BDI-II= 9, IQR 4-19).

The median time between first and second assessments was 216 days (IQR = 179-358), and the median time between second and third assessments was 204 days (IQR= 171-340). Table 3 summarizes HIV RNA detectability for plasma and CSF at each visit and Supplementary Table A,http://links.lww.com/QAI/B659 provides HIV RNA data for each participant. For plasma, 45.8% had detectable HIV RNA at the first assessment, 33.3% had detectable HIV RNA at the second assessment, and 36.5% had detectable HIV RNA at the third assessment. For CSF, 17.7% had detectable HIV RNA at the first assessment, 7.3% had detectable HIV RNA at the second assessment, and 9.4% had detectable HIV RNA at the third assessment. In the logistic regression models that incorporated the time variable as continuous (weeks from first assessment), CSF HIV RNA detection significantly decreased (p= 0.018). Plasma HIV RNA detection tended to decline over time but did not reach statistical significance (p=0.064). See Figure 1 for predicted proportions of HIV detectability over time. Detectable HIV RNA status was strongly associated between plasma and CSF over time, OR = 3.22 (95% CI: 1.42, 7.30), p=0.004. Neither CSF leukocytes nor CPE were statistically significantly associated with CSF

HIV RNA, although they trended toward significance in adjusted models (CSF leukocytes: p=0.083; CPE: p=0.084). Interactions were tested between plasma HIV RNA and either CSF leukocytes or CPE in association with CSF HIV RNA but neither was statistically significant (CSF leukocytes: p=0.19; CPE: p=0.14).

We then analyzed the relationship between detectable CSF HIV RNA and neurocognitive performance using linear mixed-effects models with neurocognitive performance as outcome. In models that adjusted only for time, detectable CSF HIV RNA was associated with worse global performance (coefficient: -1.08 [95% CI: -2.13, -0.038], p=0.043). Associations were strongest in the recall (coefficient -2.87 [95% CI: -5.26, -0.47], p=0.02) and motor (coefficient -2.48 [95% CI: -4.88, -0.094], p=0.043) domains with a trend in processing speed (coefficient -1.55 [95% CI: -3.20, 0.10], p=0.067). Multivariable analyses identified additional statistically significant associations (Table 2). Detectable CSF HIV RNA tended to be associated with worse global performance (difference in mean T-scores (Δ)= -0.96, 95% CI=[-1.99, 0.074], p=0.070). Detectable CSF HIV RNA was significantly associated with worse recall (Δ= -3.04, 95% CI=[-5.46, -0.63], p=0.014) and motor (Δ= -2.50, 95% CI=[-4.92, -0.075], p=0.045) performance. See Figure 2 for recall and motor trajectories over time. When included as an additional predictor, plasma HIV RNA did not significantly predict cognitive performance and did not improve the final models. Even though integrase inhibitor therapy was very infrequent in this cohort, it was associated with better global performance (Δ=3.89, 95% CI=[1.21, 6.57], p=0.005), specifically in the verbal (Δ= 4.58, 95% CI=[0.37, 8.79], p=0.034) and learning (Δ= 5.09, 95% CI=[0.22, 9.96], p=0.041) domains.

Discussion

Given the high prevalence of HAND in the setting of ART and the challenge of eradicating HIV, a key gap in the HIV field is understanding HIV persistence in the CNS over time. SCAs have shown that

HIV RNA is detectable in blood in a large proportion of ART-treated individuals.[37] Previously, our group and others found that HIV is also often detectable in CSF during ART.[20,21] More research is needed on low-level HIV over time during ART as well as its relationship to clinical outcomes.

In this analysis, we evaluated longitudinal data collected from 96 PWH on suppressive ART with three consecutive plasma-CSF paired specimens with an assay that detects low level HIV RNA to the level of 1 copy/milliliter (and quantifiable to 3 copies/milliliter). We found that just under one fifth of participants had detectable HIV RNA in CSF at the first assessment. This proportion declined at the second assessment but then plateaued. Early studies of adults initiating ART showed that CSF HIV RNA decay can be slow and have substantial inter-individual variability in the first few weeks of treatment.[38] Our findings support that CSF HIV RNA decay may be slow and variable in longer term follow-up as well. Studies of peripheral blood have shown that HIV RNA decay continues for many years after ART initiation when measured by single copy assay.[19] This may be from long lived cells that express virus that are never completely eradicated over time. In terms of the CNS, both macrophage tropic and CCR5 tropic virus have been identified.[39] It is possible that the cellular sources for these viruses also decay over time and could be one of the reasons that CSF HIV is less likely to be detected during extended virologic suppression. The mean time on the current regimen for this cohort at the first assessment was over two years, meaning that several years of effective ART may be required to fully suppress HIV RNA in the CNS in some PWH. The proportion of detectable plasma HIV RNA specimens also tended to decrease over time, although this did not reach statistical significance in this moderately sized analysis. HIV RNA in plasma was the strongest correlate of HIV RNA in CSF, suggesting that even low-level HIV RNA in plasma below the lower limit of quantification may continue to seed the CNS and lead to worse neurocognitive outcomes. Neither CSF leukocytes nor CPE were statistically significantly associated with HIV RNA in CSF, which may reflect true biology or, with p values below 0.20, could reflect insufficient power in this moderately sized sample. Even if the

lack of statistical significance is due to inadequate power alone, this likely means that the effect sizes between HIV RNA in CSF and these two variables are not large.

We also found associations between low-level CSF HIV and worse neurocognitive performance, suggesting that this could contribute to the persistence of HAND (and perhaps other neurological and psychiatric complications) in treated populations. This is supported by studies of symptomatic CSF viral escape in which individuals develop neuropsychiatric symptoms in the setting of isolated HIV replication in CSF[40,41] (although asymptomatic CSF viral escape also occurs). This is also supported by studies that identified a relationship between magnitude of CSF HIV RNA concentrations in CSF and severity of neurocognitive impairment.[42] Substantial evidence supports that the CNS is an HIV reservoir during ART and that HIV in the CSF can be genetically distinct from HIV in blood.[23,43] Targeting HIV in the CNS may therefore be needed for HIV eradication in at least some PWH. Based on the current study, fully suppressing HIV in the CNS may also be needed to optimize neurocognitive performance. At the same time, the association with worse performance was only present in two cognitive domains. However, the domains of memory and motor skill are crucial for daily functioning and quality of life.[4]

Our study also found that integrase inhibitor therapy was associated with better neurocognitive performance in longitudinal analyses, a finding that is particularly notable considering the small number of raltegravir users in the cohort (<10%). One published randomized controlled trial comparing raltegravir to tenofovir-emtricitabine (both in combination with darunavir-ritonavir) found evidence of improvement in one individual neurocognitive test in the raltegravir group (although not in several others).[44] In contrast, evidence of integrase inhibitor neurotoxicity has been observed in other studies.[45,46] We acknowledge that the proportion of individuals in the study on integrase inhibitors was very small and that raltegravir was the only integrase inhibitor taken. Therefore, the integrase inhibitor finding may not be generalizable to all integrase inhibitors, particularly given the differences in chemical structure and CNS pharmacokinetics that have been demonstrated with the individual

molecules in this drug class.[47,48] For example, one observational study showed that discontinuation for neuropsychiatric toxicity was more common with dolutegravir than with raltegravir or elvitegravir.[49] More research is needed to better understand the relationship between integrase inhibitor therapy and neurocognitive outcomes. Participants in this study were more likely to be on protease inhibitors, some of which are known to have relatively low CNS penetration and have been associated with CNS virologic escape.[50,51]

Our study has several limitations. Women were not well represented in the study population, which was over 85% men. HIV infection in women continues to be a significant problem in the United States and worldwide,[52] and our study would be more generalizable if more women had enrolled. Participants were enrolled between the years of 2003 and 2012, and all participants were from sites in the United States. This time frame explains the relatively low rate of integrase inhibitor use. This study may not be generalizable to the current era, during which integrase inhibitor therapy is very common. We also acknowledge that the time between visits often varied considerably (IQR = 179-358 days between first and second visit and IQR= 171-340 days between second and third visit). While the mixed modeling technique accounts for length of time between visits, we acknowledge that the varying time between visits could mean that certain periods of time may have weighed more in the models. Specifically, if some of the visits were closely spaced, then the values from those visits (which might be similar to each other) may have weighed more than values from visits that were spaced farther apart (which might be less similar to each another). It is not totally clear why no NP differences were found between the two groups at baseline. It is possible that the inclusion of only baseline data was not enough to detect an NP difference and therefore was underpowered. It is also possible that the baseline results do not reflect the detrimental effect of CNS virus over time.

Neuroimaging would have been potentially very valuable in this study. We also cannot be certain that the low levels of CSF HIV RNA in this study derived from the CNS as opposed to the periphery,

particularly in light of the strong associations between HIV RNA levels in CSF and plasma in longitudinal analyses. However, plasma HIV RNA was not statistically significantly associated with global neurocognitive performance in longitudinal analyses, which supports the idea that it is ultimately low-level HIV RNA in the CNS that influences neurocognitive performance. Given that the primary aim of the study was to evaluate the significance of low level CNS HIV, we did not include other inflammatory biomarkers. These have been shown to be collinear with CSF HIV RNA and potentially could have confounded our analysis.[53] Having said this, consideration should be given to including other biomarkers in future studies of low level CSF HIV.

We also acknowledge the differences between the findings of this longitudinal study and the previous cross-sectional study published by our group (n=220).[20] The same SCA assay was used for both studies. The assessments in the current study are almost entirely independent of the prior analysis: 253 of the 288 total assessments (87.8%) in the current analysis represent new data. In support of the independence of the two groups, the frequency of the presence of HIV RNA was lower in this study than in the prior one, particularly in CSF at the first assessment (17.7% vs. 42.3%, $p < 0.001$). This could be due to the longer total duration of ART in the current study compared with the prior one (median 89.6 months versus 70.0 months, $p < 0.01$). Our finding of continued low-level HIV RNA decay over time suggests that years on suppressive ART may be required to reach a low-level HIV RNA set point in the CNS, or that it may fluctuate with interim but unmeasured events such as medical illnesses. In the longitudinal substudy of the prior study (n=55),[20] having undetectable CSF HIV RNA over time was associated with better neurocognitive performance, which is consistent with the current study. However, the cross-sectional portion of the prior study somewhat counterintuitively showed that when analyzed as a continuous outcome, higher CSF HIV RNA was associated with better cognition. However, a significant change in HIV RNA is generally considered to be at least 0.3-0.5 log10, meaning that small numerical changes in the absolute copies/milliliter (ml) may not be meaningful.[54] The finding related to

change in the continuous copy/ml result from the first study should be interpreted in this context. In both analyses, the observational, non-randomized design is prone to bias, which would likely be reduced in a randomized clinical trial and could reduce generalizability to clinical populations. Differences between the previous and current analyses could be due to differences in integrase inhibitor use, at least in part. A few participants used integrase inhibitors in this project (17 of 288 assessments, 5.9%) but none did in the prior analysis ($p < 0.001$). Additional research is required, particularly since integrase inhibitor use has substantially expanded since these assays were performed. The lack of relationship between CPE and CSF HIV RNA detectability in the current study also contrasts with the first study, which showed a significant relationship with higher CPE and lower CSF HIV RNA levels. Again, however, the rate of CSF HIV detectability was significantly lower in the current study, which may have decreased study power to detect a relationship.

This larger longitudinal analysis supports that a) HIV RNA persists in CSF in a small proportion of PWH on long-term suppressive ART, b) this is linked to low levels of HIV RNA in blood but not to ART regimen characteristics, and c) this may contribute to the neurocognitive complications that persist in PWH. An important recently published study showed that cell-associated HIV DNA in CSF is frequently detectable despite plasma HIV suppression during ART, and that detectability is associated with worse neuropsychological performance.[25] 48% of participants in that study had detectable HIV DNA, which is even higher that the HIV RNA detection rate in the current study. The two studies were similar by including only participants with virologic suppression who then underwent testing with a comprehensive NP panel that included at least five domains. Most importantly, the findings of the two studies are similar in that both show a relationship between CNS HIV persistence and worse cognition. More research is needed on the persistence of HIV in the CNS and its contribution to clinical outcomes.

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Figure 1: Predicted proportion of participants with detectable HIV over time

Figure 2: Individual participants trajectories, and group means and 95% confidence intervals at 0, 1, 2, 3, and 4 years, for recall mean T-score (left panel) and motor mean T-score (right panel).

Table 1: Cohort characteristics grouped by low level detectable HIV on single copy assay at the first assessment

Variable	Plasma			Cerebrospinal fluid		
	HIV RNA undetectable (n = 52)	HIV RNA detectable (n = 44)	P Value	HIV RNA undetectable (n = 79)	HIV RNA detectable (n = 17)	P Value
Demographics						
Years of age	48.4 (8.87)	45.1 (7.38)	0.055	46.9 (8.16)	46.7 (9.39)	0.93
Education (years)	13.3 (2.91)	13.1 (2.02)	0.77	13.3 (2.60)	12.6 (2.12)	0.31
Male Gender	46 (88.5%)	38 (86.4%)	0.77	70 (88.6%)	14 (82.4%)	0.44
Race/Ethnicity			0.097			0.97
African	15 (28.8%)	15 (34.1%)		24 (30.4%)	6 (35.3%)	
Hispanic	12 (23.1%)	3 (6.8%)		12 (15.2%)	3 (17.6%)	
European, non-Hispanic	22 (42.3%)	25 (56.8%)		39 (49.4%)	8 (47.1%)	
Other	3 (5.8%)	1 (2.3%)		4 (5.1%)	0 (0%)	
Medical History						
History of AIDS	34 (65.4%)	30 (68.2%)	0.83	53 (67.1%)	11 (64.7%)	1.00
Current CD4+ T-cell count[a]	626 [432, 802]	498 [307,	0.020*	585 [396, 718]	481 [310,	0.17
Nadir CD4+ T-cell count[a]	144 [10, 232]	660]	0.96	139 [20, 238]	659]	0.86
HCV Seropositive	18 (40.9%)	133 [38, 249]	1.00	25 (36.2%)	137 [25, 247]	0.16
Estimated HIV duration (years)	13.9 (6.78)	16 (39.0%)	0.68	13.8 (6.61)	9 (56.2%)	0.53
ART Characteristics						
On initial regimen	12 (23.1%)	4 (9.1%)	0.099	15 (19.0%)	1 (5.9%)	0.29
Duration of current regimen (years)	2.79 (2.97)	1.87 (1.94)	0.083	2.54 (2.74)	1.56 (1.45)	0.16
Total duration on ART (years)	9.46 (6.48)	7.63 (4.70)	0.12	9.10 (5.96)	6.38 (4.32)	0.077
Number of drugs failed			0.24			0.41
0	12 (23.1%)	4 (9.09%)		15 (19.0%)	1 (5.88%)	
1-6	33 (63.5%)	29 (65.9%)		48 (60.8%)	14 (82.4%)	
7-13	7 (13.5)	11 (25.0%)		16 (20.3%)	2 (11.8%)	
Regimen by ART Drug Class						
PIs	30 (57.7%)	31 (70.5%)	0.38	49 (62.0%)	12 (70.6%)	0.64
NNRTIs	25 (48.1%)	13 (29.5%)	0.060	31 (39.2%)	7 (41.2%)	1.00
NRTIs	51 (98.1%)	42 (95.5%)	0.29	78 (98.7%)	16 (94.1%)	0.20
Integrase inhibitors	2 (3.9%)	2 (4.6%)	1.00	3 (3.8%)	1 (5.9%)	0.55
CPE	8.40 (2.23)	8.05 (2.21)	0.43	8.28 (2.29)	8.06 (1.89)	0.71
CPE ≥ median (7)	42 (80.8%)	33 (75.0%)	0.62	62 (78.5%)	13 (76.5%)	1.00
Neurocognitive testing						
Global T-score	46.2 (7.03)	47.5 (6.49)	0.34	46.7 (6.97)	47.1 (6.00)	0.86
Verbal T-score	48.1 (9.55)	50.9 (8.37)	0.14	49.2 (9.14)	50.8 (9.05)	0.53
Executive Function T-score	45.2 (10.0)	47.6 (9.17)	0.23	45.8 (9.61)	48.9 (9.90)	0.23
Processing Speed T-score	49.2 (8.53)	49.4 (10.5)	0.91	49.1 (9.53)	50.2 (9.23)	0.69
Learning T-score	44.0 (10.5)	44.8 (8.45)	0.68	44.4 (9.83)	44.5 (8.47)	0.95
Recall T-score	44.2 (10.6)	45.3 (8.78)	0.57	44.7 (10.3)	44.9 (6.40)	0.93
Working memory T-score	46.3 (10.5)	48.9 (8.78)	0.20	47.1 (10.0)	49.0 (8.87)	0.48
Motor T-score	44.7 (9.70)	45.0 (10.4)	0.92	45.7 (9.74)	40.6 (10.4)	0.064

Values are Mean (standard deviation), Median [interquartile range], or N (%). *p<0.05; [a] square-root transformed prior to comparison analysis. #=number of drugs combined; HIV= human immunodeficiency virus; RNA= ribonucleic acid; CD= Cluster of differentiation; HCV= hepatitis C virus; cART= combination antiretroviral therapy; PI= protease inhibitor; NNRTI= non-nucleoside reverse transcriptase inhibitor; NRTI= nucleoside reverse trasnscriptase inhibitor; CPE= central nervous system penetration effectiveness score; NP= neuropsychological. Detectable indicates ≥1 copy/ml

Table 2: Detectable CSF HIV RNA as a predictor of neurocognitive global and domain T-scores. Longitudinal analysis using linear mixed-effects model, adjusted for confounders.

Outcome (T score)	Predictor[a]	Coefficient (95% CI)	P Value
Global	Detectable HIV RNA, CSF	-0.96 [-1.99, 0.074]	0.070
	Integrase Inhibitor Use	3.89 [1.21, 6.57]	0.005**
	Number of drugs failed	-0.48 [-0.84, -0.12]	0.010*
	Time since baseline (years)	0.45 [0.096, 0.80]	0.013*
Recall	Detectable HIV RNA, CSF	-3.04 [-5.46, -0.63]	0.014*
	Integrase Inhibitor Use	3.88 [-1.27, 9.03]	0.14
	Duration of all ART (months)	-0.020 [-0.045, 0.0053]	0.13
	Time since baseline (years)	-0.017 [-0.83, 0.79]	0.97
Motor	Detectable HIV RNA, CSF	-2.50 [-4.92, -0.075]	0.045*
	Number of drugs failed	-0.75 [-1.27, -0.24]	0.005**
	Time since baseline (years)	0.34 [-0.43, 1.12]	0.39
Processing speed	Detectable HIV RNA, CSF	-1.40 [-3.05, 0.25]	0.098
	Integrase Inhibitor Use	4.15 [-0.065, 8.37]	0.055
	Number of drugs failed	-0.79 [-1.33, -0.25]	0.005**
	Time since baseline (years)	0.63 [0.071, 1.19]	0.028*
Learning	Detectable HIV RNA, CSF	-1.88 [-4.14, 0.38]	0.10
	Integrase Inhibitor Use	5.09 [0.22, 9.96]	0.041*
	Duration of all ART (months)	-0.018 [-0.042, 0.0055]	0.14
	Time since baseline (years)	0.14 [-0.62, 0.90]	0.71
Executive Function	Detectable HIV RNA, CSF	1.78 [-1.04, 4.61]	0.22
	Number of drugs failed	-0.52 [-1.03, -0.003]	0.051
	Time since baseline (years)	2.18 [1.27, 3.10]	<0.001**
Working Memory	Detectable HIV RNA, CSF	0.80 [-0.99, 2.59]	0.38
	Time since baseline (years)	0.25 [-0.31, 0.81]	0.39
Verbal	Detectable HIV RNA, CSF	0.034 [-1.78, 1.85]	0.97
	Integrase Inhibitor Use	4.58 [0.37, 8.79]	0.034*
	Current CD4+ T-cell Count[b]	0.18 [0.0034, 0.36]	0.047*
	Duration of all ART (months)	-0.018 [-0.044, 0.0076]	0.17
	Time since baseline (years)	-0.11 [-0.75, 0.52]	0.73

[a]CSF Detectable RNA and time were forced into the linear mixed-effects models, and current/nadir CD4 counts, duration of all ART, integrase inhibitors (IIs), and NRTIs then entered to the models as covariates; the covariates with p-value ≤ 0.2 were retained in the model; CI= confidence interval; CSF= cerebrospinal fluid; RNA=ribonucleic acid; IIs= Integrase inhibitor therapy; CD= cluster of differentiation; ART= antiretroviral therapy; NRTI= nucleoside reverse transcriptase inhibitor; [b]square-root transformation; *p<0.05; **p<0.01. For categorical predictors, the coefficient of the linear mixed-effects model is interpreted as difference of mean response (global or domain T-scores) between groups determined by the predictor, all other predictors being held constant.

Table 3: CSF and plasma HIV RNA status at each study visit. Numbers reported represent N/Total N (%)

	Detectable HIV RNA, CSF	Detectable HIV RNA, Plasma	Detectable HIV RNA, Plasma and CSF
Visit 1	17/96 (17.7%)	44/96 (45.8%)	12/96 (12.5%)
Visit 2	7/96 (7.29%)	32/96 (33.3%)	4/96 (4.17%)
Visit 3	9/96 (9.38%)	35/96 (36.5%)	5/96 (5.21%)
At least one visit	28/96 (29.17%)	68/96 (70.8%)	19/96 (19.8%)
All three visits	0/96 (0.00%)	9/96 (9.38%)	0/96 (0.00%)

Figure 1: Predicted proportion of participants with detectable HIV over time

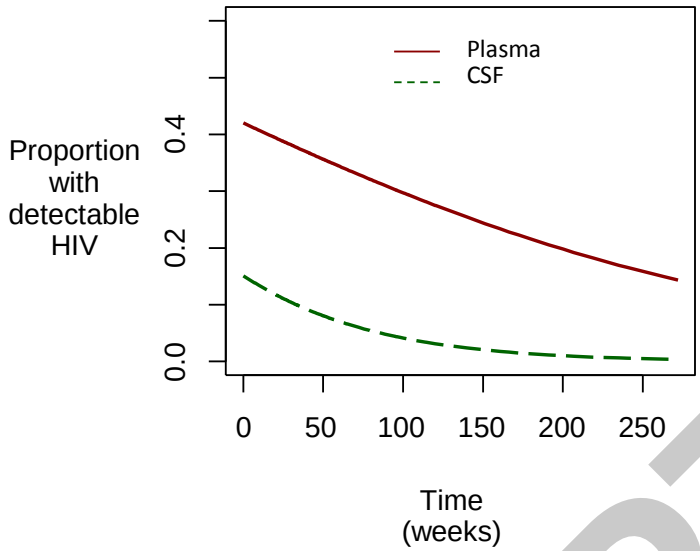

Figure 2: Individual participants trajectories, and group means and 95% confidence intervals at 0, 1, 2, 3, and 4 years, for recall mean T-score (left panel) and motor mean T-score (right panel).