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Title
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Permalink
https://escholarship.org/uc/item/1wp7979h

Journal
The Journal of Infectious Diseases, 215(1)

ISSN
0022-1899

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Publication Date
2017

DOI
10.1093/infdis/jiw505

Peer reviewed

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Prevalence and Correlates of Persistent HIV-1 RNA in Cerebrospinal Fluid During Antiretroviral Therapy

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ABSTRACT

Background: Neurocognitive disorders remain common among HIV+ adults, perhaps due to persistent HIV-1 RNA in cerebrospinal fluid (CSF) during antiretroviral therapy (ART).

Methods: Using a single copy assay, we measured HIV-1 RNA in CSF and plasma from 220 HIV+ adults who were taking suppressive ART. Fifty-five participants were tested twice.

Results: HIV-1 RNA was detected in 42.3% of CSF and 65.2% of plasma samples. Correlates of higher CSF HIV-1 RNA included higher nadir and current CD4+ counts, plasma HIV-1 RNA ≥ 1 c/mL, and lower CPE values (Model p<0.001). Worse neurocognitive (NC) performance was associated with the HIV-1 RNA discordance, lower overall CSF HIV-1 RNA, and longer ART duration among others (Model p<0.001). In the longitudinal subgroup, CSF HIV-1 RNA persisted in most (69%) participants over 7 months.

Conclusions: Low-level HIV-1 RNA in CSF is common during suppressive ART and is associated with low-level HIV-1 RNA in blood, better immune status, and lower ART drug distribution into CSF. The association between HIV-1 RNA discordance and HAND may reflect compartmentalization. The relationship between HAND, lower HIV-1 RNA in CSF, and lower CD4+ counts may reflect disturbances in the immune response to HIV-1 in the CNS.

INTRODUCTION

HIV-associated neurocognitive disorder (HAND) is common, ranging from 30% to 70% of HIV-infected adults, including those taking combination antiretroviral therapy (ART) [1-3]. Several explanations may account for this, including advancing age [4, 5], longer duration of exposure to HIV, comorbid conditions [6, 7], and more advanced immune suppression [8, 9]. Another, non-exclusive explanation for high HAND prevalence among treated individuals is incomplete effectiveness or toxicity of ART in the central nervous system (CNS) [10].

HIV-1 enters the CNS soon after infection and can be protected in this compartment from immune and drug pressure [11, 12]. Autopsy and neuroimaging studies have identified that HIV-1 can localize in the basal ganglia and hippocampus [13, 14], even during the first weeks of infection [15]. Potent ART can reduce HIV-1 in blood and cerebrospinal fluid (CSF) below the quantification limit of commercially available assays but HIV-1 might continue to replicate at low levels, increasing the risk for viral compartmentalization in the CNS [16]. Persistent low-level HIV-1 replication could also lead to glial activation and neuronal injury.

Published reports have identified that low-level HIV-1 is present in CSF in up to 28% of adults taking ART [17, 18] but have not found associations with estimated ART drug distribution into the CNS or neurocognitive (NC) outcomes. Limitations of these projects included their small sample size and the assay method. This method required ultracentrifugation of up to 12 mL of CSF, which could be prone to inaccuracy. Simpler methods, such as one that uses molecular beacons and does not require ultracentrifugation, might yield different results.

The objectives of this project were to determine how frequently HIV-1 RNA was present at low levels in CSF during suppressive ART and whether low-level HIV-1 RNA in CSF was associated with worse estimated ART drug distribution into the CNS and worse neurocognitive (NC) performance.

METHODS

Participants and Procedures

The CNS HIV-1 Antiretroviral Therapy Effects Research (CHARTER) cohort is composed of 1,555 HIV-1-infected adults who provided written informed consent for all study procedures. All

subjects completed venipuncture, neuromedical assessment, and comprehensive NC testing. The Human Subjects Protection Committees of each institution approved all procedures. 220 participants were selected for this project based on four criteria: use of 3-drug combination ART; HIV-1 RNA levels ≤ 50 copies/mL in plasma and CSF; absence of comorbid conditions of sufficient severity to account for impaired NC performance;[2] and at least 2 mL of plasma and CSF stored at -70°C. Selected participants had been assessed between October 2003 and May 2008. To assess changes in low-level HIV-1 RNA over time, a second CSF sample was assayed in 55 participants whose ART regimen was stable and who had HIV-1 RNA levels ≤ 50 c/mL in plasma and CSF at the second time point.

Laboratory Assessment

HIV-1 infection was diagnosed by enzyme linked immunosorbent assay with Western blot confirmation. Routine clinical chemistry panels, complete blood counts, rapid plasma reagin (RPR), HCV antibody, and CD4+ T-cells (flow cytometry) were performed at each site's Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. HIV-1 RNA levels were measured centrally in plasma and CSF by reverse transcriptase-polymerase chain reaction (Roche Amplicor, v. 1.5, lower limit of quantification 50 copies/mL).

To measure HIV-1 RNA levels below 50 copies/mL, a validated single copy assay (SCA) procedure that has been used in other studies of HIV-1 RNA detection from the CSF was employed[19]. The HIV-1 SuperLow Assay (bioMONTR Labs) is a proprietary, modified version of the NucliSENS EasyQ assay (bioMèrieux) and is capable of quantifying HIV-1 as low as 1 copy/mL. The standard EasyQ assay was modified by extraction of 2 mL of fluid using magnetic bead technology (miniMAG system from bioMèrieux). 25 µL of extracted eluate, 20 µL of primer, and 5 µL of 2X enzyme in place of standard kit volumes were used. Molecular beacons targeting the pol/gag region of HIV-1 RNA are utilized for amplification and detection by isothermal reactions at 41°C. HIV-1 RNA level was quantified using a proprietary reduction algorithm in conjunction with the NucliSENS EasyQ H HIV-1 v2.0 Director software[20].

Neuromedical Assessment

This assessment included medical history, structured neurological and medical examination, and body fluid collection. Participants in this project reported use of 57 different ART combinations, the most common of which are summarized in the supplemental materials. Four-day ART adherence was estimated by self-report. ART drug distribution into the CNS was estimated by the CNS penetration-effectiveness (CPE) method.[21]

Neurobehavioral Assessment

The comprehensive NC test battery assessed seven cognitive domains affected by HIV-1 disease.[2] The best available normative standards were used, which adjusted for effects of age, education, gender, and ethnicity. To classify presence and severity of NC impairment, a published objective algorithm was applied that requires presence of a least mild impairment in at least two cognitive domains; conforms to the Frascati criteria for diagnosing HAND;[1] and yielded excellent inter-rater reliability in prior studies.[22] NC performance was then summarized by the global clinical rating, a validated method that integrates relevant information about the seven NC domains and yields a value between 1 (normal performance) and 9 (severely impaired performance), with a value of 5 indicating definite, mild impairment.[23] Frascati guidelines were also used to classify comorbid neuropsychiatric conditions, the most common of which are summarized in the supplemental materials.

Data Analyses

Statistical methods included Pearson's correlation, t-tests, multivariable regression, and recursive partitioning. Data distributions were inspected and data were transformed to improve the symmetry of their distributions as needed. When transformation did not adequately improve the distribution of highly skewed data distributions, non-parametric statistical tests were used. When appropriate, Cohen's d was calculated to estimate effect size [24]. Multivariable regression was performed in four stages. First, candidate covariates were screened using a selection alpha of 0.15. Candidates included age, current and nadir CD4+ T-cell count, estimated duration of HIV-1 disease, duration of the current ART regimen, CPE of the current regimen, the total number of previous ART regimens, ART adherence, HCV serostatus, and RPR reactivity status. Second, all candidate

covariates that met the selection criterion were included in a full model. Third, the Akaike

Information Criterion (AIC) was used to identify the best model using stepwise selection. Finally,

first-order interactions were evaluated among retained covariates. When statistically significant

interactions were found, the nature of the interaction was investigated with recursive partitioning.

Analyses were performed in the total sample as well as in the subgroup that had negative HCV and

non-reactive RPR tests (n=155). Statistical analyses were performed using JMP v.12 (SAS Institute,

Cary, NC).

RESULTS

Demographics and Disease Characteristics

As summarized in **Table 1**, the median age of participants was 44 years [interquartile range

(IQR) = 39-50] and more than three-quarters were men. Subjects were from diverse racial/ethnic

backgrounds. Most subjects had been infected for longer than 10 years; had experienced advanced

immune suppression in the past; and had improved immune status during ART. About one-quarter

were taking their initial cART regimen and more than half had been on their current cART regimen

for over one year. Supplemental materials include a summary of differences between this group and

the larger CHARTER cohort.

Correlates of Low-Level HIV-1 in CSF

Primary analyses. Ninety-three (42.3%) subjects had at least 1 c/mL of HIV-1 RNA in CSF.

Since the distribution of values was highly skewed (median 0, mean 2.75, Shapiro-Wilk W=0.42,

Figure 1a), categorical analyses (binary transformation (< 1 c/mL, ≥ 1 c/mL)) were performed in

addition to analyses of continuous HIV-1 RNA values. **Table 1** summarizes the differences between

participants who had ≥ 1 c/mL of HIV-1 in CSF and those who did not.

In the initial stage of multivariable logistic regression modeling of CSF HIV-1 RNA ≥ 1 c/mL (i.e.,

the binary variable) that included all explanatory variables identified by univariate screening, only

CD4+ T-cell count had a parameter estimate p value < 0.05 (model R^2=0.07, p=0.01). In the next

stage, AIC values selected higher current CD4+ T-cell counts (p=0.005), lower CPE values

(p=0.009), HCV seronegativity (p=0.08), and shorter duration of all ART regimens (p=0.09) as

associated with CSF HIV-1 RNA ≥ 1 c/mL (model R^2=0.06, p<0.001). Interaction modeling identified

that higher CD4+ T-cell counts were only associated with CSF HIV-1 RNA ≥ 1 c/mL when CPE values < 7 (Model R^2=0.07, p<0.001).

In analyses of continuous (instead of binary) CSF HIV-1 RNA, higher current CD4+ T-cell counts (p=0.05) and lower CPE values (p<0.001) were again selected in the best models. The best model also included HIV-1 RNA levels in plasma ≥ 1 c/mL (p=0.04), race/ethnicity (higher CSF HIV-1 RNA in participants who identified as black or Hispanic, p=0.006), and higher nadir CD4+ T-cell counts (p=0.08) (model R^2=0.13, p<0.001). Interaction modeling identified that black or Hispanic participants who took lower CPE regimens (values < 7) had the highest HIV-1 RNA levels in CSF.

Since HIV-1 RNA levels in CSF were associated with the presence of HIV-1 RNA in plasma (**Figure 1b**) in the strongest models, we categorized participants into 4 groups based on the presence or absence of HIV-1 RNA in each body fluid. Two categories were concordant (detectable (+) in both fluids, n=65; or undetectable (-) in both fluids, n=48) and two were discordant (detectable in CSF but undetectable in plasma (CSF+Plasma-), n=28; or undetectable in CSF and detectable in plasma (CSF-Plasma+), n=79).

Secondary analyses. Supplemental materials include secondary analyses, including comparisons of CSF HIV-1 RNA with HCV and RPR serostatus; a subgroup analysis in participants who were HCV seronegative and RPR non-reactive; and comparisons with CSF inflammation-associated biomarkers, CSF protein, and CSF leukocytes.

Correlates of Neurocognitive Performance

Primary analyses. Table 2 summarizes the analysis of global NC performance. Univariate analyses identified that worse NC performance was associated with lower CSF HIV-1 RNA, contributing neuropsychiatric conditions, HCV seropositivity, AIDS, and longer total duration of ART. Four other variables had p values between 0.05 and 0.15 and were included as candidate covariates in multivariable modeling. The initial model identified that the strongest covariates were CSF HIV-1 RNA (**Figure 2a**), CSF+Plasma- discordance (**Figure 2b**), and HCV (Model R^2=0.13, p<0.001). The AIC selection model (AIC Model 1 in **Table 2**) identified associations between worse NC performance and five covariates (Model R^2=0.11, p<0.001), including both lower CSF HIV-1 RNA and the presence of CSF+Plasma- discordance. Interaction analysis (AIC Model 2 in **Table 2**,

Model R^2=0.18, p<0.001) identified that lower HIV-1 RNA levels correlated with worse NC
performance in HCV seronegative participants (r=-0.25, p=0.001) but not in HCV seropositive
participants (r=-0.02, p=0.86).

Among the seven cognitive domains assessed, lower HIV-1 RNA levels in CSF were most
strongly associated with worse performance in speed of information processing (r=-0.23, p=0.004),
learning (r=-0.20, p=0.02), and working memory (r=-0.20, p=0.01). In contrast, CSF+Plasma-
discordance was associated with worse performance in two other cognitive domains, verbal fluency
(p=0.03) and possibly executive functioning (p=0.09), suggesting that the mechanisms of injury for
these two conditions are distinct.

Secondary analyses. Supplemental materials include comparisons of NC performance with
HCV and RPR status, ART drug classes, and individual ART drugs.

Longitudinal Analysis of Low-Level HIV-1 in CSF over Time

In the 55 subjects who had a second CSF specimen assayed, the median duration between
visits was 7 months (IQR 5.7 – 8.9 months). Forty-three (78%) subjects had HIV-1 RNA levels in
CSF ≥ 1 c/mL at their first visit and, among this group, HIV-1 RNA remained ≥ 1 c/mL at the second
visit in 38 (88%). Among the 12 subjects who had HIV-1 RNA levels < 1 c/mL at the first visit, 3
(25%) remained < 1 c/mL at the second visit. **Figure 3** shows the four groups defined by the
presence or absence of HIV-1 RNA in CSF at the first or second visit against change in Global
Rating, identifying that the 3 (5%) subjects who maintained HIV-1 RNA levels below 1 c/mL in CSF
improved their neurocognitive performance compared with those who had HIV-1 RNA levels of more
than 1 c/mL in CSF from at least one visit. No secondary analyses were performed in this subgroup
because of the small sample size.

DISCUSSION

HIV-associated neurocognitive disorder (HAND) continues to commonly occur, even in adults
who are taking suppressive ART [2]. Consistent with this observation, substantial evidence has
accumulated that systemic and end-organ inflammation can also persist during virologic
suppression, perhaps as a result of production of HIV-1 RNA below the quantification limit of

commercial assays [25]. These findings support the need to improve understanding of the mechanisms of CNS injury in the combination ART era.

In our study of 220 HIV+ adults with HIV-1 RNA levels ≤ 50 copies/mL in both plasma and CSF, we found low-level HIV-1 RNA in the CSF of approximately 4 of 10 participants. This proportion is higher than prior studies, which have reported proportions up to 28% [17, 18, 26] Explanations for this apparent disagreement include differences in the assay used, the sample size, and cohort characteristics. Our study is the largest to date, which may make our estimates more representative of clinical populations. While we used a different assay than prior studies, its accuracy has been previously validated in CSF [19]. CHARTER was designed to generalize to U.S. clinical populations but our study participants were a subgroup of the larger CHARTER cohort. For this reason, selection bias could also explain why our findings differ from other reports.

The finding that nearly two-thirds of participants had HIV-1 RNA levels in blood ≥ 1 c/mL more closely matches prior reports from HIV+ adults taking suppressive ART[27]. This low-level circulating HIV-1 is associated with chronic inflammation in both adults who take suppressive ART and in those who spontaneously control HIV[28, 29]. A similar scenario may occur in the CNS in which low levels of HIV-1 RNA (or proteins) act as immune stimuli, resulting in chronic inflammation and injury of glia and neurons. Lymphocytes and monocytes that migrate into the CNS from the systemic circulation may be the source of low-level HIV-1 RNA in CSF in some cases, but the presence of HIV-1 RNA in CSF in the absence of detectable HIV-1 in blood suggests that viral compartmentalization in the CNS has occurred. Our study showed that approximately 1 in 8 participants (28 of 220 (12.7%)) had discordant HIV-1 RNA with levels ≥ 1 c/mL in CSF and < 1 c/mL in blood. Since these groups differ by as little as 1 copy of HIV RNA per mL of CSF or blood, the finding could theoretically occur due to differences in specimen processing or assay performance. However, the statistically significant associations between this discordant condition and several other characteristics support that it results from biological processes. Such discordance may be part of a spectrum of conditions recognized in more severe forms as CSF viral escape, which also occurs in approximately 10% of adults taking suppressive ART[26, 30]. Our study demonstrated that CSF+Plasma- discordance was independently associated with worse global NC performance, although this association weakened when participants who were either HCV+ or

RPR+ were excluded. Combined, our findings support that syphilis, HCV, and CSF viral escape could contribute to HAND in at least a subgroup of adults taking suppressive ART.

Several studies have reported that ART regimens that have greater estimated distribution into the CNS are associated with lower HIV-1 RNA levels in CSF [31, 32]. An important limitation of prior analyses, though, has been inclusion of participants who had plasma HIV-1 RNA levels > 50 c/mL. Our study directly addresses this limitation by including only participants who were taking ART with plasma HIV-1 RNA ≤ 50 c/mL. We again found that higher CPE values were statistically significantly associated with lower HIV-1 RNA levels in CSF, even in multivariable analyses. While reports linking CPE to HIV-1 RNA levels in CSF have had consistent findings, those comparing CPE to NC performance or neuroimaging findings have not. For instance, some reports have found that higher CPE regimens were associated with better NC outcomes[33] while others have found evidence of worse outcomes [34]. While differences in design and power account for at least some of these inconsistencies[35], disagreement between studies could also reflect that HIV-1 RNA levels in CSF relate differently to NC outcomes during treatment with today's potent regimens than in the past.

Our analyses also found that lower HIV-1 RNA levels in CSF were associated with worse NC performance, the direction of which is contrary to our hypothesis. Since higher CPE regimens may sometimes be prescribed for HAND and were associated with lower HIV-1 RNA levels in CSF in this project, one possible explanation for this unexpected, cross-sectional finding is that some participants had pre-existing HAND that had not fully responded to ART[36]. Consistent with this, secondary analyses in a subgroup of 109 participants who had been previously assessed supported that NC impairment was not improving (see supplemental materials). This could be due to ongoing disturbances in the CNS immune response, which may be particularly prominent in patients whose CD4+ T-cell counts have previously declined to low levels.

The relationship between low CD4+ T-cell counts and low HIV-1 RNA levels provides another clue about how low HIV-1 RNA levels in CSF might predispose to HAND. Multiple studies have found that a low nadir CD4+ T-cell count increases the risk for developing HAND and that this risk appears to persist even after immune recovery [9]. This may reflect persistent disturbances in the CNS immune response, characterized by altered migration and activity of monocytes and lymphocytes that affect HIV clearance and compartmentalization[37-39]. The correlation between

lower CSF leukocyte number (see supplemental material) and lower HIV-1 RNA level may indicate that fewer activated, replication-competent cells are being "pushed" into the CNS from the periphery or that fewer are being "pulled" into the CNS by HIV replication in CNS-resident cells (or both)[40]. While an overly robust immune response could injure the brain[41], the absence of an adequate response could favor development of compartmentalization[39]; could deprive the brain of neurotrophic factors that would support recovery from HAND[42]; and could worsen control in the CNS of other pathogens, like Treponema, HCV, Cytomegalovirus, and Toxoplasma.

Another possible and non-mutually exclusive explanation is ART neurotoxicity, either directly via neuronal or glial injury [32, 43] or indirectly via metabolic or vascular disease[44, 45]. The observed association between worse NC performance and the combination of lower HIV-1 RNA levels in CSF (as an indicator of more potent ART) and longer durations of ART support this conclusion. Weighing against the explanation of ART neurotoxicity is the absence of an association between worse NC performance and either higher CPE values or use of individual ART drugs with known neurotoxicity, such as efavirenz.[46, 47] Subgroup analyses, while modest in scope, also supported that lower HIV-1 RNA levels in CSF may be beneficial, e.g., the longitudinal analysis or the correlation with lower levels of some inflammation biomarkers in CSF (not shown: IL-6: ρ=0.49, p=0.01; TNF-α: ρ=0.36, p=0.07). Any of these effects (ART-unresponsive HAND, disturbed CNS immune response, ART toxicity) could impact patients with contributing neuropsychiatric conditions to a greater extent than those with minimal neuropsychiatric comorbidities, consistent with our findings and the concept of cognitive reserve or vulnerability[48].

The discussion thus far has focused on the detrimental effects of undetectable HIV-1 RNA levels in CSF but the converse might also be true. Could the presence of low-level HIV-1 RNA in CSF protect from HAND? If low-level HIV-1 RNA reflects persistent HIV-1 replication, then it might be due to the presence of drug resistance mutations (DRMs). If these DRMs include those that reduce viral fitness, such as M184V, then this could be associated with better NC outcomes, as we have previously found[49]. If persistent HIV-1 replication also stimulates a more effective immune response that reduces the size of the CNS reservoir, then the combination of reduced viral fitness and viral clearance from the CNS should be beneficial. The current analysis does not include data to directly test this hypothesis.

The strengths of our study include its large sample size, the comprehensiveness of the assessments, and the sensitivity of the SCA. Its weaknesses include its cross-sectional design of the primary study, the post-hoc nature of some of the analyses, the inconsistent direction of the cross-sectional and longitudinal analyses, the small longitudinal sample size, and the heterogeneity of the cohort, including nearly 30% having either a positive HCV antibody or RPR test. Our selected study population may also be less generalizable to clinical populations than the larger CHARTER cohort. In addition, our best multivariable models had relatively modest coefficients of determination, indicating that they explained less than 20% of variation in viral or neurocognitive outcomes. Validation of our findings in an independent cohort is essential.

If correct, our findings support a complex approach for HAND management in which clinicians should consider ART drug characteristics, CD4+ T-cell counts, neuropsychiatric conditions, and co-infections, among others. The best approach may be the use of ART regimens that balance sufficient potency in the CNS with the absence of neurotoxicity, similar to those currently recommended by the U.S. Department of Health and Human Services[50]. Ultimately, randomized controlled trials of ART regimens will be needed to inform the clinical management of HAND.

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CONFLICTS OF INTEREST

SLL: Research funding has been provided to the University of California, San Diego on behalf of SLL by Gilead Sciences, ViiV Healthcare, and Merck & Co. Compensation has also been paid to SLL for educational lectures or advisory boards by Cipla, Gilead Sciences, Janssen, Merck & Co., and ViiV Healthcare.

ACC: Research funding or supplies have been provided to the University of Washington, Seattle on behalf of ACC by Schering Plough, Merck & Co., and Roche Molecular Systems. Compensation has been paid to ACC for educational lectures by the International Antiviral Society-USA and by Merck & Co. for participation in a Data Safety and Monitoring Committee. ACC or her immediate family formerly owned stock in Abbott Laboratories, Bristol-Myers-Squibb, Johnson & Johnson, and Pfizer.

FIGURE CAPTIONS

Figure 1. a) Distribution of HIV-1 RNA levels among the 93 participants with values ≥ 1 c/mL. b) Although HIV-1 RNA levels in CSF did not correlate with those in plasma (not shown), participants who had HIV-1 RNA levels ≥ 1 c/mL in plasma had higher HIV-1 RNA levels in CSF. For clarity, the bars indicating mean and 95% confidence interval are shown next to the data points rather than superimposed over them.

Figure 2. a) Lower HIV-1 RNA levels in CSF correlated with worse global NC performance. b) The discordant CSF+Plasma- group had worse neurocognitive performance than the group with HIV-1 RNA ≥ 1 c/mL in both fluids. The dashed line indicates the threshold value for impairment (5). Global impairment was present in 60.7% of the CSF+Plasma- group compared with 41.5% of the CSF+Plasma+ group (p=0.09).

Figure 3. Having HIV-1 RNA < 1 c/mL in CSF at both visits was associated with improved neurocognitive performance over time.

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50. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescen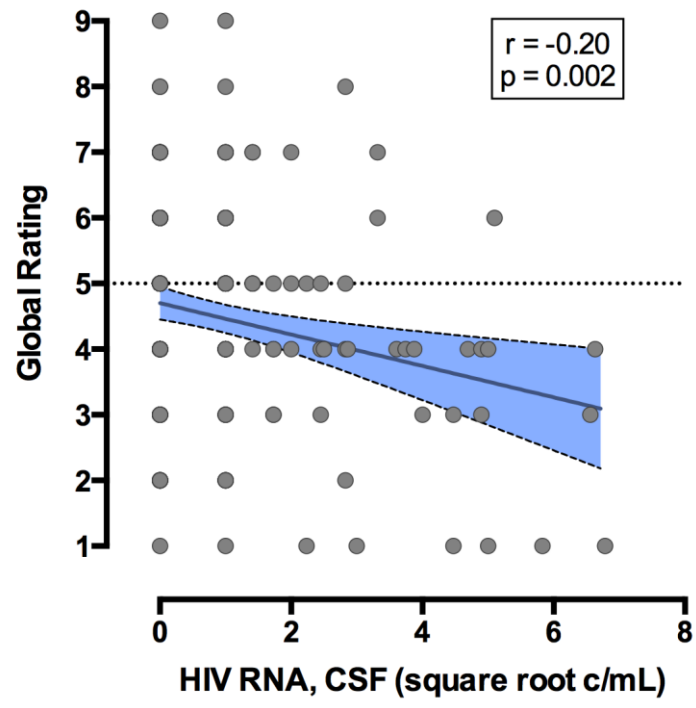ts. Department of Health and Human Services. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf. Section accessed 13 July 2015.
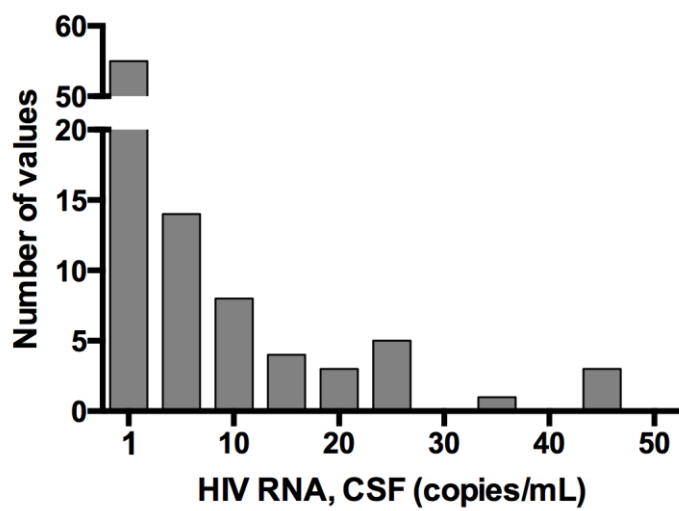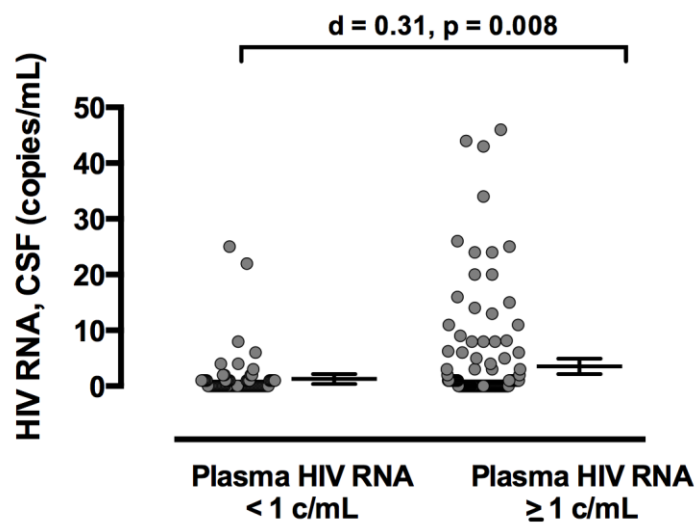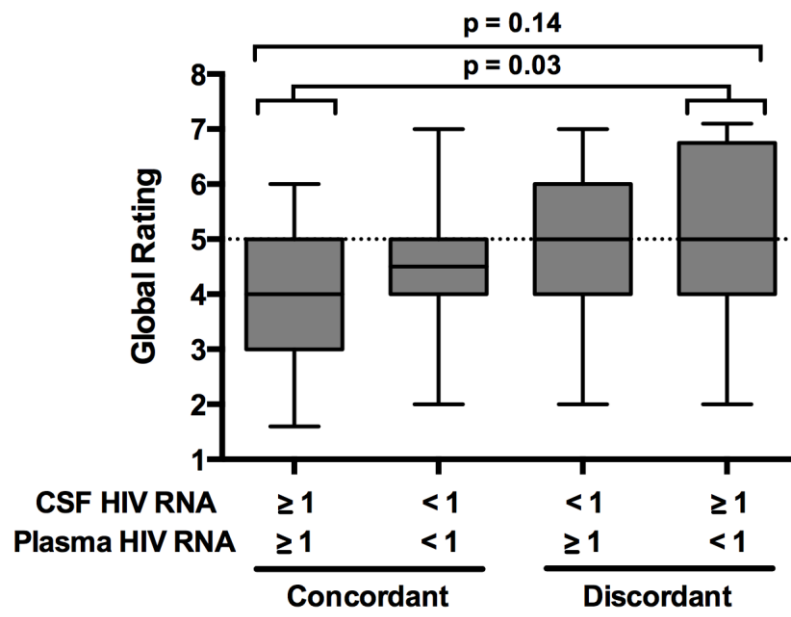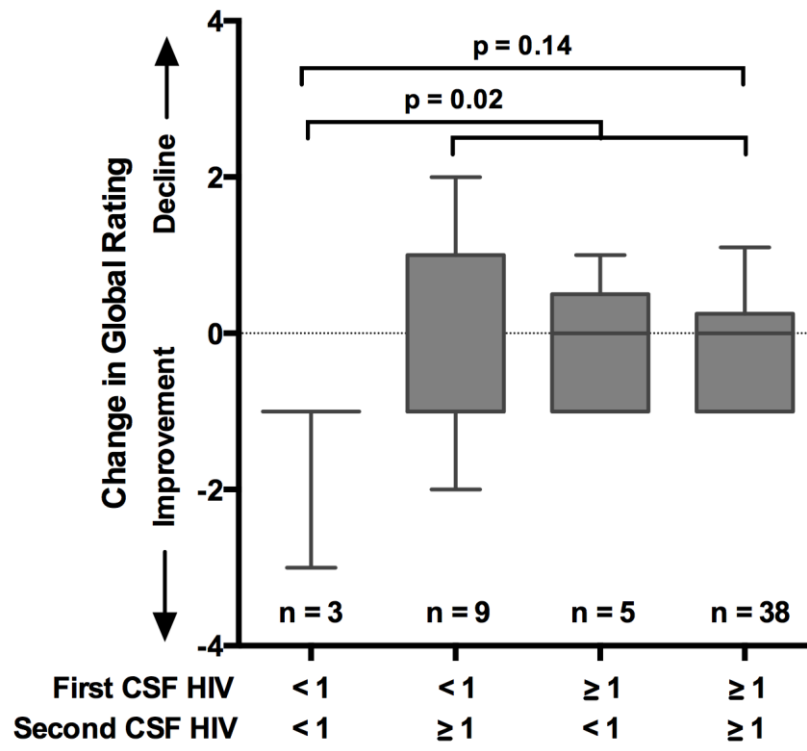
Table 1. Demographic and clinical characteristics of the sample. Values are expressed as median (interquartile range) unless otherwise specified. Characteristics that have bolded p values were included in multivariable analyses. Abbreviations: HIV= Human Immunodeficiency Virus; RNA = Ribonucleic Acid; ml=milliliter; CD = Cluster of differentiation; µL= microliter; AIDS = Acquired Immunodeficiency Syndrome; RPR = Rapid Plasma Reagin; HCV = Hepatitis C Virus; ART = combination antiretroviral therapy; NNRTI = non-nucleoside reverse transciptase inhibitor; PI = protease inhibitor; CPE = Central Nervous System Penetration Effectiveness; SD = Standard Deviation.

	All subjects (n=220)	CSF HIV-1 RNA ≥ 1 copy/mL (n=93)	CSF HIV-1 RNA < 1 copy/mL (n=127)	p value
Demographic characteristics				
Age, years	44.0 (39-50)	44.0 (38-51)	44.0 (40-50)	0.31
Gender, Women	22.6%	24.5%	21.3%	0.57
Race/Ethnicity				0.70
Black	36.2%	38.3%	34.6%	
Hispanic	11.3%	11.7%	11.2%	
White	50.2%	48.9%	51.2%	
Other	2.3%	1.1%	3.2%	
HIV-1 disease and co-infection characteristics				
Time since HIV-1 diagnosis, months	121.5 (69-198)	111.0 (66-190)	139.9 (73-202)	0.39
Current CD4+ T-cell count, /µL	503.0 (326-728)	575.5 (348-788)	451.0 (269-679)	**0.01**
Nadir CD4+ T-cell count, /µL	150.0 (36-261)	176.5 (50-280)	133.0 (27-225)	**0.06**
Nadir CD4+ T-cell count < 200/µL	62.4%	53.2%	69.3%	**0.01**
Plasma HIV-1 RNA ≥ 1 copy/mL	65.2%	69.2%	62.2%	0.28
AIDS diagnosis	68.8%	61.7%	74.0%	**0.05**
RPR Reactive	7.7%	7.4%	7.9%	0.90
HCV Seropositive	25.8%	19.2%	30.7%	**0.05**
ART characteristics				
First ART regimen	22.2%	22.3%	22.0%	0.96
Duration of current ART regimen, months	16.1 (7-32)	19.4 (5-33)	14.9 (7-31)	0.56

Total duration of all ART, months	70.0 (35-106)	59.6 (32-104)	73.5 (41-108)	**0.14**
Current ART regimen type				
NNRTI-containing	44.8%	46.8%	43.3%	0.60
PI-containing	54.3%	54.3%	54.3%	0.99
Took ≥ 95% of doses in past 4 days	91.9%	92.6%	91.3%	0.74
CPE (mean ± SD)	7.1 ± 1.3	6.8 ± 1.2	7.2 ± 1.3	**0.02**
CPE ≥ median (7)	72.2%	59.6%	71.6%	**0.06**

Table 2. Correlates of Worse Neurocognitive Performance in all 220 Participants. *Covariate is included in a first-order interaction term. An interaction with HCV was the only one to include CSF HIV RNA. The others were between HCV and either a) Duration of the current regimen or b) Neuropsychiatric conditions. Abbreviations: AIC= Akaike Information Criterion; CSF = cerebrospinal fluid; HCV = Hepatitis C Virus; ART = combination antiretroviral therapy; RPR = Rapid Plasma Reagin; AIDS = Acquired Immunodeficiency Syndrome; CPE = Central Nervous System Penetration Effectiveness; CD = Cluster of differentiation; PI = protease inhibitor; NNRTI = non-nucleoside reverse transciptase inhibitor; ml=milliliter; µL= microliter

	Risk Direction	Univariable Analysis p Value	Full Model p Value	AIC Model 1 p Value	AIC Model 2 p Value
HIV-1 RNA in CSF	Lower	**0.002**	**0.003**	**0.001**	**0.06***
HCV Serostatus	Positive	**0.01**	**0.07**	**0.02**	**0.02***
Neuropsychiatric Conditions	Contributing	**0.02**	0.16	**0.09**	**0.05***
Duration of all ART regimens	Longer	**0.03**	0.28	-	-
Duration of current regimen	Longer	**0.04**	0.14	**0.08**	**0.05***
RPR	Reactive	**0.07**	0.28	-	-
AIDS Diagnosis	Present	**0.11**	0.35	-	-
Plasma HIV-1 RNA	< 1 c/mL	**0.14**	0.72	-	-
CSF-Plasma Group	CSF+Plasma-	**0.14**	**0.07**	**0.03**	**0.04**
CPE	-	0.18	-	-	-
Nadir CD4+ T-cell count	-	0.20	-	-	-
Duration of HIV-1 disease	-	0.25	-	-	-
Number of ART regimens	-	0.30	-	-	-
PI-Containing Regimen	-	0.90	-	-	-
Current CD4+ T-cell count	-	0.91	-	-	-
NNRTI-Containing Regimen	-	0.91			
Four-day adherence	-	0.99	-	-	-
Multivariable Model Statistics	-	-	R^2=0.13 p<0.001	R^2=0.11 p<0.001	R^2=0.18 p<0.001