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Authors

Livelli, Alessandro
Vaida, Florin
Ellis, Ronald J
[et al.](#)

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Correlates of HIV RNA concentrations in cerebrospinal fluid during antiretroviral therapy: a longitudinal cohort study

Alessandro Livelli, Florin Vaida, Ronald J Ellis, Qing Ma, Micol Ferrara, David B Clifford, Ann C Collier, Benjamin B Gelman, Christina M Marra, Justin C McArthur, J Allen McCutchan, Susan Morgello, Ned Sacktor, David M Simpson, Igor Grant, and Scott L Letendre for the CHARTER Group*

Summary

Background Few large projects have evaluated the factors that influence the HIV RNA concentrations (viral load) in cerebrospinal fluid (CSF) during antiretroviral therapy (ART) over time. We aimed to determine the correlates of HIV RNA in CSF in a large cohort.

Methods We analysed longitudinal data from adults living with HIV in the US CHARTER cohort. Participants in the CHARTER study were recruited from six US academic medical centres—in Baltimore (MD), Galveston (TX), New York (NY), St Louis (MO), San Diego (CA), and Seattle (WA). Participants in this study had been assessed at least three times between Sept 4, 2003, and Sept 14, 2010, and were taking ART and underwent venous and lumbar puncture with measurement of HIV RNA concentration at all assessments. The lower limit of quantification of the HIV RNA assays was 50 copies per mL. Data were analysed with longitudinal mixed effects logistic regression to identify correlates of HIV RNA concentration (as a binary [detectable or not] and as a continuous variable) in CSF over time. We tested demographic characteristics, plasma HIV RNA, nadir and current CD4 cell count in blood, current CD8 cell count in blood, estimated duration of HIV infection, AIDS diagnosis, duration of ART, adherence to ART, ART characteristics, and CSF characteristics as potential correlates.

Findings At the time of analysis, 2207 assessments from 401 participants met the criteria for inclusion in this study. Mean duration of observation was 33·7 months (range 12–84). HIV RNA concentrations in 710 (32·2%) plasma specimens and in 255 (11·6%) CSF specimens were greater than the lower limit of quantification. The best multivariate model of HIV RNA concentration in CSF greater than the lower limit of quantification over time included increased plasma HIV RNA concentration (odds ratio 18·0 per 1 log₁₀ copy per mL, 95% CI 11·3 to 28·8; $p < 0·0001$), increased CSF leucocyte count (2·01 per 5 cells per μL , 1·61 to 2·39; $p < 0·0001$), decreased CD4 cell count (0·53 per 5 square-root cells per μL , 0·35 to 0·79; $p = 0·0025$), decreased CNS penetration-effectiveness value (0·71 per unit, 0·56 to 0·92; $p = 0·0078$), increased CD8 cell count (1·51 per 5 square-root cells, 1·11 to 2·06; $p = 0·0089$), and protease inhibitor use (3·26, 1·04 to 10·23; $p = 0·039$; model $R^2 = 0·22$, $p < 0·0001$). Analyses of continuous HIV RNA concentration in CSF that accounted for censoring below the lower limit of quantification had similar findings, although increased HIV RNA concentrations in CSF were also associated with black ethnicity (change in log₁₀ HIV RNA concentration in CSF 0·205, 0·0367 to 0·3733; $p = 0·017$), increased total protein in CSF (0·0025, $-0·0002$ to 0·0052; $p = 0·069$), and the presence of addictive-drug metabolites in urine (0·103, $-0·013$ to 0·219; $p = 0·081$).

Interpretation The identified correlates of HIV RNA concentration in CSF during ART could strengthen clinical prediction of risk for failure to achieve or maintain HIV RNA suppression in CSF. Because most participants in this analysis were ART-experienced and were taking a three-drug regimen that did not include an integrase inhibitor, future research should focus on participants who are taking their first ART regimens or regimens that include integrase inhibitors or two drugs.

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Introduction

Combination antiretroviral therapy (ART) suppresses HIV RNA in the cerebrospinal fluid (CSF) in most patients but persistently detectable HIV RNA during ART has been linked to the risk for HIV-associated neurocognitive disorder (HAND)¹ and depression.² For example, early analyses identified that higher HIV RNA concentrations in CSF, but not in plasma, were associated with HAND.^{3–5} Specifically, elevated HIV RNA concentrations in CSF predicted impairment in the

domains of learning, motor, attention, and working memory function.⁶ Analyses in the era of potent combination ART, however, have generally found weakened associations between HIV RNA concentration in CSF and neurocognitive outcomes.⁷

HIV RNA in CSF might also be important for other reasons. First, low concentrations of HIV RNA in CSF could lead to inflammation in the CNS.⁸ Second, HIV RNA in CSF could indicate viral escape in the CNS, which occurs in about 5% of otherwise effectively treated

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*Other members listed at end of Article

Department of Psychology (A Livelli PhD) and Department of Medical Sciences (M Ferrara MD), Università degli Studi di Torino, Torino, Italy; Department of Psychiatry (Prof F Vaida PhD,

Prof R J Ellis MD, Prof I Grant MD,

Prof S L Letendre MD),

Department of Neurosciences (Prof R J Ellis), and Department of Medicine

(Prof J A McCutchan MD,

Prof S L Letendre), University of California San Diego, San Diego,

CA, USA; Department of Pharmacy Practice, University at Buffalo, Buffalo, NY, USA

(Q Ma PharmD); Department of Neurology, Washington University, St Louis, MO, USA

(Prof D B Clifford MD);

Department of Medicine (Prof A C Collier MD) and

Department of Neurology (Prof C M Marra MD), University of Washington, Seattle, WA,

USA; Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA

(Prof B B Gelman MD);

Department of Neurology, Johns Hopkins University,

Baltimore, MD, USA

(Prof J C McArthur MPH,

Prof N Sacktor MD); and

Department of Neurology (Prof S Morgello MD,

Prof D M Simpson MD),

Department of Pathology, and

Department of Neuroscience (Prof S Morgello), Mount Sinai School of Medicine, New York,

NY, USA

Correspondence to:
Prof Scott L Letendre,
Department of Psychiatry and
and Department of Medicine,
University of California San Diego,
San Diego, CA 92103, USA
sletendre@ucsd.edu

Research in context

Evidence before this study

Before doing this study, we considered the extent of previously published data on the correlates of HIV RNA concentrations in cerebrospinal fluid (CSF) in adults living with HIV and taking combination antiretroviral therapy (ART). We searched PubMed for analyses of longitudinal data published between Jan 1, 2002, and Dec 31, 2017, using terms “HIV RNA” AND “cerebrospinal fluid” AND “antiretroviral” AND “adult” AND “longitudinal” NOT “opportunistic” NOT “monotherapy”. The results of our search are summarised in the appendix. In brief, many of these longitudinal studies focused on the change in HIV RNA in CSF in response to ART and some identified correlates of HIV RNA in CSF over time, which included HIV RNA in plasma, CD4 cell count, duration of ART, and characteristics of the ART regimen. A separate, large analysis from our group focused solely on cross-sectional data and confirmed some of these correlates of HIV RNA in CSF (eg, HIV RNA in plasma and ART characteristics) and also identified other contributors (eg, race and depression).

Added value of this study

The publications we found in our search (appendix) have a mean sample size of 41 participants. By contrast, this Article

summarises longitudinal data from more than 2200 lumbar puncture samples from nearly ten times as many participants (n=401), which adds substantially to the existing HIV literature. In addition to confirming previous findings linking HIV RNA levels in CSF to HIV RNA levels in plasma, CSF leukocyte number, and ART drug characteristics (eg, number of neuroactive drugs), we found evidence of associations with CD4 and CD8 cell counts. This finding adds to mounting evidence linking the low ratio of these T-cell subsets to persistent immune dysfunction, reservoir size, and complications in adults living with HIV during ART.

Implications of all the available evidence

The longitudinal data in this Article strengthen existing data from previous small studies. With the increasing recognition that CSF viral escape occurs in about 5% of treated adults living with HIV, our findings might help identify patients at risk for CSF viral escape and other complications of the CNS in the clinic. The data could also have implications for efforts to eradicate HIV from the CNS.

adults and can be associated with neurological symptoms.^{9,10} Third, failure to suppress HIV RNA fully in CSF could lead to development of drug-resistance mutations and limit the potential for a functional HIV cure. Finally, HIV RNA concentrations in CSF might reflect those in brain tissue and thus might be useful for studies that are focused on eradicating HIV.

Although many studies of HIV RNA in CSF have been published (appendix), they were either cross-sectional in design or had relatively modest sample sizes. Large, longitudinal analyses are better suited for inferring causality than smaller, cross-sectional ones. An observational cohort, CNS HIV AntiRetroviral Therapy Effects Research (CHARTER), sampled CSF in a large number of volunteers over several years, enabling such analyses.⁶ Our objective was to understand the correlates of HIV RNA concentration in CSF over time in adults who are taking ART. We hypothesised that multiple characteristics, including HIV RNA in plasma and estimated drug distribution in the CNS, would be associated with HIV RNA concentration in CSF over time.

Methods

Study design

The CHARTER study is a six-site, prospective, observational study that was designed to comprehensively assess a representative clinical population of adults living with HIV in the USA. The six sites were in Baltimore (MD), Galveston (TX), New York (NY), St Louis (MO), San Diego (CA), and Seattle (WA). All sites were based at US academic medical centres and were selected by a

competitive, peer-review process. Participants were recruited from the clinics located at these US academic medical centres and via community centres, advocacy groups, and community advisory boards.

Study population

We analysed data from all participants in the US CHARTER cohort who underwent at least 3 semi-annual assessments between Sept 4, 2003, and Sept 14, 2010; reported use of ART at all visits; successfully underwent venous and lumbar puncture; and had HIV RNA concentration measured in collected blood and CSF.

The Institutional Review Board at each site approved all study procedures and all participants provided written informed consent.

Procedures

Comprehensive neuromedical assessments were done, including medical and treatment history, physical examination, and venous and lumbar puncture. Participants were assessed by investigators or trained study personnel. All participants underwent all assessments at each visit with the exception of lumbar puncture (not all participants consented) and neuro-imaging (done in only a subgroup by design).

ART drug distribution into the CNS was estimated by the CNS penetration-effectiveness method.¹¹ ART non-adherence was estimated by self-report of taking fewer than 95% of doses in the 4 days before assessment (AIDS Clinical Trials Group 4-day method). HIV RNA was quantified by commercial RT-PCR (Amplicor

See Online for appendix

For the CHARTER study website
see <https://charternmtc.org>

version 1.5, Roche Diagnostics, Indianapolis, IN, USA; lower limit of quantification 50 copies per mL). Other biomarkers (eg, CD4 cell count, electrolytes, complete blood count, and CSF leucocytes) were measured by routine clinical methods. Nadir CD4 cell count was defined as the lowest value of either self-reported count or study measurement. HIV-associated neurocognitive disorders were diagnosed according to the Frascati criteria.¹² All participants completed the Beck Depression Inventory and major depression was diagnosed by Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria.

Statistical analysis

Because all participants were taking ART, the distribution of HIV RNA in CSF was highly skewed with many tied, undetectable values. For this reason, HIV RNA concentration in CSF was analysed by two methods. First, we analysed HIV RNA in CSF as a binary variable (greater than the lower limit of quantification [detectable] or less than or equal to the lower limit of quantification [undetectable]) using longitudinal modelling with logistic regression. Then, we analysed HIV RNA in CSF as a continuous variable and accounting for censoring below the limit of detection by a published method¹³ using linear mixed models. We screened demographic, HIV disease, treatment, and CSF characteristics, for inclusion in multivariate models by using individual mixed effects models. Candidate covariates were selected for multivariate analysis using an α of 0.10. Multivariate analysis was then done, also using longitudinal mixed effects logistic regression. When the distribution of independent variables were highly skewed, we transformed them to reduce skewness (eg, $\log_{10}$ transformation of plasma HIV RNA and square-root transformation of cell counts). Regression-derived estimates were converted to odds ratios (ORs) to ease interpretation. For this same reason, ORs were adjusted for plasma HIV RNA (per 1 $\log_{10}$ copies per mL) and cell counts (per 5 square-root cells). Correlation within participants was modelled via a participant-specific random effect. We built multivariate models with backward elimination with the goal of deriving a concise combination of predictors based on maximal explanatory value after eliminating variables that did not contribute to a better fit.

Data were analysed using R statistical software program (version 3.1.1), including the Imec package.

Role of the funding source

The funder of the study played no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author and the principal investigator had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

At the time of analysis, 2207 assessments from 401 participants met the criteria for inclusion in this study. At first visit, participants were mostly middle-aged (mean 44 years [SD 8.2]) men (318 [79%] of 401) who reported their ethnicity as either black (176 [44%]) or white (170 [42%]). 286 (71%) participants had been diagnosed with AIDS. Median nadir CD4 cell count was 135 cells per μ L (IQR 28–243) and median CD4 cell count was 446 cells per μ L (276–635). 109 (27%) participants were seropositive for hepatitis C virus (table 1).

For the Beck Depression Inventory see <https://beckinstitute.org/get-informed/tools-and-resources/professionals/patient-assessment-tools/>
For the DSM-IV see <https://www.psychiatry.org/psychiatrists/practice/dsm>

(n=401)	
Demographics and other characteristics	
Age (years)	44 (8.2)
Sex	
Female	83 (21%)
Male	318 (79%)
Ethnicity	
Black	176 (44%)
White	170 (42%)
Hispanic	45 (11%)
Other	10 (2%)
Disease and treatment characteristics	
Plasma HIV RNA concentration $\leq$ 50 copies per mL	240 (60%)
CD4 count (cells per μ L)	446 (276–635)
CD8 count (cells per μ L)	867.5 (628–1170)
Nadir CD4 cell count (cells per μ L)	135 (28–243)
Duration of HIV disease, months	143.0 (68–189)
AIDS diagnosis	286 (71%)
Number of current antiretroviral drugs	3.2 (1–6)
Duration of ART, months	18.0 (7.6–34.2)
CPE of current regimen	7.5 (2.02)
Current protease inhibitor use	242 (60%)
Number of past antiretroviral drugs	3 (1–6)
Adherence $>$ 95%	351 (88%)
HCV seropositive	109 (27%)
CSF characteristics	
CSF white blood cells (cells per μ L)	2 (1–4)
CSF protein (mg/dL)	42 (33–53)
CSF HIV RNA concentration $\leq$ 50 copies per mL	347 (87%)
HAND and depression characteristics	
HAND diagnosis	
Unimpaired	206 (51%)
Asymptomatic neurocognitive impairment	114 (28%)
Mild neurocognitive disorder	34 (8%)
HIV-associated dementia	8 (2%)
Impaired because of a cause other than HIV	39 (10%)
Major depression	
Baseline	49 (12%)
Lifetime	184 (46%)

Data are n (%), median (IQR), or mean (SD). ART=antiretroviral therapy. CPE=central nervous system penetration effectiveness. HCV=hepatitis C virus. CSF=cerebrospinal fluid. HAND=HIV-associated neurocognitive disorder.

Table 1: Baseline characteristics at first visit

Figure: Modelled longitudinal regression curve of binary HIV RNA in CSF and CPE and other longitudinal correlates of binary HIV RNA in CSF
 (A) Data points are point estimates (95% CI) and the solid line was modelled from a longitudinal analysis of the proportion of samples with HIV RNA concentrations of 50 copies per mL or more. (B) Significant covariates of binary HIV RNA concentration in CSF in the best multivariable model. CPE=CNS penetration effectiveness. CSF=cerebrospinal fluid. OR=odds ratio.

The mean duration of observation was 33.7 months (range 12–84). The mean number of lumbar punctures per subject was 5.3 (SD 3.0). All participants reported ART use at all visits. For all visits combined, the median duration of the current (ie, at the time of each visit) ART regimen was 18.0 months (IQR 7.6–34.2). 282 (70.3%) participants took ART regimens containing three drugs and 89 (22%) were taking their first regimen. The most common regimens across all 2207 visits were efavirenz, tenofovir, and emtricitabine (378 visits [17.1%]), ritonavir-boosted atazanavir, tenofovir, and emtricitabine (363 [16.4%]), ritonavir-boosted lopinavir, tenofovir, and emtricitabine (115 [5.2%]), and efavirenz, abacavir, and lamivudine (62 [2.8%]). The most common individual drugs were tenofovir (1681 [76.2%]), low-dose ritonavir (1394 [63.2%]), emtricitabine (1209 [54.8%]), lamivudine (1054 [47.8%]), atazanavir (787 [35.7%]), efavirenz (706 [32.0%]), abacavir (667 [30.2%]), zidovudine (512 [23.2%]), and lopinavir (433 [19.6%]). Raltegravir was used at 67 visits (3.0%). The median CNS penetration-effectiveness value was 7 (IQR 6–9).

HIV RNA concentrations were undetectable in plasma at 1444 (65.4%) of 2207 visits and in CSF at 1955 (88.6%) visits. When HIV RNA concentrations were detectable in plasma, the median value was 544 copies per mL (IQR 137–7265). When HIV RNA concentrations were detectable in CSF, the median value was 535 copies per mL (145–2498).

In analyses of the binary CSF HIV RNA (detectable vs undetectable) over time, screening analyses identified 15 variables that were associated with the outcome, the strongest of which was HIV RNA in plasma (OR 19.7, 95% CI 12.8–30.5; $p<0.0001$; figure; table 2). Variables that had p values greater than 0.10 with HIV RNA in CSF over time included sex, AIDS, current or lifetime depression, nadir CD4 cell count, duration of HIV infection, hepatitis C virus serostatus, and CSF erythrocyte number.

The best multivariate mixed effects model included six covariates (table 2). In descending order of effect size, the covariates of detectable HIV RNA in CSF were increased HIV RNA concentrations in plasma (OR 18.0 per 1 log₁₀ copies per mL, 95% CI 11.3–28.8), protease inhibitor use (3.26, 1.04–10.23), increased CSF leucocyte number (2.01 per 5 cells per μ L, 1.61–2.39), decreased CD4 cell count (0.53 per 5 square-root cells per μ L, 0.35–0.79), increased CD8 cell count (1.51 per 5 square-root cells per μ L, 1.11–2.06), and decreased CNS penetration-effectiveness value (0.71 per unit, 0.56–0.92; model $R^2=0.22$, $p<0.0001$).

When a binary transformation of CNS penetration effectiveness (CNS penetration effectiveness $\leq$ or $>$ than the median value of 7) was included in the model instead of the untransformed value, protease inhibitor use was no longer significantly associated with HIV RNA in CSF over time.

In analyses of continuous HIV RNA in CSF that accounted for censoring below the lower limit of quantification (table 3), the covariates were the same with four exceptions: CD8 cell count was not included in the best multivariate model, whereas increased CSF total protein, presence of addictive drug metabolites in urine, and black ethnicity were (model $p<0.0001$).

In secondary analyses, 148 (37%) of 401 participants had HIV RNA concentrations in plasma of 50 copies per mL or less at all visits. Nine (6%) of these participants had HIV RNA concentrations in CSF greater than 50 copies per mL at one or more visits, consistent with CSF viral escape.⁹ These nine participants had a total of 63 visits and CSF viral escape occurred at 13 (21%) of them. Analyses of these 148 participants identified that CSF viral escape was associated with the presence of increased CSF leucocyte counts ($p=0.035$) across all visits. Limiting the analysis to the 63 visits from the nine participants who had a CSF viral escape event identified, we found that CSF viral escape was associated with both increased CSF leucocyte counts ($p=0.029$) and decreased CNS penetration-effectiveness values ($p=0.022$).

Discussion

In this study, we modelled longitudinal data from 401 adults observed over an average of 2.5 years. We found that several viral, immune, and treatment variables were associated with HIV RNA in CSF over time. As expected, failure to suppress HIV RNA fully in plasma was the most influential factor in both linear and logistic models of HIV RNA in CSF. Fewer CD4 cells were also associated with HIV RNA in CSF over time in both models, consistent with the known link between low CD4 cell count and risk of HAND.¹⁴ CD8 cells have not previously been clearly linked to HIV RNA in CSF, although reports¹⁵ of CD8 encephalitis suggest at least an indirect link. The combination of low CD4 and high CD8 cell count is consistent with a persistently inverted CD4-to-CD8 ratio during ART, which might be a marker of immune senescence,¹⁶ persistent T-cell activation,¹⁷ and risk for non-AIDS comorbidities.¹⁸ Our data suggest that a greater degree of CD4-to-CD8 ratio inversion also indicates increased risk for detectable HIV RNA in CSF during ART, which could increase the risk of CNS inflammation and vulnerability to HAND.¹⁹ Of note, we did not find an association between CD8 cells and HIV RNA in CSF in analyses of continuous HIV RNA concentration in CSF that accounted for censoring below the lower limit of quantification.

CSF leucocytes have long been linked to HIV RNA concentrations in CSF.²⁰ Our analysis independently validates this historical association and further supports a role for leucocyte migration in CSF viral escape. The combination of low CD4 cell counts and high CSF leucocyte counts is consistent with reports linking these factors with greater autonomous CNS production of HIV²¹ and viral adaptation in CSF.²²

Decreased estimated effectiveness of ART in the CNS has previously been reported to correlate with HIV RNA in CSF in cross-sectional analyses. Our data lend further support to the role of ART in the pathogenesis of CSF viral escape.^{9,23} Although ART characteristics might be more clearly linked to the presence of HIV RNA in CSF, their relation to neurocognitive disorders remains controversial.²⁴ The inclusion of both CNS penetration effectiveness and protease inhibitor use in multivariate models suggests that protease inhibitors (primarily atazanavir, lopinavir, and ritonavir, in this analysis) might influence the concentration of HIV RNA in CSF by mechanisms that are not adequately described by the CNS penetration-effectiveness method. Although the protease inhibitor effect size was large, the confidence interval of the estimate was broad (OR 3.26, 95% CI 1.04–10.23) and the effect was not selected in models that included a binary transformation of CNS penetration effectiveness (data not shown). If protease inhibitor use were truly clinically associated with increased HIV RNA concentrations in CSF, several explanations would be possible. Protease inhibitors can modulate molecular drug transporters,²⁵ which could reduce protease

	Univariable		Multivariable	
	OR (95% CI)	p value	OR (95% CI)	p value
HIV disease characteristics				
Plasma HIV RNA (per 1 log ₁₀ counts per mL)	19.7 (12.8–30.5)	<0.0001	18.0 (11.3–28.8)	<0.0001
Current CD4 cell count (per 5 square-root cells per µL)	0.29 (0.21–0.40)	<0.0001	0.53 (0.35–0.79)	0.0025
Current CD8 cell count (per 5 square-root cells per µL)	1.04 (0.81–1.31)	0.0001	1.51 (1.11–2.06)	0.0089
Treatment characteristics				
CPE (per 1 unit)	0.75 (0.63–0.89)	0.0003	0.71 (0.56–0.92)	0.0078
Protease inhibitor use	2.83 (1.17–6.83)	0.0059	3.26 (1.04–10.23)	0.039
Adherence <95% doses in 4 days before assessment	2.97 (1.49–5.94)	0.0011	..	..
Number of current antiretrovirals	0.62 (0.37–1.02)	0.046	..	..
Number of past antiretrovirals	1.14 (0.98–1.32)	0.052	..	..
Duration of current ART regimen (per 1 month)	0.92 (0.83–1.03)	0.096	..	..
Demographics and other characteristics				
Age (per 1 year)	1.06 (0.99–1.14)	0.038	..	..
Ethnicity (black)	5.90 (1.71–20.4)	0.0017	..	..
Additive drug metabolites in urine	2.28 (1.35–3.85)	0.0024	..	..
Additive drug use within previous 30 days	2.26 (1.04–4.92)	0.041	..	..
CSF characteristics				
CSF leucocytes (per 5 cells per µL)	1.90 (1.61–2.25)	<0.0001	2.01 (1.61–2.39)	<0.0001
CSF total protein (per 10 mg/L)	1.02 (1.00–1.03)	0.047	..	..
CSF=cerebrospinal fluid. OR=odds ratio. CPE=central nervous system penetration effectiveness. ART=antiretroviral therapy.				

Table 2: Correlates of binary HIV RNA in CSF

inhibitor concentrations in the CNS in a way that is not captured by the CNS penetration-effectiveness method. Consistent with this, some studies have confirmed that CSF concentrations of several protease inhibitors appear to be lower than expected on the basis of the unbound fraction in blood.²⁶ Protease inhibitors have also recently been linked to cerebral small vessel disease,²⁷ which could alter drug distribution, adhesion molecule expression,²⁸ and cell migration.²⁹ Protease inhibitor use could also be an indicator of unmeasured factors, such as clinical channelling. For example, clinicians tend to choose protease inhibitors for patients who have high HIV RNA concentrations in blood, more advanced immune suppression, increased ART experience, or increased ART drug resistance.

Although our study was large and longitudinal, it does have limitations that could affect the accuracy and interpretation of the findings. The most important is the observational nature of the CHARTER study, meaning that ART initiation was not randomised or synchronised across participants, which can be a source of bias. Another limitation is that the analysis included participants who had detectable HIV RNA in plasma, which could reduce generalisability since suppressing HIV is the chief goal of ART in the clinic. However, most

	Univariable		Multivariable	
	Change in log ₁₀ HIV RNA concentration in CSF (95% CI)	p value	Change in log ₁₀ HIV RNA concentration in CSF (95% CI)	p value
HIV disease characteristics				
Plasma HIV RNA (per 1 log ₁₀ counts per mL)	0.834 (0.464 to 1.204)	<0.0001	0.696 (0.364 to 1.029)	<0.0001
Current CD4 cell count (per 5 square-root cells per µL)	-0.518 (-0.748 to -0.289)	<0.0001	-0.0925 (-0.1412 to -0.0438)	0.0002
Treatment characteristics				
CPE (per 1 unit)	-0.116 (-0.163 to -0.069)	<0.0001	-0.0311 (-0.0587 to -0.0035)	0.027
Protease inhibitor use	0.481 (0.248 to 0.714)	0.0001	0.219 (0.0886 to 0.3494)	0.0013
Adherence <95% doses in 4 days before assessment	0.493 (0.288 to 0.697)	<0.0001	..	..
Number of past antiretrovirals	0.0324 (-0.013 to 0.078)	0.16	..	..
Number of current antiretrovirals	-0.184 (-0.326 to -0.042)	0.011	..	..
Demographics and other characteristics				
Age (per 1 year)	-0.0261 (-0.047 to -0.005)	0.014	..	..
Ethnicity (black)	-0.284 (-0.403 to -0.164)	<0.0001	0.205 (0.0367 to 0.3733)	0.017
Addictive drug metabolites in urine	0.429 (0.240 to 0.619)	<0.0001	0.103 (-0.013 to 0.219)	0.081
Addictive drug use within previous 30 days	0.393 (0.169 to 0.617)	0.0006	..	..
CSF characteristics				
CSF leucocytes (per 5 cells per µL)	0.0389 (0.0325 to 0.0452)	<0.0001	0.0247 (0.0133 to 0.0361)	<0.0001
CSF total protein (per 10 mg/L)	0.00916 (0.00479 to 0.01353)	<0.0001	0.0025 (-0.0002 to 0.0052)	0.069
CSF=cerebrospinal fluid. CPE=central nervous system penetration effectiveness.				
Table 3: Correlates of continuous HIV RNA concentration in CSF accounting for censoring below the lower limit of quantitation				

participants who had detectable HIV RNA in plasma had concentrations less than 1000 copies per mL (59.3%), which does not necessarily portend ART failure.³⁰ Focusing solely on participants taking suppressive ART would require that we re-assay all specimens with a more sensitive assay, such as a single copy assay. Another limitation related to the inclusion of participants who had detectable HIV RNA in plasma is the absence of data for ART drug-resistance mutations in these analyses. The participants were mostly ART-experienced, having previously used a median of five ART drugs. Detectable HIV RNA in CSF could be due to unmeasured ART drug resistance, for example, rather than other factors, such as CNS penetration effectiveness, which could theoretically be colinear with it in analyses of HIV RNA. Finally, by design, this analysis focused on HIV RNA in CSF, not on other neurological outcomes. As noted, we have previously reported links between increased HIV RNA in CSF and two common neurological complications: neurocognitive decline¹ and incident depression.²

In this large analysis of ART users over the course of up to about 3.5 years, we found that HIV RNA was rarely detectable in CSF (<12% of visits) and that it was associated with several factors over time, most importantly high HIV RNA in plasma. The associations with CD4 cell counts and CSF leucocyte counts are consistent with previous reports,³¹ but the association with the concentration of CD8 cells is a new finding that might help identify patients at risk for CSF viral escape and neurological complications. Only two correlates, CNS penetration-effectiveness values and protease inhibitor use, were modifiable, which might have

implications for CSF viral escape and achieving functional cure.

Contributors

RJE, DBC, ACC, BBG, CMM, JMca, AMcC, SM, NS, DMS, IG, and SLL designed and implemented the study, obtained funding for it, and collected the data. AL, FV, MF, and SLL analysed the data. All authors contributed to the writing and editing of the manuscript.

Other CHARTER group members

In addition to the authors, the CHARTER Group includes Ian Abramson PhD, Muhammad Al-Lozi MD, Sarah Archibald, J Hampton Atkinson MD, Brookie Best PharmD, Clint Cushman, Matthew Dawson, Christine Fennema-Notestine PhD, Donald Franklin Jr, Eleanor Head RN, BSN, Robert K Heaton, Trudy Jones MN, ARNP, Kenneth Maravilla MD, Thomas D Marcotte PhD, Letty Mintz NP, Thomas Naidich MD, David M Smith MD, K C Stegbauer PhD, Cheuk Tang PhD, and Mengesha Teshome MD.

Declaration of interests

DBC reports consultant fees from Biogen, Millennium/Takeda, Bristol-Myers Squibb, Genzyme (Sanofi), Pfizer, Amgen, Genentech/Roche, GlaxoSmithKline, Merck & Co/Serono, Inhibikase, Protagonist Therapeutics, and Shire Pharmaceuticals, and compensation as a Data Safety Monitoring Board member for Wave Pharmaceuticals. ACC reports compensation from Merck & Co as a Data Safety Monitoring Board member and by the International AIDS Society-USA for an educational lecture, and that research funding has been provided on her behalf to the University of Washington (WA, USA) by Bristol-Myers Squibb. SLL reports advisory board compensation from ViiV Healthcare and that research funding has been provided to the University of California, San Diego (CA, USA) on his behalf by Gilead Sciences and ViiV Healthcare. All other authors declare no competing interests.

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Johns Hopkins University Mount Sinai School of Medicine (NY, USA), University of California, San Diego (CA, USA), University of Texas, Galveston (TX, USA), University of Washington (WA, USA), and Washington University, St Louis (MO, USA), and is headquartered at the University of California, San Diego.

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