Title

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Journal

Journal of Medical Virology, 96(3)

ISSN

0146-6615

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Publication Date

2024-03-01

DOI

10.1002/jmv.29550

Received: 15 December 2023 | Accepted: 10 March 2024

DOI: 10.1002/jmv.29550

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Longitudinal analysis of CSF HIV RNA in untreated people with HIV: Identification of CSF controllers

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Funding information
National Institute of Mental Health, Grant/Award Numbers: P30 MH62512, R01 MH07345, R25 MH081482; National Institute on Drug Abuse, Grant/Award Number: P50 DA26306

Abstract

Interindividual variation of human immunodeficiency virus (HIV) RNA setpoint in cerebrospinal fluid (CSF) and its determinants are poorly understood, but relevant for HIV neuropathology, brain reservoirs, viral escape, and reseeding after antiretroviral interruptions. Longitudinal multicentric study on demographic, clinical, and laboratory correlates of CSF HIV RNA in 2000 follow-up visits from 597 people with HIV (PWH) off antiretroviral therapy (ART) and with plasma HIV RNA > the lower limit of quantification (LLQ). Factors associated with CSF control (CSFC; CSF HIV RNA < LLQ while plasma HIV RNA > LLQ) and with CSF/plasma discordance (CSF > plasma HIV RNA > LLQ) were also assessed through mixed-effects models. Posthoc and sensitivity analyses were performed for persistent CSFC and ART-naïve participants, respectively. Over a median follow-up of 2.1 years, CSF HIV RNA was associated with CD4+ and CD8+ T cells, CSF leukocytes, blood–brain barrier (BBB) integrity, biomarkers of iron and lipid metabolism, serum globulins, past exposure to lamivudine, and plasma HIV RNA (model $p < 0.0001$). CSFC (persistent in 7.7% over 3 years) and CSF/plasma discordance (persistent in <0.01% over 1 year) were variably associated with the same parameters (model $p < 0.001$). Sensitivity analyses confirmed most of the previous associations in participants never exposed to ART. Persistent CSFC was associated with higher $CD4^+$ T-cell count nadir ($p < 0.001$), lower serum globulins ($p = 0.003$), and lower CSF leukocytes ($p < 0.001$). Without

ART, one in 13 PWH had persistently undetectable CSF HIV RNA, while persistent CSF/plasma discordance was extremely rare over years. Immune responses, inflammation, BBB permeability, and iron and lipid metabolism were all associated with HIV replication in CSF.

KEYWORDS

antiretroviral naïve, blood–brain barrier, central nervous system, CSF control, CSF/plasma discordance, HIV viral load

1 | INTRODUCTION

In people with HIV (PWH) not on antiretroviral therapy (ART), CD4+ T cells typically decline over time, while plasma HIV RNA levels off to a setpoint that has substantial interindividual variability and, on average, slowly increases over time.[1]

Early during the infection, HIV rapidly disseminates into tissues, including the central nervous system (CNS). HIV RNA is below quantification in blood and cerebrospinal fluid (CSF) of elite controllers[2] (<1% of PWH), whose robust antiviral immune response suppresses HIV without ART.[3] Whether a subgroup of PWH has CSF HIV RNA below quantification when HIV RNA is quantifiable in plasma is unknown. The existence of such "CSF controllers" (CSFC) is supported by the rare identification of PWH who had no detectable HIV RNA, HIV DNA, or HIV p24 antigen in the brain at autopsy despite being off ART.[4] At the opposite extreme of the spectrum, a small proportion of PWH can have CSF/plasma discordance (i.e., CSF HIV RNA > plasma HIV RNA in the absence of ART or coinfections).[5,6] This group of PWH could be at greater risk for CSF viral escape on ART and for neuropsychiatric complications over time.[7]

CSF HIV RNA derives from CNS and systemic sources.[8] Substantial interindividual variability of CSF HIV RNA without ART was described in the 1990s[9–11] and in a more recent analysis of more than 1000 PWH.[5] These studies linked higher CSF HIV RNA to an AIDS diagnosis, HIV encephalitis, HIV-associated dementia, or concurrent CNS coinfections.[5,6,9,11] Much less is known about other characteristics that may determine the CSF HIV RNA setpoint. Identifying different CSF phenotypes could be relevant since their determinants, prognosis, and treatment might differ.[12,13] Furthermore, CNS is one of the main reservoirs contributing in the reactivation and peripheral reseeding of HIV when ART is interrupted,[14] and lastly, the identification of a subgroup of CSFC could be relevant to experimental cure strategies: PWH who better control HIV replication in CSF without ART might respond better to cure interventions and be less vulnerable to CNS reactivation during analytical treatment interruption.

Compared with earlier in the HIV pandemic, modern cohorts have now collected data over time, which enables more powerful analyses. For example, observational cohorts coordinated by the HIV Neurobehavioral Research Program (HNRP) at the University of California, San Diego (UCSD) have collected longitudinal data from PWH who were not taking ART over 28 years. Here we leverage this wealth of data to better understand interindividual differences in CSF HIV RNA in the absence of ART. We determined the demographic, clinical, and laboratory correlates of CSF HIV RNA and of the groups defined across the spectrum of the relationship between blood and CSF HIV RNA (from CSFC to CSF/plasma discordance) over time.

2 | METHODS

2.1 | Study design

This retrospective study evaluated CSF HIV RNA of 597 PWH who were assessed in observational research projects coordinated by the HNRP (UCSD) at five additional sites (Johns Hopkins University, Icahn School of Medicine at Mount Sinai, University of Texas Medical Branch, University of Washington, Washington University), between 1990 and 2018.

All participants were not taking ART, had plasma HIV RNA > the lower limit of quantification (LLQ) of the Roche Amplicor assay, and had two to six research lumbar punctures separated by at least 30 days. The total number of lumbar punctures was 2000. All lumbar punctures were performed solely for research purposes, and no participant had clinical indication (e.g., untreated CNS and systemic infections, untreated CNS disorders). Although no participants reported ART use at the time of the assessments, some took it previously or between assessments. For this reason, we included past ART exposure, its duration, and ART class in the analyses. The four principal cohorts that contributed data to our analyses were the HNRC cohort (1074 lumbar punctures from 318 participants between 1990 and 2013[15]), the CHARTER cohort (569 lumbar punctures from 156 participants between 2003 and 2018[16]), the CNTN cohort (156 lumbar punctures from 60 participants between 1998 and 2012[17]), and the TMARC cohort (155 lumbar punctures from 48 participants between 1999 and 2008[18]). The remaining 46 lumbar punctures were performed in 15 participants of multiple, smaller projects between 2002 and 2013. All cohorts shared the common focus on investigating the neuropsychiatric effects of HIV infection.

Three CSF groups were defined: (A) CSF/plasma discordance: CSF HIV RNA > plasma HIV RNA > LLQ, (B) CSFC: CSF HIV RNA < LLQ

while plasma HIV RNA > LLQ, and (C) Plasma HIV RNA > CSF HIV RNA > LLQ.

All projects were approved by local Institutional Review Board and all participants provided written informed consent for the research procedures and future use of their data in accordance to the Declaration of Helsinki.

2.2 | Medical and laboratory assessment

Demographics, HIV-related parameters, and clinical data were collected. Laboratory tests included complete blood count and CSF and blood biochemistry. The biochemistry panel also included erythrocytes indices linked with iron metabolism (hemoglobin, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and MCH concentration MCHC), serum total cholesterol, and triglycerides. The reason for investigating also these variables was based on previous evidence linking neuro-inflammation, blood–brain barrier (BBB), cognitive performance, and iron metabolism (as erythrocytes indices) in PWH,[19–22] and on previous evidence of an interplay between lipids, BBB, and neurodegenerative processes in HIV-negative populations.[23–26] Similarly, platelet count was also analyzed as previous studies have shown associations between platelets, HIV RNA replication, BBB dysfunction, and neuro-inflammation.[27–30]

CSF albumin was estimated based on the intercept and parameter estimates for sex, age, race, and CSF total protein obtained through multivariable linear regression in 164 PWH with paired CSF and serum albumin measurements (71% explained variance). Serum and CSF globulins were estimated by subtracting serum and CSF albumin from serum and CSF total protein, respectively. CSF-to-serum albumin ratio (CSAR) was used as index of BBB integrity. Neurocognitive assessment was available for only a subgroup of participants and was therefore excluded from the current study.

2.3 | Statistical analysis

Due to the assay LLQ, CSF HIV RNA values in the database had been censored at the LLQ (which ranged from 30 to 400 copies/mL during the study period and across the study sites). Censored values were imputed for an exact point-value quantification using Markov chain Monte Carlo algorithm.[31] The imputation of the 281 values (14.0%) below the LLQ allowed us to classify these participants into the CSF groups and provided an exact estimation to be included in linear models for CSF HIV RNA.

Plasma and CSF HIV RNA and other non-normally distributed variables were $\log_{10}$-transformed to reduce skewness. Data were summarized with mean (SD) or median (interquartile range) for continuous variables and number (%) for categorical variables. These were compared between the CSF groups using Kruskal–Wallis H test, Mann–Whitney U test, or χ^2 test as appropriate, together with Pearson's correlations at baseline. Pairwise comparisons were adjusted using Bonferroni correction ($k = 3$, $\alpha = 0.0167$) for type I errors.

Longitudinal analyses for CSF HIV RNA levels and CSF groups were performed using linear mixed-effects models and mixed-effects multinomial logistic regression for repeated measures (visits) that included fixed effects of a variable, time (cumulative days), and subject-specific random intercept. Variables showing significant association with the outcome ($p < 0.05$) were included as candidate covariates and retained in a multivariable model if still associated with the outcome after adjusting for age, sex, race, and intercurrent exposure to ART. The final models were selected by backward selection (Akaike Information Criterion). Unadjusted and adjusted β coefficients (aβ), adjusted relative risk ratios, and adjusted odds ratios were reported; z values (regression coefficient divided by its standard error) were used to ease comparisons of effect estimates. Missing data were handled in mixed-effects models through maximum likelihood estimation method. Multicollinearity was assessed through variance inflation factor (VIF). The highest VIF among models was <5 (threshold for problematic collinearity[32]), except for CSAR, serum, and CSF albumin; for this reason, only CSAR was included in multivariable models when significant.

Posthoc analyses for CSFC were performed comparing participants with persistent CSF control with plasma HIV RNA-matched (±0.5 $\log_{10}$ copies/mL) participants that had detectable CSF HIV RNA at all the assessments (1:2 ratio). Sensitivity analyses were performed after excluding all the visits of participants that followed ART exposure at any time (before or during follow-up).

Analyses were performed with STATA SEv.18 (Stata Corp LLC).

3 | RESULTS

3.1 | Study population and CSF HIV RNA at baseline

As shown in Table 1, participants were mostly White or Black, men, younger than 40 years. The mean estimated duration of HIV disease was 7.5 years and 42.0% had been diagnosed with AIDS. Approximately half were ART-naïve, while the others had previously taken ART for a median of 2 years. About a quarter (137, 22.9%) were CSFC and 24 (4.0%) had CSF/plasma discordance.

CSF HIV RNA positively correlated with plasma HIV RNA and several blood/CSF variables (Figure 1A,B) and was lower by 1.5 $\log_{10}$ copies/mL (0.9–2.2). In participants with CSF/plasma discordance, CSF HIV RNA exceeded plasma HIV RNA by a median of 0.2 (0.05–0.3) $\log_{10}$ copies/mL, while CSFC had median plasma HIV RNA of 2.4 (1.7–2.9) $\log_{10}$ copies/mL (Table 1).

CSFC had lower plasma HIV RNA, $CD8^+$ T cells %, serum and CSF globulin, CSF leukocytes, and CSAR, and higher CD4/CD8 ratio, among other differences (Table 1). Participants with CSF/plasma discordance had the highest levels of CSF globulin and leukocytes,

TABLE 1 Baseline participants characteristics by CSF group.

	(A) CSFCs (n = 137)	(B) Plasma > CSF HIV RNA (n = 436)	(C) CSF/plasma discordance (n = 24)	p	Bonferroni-adjusted pairwise comparisons		
					A vs. B	A vs. C	B vs. C
Demographics							
Age, years[a]	38.1 (8.5)	38.1 (8.5)	40.7 (7.7)	0.180	–	–	–
Sex, male[b]	115 (83.9%)	362 (83.0%)	18 (75.0%)	0.541	–	–	–
Education, years[a]	13.0 (2.6)	12.7 (2.6)	13.2 (1.9)	0.480	–	–	–
Ethnicity[b]							
White	91 (66.4%)	266 (61.0%)	15 (62.5%)	0.610	–	–	–
Black	31 (22.6%)	132 (30.3%)	8 (33.3%)	0.258	–	–	–
HCV co-infection[b]	26 (19.0%)	96 (22.0%)	2 (8.3%)	0.246	–	–	–
HIV-related characteristics							
AIDS diagnosis[b]	59 (43.1%)	182 (41.7%)	10 (41.7%)	0.917	–	–	–
Est. duration of HIV infection, years[a]	6.8 (5.8)	7.6 (6.1)	9.3 (4.2)	0.099	–	–	–
CD4+ T cell, /µL[c]	469 (207; 698)	347 (180; 511)	320 (182; 488)	**0.029**	*	*	–
CD4+ T cell, %[c]	23 (16; 35)	21 (14; 28)	22 (15; 30)	**0.010**	*	–	–
CD8+ T cells, /µL[c]	829 (592; 1173)	886 (625; 1155)	777 (542; 1230)	0.619	–	–	–
CD8+ T cell, %[c]	50 (42; 58)	55 (47; 64)	54 (48; 62)	**<0.001**	*	*	–
CD4/CD8 ratio[c]	0.46 (0.31; 0.75)	0.40 (0.23; 0.57)	0.42 (0.22; 0.56)	**0.002**	*	*	–
Nadir CD4+ T cell, /µL[c]	272 (156; 500)	271 (140; 403)	249 (202; 299)	0.323	–	–	–
HIV RNA, Plasma $\log_{10}$[c]	3.5 (2.7; 4.1)	4.6 (4.1; 5.1)	4.3 (3.8; 4.6)	**<0.001**	**	*	*
HIV RNA, CSF $\log_{10}$[c]	1.1 (0.6; 1.4)	3.1 (2.5; 3.7)	4.4 (3.9; 4.8)	**<0.001**	**	**	**
CSF-plasma HIV RNA, $\log_{10}$[c]	−2.4 (−2.9; −1.7)	−1.4 (−2.0; −0.8)	0.2 (0.05; 0.3)	**<0.001**	**	**	**
ART naïve[b]	62 (45.2%)	216 (49.5%)	12 (50.0%)	0.419	–	–	–
Prior duration of ART, months[c,d]	27.9 (8.8; 65.2)	25.8 (8.9; 54.7)	76.6 (32.0; 119.0)	**0.044**	–	*	*
3TC exposure[b]	55 (40.1%)	146 (33.5%)	9 (37.5%)	0.388	–	–	–
ZDV exposure[b]	44 (32.1%)	139 (31.9%)	9 (37.5%)	0.834	–	–	–
ABV exposure[b]	10 (7.3%)	49 (11.2%)	4 (16.7%)	0.259	–	–	–
EFV exposure[b]	13 (9.5%)	59 (13.5%)	3 (12.5%)	0.582	–	–	–
NVP exposure[b]	12 (8.7%)	41 (9.4%)	2 (8.3%)	0.934	–	–	–
TDF exposure[b]	9 (6.6%)	47 (10.8%)	3 (12.5%)	0.259	–	–	–
LPV exposure[b]	5 (3.6%)	37 (8.5%)	1 (4.2%)	0.136	–	–	–
SQV exposure[b]	13 (9.5%)	25 (5.7%)	3 (12.5%)	0.170	–	–	–
IDV exposure[b]	14 (10.2%)	41 (9.4%)	2 (8.3%)	0.940	–	–	–
Blood and CSF biochemistry							
Serum glucose, mg/dL[c]	89 (76; 104)	89 (76; 104)	91 (74; 106)	0.998	–	–	–
Serum creatinine, mg/dL[c]	0.9 (0.8; 1.0)	0.9 (0.8; 1.0)	0.9 (0.8; 0.9)	0.578	–	–	–
Serum total protein, g/dL[c]	7.5 (7.0; 7.8)	7.8 (7.3; 8.2)	8.0 (7.5; 8.5)	**<0.001**	*	*	–
Serum albumin, g/dL[c]	4.2 (4.0; 4.5)	4.0 (3.8; 4.3)	4.1 (3.8; 4.4)	**<0.001**	*	–	–

TABLE 1 (Continued)

	(A) CSFCs (n = 137)	(B) Plasma > CSF HIV RNA (n = 436)	(C) CSF/plasma discordance (n = 24)	p	Bonferroni-adjusted pairwise comparisons A vs. B	A vs. C	B vs. C
Serum globulin, g/dL[c]	3.2 (2.7; 3.8)	3.7 (3.1; 4.1)	3.8 (3.2; 4.4)	**<0.001**	**	*	–
Serum total cholesterol, mg/dL[c]	166 (151; 195)	164 (138; 184)	170 (165; 213)	**0.002**	*	–	*
Serum triglycerides, mg/dL[c]	188 (99; 309)	145 (96; 212)	118 (83; 213)	**0.005**	*	*	–
CSF glucose, mg/dL[c]	62 (58; 66)	60 (55; 65)	57 (54; 63)	**<0.001**	**	*	–
CSF total protein, mg/dL[c]	38 (29; 49)	40 (32; 50)	53 (37; 69)	**<0.001**	–	*	–
CSF albumin, mg/dL[c]	15.9 (12.9; 20.2)	16.7 (14.3; 20.3)	21.3 (15.5; 26.3)	**0.002**	–	*	*
CSF globulin, mg/dL[c]	0.37 (0.34; 0.40)	0.38 (0.36; 0.41)	0.40 (0.38; 0.42)	**<0.001**	*	**	*
CSAR[c]	0.59 (0.47; 0.67)	0.61 (0.54; 0.71)	0.73 (0.58; 0.83)	**<0.001**	*	*	*
Blood Leukocytes, $\times 10^9$/L[a]	5.1 (1.6)	5.0 (2.1)	4.2 (1.5)	**0.019**	–	–	–
Red blood cells, million/µL[a]	4.4 (0.6)	4.5 (0.6)	4.7 (0.4)	**0.040**	–	–	–
Hemoglobin, g/dL[a]	14.2 (1.4)	13.9 (1.5)	13.8 (1.4)	0.228	–	–	–
MCV, fL[a]	95.4 (10.8)	91.1 (7.4)	89.5 (6.6)	**<0.001**	**	*	–
MCH, pg/cell[a]	32.3 (3.9)	30.8 (2.6)	29.6 (2.4)	**<0.001**	**	*	–
MCHC, g/dL[a]	33.8 (0.9)	33.7 (0.9)	33.4 (1.4)	0.115	–	–	–
Platelet count, $\times 10^9$/L[a]	237 (60)	215 (74)	218 (53)	**0.002**	**	–	–
CSF leukocytes, /µL[c]	1 (1; 2)	4 (2; 9)	23 (6; 33)	**<0.001**	**	**	*
CSF erythrocytes, /µL[c]	2 (0; 22)	2 (0; 12)	2 (0; 14)	0.584	–	–	–

Note: Significant p values are highlighted in bold.

Abbreviations: 3TC, lamivudine; ABV, abacavir; ART, antiretroviral therapy; CSAR, CSF-to-serum albumin ratio; CSF, cerebrospinal fluid; EFV, efavirenz; IDV, indinavir; LPV, lopinavir; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; NVP, nevirapine; SQV, saquinavir; TDF, tenofovif; ZDV, zidovudine.

[a]Mean (SD).

[b]Number (%).

[c]Median (interquartile range).

[d]Only in participants previously exposed to ART (n = 307).

*Bonferroni corrected $p < 0.05$.

**Bonferroni corrected $p < 0.001$.

and the highest CSAR values, even though, compared to participants with plasma > CSF HIV RNA, they had similar HIV-related parameters and lower plasma HIV RNA (Table 1). Of note, CSF erythrocytes were similar across all groups suggesting low-null risk of blood contamination of CSF samples.

3.2 | CSF HIV RNA and CSF groups over time

The 2000 assessments covered a median time of 2.1 (1.0–3.9) years and each participant contributed with a median of 3 (2–4) visits. The mean period of observation per participant was 455 days (±521; range 31–5700 days). Either during the period of observation or previously, most participants (427, 71.5%) reported ART use at least once (for 46 months ± 40; range 0.1–219).

Most participants (60.1%) remained in the same baseline CSF group during the follow-up (Figure 2). Among these, most had plasma HIV RNA > CSF HIV RNA (52.1%), 46 participants (7.7%) remained CSFC over 2.9 (1.1–4.5) years, whereas only two had persistently CSF/plasma discordance and their follow-up was the shortest (1 year, two visits each). Of note, nine participants transitioned between CSFC and CSF/plasma discordance: after accounting for the possible prolonged effects of previous ART exposure, only three participants plausibly progressed from CSFC to CSF/plasma discordance (Figure 2; Case 5, 8, and 9 in Supporting Information S1: Table 1).

Supporting Information S1: Tables 2 and 3 show the results of univariable and multivariable mixed-effects models for CSF HIV RNA and for CSF groups at the 2000 assessments. Figure 3 summarizes the factors retained in the final models. Specifically,

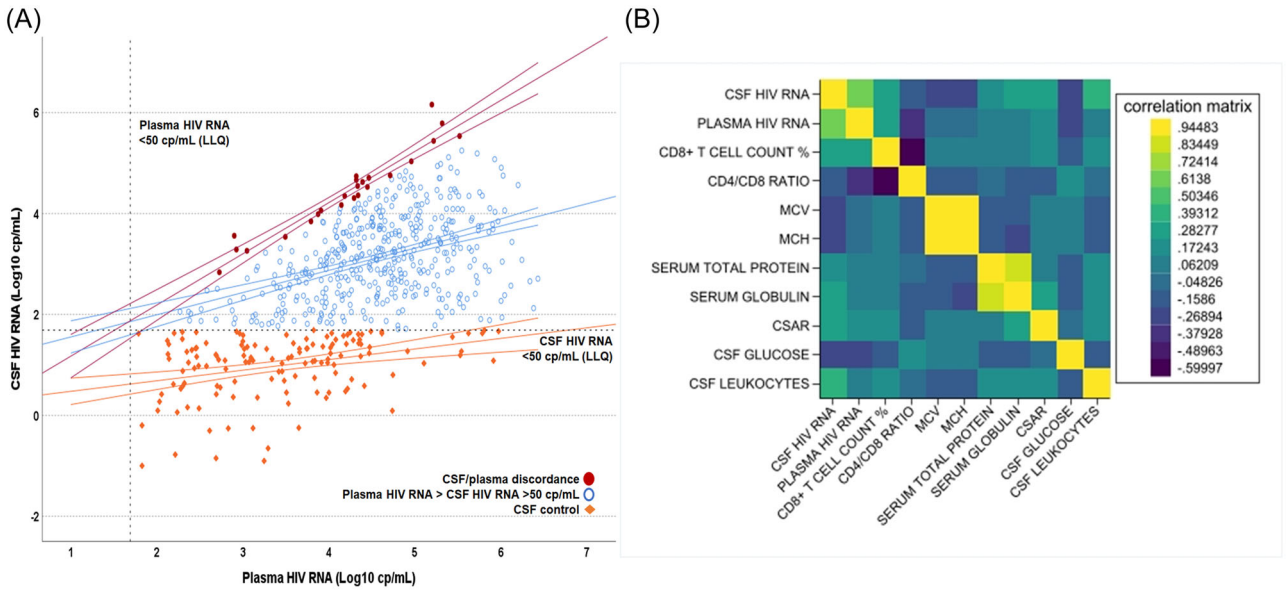

FIGURE 1 Correlations between CSF and blood HIV RNA by CSF group (A) and between CSF HIV RNA and biomarkers in blood and CSF (B) at baseline. (A) Pearson's correlation between CSF and plasma HIV RNA in participants with CSF/plasma discordance (red dots $n = 24$; $\rho = 0.956$, $p < 0.001$), in CSF controllers (orange diamonds $n = 137$; $\rho = 0.337$, $p < 0.001$) and in participants with plasma HIV RNA higher than CSF HIV RNA (empty blue dots $n = 436$; $\rho = 0.432$, $p < 0.001$). The heatmap in panel B shows the variables that were significantly correlated with CSF HIV RNA at baseline, after excluding correlations with very weak effect size ($\rho < 0.2$). Viro-immunological parameters: plasma HIV RNA ($\rho = 0.585$, $p < 0.001$), CD4/CD8 ratio ($\rho = -0.207$, $p < 0.001$), $CD8^+$ T-cell % ($\rho = 0.242$, $p < 0.001$). Blood biochemistry: serum total protein ($\rho = 0.215$, $p < 0.001$), serum globulins ($\rho = 0.286$, $p < 0.001$). Hematology: MCV ($\rho = -0.224$, $p < 0.001$), MCH ($\rho = -0.220$, $p < 0.001$). CSF parameters: CSAR ($\rho = 0.271$, $p < 0.001$), CSF leukocytes ($\rho = 0.442$, $p < 0.001$), CSF glucose ($\rho = -0.236$, $p < 0.001$). CSAR, CSF-to-serum albumin ratio; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; LLQ, lower limit of quantification; MCH, mean cell hemoglobin; MCV, mean cell volume.

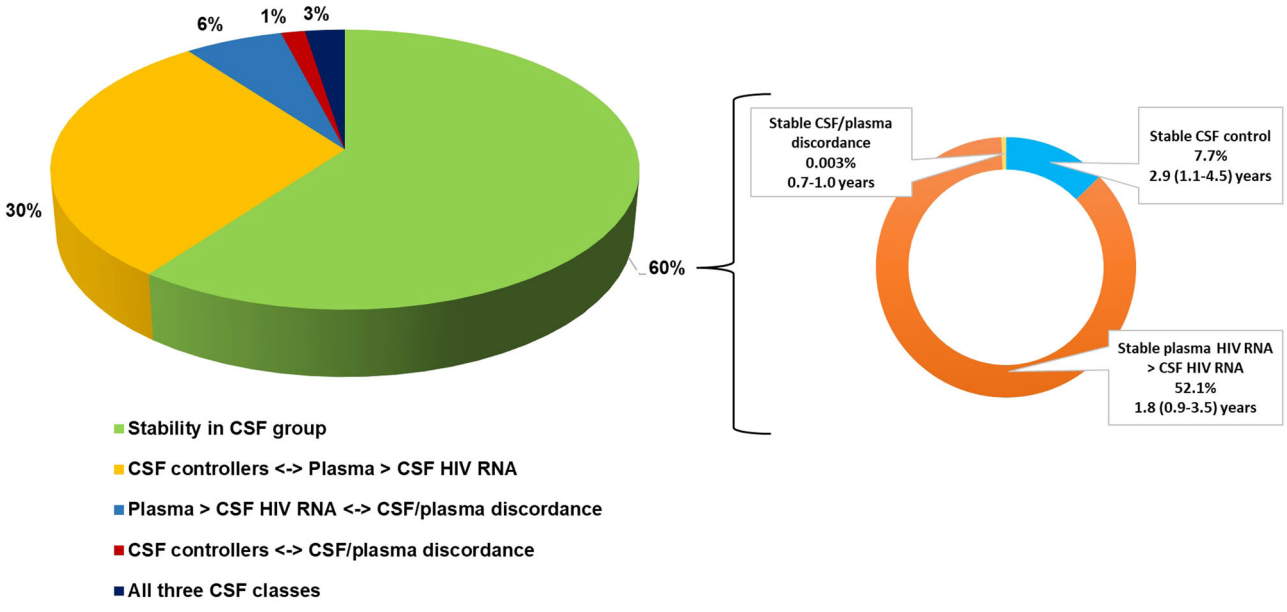

FIGURE 2 Migration of baseline cerebrospinal fluid (CSF) groups at follow-up visits. (A) The piechart shows the proportion of participants who consistently belonged to the same CSF group during the follow-up (green $n = 359$, 60.1%) and the proportions of those who migrated across different CSF groups: between CSF control and plasma > CSF human immunodeficiency virus (HIV) RNA (yellow $n = 177$, 29.6%), between plasma > CSF HIV RNA and CSF/plasma discordance (light blue $n = 37$, 6.2%), between CSF control and CSF/plasma discordance (red $n = 9$, 1.5%), and the propotion of those who were classified in all the CSF groups at least once (dark blue $n = 15$, 2.5%). (B) The subproportions composing the group of participants who remained consistently in the same CSF group: 311 participants persistently showed plasma > CSF HIV RNA (52.1%) for a median length of follow-up of 1.8 years; 46 participants were persistently classified as CSF controllers (7.7%) for a median length of follow-up of 2.9 years; only two participants had persistent CSF/plasma discordance over 0.7 and 1.0 year of follow-up (0.003%).

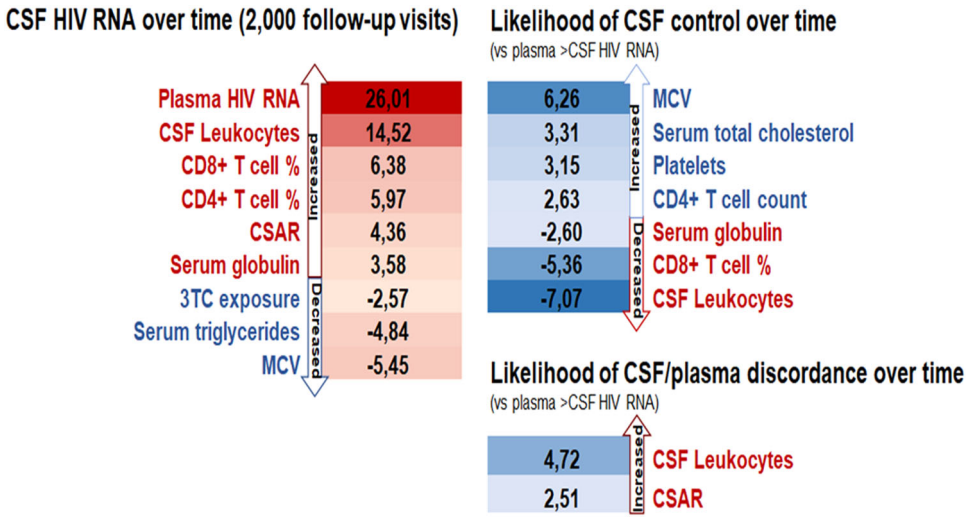

FIGURE 3 Summary of the final models for cerebrospinal fluid (CSF) human immunodeficiency virus (HIV) RNA and for CSF control and CSF/plasma discordance at 2000 lumbar punctures. The figure summarizes the significant variables retained in the final multivariable linear mixed-effects model for CSF HIV RNA (red heatmap, left) and in the final multivariable mixed-effects multinomial model for the likelihood of presenting CSF control or CSF/plasma discordance (vs. plasma > CSF HIV RNA; blue heatmaps, right). In the heatmaps, the sign of the z-value (regression coefficient divided by its SE) indicates the direction (highlighted also by the lateral arrows) of CSF HIV RNA levels and of the likelihood of belonging to the specific CSF group when the corresponding variable increases. 3TC, lamivudine; CSAR, CSF-to-serum albumin ratio; MCV, mean cell volume.

higher CSF HIV RNA was associated with higher plasma HIV RNA, CSF leukocytes, $CD8^+$ and $CD4^+$ T-cell %, CSAR, and serum globulins. On the other hand, lower CSF HIV RNA was associated with higher MCV, higher serum triglyceride, and with past exposure to lamivudine (model $p < 0.001$). The effect sizes for clinical threshold values for continuous covariates with dichotomic (above/below) cutoffs were as follows: CSF leukocytes (>5 cells/μL, aβ: 0.71 [0.61; 0.79], $p < 0.001$), serum globulins (>35 g/dL, aβ: 0.19 [0.087; 0.28], $p < 0.001$), and serum triglycerides (≥150 mg/dL, aβ: −0.20 [−0.28; −0.11], $p < 0.001$). The probability of CSFC was independently associated with higher MCV, higher platelets, higher serum total cholesterol, higher $CD4^+$ T-cell count, and with lower serum globulin levels, $CD8^+$ T-cell %, and CSF leukocytes. CSF/plasma discordance was independently associated with higher CSF leukocytes and worse BBB integrity.

3.3 | Posthoc analysis for persistent CSFCs

To further characterize factors associated with CSF control, we compared the 46 participants who were persistently CSFC with matched controls (1:2). CSFC and controls were matched by plasma HIV RNA, and controls were sampled among all participants who had CSF HIV RNA > LLQ at all the assessments. Table 2 shows the comparisons between CSFC and matched controls. After adjusting for demographics and ART exposure, only higher $CD4^+$ T-cell count nadir, lower serum globulins, and lower CSF leukocytes retained an independent association with persistent CSFC (Table 2).

3.4 | Sensitivity analysis in ART-naïve participants

To further account for potential confounding effects of past/intercurrent ART exposure, we considered only participants who remained ART naïve over time. We censored all visits after the first lifetime ART exposure, and kept participants with at least two visits, which left 238 participants with a total of 764 assessments (Supporting Information S1: Tables 4 and 5). Among these, 22 participants (9.2% and 3.7% of the study population) were persistent CSFC over a median follow-up of 2.3 (1.0–3.9) years. Higher CSF HIV RNA was associated with higher plasma HIV RNA, higher CSF leukocytes, higher $CD4^+$ T-cell %, higher CSAR, and longer duration of HIV infection, as well as with lower CD4/CD8 ratio, lower $CD4^+$ T-cell nadir, and lower serum triglycerides (model $p < 0.001$; Supporting Information S1: Table 4 and Figure 1). CSFC was associated with higher values of $CD4^+$ T-cell count and with lower values of CSAR, $CD8^+$ T-cell %, and of CSF leukocytes; CSF/plasma discordance was associated with higher CSF leukocytes (Supporting Information S1: Table 5 and Figure 1).

4 | DISCUSSION

This longitudinal study on PWH off ART is the largest analysis of CSF HIV RNA ever published. Overall, about two in three PWH had stable CSF/plasma relationship over several years and about one in 13 (~7.5%) persistently had CSF HIV RNA below quantification, which is consistent with the concept that a subgroup of PWH may have spontaneous control of HIV replication in CSF.[2,4]

TABLE 2 Comparison between CSFCs and plasma HIV-RNA-matched participants with CSF HIV RNA > LLQ at every assessment.

	CSFC (*n* = 46)	Controls (*n* = 92)	OR (95% CI)	*p*	aOR (95% CI)	*p*
Age, years[a]	38.3 (8.4)	40.3 (8.2)	0.97 (0.93; 1.02)	0.198	1.02 (0.95; 1.09)	0.652
Male sex[b]	39 (84.8%)	66 (71.7%)	0.46 (0.18; 1.15)	0.095	0.39 (0.09; 1.69)	0.206
White race[b]	19 (41.3%)	35 (38.0%)	1.15 (0.56; 2.36)	0.711	0.95 (0.25; 3.60)	0.945
Black race[b]	13 (28.3%)	27 (29.3%)	0.95 (0.43; 2.08)	0.894	–	–
HCV coinfection[b]	9 (19.6%)	28 (30.4%)	0.56 (0.24; 1.31)	0.177	–	–
AIDS diagnosis[b]	16 (34.8%)	41 (44.6%)	0.66 (0.32; 1.38)	0.272	–	–
Est. duration of HIV, years[a]	7.4 (6.6)	10.6 (6.1)	0.92 (0.86; 0.99)	**0.019**	Excluded[c]	–
Absolute CD4+ T-cell count, /μL[d]	451 (370; 710)	381 (308; 549)	1.01 (1.00; 1.01)	**0.038**	Excluded[c]	–
CD4+ T cells, percentage[d]	25 (18; 35)	25 (18; 32)	1.01 (0.98; 1.05)	0.425	–	–
Absolute CD8+ T-cell count, /μL[d]	955 (720; 1154)	948 (711; 1084)	1.00 (1.00; 1.00)	0.906	–	–
CD8+ T cells, percentage[d]	50 (40; 59)	55 (47; 64)	0.96 (0.93; 0.99)	**0.013**	Excluded[c]	–
CD4/CD8 ratio[d]	0.52 (0.32; 0.80)	0.45 (0.27; 0.65)	2.35 (0.82; 6.76)	0.113	–	–
Nadir CD4+ T-cell count, /μL[d]	317 (178; 600)	256 (159; 398)	1.00 (1.00; 1.01)	**0.006**	1.01 (1.00; 1.01)	**0.001**
ART naïve[b]	23 (50.0%)	57 (62.0%)	0.61 (0.30; 1.25)	0.181	0.60 (0.16; 2.22)	0.447
Exposure to ART, months[d]	1 (0; 30)	5 (0; 45)	0.99 (0.99; 1.00)	0.212	–	–
3TC exposure[b]	17 (37.0%)	51 (55.4%)	0.47 (0.23; 0.97)	**0.042**	Excluded[c]	–
ZDV exposure[b]	13 (28.3%)	41 (44.6%)	0.49 (0.23; 1.05)	0.067	–	–
EFV exposure[b]	1 (2.2%)	11 (12.0%)	0.16 (0.02; 1.31)	0.088	–	–
TDF exposure[b]	2 (4.3%)	10 (10.9%)	0.37 (0.08; 1.78)	0.216	–	–
NVP exposure[b]	1 (2.2%)	8 (8.7%)	0.23 (0.03; 1.92)	0.176	–	–
Serum total protein, g/dL[d]	7.4 (7.0; 7.8)	7.8 (7.3; 8.2)	0.53 (0.30; 0.92)	**0.025**	Excluded[c]	–
Serum albumin, g/dL[d]	4.2 (4.0; 4.6)	4.0 (3.7; 4.3)	4.11 (1.66; 10.22)	**0.002**	–	–
Serum globulin, g/dL[d]	3.0 (2.6; 3.8)	3.8 (3.4; 4.2)	0.37 (0.22; 0.64)	**<0.001**	0.24 (0.09; 0.61)	**0.003**
Serum total cholesterol, mg/dL[d]	166 (133; 195)	166 (139; 192)	1.00 (0.99; 1.01)	0.964	–	–
Serum triglycerides, mg/dL[d]	152 (85; 299)	131 (74; 218)	1.00 (1.00; 1.00)	0.152	–	–
CSF glucose, mg/dL[d]	63 (59; 68)	59 (55; 64)	1.04 (1.00; 1.09)	**0.037**	Excluded[c]	–
CSF total protein, mg/dL[d]	36 (28; 49)	42 (31; 51)	0.98 (0.96; 1.00)	0.088	–	–
CSF albumin, mg/dL[d]	15.8 (12.3; 20.4)	17.4 (13.5; 20.4)	0.95 (0.88; 1.01)	0.097	–	–
CSF Globulin, mg/dL[d]	2.3 (2.2; 2.5)	2.4 (2.3; 2.5)	0.26 (0.05; 1.48)	0.129	–	–
CSAR[d]	0.59 (0.45; 0.66)	0.64 (0.55; 0.73)	0.03 (0.002; 0.38)	**0.007**	Excluded[c]	–
Blood Leukocytes, $\times 10^9$/L[d]	5.1 (4.1; 6.4)	4.6 (4.0; 5.8)	1.04 (0.86; 1.25)	0.682	–	
Blood erythrocytes, million/μL[d]	4.5 (4.2; 4.8)	4.5 (4.1; 4.9)	0.92 (0.48; 1.77)	0.813	–	
Hemoglobin, g/dL[d]	14.3 (13.4; 15.1)	13.8 (12.8; 15.0)	1.23 (0.95; 1.59)	0.110	–	–
MCV, fL[d]	93 (86; 101)	90 (86; 94)	1.05 (1.00; 1.09)	**0.037**	Excluded[c]	–
MCH, pg/cell[d]	31.6 (29.0; 34.4)	31.6 (29.0; 34.4)	1.12 (1.00; 1.24)	**0.048**	Excluded[c]	–
MCHC, g/dL[d]	33.7 (33.3; 34.4)	33.8 (33.1; 34.6)	1.03 (0.72; 1.46)	0.886	–	–

TABLE 2 (Continued)

	CSFC ($n = 46$)	Controls ($n = 92$)	OR (95% CI)	p	aOR (95% CI)	p
Platelets, $\times 10^9/L$[d]	230 (200; 278)	221 (181; 260)	1.00 (0.99; 1.01)	0.390	–	–
CSF leukocytes, /μL[d]	2 (1; 2)	3 (2; 8)	0.73 (0.61; 0.88)	**<0.001**	0.56 (0.39; 0.81)	**0.002**

Note: Significant p values are highlighted in bold.

Abbreviations: 3TC, lamivudine; aOR, adjusted odds ratio; AIC, Akaike Information Criterion; ART, antiretroviral therapy; CSAR, CSF-to-serum albumin ratio; CSF, cerebrospinal fluid; CSFC, cerebrospinal fluid controllers; EFV, efavirenz; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; NVP, nevirapine; OR, odds ratio; TDF, tenofovir; ZDV, zidovudine. 2 (a) Mean (SD); 1 (b) Number (%); 3 (c) Median (interquartile range).

[a]Mean (SD).

[b]Number (%).

[c]Variable excluded from the final model by backward selection method based on AIC.

[d]Median (interquartile range).

The compartment specificity of such spontaneous control is suggested by the fact that the concurrent plasma HIV RNA was up to 450 604 copies/mL. Even after excluding ART-exposed participants, 3.7% of the participants were persistent CSFC. Our findings suggest that CSFC have less pro-inflammatory immune responses, better BBB integrity, and possibly less cell migration into CSF (as suggested by the lower values of CSF leukocytes, proteins, and CSAR, lower proportion of $CD8^+$ T cells, and lower globulins levels). Of note, CSFC had higher $CD4^+$ T-cell absolute counts in blood, but higher $CD4^+$ T-cell percent was also associated with higher CSF HIV RNA, in line with the acknowledged dual role played by $CD4^+$ T cells: effector of protective immune responses but also HIV carriers trafficking into the CNS.[33–35]

At the other end of the spectrum, 4.0% of PWH showed CSF/plasma discordance at the first assessment, and 82 (4.1%) episodes of discordance occurred over 2000 visits, although the persistence of this condition over time was almost null (<0.01%). The period prevalence of 4.1% is similar to cross-sectional estimates of CSF viral escape in treated PWH.[36–38] Up to 8% of cases of CSF viral escape are not explained by pharmacological issues or resistance to ART in the CNS,[39] suggesting that other factors can contribute to this risk. Whether PWH who have CSF/plasma discordance off ART are at greater risk for CSF viral escape during ART because of less effective antiviral immune responses in the CNS, because of the establishment of larger and more diverse CNS reservoirs, or because of greater trafficking of infected lymphoid and myeloid cells within the CNS (or because of all) need to be elucidated. However, our longitudinal data showed that persistent CSF/plasma discordance is extremely rare, suggesting that this finding is more incidental than consistent in nature, and its neuropathogenic potential should be re-assessed longitudinally. Furthermore, prior exposure to lamivudine was associated with lower CSF HIV RNA. Lamivudine has low genetic barrier which, coupled with its CNS pharmacokinetics,[40] may facilitate the selection of resistance-associated mutations. Without pharmacological pressure, such mutations (e.g., M184V) can substantially reduce viral fitness,[41] explaining our finding. This may also suggest that higher CSF HIV RNA before ART may be a poor predictor of CSF escape during ART, considering the complexity of determinants affecting the CSF setpoint.

Our results identified multiple characteristics that were associated with CSF HIV RNA and its relationship with plasma HIV RNA off ART; these included direct and indirect indicators of systemic and CNS inflammation, but also biomarkers of iron and lipid metabolism. The association with indicators of iron metabolism (MCV) may result from the link between HIV replication and inflammation, as iron deficiency/reduced iron uptake depend on increased production of hepcidin, an inflammatory response protein.[42] Although inflammation may affect iron metabolism and BBB integrity separately,[43] iron may also directly alter endothelial functioning and iron-dependent oxidative stress may damage the BBB,[20] which in turn might equilibrate plasma and CSF HIV RNA, alternatively explaining our results. Only one-quarter of MCV measurements were outside the normal range, suggesting that the relationship between this biomarker and HIV RNA may not be driven by pathological conditions such as anemia, but by variation within the normal range.

Lower serum triglycerides were associated with higher CSF HIV RNA and higher total cholesterol with higher likelihood of CSFC. Our study was not designed to disentangle specific mechanisms, although many can be postulated. As a premise and similarly to MCV, the range of triglycerides and cholesterol in our cohort was mostly within normal values and the prevalence of hyperlipidemia was 4.8%. Serum lipids have been implicated in maintaining BBB integrity in Alzheimer's disease, and elevation of serum triglycerides has been linked to cerebrovascular inflammation.[24,26] In our study population, higher serum triglycerides correlated with higher CSAR ($p = 0.15$, $p < 0.001$), but the association between triglycerides and CSF HIV RNA was independent from BBB in the multivariable models. Therefore, while it is possible that lipids could mediate HIV entry and replication within the CNS by shaping BBB and CNS inflammation, future studies should address these hypotheses.

Among the novel findings, higher platelet count correlated with lower CSF HIV RNA and CSFC. Platelets regulate both leukocyte activities and vascular permeability,[44] aligning this finding along the previous suggesting that CSFC may have distinct immune responses

and better BBB integrity. Of note, the association between platelets and CSFC was independent from biomarkers of immune status, BBB, and inflammation. Platelets can bind HIV virions, facilitate the inhibition of HIV infection via endogenously produced inhibitors of viral replication,[30] are involved in virus-mediated BBB dysfunction,[27] represent a viral reservoir with no clear replication competence,[30] and can form complexes with monocytes that showed enhanced ability to adhere to brain endothelial cells.[28] In line with this, drugs that modulate platelet activation may improve neurocognitive impairment in PWH.[27,28] Future studies should address the role of platelets and lipids in HIV replication in the CNS, also considering the large armamentarium of available drugs for both.

This study has important limitations. First, the participants were relatively young, and the findings may not generalize well to older PWH, but this also means that they are less likely to be confounded by aging-related conditions (e.g., vascular disease). We were unable to adjust for time since ART discontinuation in those previously ART exposed, but the sensitivity analyses in ART-naive participants had mostly consistent results, except that MCV, cholesterol, platelets, and globulins were not retained in final models. Their absence may be due to the reduced power in this subgroup, or because past ART exposure influences some of these relationships, such as intermittent interruption of antiviral responses or the long-term effect of some antiretrovirals on iron metabolism.[45] The definition of CSFC was arbitrary and may not indicate the absence of HIV RNA; the LLQ of 30–400 cp/mL, higher than most current assays, might miss low-level HIV RNA values. Imputation of CSF HIV RNA below the LLQ was based on Bayesian inference and the accuracy of the exact point-value quantification cannot be always assured. However, only 39 of the 281 imputed values (13.9%) were assigned within a range that might change the CSF group assignment. The neuropsychiatric characteristics (e.g., cognitive performance, depressive symptoms) of participants were only available for a subgroup of participants. For this reason, they were not analyzed in this manuscript and will be the focus for future analyses to assess the clinical relevance and the impact of the observed associations on mental health outcomes. Finally, although the longitudinal analyses strengthen causal inference, the project is not interventional so conclusions regarding causation should be appropriately conditioned.

In conclusion, off ART, about two in three PWH had stable CSF-plasma dynamics over years, one in 13 had spontaneous CSF control, and persistent CSF/plasma discordance was extremely rare. Biomarkers of disease progression, systemic and CSF inflammation, iron and lipid metabolism were associated with CSF HIV RNA and its relationship with plasma viremia. While few PWH remain off ART in modern clinics, these findings inform neuropathogenesis and future studies to better understand CSF and systemic HIV control, viral escape, and the effects of analytical or patient-driven ART interruptions in the CNS.

AUTHOR CONTRIBUTIONS

Study conception and design: Mattia Trunfio, Bin Tang, Oluwakemi Okwuegbuna, Scott L. Letendre; data collection: all the authors; analysis and interpretation of results: all the authors; draft manuscript preparation: Mattia Trunfio, Oluwakemi Okwuegbuna, Scott L. Letendre All authors reviewed the results and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of the research volunteers and participants. This work was supported by the National Institute of Mental Health (grant numbers P30 MH62512, R01 MH07345, R25 MH081482) and the National Institute on Drug Abuse (grant number P50 DA26306).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Scott L. Letendre, upon reasonable request.

ETHICS STATEMENT

All projects were approved by local IRB of each center.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Trunfio M, Tang B, Okwuegbuna O, et al. Longitudinal analysis of CSF HIV RNA in untreated people with HIV: identification of CSF controllers. *J Med Virol*. 2024;96:e29550. doi:10.1002/jmv.29550