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Prevalence of detectable HIV-DNA and HIV-RNA in cerebrospinal fluid of youth with perinatal HIV and impaired cognition on antiretroviral therapy

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Objective: Central nervous system (CNS) HIV infection can impact cognition and may be an obstacle to cure in adolescents and young adults with perinatal HIV (AYAPHIV). IMPAACT2015 enrolled AYAPHIV on suppressive antiretroviral therapy (ART) with cognitive impairment to detect and quantify HIV in blood and cerebrospinal fluid (CSF).

Design: IMPAACT2015 was a U.S.-based multi-site, exploratory, observational study.

Methods: Cognitive impairment was defined as NIH Toolbox Fluid Cognition Composite score (FCCS) more than 1 standard deviation below age-adjusted normative group mean. Cell-free HIV-RNA and cell-associated HIV*pol*/*gag*-DNA and 10 biomarkers of inflammation/neuronal injury were measured in paired CSF and blood. ART exposure concentrations were quantified in hair.

Results: Among 24 participants, 20 had successful CSF collection and 18 also met viral suppression criteria. Nine of 18 (50%) were female sex-at-birth, and 14 of 18 (78%) were black. Median (range) age was 20 years (13–27), time on ART was 18.3 years (8.0–25.5), and FCCS was 68 (53–80). HIV-DNA was detected in PBMCs from all participants. In CSF, two of 18 (11%, 95% CI: 1.4–34.7%) participants had detectable cell-free HIV-RNA, while HIV*gag* or *pol*-DNA was detectable in 13 of 18 (72%, 95% confidence interval: 47–90). Detectable HIV-DNA in CSF was associated with male sex-at-birth ($P = 0.051$), lower $CD4^+$ cell count at enrollment ($P = 0.016$), and higher PBMC HIV*pol*-DNA copies ($P = 0.058$). Hair antiretroviral concentrations and biomarkers were not associated with CSF HIV-DNA detection.

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Conclusion: We found that a high proportion of AYAPHIV with neurocognitive impairment had CSF cells harboring HIV-DNA during long-term virologic suppression. This evidence of persistent HIV-DNA in CSF suggests that the CNS should be considered in treatment and cure studies.

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Keywords: central nervous system, HIV, perinatal, reservoir

Introduction

Despite recent progress in understanding HIV persistence, a definitive strategy to induce HIV cure remains elusive. The central nervous system (CNS) represents a reservoir for HIV that may need to be targeted by curative approaches [1,2]. HIV invades the CNS early in the course of infection and establishes infection predominantly in immune cells like microglia and perivascular macrophages [3]. The long half-life of these cells, limited immunosurveillance, and reduced penetration of some antiretrovirals into the CNS support the CNS as a sanctuary site of HIV persistence. As a result, the CNS is hypothesized to represent a barrier to HIV eradication [4].

Apart from autopsy studies and nonhuman primate models in which the brain can be directly studied, collection of cerebrospinal fluid (CSF) provides the most accessible insight into the CNS in living individuals. HIV-RNA in plasma and CSF are correlated and decline in parallel after starting antiretroviral therapy (ART) [5–7]. However, many cases of neurological symptoms associated with quantifiable HIV-RNA in CSF and undetectable HIV in plasma have been described [8–10]. In asymptomatic adults on suppressive ART, 4–23% have HIV-RNA in CSF at levels quantifiable by standard viral load assays [11–16]. The frequency of HIV-RNA detection in CSF increases with inclusion of adults with neurocognitive impairment and use of more sensitive assays [10,17]. HIV-DNA has been detected in CSF cell pellets in 48–81% of ART-suppressed adults [6,18,19], and is associated with impaired neurocognition [18]. The replication-competence of HIV-DNA in CSF cells has not been assessed, although HIV-RNA transcripts were found in CSF cells in 88% of participants in one study [19]. The critical question as to whether compartmentalized HIV in CSF contributes to systemic viral rebound after ART interruption warrants further research, as results to date are conflicting [20,21]. Understanding the significance of HIV-DNA in CSF cells during ART would help to elucidate the importance of CNS HIV persistence for HIV neuropathogenesis and cure.

Children with perinatally acquired HIV (PHIV) are at an increased risk for lower neurocognitive functioning [22–35]. Neuroimaging studies in this population showed white matter microstructure and functional connectivity abnormalities that correlated with HIV disease severity, nadir $CD4^+$ percentage, and peak viremia [36–40]. Blood–brain barrier differences in neonates and infants compared to adults may contribute to increased entry of HIV into the CNS prior to ART initiation [41]. Indeed, compartmentalization of HIV sequence evolution between peripheral blood and CSF has been demonstrated in ART-naive children with PHIV [42]. However, HIV persistence in the CSF of ART-suppressed youth with PHIV has not been described. The IMPAACT2015 study sought to address this gap in knowledge by determining the prevalence of HIV-RNA and -DNA in the CSF of PHIV on suppressive ART. Markers of inflammation and neuronal injury in circulation and CSF, neurocognitive performance, and ART drug levels in hair were evaluated for associations with CSF HIV.

Materials and methods

Study approval

The institutional review boards at all participating sites approved IMPAACT2015. Written informed consent was received from all participants or their guardians when appropriate; assent was received from all minors.

Study participants and procedures

AYAPHIV (13–30 years old) on ART for at least 12 consecutive months with at least two consecutive viral loads less than 40 copies/ml obtained more than 3 months apart, with one specimen within 60 days of enrollment, and NIH Toolbox Fluid Cognition Composite score (FCCS) less than 85 were enrolled in IMPAACT2015. Known ART interruption for more than 7 days, any viral load more than 200 copies/ml in the last year, non-HIV related neurologic conditions, contraindications to lumbar puncture, immunomodulatory medications, and recent infections or vaccines were exclusion criteria. HIV history was obtained by chart review. Blood, CSF, and hair strands were collected on the same day. Pencil-point needles (e.g., Sprotte) were recommended and used for 71% of lumbar punctures. Collection of 17 ml CSF from participants less than 18 years old and 25 ml from participants at least 18 years old was planned, with a minimum of 8 ml accepted. CSF samples were promptly centrifuged, and supernatants and cell pellets stored at −80°C according to established ACTG CSF processing

protocols. Participants were instructed to report any signs or symptoms following lumbar puncture; to facilitate reporting, study staff contacted participants the day after lumbar puncture.

Cognitive assessments

Cognitive functioning was assessed using the NIH Toolbox for the Assessment of Neurological and Behavioral Function. The NIH Toolbox includes brief subtests that assess Executive Function, Attention, Episodic Memory, Language, Processing Speed and Working Memory. Performance is compared to a nationally representative sample of persons of the same age and/or ethnicity; standardized subtest scores are combined to form Fluid, Crystallized and Total Cognition Composite scores (mean = 100, SD = 15) [43]. The FCCS was selected to screen participants for IMPAACT2015 with FCCS less than 85 (≤1 SD below the mean) required to be eligible. Auditory Verbal Learning scores were not normalized. High Crystallized Composite and Oral Reading Cognition scores for two participants and a high Auditory Verbal Learning score for one participant were considered outliers and excluded per team decision.

HIV-RNA quantification

Levels of HIV-RNA in plasma and CSF were measured by commercially available quantitative RT-PCR at Quest Diagnostics with limit of detection of 20 copies/ml.

HIV-DNA quantification

Levels of HIV-DNA in PBMCs and CSF cells were measured using droplet digital PCR (ddPCR) as previously described [44]. Briefly, DNA was extracted from cryopreserved cells using the AllPrep DNA/RNA minikit (Qiagen, Germantown, MD, USA). One thousand nanogram of DNA per replicate were digested with BSAJ1 enzyme (New England BioLabs, Ipswich, MA, USA) before ddPCR quantification. HIV-DNA PCRs were performed with a duplex assay using HEX (*gag)* and FAM (*pol*) probes, with the following cycling conditions: 10 min of initial enzyme activation at 95°C, 40 cycles of 30 s denaturation at 94°C followed by 60°C extension for 60 s, and final inactivation of 10 min at 98°C. Host cell RNase P/MRP 30 kDA-subunit gene (RPP30) was used as a cellular normalizer. Primer and probe sequences used were as follows: *joPOL_FAM:* Forward 5'-TACAGTGCAGGGGAAAGAATA-3', Reverse 5'-CTGCCCCTTCACCTTTCC- 3', Probe 5'-TTTCGGGTTTATTACAGGGACAGCAG-3' (5' FAM/ZEN/IBFQ 3'); *skGAG_HEX:* Forward 5'-AGTTGGAGGACATCAAGCAGCCATGCAAAT-3', Reverse 5'-TGCTATGTCAGTTCCCCTTGGTTCTCT-3', Probe 5'-AGACCATCAATGAGGAAGCTGCAGAATGGGAT-3' (5' HEX/ZEN/IBFQ 3'); *RPP30_HEX:* Forward 5'-GATTTGGACCTGCGAGCG-3', Reverse 5'- GCGGCTGTCTCCACAAGT-3', Probe 5'-CTGACCTGAAGGCTCT-3' (5' HEX/ZEN/IBFQ 3'). Copy numbers were calculated as the mean of three replicate wells. A range of 71 800–99 900 PBMCs [lower limit of detection (LLD) 3.34–4.64 copies/million PBMCs] and 159–5850 CSF cells (LLD 170.94–6289.31 copies/million cells) were interrogated. The LLD was set as the input cell number.

Biomarker assessments

Levels of IL-6, MCP-1, sCD14, TNFa, IP-10, sCD163, CRP (all from R&D), neopterin (GenWay BioTech, San Diego, CA, USA), and sICAM-5 (Cloud-Clone, Katy, TX, USA) were measured using ELISA per manufacturers' instructions. Duplicates of 15% of samples were included and results analyzed using BioTek ELx800 ELISA reader using KCjunior software (version 1.6). For sICAM-5 in plasma and TNFa in CSF, few samples were above the lower limit of quantification [LLQ; values ≥LLQ for sICAM-5 in plasma ($n = 3$) were: 0.25, 2.23, and 6.85 μg/l; value ≥LLQ for TNFa in CSF ($n = 1$) was 0.20 ng/l]. All CSF samples were <LLQ for sICAM-5. Neurofilament light chain (NF-L) was measured by Monogram Biosciences. Five values of NF-L in plasma were <LLQ of 2.74 pg/ml.

Hair antiretroviral measurements

Fifty to sixty strands of hair were cut close to the occipital scalp, placed in tin foil, labeled at the distal end, and stored in a plastic bag including desiccant pellet in the dark at room temperature prior to shipping to the UCSF Hair Analytical Laboratory (HAL). The proximal section of the sample (~1.5 cm) was then chopped to 1–2 mm segments with scissors and 5 mg were weighed, processed, and analyzed using liquid chromatography/tandem mass spectrometry (LC/MS-MS) as described [45,46]. The drugs measured were dolutegravir, lamivudine, and tenofovir disoproxil fumarate/tenofovir alafenamide. Each sample was analyzed once. For one participant, both TFV and FTC hair concentrations were <LLQ. The UCSF HAL's methods are peer-reviewed and approved by the NIH-supported Clinical Pharmacology and Quality Assurance (CPQA) program.

Statistical analysis

The primary outcome was a binary measure for quantifiable HIV-RNA (≥20 copies/ml) in CSF; detection of HIV-DNA in CSF cells was secondary. Confidence intervals (95% CI) for proportions were calculated using exact binomial distribution (Clopper Pearson). In descriptive figures and summaries of continuous measures of HIV-1 DNA, biomarker and drug concentration data, results below the LLD/LLQ were plotted at the lower limit and set to the lower limit if imputed. Associations with HIV-DNA detection in CSF cells were assessed using complementary log-log binary regression incorporating the input cell number [44,47]. When possible, associations between continuous measures were assessed with Spearman rank correlation, assigning results below the assay LLD/LLQ the lowest rank. For associations with HIV-DNA level in CSF cells,

the large overlap of LLDs and quantified HIV-DNA levels could not resolve ranking and Pearson correlation was estimated. Estimation was by maximum likelihood of a bivariate normal distribution for log-transformed CSF HIV-DNA level and the second variable, incorporating left-censoring of values below LLD/LLQ as needed [48,49]. Associations with CSF HIV-DNA detection and level were assessed with likelihood ratio tests. *P* values are presented for two-sided tests at 0.05 significance level. Analyses were carried out using Statistical Analysis System (SAS), version 9.4 (Cary, North Carolina, USA).

Results

Characteristics of study participants

Twenty-four AYAPHIV with cognitive impairment on long-term ART and plasma HIV-RNA less than 40 copies/ml were enrolled from nine IMPAACT sites across the U.S. Twenty-two participants underwent lumbar puncture, of which 20 of 22 (91%) were successful (defined as a collection of $\geq$8 ml CSF). Two participants underwent a second lumbar puncture due to insufficient CSF collected on the first attempt. Eighteen participants maintained plasma HIV-RNA less than 20 copies/ml from entry through the day of successful lumbar puncture. Characteristics of these 18 evaluable study participants, seven of whom were 18 years of age or less, are summarized in Table 1 and Supplementary Table 1, http://links.lww.com/QAD/D231. Median CSF volumes obtained were 18 ml in participants 13–18 years of age and 25 ml in those 19–26 years of age. Lumbar punctures were generally well tolerated with only two participants reporting symptoms considered to be Grade 3 events: post lumbar puncture syndrome and dehydration in the first and neck pain and headache in the second (Supplementary Table 2, http://links.lww.com/QAD/D231).

HIV persistence in the blood and cerebrospinal fluid

HIV-RNA in CSF supernatant was detected in two of 18 participants (11%; 95% CI: 1.4–34.7). CSF HIV-RNA was quantifiable at more than 20 copies/ml in only one of these two participants (5.6% of the 18 evaluable; 95% CI: 0.1–27.3) at 25 copies/ml (Fig. 1a). HIV-RNA in plasma was less than 20 copies/ml in all 18 participants as per study inclusion criteria (Fig. 1a).

HIV-DNA was evaluated in PBMCs and CSF cell pellets from the 18 virologically suppressed participants using both *gag* and *pol* targets. HIV-DNA by either *gag* or *pol* was detected in PBMCs from 100% of participants (95% CI: 81–100; Fig. 1a), with both targets detected in 16/18. A median of 223 (range: 4–984) and 202 (range: 3–780) HIV-DNA copies/10^6 PBMCs were measured for *gag* and *pol*, respectively (Fig. 1b). In CSF cells, HIV*gag* or *pol*-DNA was detected in 13 of 18 participants (72%; 95% CI: 47–90; Fig. 1a). There were two participants with only HIV*gag*-DNA and one participant with only HIV*pol*-DNA detected in CSF cells (Fig. 1b). In the 12 individuals with HIV*gag*-DNA detected, median levels were 1563 copies/10^6 CSF cells. In the 11 individuals with HIV*pol*-DNA detected, median levels were 2353 copies/10^6 CSF cells. The number of CSF cells analyzed ranged from 159 to 5850 (Fig. 1b). In the five of 18 participants without HIV*gag* or *pol*-DNA detected in CSF, cell numbers assayed ranged from 159 to 780 (Fig. 1b and Supplementary Fig. 1, http://links.lww.com/QAD/D231). Comparison of HIV*gag* and *pol*-DNA levels in PBMCs and CSF cells, respectively, showed positive, nonsignificant correlations (Supplementary Fig. 2, http://links.lww.com/QAD/D231). Restricting this analysis to the 17 participants with HIV*gag*-DNA detected in PBMCs, a strong correlation between HIV*gag*-DNA levels in PBMCs and CSF cells was observed ($r = 0.92$, $P < 0.001$).

Detectable HIV*gag* or *pol*-DNA in CSF cells was associated with higher levels of HIV*pol*-DNA copies in PBMCs ($P = 0.058$; Fig. 1c). The two participants with detectable CSF HIV-RNA had the highest levels of HIV*gag* and *pol*-DNA in PBMCs (#17 and #18; Fig. 1b). Detectable HIV-DNA in CSF cells was associated with male sex at birth ($P = 0.051$) and lower $CD4^+$ cell count at enrollment ($P = 0.016$). We note that the five men in the study tended to have lower $CD4^+$ cell counts at enrollment (median of 628 versus 797 cells/μl) with later

Table 1. Characteristics of virologically suppressed study participants.

Characteristic ($n = 18$)	Median (Q1, Q3)	Min, Max
Age (years)	20 (18, 23)	13, 27
Sex, proportion (% female sex at birth)	9/18 (50%)	na
Race, proportion (% Black or African–American)	14/18 (78%)	na
Duration of ART (years)	18.3 (16.8, 20.4)	8, 25.5
Pre-ART nadir $CD4^+$ T cell count (cells/μl) ($n = 10$)	786 (550, 1110)	0, 1308
Pre-ART highest HIV-RNA (log10 copies/ml) ($n = 10$)	5.5 (4.9, 6.0)	4.7, 6.5
Enrollment $CD4^+$ T cell count (cells/μl)	701 (430, 1012)	143, 1342
CSF leukocytes (cells/μl)	1 (0, 2)	0, 5
CSF protein (mg/dl)	24.55 (18.0, 27.0)	14.1, 55.0
CSF/plasma albumin ratio	3 (2, 4)	0, 35
Fluid Cognition Composite Score	68.0 (59.0, 75.0)	53.0, 80.0

na, not applicable.

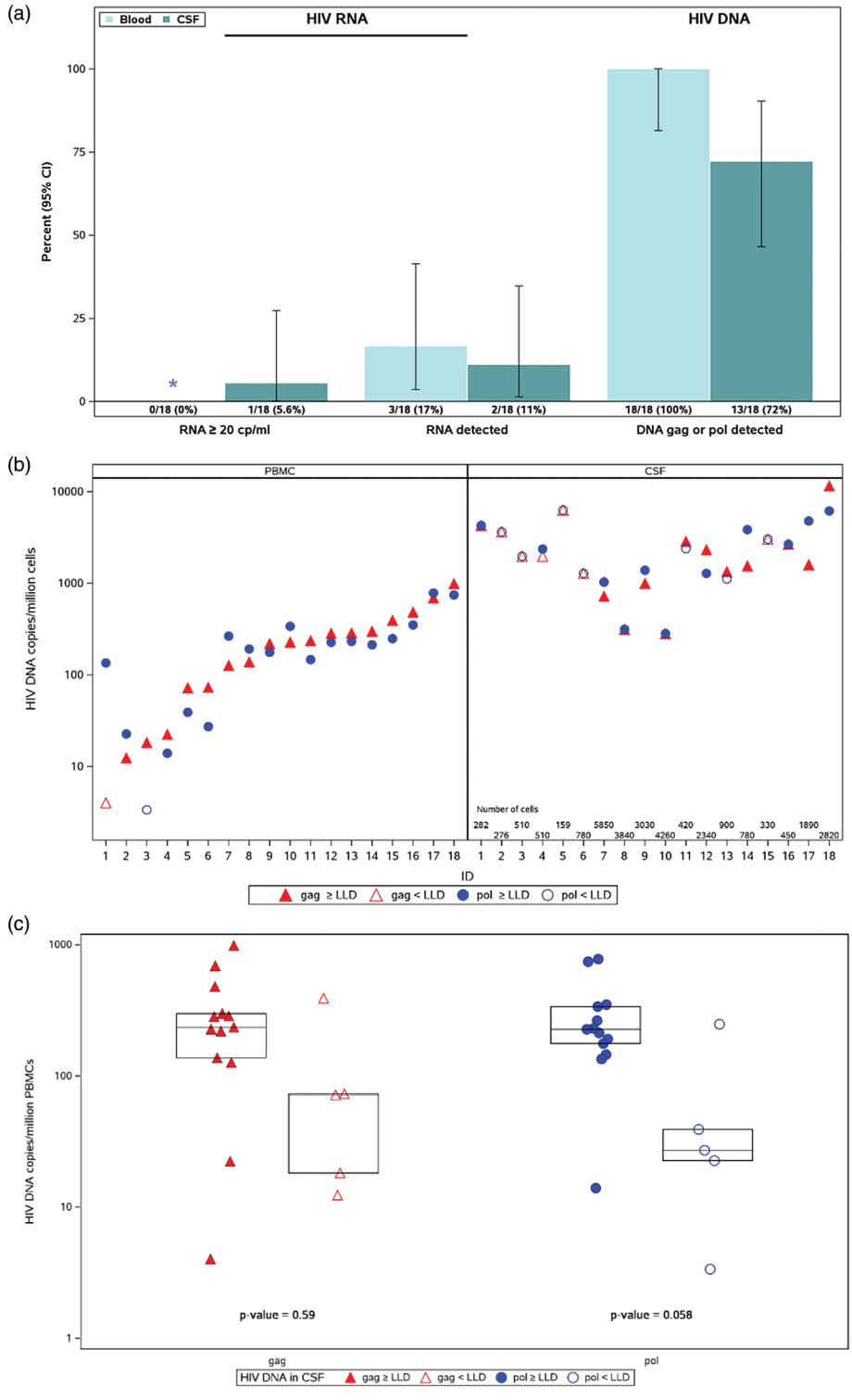

Fig. 1. Detection of HIV in blood and cerebrospinal fluid. (a) Percentage of participants with detectable HIV-RNA in plasma and CSF and HIV-DNA in PBMCs and CSF cells. 95% confidence intervals are provided where possible. *Per study design no participants had HIV-RNA in peripheral blood > 20 copies/ml. (b) HIV*gag* (red) and *pol* (blue) DNA copies/million cells for each participant. Open symbols reflect results below the LLD. CSF cell count is indicated above each participant, ordered based on the quantity of *gag* detected in PBMCs. (c) The quantity of HIV*gag* (left) and *pol* (right) DNA in PBMCs grouped based on whether HIV-DNA was detected (filled symbol) or not detected (open symbols) for both *gag* and *pol* in CSF cells; box shows 25th percentile, median and 75th percentile. The two undetectable HIV-DNA measurements in PBMCs are plotted at the LLD.

age at ART initiation (median of 2.1 versus 0.6 years). There were no associations between the detection of HIV-DNA in CSF cells and race, age, years on ART, or age at ART initiation (Supplementary Table 3, http://links.lww.com/QAD/D231).

Biomarkers of inflammation and neuronal injury

Biomarkers of inflammation and neuronal injury were measured in paired plasma and CSF and were relatively homogeneous, with CRP demonstrating the greatest variability in both fluids (Fig. 2). Concentrations of CRP, sCD14, and sCD163 were higher in plasma compared to CSF, and MCP-1 and NF-L were higher in CSF compared to plasma (Fig. 2). CRP concentrations in blood were strongly positively correlated with both CRP ($r=0.86$, $P<0.001$) and sCD163 ($r=0.63$, $P=0.005$) concentrations in CSF, and CRP levels in CSF were negatively associated with MCP-1 levels in blood ($r=-0.53$, $P=0.023$) (Supplementary Fig. 3, http://links.lww.com/QAD/D231). Positive correlations between the concentrations of CSF IP-10 with blood MCP-1 ($r=0.56$, $P=0.017$) and blood neopterin ($r=0.51$, $P=0.030$) were also found.

To uncover relationships between measured analytes and infected cells, we used both quantitative and qualitative measures of HIV-DNA. Accordingly, concentrations of biomarkers in blood and CSF were assessed for correlations with the levels of HIV-DNA in PBMCs or CSF cells (quantitative measure) and the presence of detectable HIV-DNA in CSF cells (qualitative measure). Levels of HIV*gag* and *pol*-DNA in PBMCs were positively correlated with levels of NF-L in CSF ($r=0.62$, $P=0.006$ and $r=0.46$, $P=0.058$; Supplementary Fig. 4A, http://links.lww.com/QAD/D231). A negative correlation between neopterin levels in CSF and HIV*pol*-DNA levels in CSF cells was found ($r=-0.59$, $P=0.014$; Supplementary Fig. 4B, http://links.lww.com/QAD/D231). Upon qualitative analysis, there was no apparent association of any biomarker with the presence of detectable HIV*gag* or *pol*-DNA in CSF cells (Fig. 2). A similar qualitative analysis of biomarker concentrations and PBMCs was not performed as 100% of participants had detectable HIV-DNA in PBMCs (Fig. 1a).

Antiretroviral measurements in hair

We next assessed antiretroviral drug levels in hair follicles, which provides a measure of cumulative adherence. The 18 participants were on 11 different ART regimens and we measured levels for at least two drugs for 15 of 18 participants and a single drug for three of 18 participants. One participant (#15) had both tenofovir and lamivudine levels <LLQ and the fourth highest level

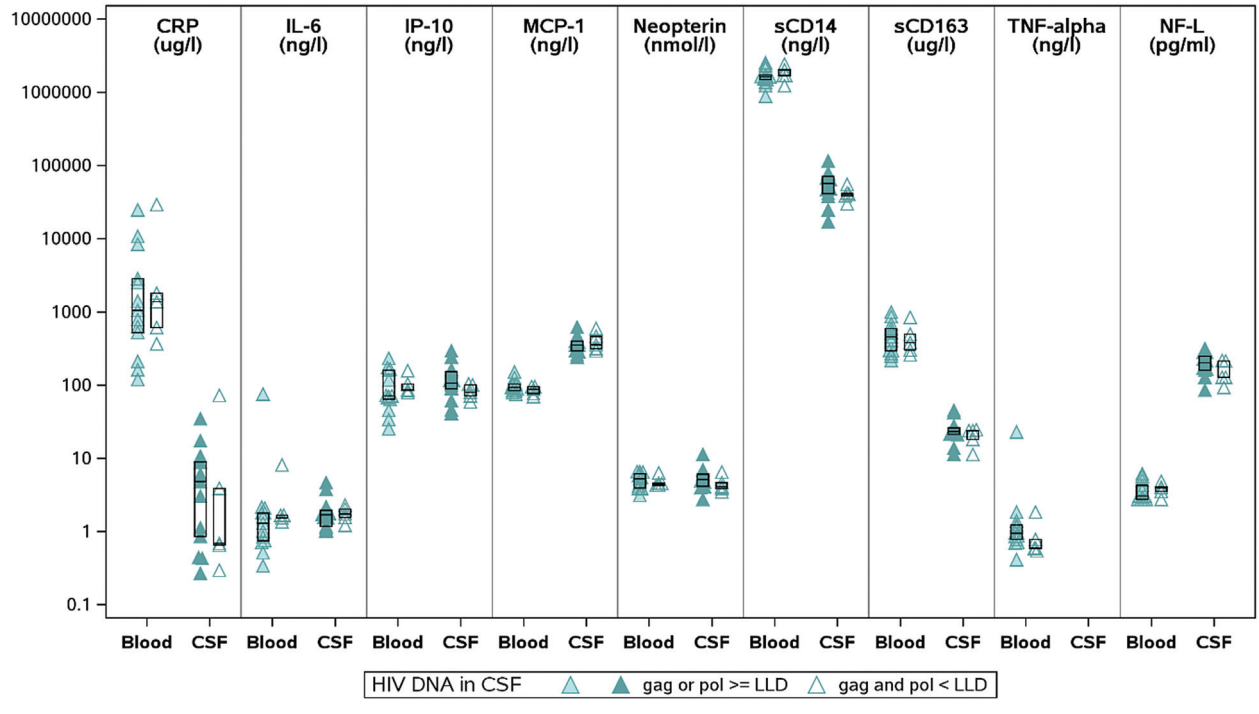

Fig. 2. Biomarkers of inflammation and neuronal injury. Concentrations of biomarkers in plasma and CSF from each participant are shown as separate triangles. Filled or open triangles reflect participants with or without HIV-DNA detected in CSF cells, respectively. Five values of NF-L in plasma <LLQ were plotted at the LLQ of 2.74 pg/ml. Only a single value was >LLQ for TNFα in CSF and only three values were >LLQ for sICAM-5 in plasma so these data are not presented. sICAM-5 was not detected in CSF from any participant.

of level of HIV-DNA in PBMCs; however, this individual also had undetectable HIV-DNA and -RNA in CSF (and HIV-RNA <20 copies/ml in plasma, per study inclusion criteria). Antiretroviral drug concentrations were quantifiable in all other participants, including the two with HIV-RNA detected in CSF. We compared hair levels of dolutegravir, lamivudine and tenofovir in participants with and without detectable HIV-DNA in CSF cells and did not find significant differences (Fig. 3).

Neurocognitive assessments

Participants were included if they had a FCCS at least 1 SD below the normative group mean and scores spanned a relatively restricted range from 53 to 80. Participants with detectable HIV-DNA in CSF cells had median Total Composite score of 68 compared to 72 in those without HIV-DNA detected in CSF, although this association was not statistically significant ($P = 0.45$; Fig. 4). Levels of HIV-DNA (*gag* or *pol*) in PBMCs did not correlate with any of the individual neurocognitive test domain scores (Supplementary Fig. 5A, http://links.lww.com/QAD/D231); however, levels of HIV*pol*-DNA in CSF cells were negatively correlated with scores on the Crystallized Cognition Oral Reading Recognition Subtest ($r = -0.68$, $P = 0.012$; Supplementary Fig. 5B, http://links.lww.com/QAD/D231). With this exception, neurocognitive scores were not consistently associated with HIV in CSF or peripheral blood cells.

Discussion

This study assessed CSF HIV persistence in AYAPHIV and neurocognitive impairment on long-term suppressive ART. Despite a median of 18.3 years on ART, a high proportion of participants (13/18, 72%) had HIV-infected cells in their CSF. This proportion of detectable HIV-DNA in CSF is consistent with the 48–81% reported in prior studies of adults on ART for more than 8 years [18,19], and similar to the previously reported proportion among untreated individuals [50–53]. An association between HIV*pol*-DNA copies in PBMCs and detectable HIV-DNA in CSF cells was found, which may reflect trafficking of infected cells from the periphery into the CSF and/or CNS. Indeed, the two participants with HIV-RNA detected in CSF had the highest HIV*gag* and *pol*-DNA in PBMCs. One notable observation of this study is that the HIV-DNA level in CSF cells was on average one-log higher than in PBMCs, consistent with prior findings from adults with horizontally acquired HIV [18,19]. Differences in the cellular composition of CSF versus peripheral blood and/or selective trafficking of HIV-infected cells into the CSF may lead to enrichment of infected cells in this compartment. It is possible that HIV-DNA in PBMCs could be used as an indirect measure of the CSF HIV reservoir, although larger studies would be needed to better delineate this relationship. These results provide strong evidence that a substantial

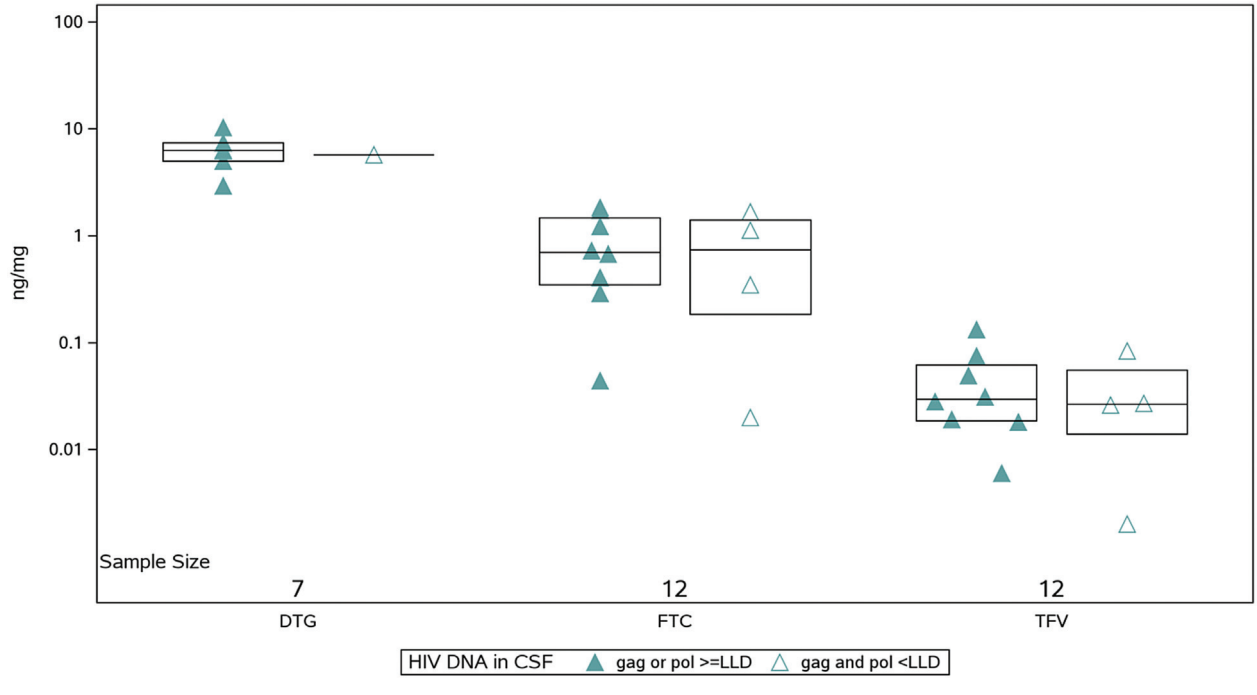

Fig. 3. Antiretroviral drug levels. Concentrations of dolutegravir (DTG), emtricitabine (FTC), and tenofovir (TFV) in hair follicles. Sample size indicates the number of participants taking each drug. Filled or open symbols reflect participants with or without HIV-DNA detected in CSF cells, respectively. For one participant without HIV-DNA detected in CSF cells, both TFV and FTC hair concentrations were below the LLQ and values were plotted at the quantification limit.

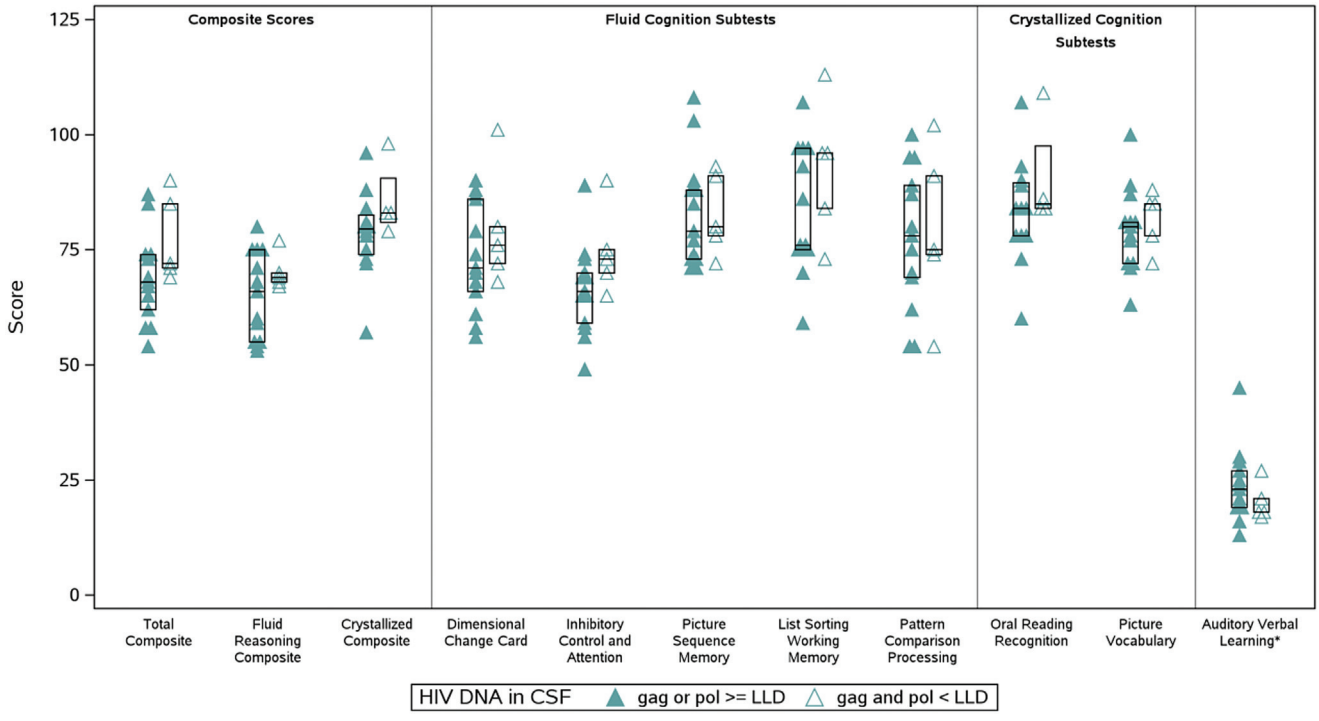

Fig. 4. Neurocognitive assessments. Composite scores are on the left side, individual component scores are on the right side, grouped by fluid and crystallized subsets ($n = 18$). *Auditory verbal learning scores were not normalized. Filled or open symbols reflect participants with or without HIV-DNA detected in CSF cells, respectively. For two participants, high Crystallized Composite and Oral Reading Cognition scores and for one participant a high Auditory Verbal Learning score were considered outliers and excluded per team decision.

proportion of youth with PHIV and neurocognitive impairment harbor cells in the CSF with HIV-DNA, warranting further study to determine whether these CSF cells are infected with replication-competent proviruses.

In a prior study of adults that did not include cognitive impairment as an inclusion criterion, individuals with detectable HIV-DNA in CSF cells had worse neurocognitive performance [18]. A similar pattern was seen here, where AYAPHIV with detectable HIV-DNA in CSF had a median NIH Toolbox Total Composite score 4 points lower than those without HIV-DNA detected in CSF cells. This difference was not statistically significant and may have limited clinical significance, however. A second study in adults on ART for a median of 22 years did not identify an association between HIV in CSF and a prior or current diagnosis of HIV-associated neurocognitive disorder [19]. Neurocognitive impairment was an inclusion requirement for this study thereby reducing our ability to draw rigorous conclusions regarding neurocognitive function and HIV persistence in CSF. It is important to note that the few published studies of older adolescents and young adults with PHIV have demonstrated reduced cognitive functioning and high rates of impairment, especially among those with histories of severe HIV disease [54]. For example, young adults with PHIV aged 20–30 years had higher rates of cognitive impairment (85%) compared with age-matched people with horizontally acquired HIV (38%) and older adults with a similar duration of HIV infection (62%) [55]. YAPHIV displayed worse performance in global cognition, information processing speed, working memory, and verbal fluency compared to the other groups. However, rates of impairment in AYAPHIV and early ART initiation with consistent viral suppression are currently unknown. Further investigation of the impact of HIV-infected cells in CSF on neurocognitive outcomes should be researched in a larger participant group with a broader range of cognitive function.

Cell-free HIV-RNA in CSF was detectable using a standard assay with 20 copies/ml as the LLQ in 11% of participants, although the HIV-RNA concentration was low (25 copies/ml in one participant and <LLQ in the other participant). This percentage is consistent with studies in adults (with and without cognitive impairment) in the recent ART era using the same assay [11,56]. The presence of HIV-RNA reflects persistence of virus-producing cells, and even intermittent production of HIV-RNA has the potential to cause inflammation and contribute to comorbidities. Interestingly, a recent study of HIV-RNA in CSF in the setting of ART interruption suggests that this virus originates from $CD4^+$ T-cells and is associated with transient CSF pleocytosis [21]. Further, adults with HIV and symptomatic CSF escape showed evidence of a CD26+CD4+ T-cell origin of

CSF HIV-RNA [57]. These studies argue against long-lived CNS myeloid-lineage cells as major contributors to HIV-RNA in CSF, although brain microglia have been convincingly demonstrated to harbor replication-competent HIV [2]. Similar investigations have yet to be performed following PHIV infection but are the subject of future work by our group.

A secondary objective of this study was to identify biomarkers associated with CSF HIV persistence. However, none of the 10 potential biomarkers we evaluated were associated with qualitative detection of CSF HIV-DNA. The levels of HIV*gag* and *pol*-DNA in PBMCs positively correlated with CSF NF-L concentrations, known to be elevated in individuals with HIV on ART compared to people without HIV [58], suggesting an association between neuroaxonal injury and increased HIV persistence in circulation. In adults (median age 42 years) on ART, CSF NF-L levels positively correlated with CSF IP-10, and higher CSF IP-10 levels were associated with the presence of HIV-RNA in CSF [59]. Detectable CSF HIV-RNA has also been associated with higher levels of CSF neopterin [11]. We found that CSF neopterin levels negatively correlated with HIV*pol*-DNA levels in CSF cells, with a similar trend for HIV*gag*-DNA levels. Interestingly, CSF neopterin has been proposed to be cytoprotective [60], perhaps suggesting a response to inflammation under ART that leads to a reduced concentration of HIV-infected cells in the CSF. Another study of plasma and CSF biomarkers of immune activation, including neopterin and IP-10, did not identify significant relationships with HIV-DNA or HIV-RNA in CSF of ART-suppressed adults [18], highlighting the need for further investigation to identify and validate predictors of HIV persistence in CSF.

In this work, ART exposure assessed in hair was not associated with HIV-DNA detectability in CSF cells, but we note that hair levels reflect systemic and not CNS exposure. The finding that detectable HIV-DNA in CSF cells was associated with male sex at birth and lower $CD4^+$ cell count at enrollment should be evaluated in larger studies, particularly given previously described sex differences in HIV pathogenesis and persistence. These include higher type I interferon responses and lower viral loads in untreated infection, with increased residual immune activation yet decreased residual viremia and cell-associated HIV-RNA levels during ART in women [61]. How these differences influence CSF HIV-DNA in men and women is not known.

There were limitations to this study. These included the small sample size, which was further reduced due to early accrual closure related to the COVID-19 pandemic. Exclusion of individuals without cognitive impairment limits generalizability to this group. There were also technical limitations, in particular the sensitivity of detection of HIV-DNA in CSF that was dependent on the number of available cells. Still, as the first study of CSF HIV in AYAPHIV on suppressive ART, IMPAACT2015 successfully enrolled a diverse group of participants who were 78% black and 50% female sex at birth. By study design, half of the enrolled participants were targeted to be aged 13–18 years and half to be more than 18 years. IMPAACT2015 showed that performing lumbar punctures as part of a study protocol is feasible in participants as young as 13 years of age and results in relatively few adverse events.

In summary, we demonstrate that there is persistence of cells with HIV-DNA in the CSF of AYAPHIV on long-term suppressive ART, with potential to serve as important reservoirs of HIV. These data support evaluation of the CNS as a reservoir for HIV in treatment and cure studies enrolling children and youth with PHIV.

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Conflicts of interest

There are no conflicts of interest.

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