Cerebrospinal Fluid viral load across the spectrum of untreated HIV-1 infection: a cross-sectional multi-center study

CSF HIV-RNA load across the spectrum of untreated HIV-1 infection: a cross-sectional multi-center study

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Abstract

Background: HIV-1 is a neurotropic virus that enters the central nervous system (CNS) shortly after transmission and persists throughout the course of the disease. By establishing a unique, large, multicenter cohort our aim was to determine variations in cerebrospinal fluid (CSF) HIV-RNA in different phases of untreated HIV infection and its associations with plasma HIV-RNA and other biomarkers.

Methods: Retrospective data from 1,018 patients at eight different medical centers were collected. All participants were treatment naïve and had undergone CSF examination for clinical indications or as part of research studies. Participants were divided into pre-defined groups according to significant phases of HIV disease: primary infection (PHI, n=136); four groups of neuroasymptomatic patients with chronic HIV infection defined by $CD4^+$ T-cell count thresholds (n=545); HIV-associated dementia (HAD, n=144); opportunistic CNS infections (CNS OI, n=174) and a small group of elite controllers (n=19). Measurements of plasma HIV-RNA, CSF white blood cell count (CSF WBC), serum and CSF neopterin, and CSF/serum albumin ratio (CSAR) were included when available.

Results: CSF HIV-RNA load was characteristically lower than plasma HIV-RNA, with a median (IQR) difference of 1.03 $\log_{10}$ (0.37–1.86) copies/mL and it correlated with plasma HIV-RNA (r=0.44, $p<0.01$), neopterin concentration in CSF (r=0.49, $p<0.01$) and in serum (r=0.29, $p<0.01$), CSF WBC (r=0.34, $p<0.01$) and CSAR (r=0.25, $p<0.01$). CSF HIV-RNA paralleled plasma HIV-RNA in all groups except neuroasymptomatic patients with advanced immunodeficiency (CD4 <200) and patients with HAD or CNS OIs.

Patients with HAD had the highest CSF HIV-RNA (median [IQR] 4.73 (3.84–5.35) $\log_{10}$ copies/mL), while none of the elite controllers had detectable CSF HIV-RNA. CSF viral load discordance (CSF>plasma HIV-RNA) was found in 126 of 972 individuals (13%), with a wide variation between groups, namely 1% of patients with PHI, 11% of those in the neuroasymptomatic groups, and 30% of patients with HAD.

Conclusions: Our study confirms substantial variations in CSF HIV-RNA between different stages of HIV disease, as previously found in smaller studies. Overall, CSF HIV-RNA was approximately 1 $\log_{10}$ copies/mL lower in CSF than in plasma, but 11% of neuroasymptomatic subjects and 30% of patients with HAD had higher HIV-RNA in CSF than in plasma.

Background

It was previously observed that asymptomatic, untreated HIV-infected individuals with advanced immune dysfunction usually have relatively low cerebrospinal fluid (CSF) HIV RNA, in contrast to plasma HIV RNA, which normally increases as HIV disease progresses [1-6]. In a large multi-center study, we aimed to a) investigate whether these observations could be confirmed, b) compare the CSF HIV RNA in various stages of the course of infection and during opportunistic complications of HIV disease, and c) hypothesize possible explanations.

HIV invades the central nervous system (CNS) shortly after acquisition of infection. HIV RNA can be detected in CSF almost simultaneously as in plasma during primary infection, and it remains detectable in the CSF throughout the untreated course of the disease [1-3]. The dynamics of CSF HIV RNA during the chronic asymptomatic phase of infection are generally parallel to plasma HIV RNA, characteristically increasing over time although less so than plasma. In patients with severe immunodeficiency but no neurological complications, surprisingly low levels of CSF HIV RNA have been reported [1, 4, 7].

In a subset consisting of approximately 20% to 30% of all patients with untreated infection but having HIV-associated dementia (HAD), formerly termed AIDS dementia complex (ADC), or diagnosed with other processes affecting the CNS such as opportunistic infections and malignancies, CSF viral load often exceeds that in plasma [1, 8, 9].

By establishing a uniquely large multicenter cohort we were able to study variations in CSF HIV RNA at different phases of untreated HIV infection and observe how these variations correspond to plasma HIV RNA. In addition, we examined the relationship between CSF HIV RNA and markers of CSF inflammation and blood brain barrier integrity, which may assist our understanding of the biological processes involved.

Methods

Study design and participants

By collecting retrospective data from eight different centers in the cities of Gothenburg, Rome, Sydney, Turin, Milan, Bucharest, San Francisco and London between 1982 and 2017, we established a cohort of 1,018 participants. All were treatment naïve and had undergone CSF examination for CSF HIV-1 RNA quantification, either due to CNS symptoms or in the course of clinical studies. Other analyses, described in detail below, were included if available. The participants were divided into pre-defined groups similar to those used in previous studies [10, 11], each group representing a significant phase of HIV disease: primary infection (PHI, defined as within the first twelve months of an initial HIV-1 infection [12, 13]); five groups of neuroasymptomatic (NA) subjects defined by $CD4^+$ T-cell count thresholds (> 500 cells/μL; 350–499 cells/μL, 200–349 cells/μL, 50–199 cells/μL and < 50 cells/μL); HAD; opportunistic CNS infections and malignancies (CNS OI); and ´elite´ controllers (HIV positive ≥ 1 year, with ≥ 2 HIV RNA < 50 copies/mL despite no antiretroviral therapy [ART] [14]). The CNS OI group included patients with cytomegalovirus (CMV) encephalitis, Epstein-Barr virus-associated primary CNS lymphoma (PCNSL), cryptococcal meningitis, tuberculous meningitis, progressive multifocal leukoencephalopathy (PML), and CNS toxoplasmosis.

The diagnoses of ADC/HAD and each of the opportunistic neurological CNS complications were based on the Centers for Disease Control and Prevention (CDC) and American Academy of Neurology AIDS Task force criteria, using standard clinical and laboratory evaluations [15-17].

Our study was approved by Institutional Review Boards at each study site and informed consent was obtained from all participants.

CSF and blood measurements

CSF and blood samples were collected and analyzed according to current clinical routine practice.

Blood CD4+ T-cell counts, serum and CSF albumin levels, and CSF white blood cell counts (WBC) were performed using routine methods. Pleocytosis was defined as CSF WBC ≥ 5 cells/µL. Albumin ratios were calculated as CSF albumin (mg/L)/plasma albumin (g/L) and used to evaluate BBB function. Reference values were < 6.8 for individuals age 45, and < 10.2 for those ≥ 45 years old [18].

HIV RNA levels were quantified by real-time polymerase chain reaction (PCR) using the assay available at the respective site and time.

Neopterin was analyzed in CSF and serum using a commercially available immunoassay (NEOPT-SCR.EIA 384 Det., Thermo Fisher Scientific – BRAHMS GmbH, Henningsdorf, Germany) with an upper normal reference value of 8.8 nmol/L in plasma and 5.8 nmol/L in CSF [6].

Samples collected prior to using HIV RNA PCR in clinical routines were retrospectively analyzed in stored aliquots of CSF and blood (frozen at –70ºC after centrifugation) as part of clinical studies or as a result of clinical procedures independent of this study.

Statistical methods

Descriptive statistics were performed using SPSS (IBM SPSS version 24 software, Armonk, NY, USA) or Prism 9.0 (GraphPad Software, San Diego, CA, USA). Continuous variables

are reported as median (interquartile range) and were $\log_{10}$ transformed where appropriate for the tests used. Comparisons between groups were done using either Mann-Whitney test or one-way ANOVA with Dunnett's T3 post hoc test. Correlations were explored using Spearman's rank correlation.

Results

Background clinical and laboratory characteristics for each subject group are summarized in Table 1.

Study population

The cohort of 1,018 participants (76% male) had a median age of 38 (32–47) years. The lowest age was seen in the PHI group: 34 years (28–43), the highest was found among the elite controllers': 51 years (38–59).

Table 2 shows the number of participants in each group and the center from which they were recruited.

CSF and blood measurements

CSF HIV RNA, plasma HIV RNA, and plasma:CSF HIV RNA ratio ($\log_{10}$ plasma HIV RNA – $\log_{10}$ CSF HIV RNA) in the various groups are shown in Figure 1. Scatter plots showing the relationship between CSF and plasma HIV RNA in the groups appear in Figure 2. Group level comparisons of HIV RNA, CSF WBC, neopterin, and albumin ratios using Dunnett's T3 post hoc test following ordinary one-way ANOVA are listed in Table 3. Supplemental

Figure 1 shows the level of neopterin in CSF and serum, CSF WBC counts, and albumin ratios in each group.

HIV RNA

CSF HIV RNA was characteristically lower than plasma HIV RNA, with a difference of 1.03 (0.37–1.86) $\log_{10}$ copies/mL. CSF HIV RNA discordance, defined as ≥ CSF than plasma HIV RNA in untreated individuals[19], i.e, a plasma:CSF ratio ≤ 0 was found in 126 of 972 patients (13%) with a wide variation between the various groups. Table 4 shows the proportion of patients with CSF HIV RNA > plasma HIV RNA by groups.

CSF HIV RNA was significantly higher in patients with HAD compared to all other subgroups (4.73 $\log_{10}$ copies/mL [3.84–5.31]). The same group demonstrated the lowest plasma:CSF ratio: 0.54 (0.18–1.34); 30% (n = 38) exhibited CSF discordance, having higher HIV RNA in CSF than in plasma. Neuroasymptomatic patients with CD4 < 50 demonstrated the highest plasma HIV RNA (5.35 $\log_{10}$ copies/mL [4.91–5.76]), and the largest plasma:CSF ratio (2.13 [1.40–2.76]). The PHI group also showed a high plasma:CSF ratio: 1.77 (1.22–2.30), with only 1% (n = 2) having CSF discordance. These comparisons support the visual impression that viral load increases in parallel in both compartments in the chronic neuroasymptomatic stage (Fig. 1), but that CSF HIV RNA decreases in the most immunocompromised stage (CD4 < 50), except in the case of neurosymptomatic patients (HAD and some CNS OI), where despite low CD4 cell counts, CSF HIV RNA often exceeds that in plasma.

The heterogenous group with CNS OI had an overall median plasma:CSF ratio of 0.81 (0.26–1.81), with 17% of cases (n = 26) of CSF discordance. Viral loads in both compartments did

not vary significantly across the different CNS OIs, but for patients with PML who had significantly lower CSF HIV RNA levels than those with cryptococcal meningitis ($p < 0.01$) and CMV encephalitis ($p < 0.05$) (Fig. 3).

Background biomarkers

Pleocytosis was a common finding in all groups (total 42%, n =404), except for NA patients with CD4 < 50 and elite controllers (Table 1 and Suppl. Fig. 1).

The vast majority of subjects had elevated neopterin levels in both compartments: 92% in CSF and 87% in plasma (Table 1 and Suppl. Fig. 1 C–D). The highest values of CSF neopterin were found in HAD: 63.5 (33.0–120) nmol/L, whereas the highest level of serum neopterin was found among patients with CNS OI: 32.6 (16.5–212.1) nmol/L. Approximately half of elite controllers had normal neopterin levels in both compartments. CSF neopterin was significantly ($p < 0.001$) higher in subjects with CSF discordance versus those with non-discordance: 33.4 (23.0–83.1) and 17.8 (9.6–32.4), respectively (Suppl. Fig 4).

The highest CSF/plasma albumin ratio was observed in the HAD group: 9.80 (5.69–13.25). This was approximately twice as high as all other groups, with the exception of CNS OI: 7.50 (5.15–9.45). Sixty-three percent of patients with HAD had an elevated albumin ratio that was approximately five times higher than NA patients. Forty-eight percent of those in the CNS OI group had elevated albumin ratios, while albumin ratios were normal in all elite controllers (Table 1 and Suppl. Fig. 1).

Correlations across groups

Correlations between HIV RNA, CSF WBC, neopterin, and albumin ratios are shown in a heat map (Suppl. fig. 2). Overall, CSF HIV RNA was most closely associated with CSF neopterin (r = 0.49, $p < 0.01$) and plasma HIV RNA (r = 0.44, $p < 0.01$). We also explored correlations among the same biomarkers after dividing the patients into four groups: PHI, NA, HAD and CNS OI (Suppl. Fig. 3 A–D). In the NA group, CSF neopterin (r = 0.43, $p < 0.01$), CSF WBC (r = 0.42, $p < 0,01$), and plasma HIV RNA (r = 0.35, $p < 0,01$) were associated with CSF HIV RNA, while the plasma:CSF ratios were negatively associated with CSF WBC counts (r = 0.61, $p < 0.01$). The HAD group showed a somewhat different pattern, with no significant correlation between CSF HIV RNA and plasma HIV RNA (r = 0.14, $p = 0.11$). Still, a weak but significant association between CSF HIV RNA,CSF neopterin (r = 0.25, $p < 0.05$), and CSF WBC (r = 0.21, $p < 0.05$) was found. In the CNS OI group, CSF HIV RNA was associated with the albumin ratio only (r = 0.47, $p < 0.01$).

Discussion

On the basis of our study on the relationship between plasma and CSF HIV RNA, we were able to confirm parallel dynamics between these two compartments during the untreated infection. One exception however, was the low level of CSF HIV RNA in NA HIV-infected patients who had advanced immunodeficiency despite high plasma HIV RNA. This low CSF HIV RNA was accompanied by a low CSF WBC count. Moderate CSF pleocytosis is a common finding in early stages of HIV infection [1, 2], implying that CSF HIV RNA is closely associated with meningeal inflammation in NA HIV. Nevertheless, CSF neopterin concentrations were also elevated in patients whose CD4 cell count was < 50 x 10^6/mL, probably reflecting extensive macrophage and microglial activation[6]. CSF neopterin has previously been described as elevated in all phases of infection, and said to increase

progressively as CD4 cell counts drop, with the highest CSF neopterin levels found in HAD and CNS OI [4, 6, 20]. An association between CSF neopterin and the light unit of neurofilament protein (NfL) in CSF, signaling neuronal injury in untreated NA HIV-infected patients, has previously been shown. It supports the theory of progressive immune activation with increased risk of subsequent neuronal injury as immunosuppression worsens [21]. Although there is usually a significant rise in CSF HIV RNA in the case of HAD, because high levels of CSF RNA are also commonly found in NA HIV-infected patients, it makes CSF RNA of limited use as a single predictor of HIV-related CNS disease[22].

A possible explanation for the shifting of plasma:CSF viral load ratios at various CD4 cell strata during the chronic NA phase of HIV infection may be that CSF HIV RNA is mainly a reflection of a continuous re-seeding of immune cells, predominantly CD4+ T lymphocytes. These cells traffic into the CSF from the blood stream while a patient still has a somewhat restored immune function. However, in the later stages of disease, and in severely impaired T-cell deficiency, this trafficking might be severely reduced if the patient is not suffering from an opportunistic infection.

By contrast, severely immunodeficient patients with HAD have high CSF HIV RNA, but often no CSF pleocytosis. Under those circumstances CSF HIV RNA is most likely dominated by virions produced by cells in the CNS compartment itself[1, 23, 24], while in asymptomatic patient's peripheral virus has a greater influence on the CSF viral load. Thus, the infection is probably compartmentalized to varying degrees in the CNS throughout the course of the disease[25], a theory further supported by the lack of association between CSF HIV RNA and plasma HIV RNA, CSF WBC, and albumin ratio in the HAD group. On the other hand, the degree of HIV CNS compartmentalization is low during PHI [26], and so we found CSF discordance to be a rare phenomenon (1%) in patients with PHI.

The third exception to parallel CSF/plasma viral replication were patients with CMV encephalitis, tuberculous, or cryptococcal meningitis where CSF viral loads often exceeded plasma levels. In these patient groups CSF HIV RNA was also associated with CSF pleocytosis, a finding previously observed in smaller studies [27, 28] and in other CNS co-infections such as neuroborreliosis and herpes zoster [19, 29]. By contrast, PML, an opportunistic CNS infection linked to minor CNS inflammation, was not associated with an increased CSF viral burden.

A fourth exception to parallel CSF/plasma HIV RNA development during untreated HIV infection is the finding of higher CSF than plasma HIV RNA in approximately 10% of all NA patients in our study, a condition which has been designated discordant CSF/plasma HIV RNA. CSF discordance was lower in our investigation in comparison to what has been found in previous, smaller studies [4, 30]. Perhaps this is because we included more NA patients in our cohort whereas earlier research has mainly focused on patients presented with neurological or cognitive symptoms. CSF discordance might be accounted for by greater activity of the infection in the CNS compartment than in the peripheral system in the case of NA disease, a theory supported by the significantly higher CSF neopterin levels found in that subgroup. Whether this is mainly attributable to variation in HIV entry phenotypes and cell tropism, meningeal inflammation, astrocyte involvement, or host factors is still unclear [3, 31, 32].

The present study included HIV-infected patients who had not received ART. The widespread availability of effective ART has resulted in a dramatic effect on reducing the risk of HAD and CNS OI. After initiation of ART CSF HIV RNA usually decays as rapidly as plasma HIV RNA, and CSF pleocytosis then disappear [33, 34].

The limitations of our study include the large number of study sites involved, the retrospective design, the extensive period of time for patient assessment and sampling, and the different PCR methods used at various locations and times. A major strength is the large number of patients examined by means of lumbar puncture, allowing us to stratify large, pre-defined immunodeficiency CD4 groups.

In clinical situations, such as unclear neurological symptoms in HIV-infected patients, it is important to understand how HIV affects the CNS and the HIV RNA and WBC counts that are normally found in various phases of the disease so that practitioners can evaluate laboratory CSF findings in relation to HIV CNS symptoms and disease. Data from this large study may be helpful in that respect.

We believe our study confirms previous research that indicates HIV RNA is detectable in CSF at all stages of HIV infection. However, we found that CSF HIV RNA does not parallel plasma HIV RNA in NA patients with advanced immunodeficiency, and in patients with HAD. Instead, CSF discordance with higher HIV RNA in CSF than in plasma, could be found in approximately 10% of all NA patients without ART and in 30% of those with HAD.

Legends to figures

Figure 1. HIV RNA concentration in CSF and plasma in subject groups

Boxes depict interquartile ranges with median (line) and mean ('+'), while whiskers represent 10^{th} to 90^{th} percentiles. The HIV RNA concentrations are displayed as $\log_{10}$ copies/mL whereas the dotted line in panels A and B indicates 50 (1.70 $\log_{10}$) copies/mL. The findings and statistical comparisons between the groups are described in the text and Tables 1,3, and 4. Panel C visualizes the difference in viral load between plasma and CSF ($\log_{10}$ plasma HIV RNA – $\log_{10}$ CSF HIV RNA); the grid line represents the difference of zero $\log_{10}$ copies/mL. The number of subjects (n) in each group is showed in brackets following the group name.

Abbreviations: PHI, primary HIV infection; NA, HIV+ neuroasymptomatic patients; CD4, $CD4^+$ T lymphocyte count; HAD, HIV dementia complex; CNS OI, CNS opportunistic infections; Elites, elite controllers; n, number of subjects

Figure 2. Relationship between CSF HIV RNA and plasma HIV RNA among groups

Figures A–H show the relationship between plasma- and CSF HIV RNA in eight pre-defined groups. Red circles represent patients with plasma HIV RNA > CSF HIV RNA; blue circles indicate patients with CSF HIV RNA > plasma HIV RNA.

Thick black lines represent the median HIV-1 RNA levels in CSF and plasma. The dotted line is an aid to visualize the median difference in each of the two body compartments.

Abbreviations: PHI, primary HIV infection; NA, HIV+ neuroasymptomatic patients; CD4, CD4+ T lymphocyte count; HAD, HIV dementia complex; CNS OI, CNS opportunistic infections; Elites, elite controllers; VL viral load; CSF, cerebrospinal fluid; P, plasma

Figure 3. Comparison of viral load among various CNS opportunistic infections

Boxes depict interquartile ranges with median (line) and mean ('+'), while whiskers represent 10^{th} to 90^{th} percentiles. Dotted lines represent upper normal reference values and the grid line (Panel C) represents the HIV RNA difference of zero $\log_{10}$ copies/mL. Horizontal brackets show *p* values for significant group differences by Dunnett's T3 post hoc test after ordinary one-way ANOVA test: * = < 0.05, ** = < 0.01.

Abbreviations: CMV, cytomegalovirus; CNS, central nervous system; PML, progressive multifocal leukoencephalopathy

Supplemental Figure 1. Concentrations of CSF WBC, albumin ratios and neopterin in subject groups

Boxes depict interquartile ranges with median (line) and mean ('+'), while whiskers represent 10^{th} to 90^{th} percentiles. Dotted lines represent upper normal reference values in panels A, C and D. No age adjustment was made according to albumin ratio. The dotted lines in panel B represent the upper normal reference values for individuals < 45 years (red) and those ≥ 45 years of age (blue).

The findings and statistical comparisons between the group are described in the text and Tables 1 and 3. The number of subjects (n) in each group is showed in brackets following the group name.

Abbreviations: PHI, primary HIV infection; NA, HIV+ neuroasymptomatics; CD4, $CD4^+$ T lymphocyte count; HAD, HIV dementia complex; CNS OI, CNS opportunistic infections; Elites, elite controllers; n, number of subjects; WBC, white blood cell count

Supplemental Figure 2. Heat map showing correlations between variables

Numbers in boxes denote Spearman's rank correlation coefficient.

Supplemental Figure 3. Heat map showing correlations between variables among four pre-defined groups

Numbers in boxes denote Spearman's rank correlation coefficient.

Supplemental Figure 4. CSF neopterin level and CSF discordance

CSF neopterin level in subjects with CSF HIV RNA > plasma HIV RNA, and plasma HIV RNA > CSF HIV RNA. Boxes depict interquartile ranges with median (line) and mean ('+'), while whiskers represent 10^{th} to 90^{th} percentiles. Dotted line represents the upper normal reference value of 5.8 nmol/m

Table 1. Subject characteristics, including subgroups

* Percentage of subjects with biomarker concentrations above normal upper reference value

Abbreviations: ND, no data; N/A, not applicable

Table 2: Study population centers in cohort

GOT, Gothenburg; ROM, Rome; SF, San Francisco; MIL, Milan; BUCH, Bucharest; TOR, Turin; LON, London; SYD, Sydney.

Table 3. HIV RNA and background biomarker concentrations among subject groups

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