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Cerebrospinal fluid pleocytosis as a predictive factor for CSF and plasma HIV RNA discordance and escape

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Abstract

Background: The aims of this study were: investigate the frequency of HIV-1 RNA levels discordance between the cerebrospinal fluid (CSF) and plasma and of CSF viral escape (CVE) in patients HIV-1 subtype C on antiretroviral therapy; evaluate the CSF white blood cell (WBC) performance characteristics in predicting CSF discordance in HIV+ group; and the frequency of cognitive impairment in individuals with CSF HIV discordance or escape.

Methods: HIV-1 RNA levels were assessed in plasma and CSF samples from 68 HIV+ participants without opportunistic infection.

Results: CSF discordance was found in 7.4% and CVE in 10%, with comparable frequencies between HIV-1B and C. Twenty samples (29%) showed increased CSF WBC counts. This group had higher CSF and plasma HIV-1 RNA levels than the group with normal WBC counts ($p < 0.0001$ and 0.006, respectively). The odds of CSF discordance were 18 times higher for a person with CSF WBC count of >5cells/mm^3 than the group with normal CSF WBC count. CSF WBC counts (cut-off of 15 cells/mm^3) showed high-performance characteristics as a predictive biomarker of CSF discordance (AUC the ROC curve 0.98). The frequency of cognitive impairment for CSF escape or discordance, was 83%, and 80%. The odds of cognitive impairment in these groups were 19 and 15 times higher, than those for a HIV(−) person.

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Conflicts of Interest

The authors state that there are no conflicts of interest regarding the publication of this article.

Conclusion: Viral discordance or escape in the CNS occurs at a comparable frequency for HIV-1C and HIV-1B. The CSF WBC count was effective as a predictive biomarker of CSF and plasma discordance.

Keywords

HIV-1; cerebrospinal fluid (CSF); central nervous system (CNS); white blood cell; scape; discordance

CSF inflammation begins shortly after HIV infection and persists throughout the early stages of immunosuppression (Hollander et al. 1994). The CSF white blood cell (WBC) count is a sensible biomarker of intrathecal inflammation (Hollander et al. 1994; Peluso et al. 2012; Manesh et al. 2019; Monteiro de Almeida et al. 2006). The CSF HIV RNA level was found to be significantly associated with the CSF WBC count (Buffet et al. 1991), supporting the hypothesis that a substantial part of the virus in the CSF of people with HIV (PWH) is locally produced by mononuclear cells (Martin et al. 1998) or that CSF WBC traffic into CSF when CSF HIV RNA is elevated.

Previous studies have reported a subset of individuals (~5% to 21%) presenting with CSF viral escape (CVE), regardless of sustained HIV suppression in the blood during antiretroviral therapy (ART) (Clifford 2010; Eden et al. 2010; Lescure et al. 2013). Despite effective suppression of viremia with ART, HIV can still replicate in the CNS, with the development of quasispecies in patients with acute or subacute neurological manifestations (Haggert and Stevenson 1991; Zarate et al. 2007; Harrington et al. 2009). CSF pleocytosis was presumed to reflect virological control failure in the CNS (Eden et al. 2010; Cusini et al. 2013; Ferretti et al. 2015; Pérez-Valero et al. 2019).

The majority of research studies on CSF and plasma HIV-1 RNA discordance (hereinafter CSF discordance) and CVE have been carried out in settings where HIV-1B predominates (Peluso et al. 2012; Canestri et al. 2010; van Lelyveld et al. 2010; Katlama et al. 2010; Bogoch et al. 2011; Bingham et al. 2011; Del Palacio et al.2012), and little is known about the CSF discordance and CVE of the non-B HIV subtypes. HIV-1 subtype C has been proposed to be less neuropathogenic than subtype B (Satishchandra et al. 2000), on the basis of an *in vitro* defective transactivator of transcription (Tat) chemokine dimotif in the position (C30C31) that might influence cellular trafficking and CNS inflammation (Ranga et al. 2004). The frequency of CVE in PWH infected with subtype C is largely unknown. Therefore, this cross-sectional study was performed to investigate the impact of the defective Tat chemokine dimotif on the frequency of CSF discordance and CVE among PWH infected with HIV-1C and HIV-1B.

The aims of this study were to investigate the frequency of CSF discordance and CVE in participants chronically infected with HIV-1C and who are on ART. In addition, we evaluated the diagnostic characteristics of the CSF WBC count to ascertain its performance in predicting CSF discordance in PWH without opportunistic CNS infections. We also investigated the frequency of cognitive impairment in participants with CSF discordance or CVE.

RESEARCH DESIGN AND METHOD

Samples

The HIV-positive (HIV+) participants were recruited at Hospital de Clínicas, Universidade Federal do Paraná, HC-UFPR, Brazil. This study was approved by the UCSD Institutional Review Board (IRB), the HC-UFPR IRB, and the Brazil National IRB (CONEP). Individuals with opportunistic CNS infections were not included in this study. All volunteers provided paired blood and CSF samples, and underwent serological testing to confirm the HIV status before enrollment (BRASIL, 2018).

A total of 68 paired CSF and blood samples from the HIV+ participants were tested. The CSF samples were collected by lumbar puncture (LP), which was performed using an atraumatic spinal needle and aseptic technique. For participants with a clinically resistant infection, the infecting HIV subtype was genotyped with *pol* sequences, whereas *env* sequences were used for all other participants. Genotyping revealed that 27 individuals were infected with HIV-1B and 40 with non-B HIV-1 subtypes (C, n = 26; BF, 10; BC, 1; CF, 1; and F, 2). The HIV-1 subtype could not be genotyped in one participant.

The HIV-negative (HIV–) participants (n = 48) were recruited from the HC-UFPR blood bank, described previously (de Almeida et al. 2013).

Methods

The total CSF WBC count (cells/mm^3) and cell-type differentials were quantified using fresh non-centrifuged CSF samples immediately after the LP. CSF pleocytosis was defined as a WBC count of >5 cells/mm^3.

Quantification of plasma and CSF HIV RNA levels

The VERSANT® HIV-1 RNA 3.0 bDNA Kit (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) was used for CSF or plasma samples. It was used 1 mL of CSF or plasma. The assays were performed immediately after sample collection. Samples with an HIV RNA level of <50 copies/mL were considered under the detection limit.

The definitions of the following terms are based on the quantification of CSF HIV RNA in paired CSF and plasma samples. (1) Discordance between the CSF and plasma HIV RNA levels was defined as follows: (a) CSF HIV RNA level greater than 1 Log_{10} of the plasma viral load (Canestri et al. 2010). (b) Additionally, a separate group of CSF samples with HIV RNA levels higher than the plasma levels (regardless of the magnitude of the difference) was studied. (2) CVE was defined as any HIV RNA in the CSF above the limit of detection of the assay used, despite undetectable plasma levels by the same assay. It reflects the loss of control of brain HIV infection in a patient on effective ART (Peluso et al. 2012). In three participants for whom serial CSF and plasma samples were collected, CVE was classified into the following subtypes, on the basis of longitudinal analysis of the CSF and plasma HIV-1 RNA levels: (1) CSF blip, a single occurrence of CVE while suppressed in the plasma; (2) CSF slow suppression, a CVE with preceding lack of suppression in the plasma;

and (3) persistent CVE, ≥2 consecutive CVEs while suppressed in the plasma (Joseph et al. 2016; Mukerji et al. 2017).

Neuropsychological assessments

All participants underwent a neuropsychological assessment, which covered seven ability domains with 18 individual tests. Global deficit scores (GDS), a measure of cognitive impairment, was calculated. Neuropsychological assessments was described in detail previously (de Almeida et al. 2013).

Data analyses

The results are presented as the median and interquartile range (IQR) or the percentage (%), as appropriate. Comparisons between groups were made using the chi-square test, Fisher's exact test, or Mann-Whitney nonparametric test (unadjusted analysis), where appropriate. Comparisons between HIV RNA (Log_{10}) in matched CSF and plasma samples was done with Wilcoxon signed rank test. Differences were considered statistically significant at the 5% alpha level. The 95% confidence intervals (95% CIs) for impaired proportions were calculated using the normal distribution with no continuity correction. For the correlation analysis, the correlation coefficients (r_s) were estimated using Spearman's rank-order method.

The performance characteristics of the increased CSF WBC count (index test) in predicting CSF discordance were evaluated. The CSF and plasma HIV RNA levels were used as the reference. The following performance characteristics of the CSF WBC count were calculated: sensitivity; specificity; accuracy (efficiency); positive and negative predictive values (PPV, NPV); Youden index (Galen and Gambino, 1975); positive and negative clinical utility index (CUI+, CUI−). The CUI values were classified as follows: excellent, ≥0.81; good, ≥0.64; fair, ≥0.49; poor, ≤0.49; and very poor, ≤0.36 (Mitchell 2008; Mitchell 2011). The positive and negative likelihood ratio (LR+, LR−) were calculated, where an LR + value of ≥10.0 indicates that a positive test almost confirmed the disease, a value of ~6.0 indicates that the disease was present, and a value of ~1.0 indicates that the test was not able to show whether the disease was present or not. An LR+ value of ≤0.1 indicates that the disease was practically absent (McGee 2002; Akobeng 2007A). The receiver operating characteristic (ROC) curve was used to evaluate the ability of the CSF WBC count to accurately classify impaired and normal participants and to establish the best cut-off of the CSF WBC count increase (Akobeng 2007B).

RESULTS

The demographic, clinical, and laboratory characteristics of the groups studied (viz., HIV-1 as a whole and categorized by subtypes B or C, and CSF WBC cell counts) are presented in Table 1; the groups with CSF discordance or CVE and virological suppression in the CSF and plasma (aviremia) are summarized in Table 2.

The individuals infected with either HIV-1B or HIV-1C were similar in age, gender, years of education. Participants with subtype B were significantly more likely to have undetectable plasma HIV RNA, participants with HIV-1C had lower CSF glucose levels than those with

subtype B, although CSF glucose levels in both groups were between reference range. The relationship of the CSF HIV RNA level in paired CSF and plasma samples (escape, discordance, and CSF and plasma aviremia) are summarized in Table 2. Participants with discordance had significantly higher CSF WBC and higher protein, and albumin quotient than those who were undetectable in both compartments. In the HIV+ group, combination antiretroviral therapy (CART), mostly protease inhibitors, was prescribed to 55 participants (81%). The median (IQR) CNS penetration effectiveness rank of the antiretrovirals was 8 (6; 9), and adherence (AIDS Clinical Trials Group, adherence questionnaire) was maintained by 51 (93%) participants. In the HIV+ group as a whole, there were positive correlations between the CSF WBC count and the CSF HIV RNA level (Log_{10}) [r_s = 0.614 (95%CI: 0.434–0.747), $p < 0.0001$] and the plasma HIV RNA level (Log_{10}) [r_s = 0.386 (95%CI: 0.155–0.577), p = 0.001]. For the HIV-1B and HIV-1C groups, there was correlation between the CSF WBC count and CSF HIV RNA level (Log_{10}) [r_s = 0.586 (95%CI: 0.254–0.795), p = 0.001; and r_s = 0 656 (95%CI: 0.350–0.836), p = 0.0003, respectively], but not between the CSF WBC count and the plasma HIV RNA level (Log_{10}).

Twenty samples (29%) showed increased CSF WBC counts (>5 cells/mm^3), with predominantly lymphocytes (85–100%), the median (IQR) WBC count was 13 (9.5; 31.5) cells/mm^3 (Table 1). Participants with CSF WBC counts of >5 cells/mm^3 had higher HIV RNA levels both in the CSF and plasma than the group with normal WBC counts ($p < 0.0001$ and 0.006, respectively). Participants with pleocytosis had significantly higher quotient of albumin and were less likely to have undetectable plasma and CSF HIV RNA than those with normal CSF WBC (Table 1; Figure 1A). Four participants (20%) have CSF pleocytosis with undetectable CSF HIV viral load, median (minimum; maximum) of CSF WBC in this group 14.65 (6.3; 27) cells/mm^3. Whereas 31 (64.5%) participants in the group without CSF pleocytosis have undetectable CSF HIV viral load (p=0.0012) (Table 1; Figure 1A).

Group with CSF and plasma HIV RNA discordance

CSF discordance (Canestri et al. 2010) in the HIV+ group as a whole was found in 5 (7.35%) individuals (one of these participants was not on CART). The frequency of CSF discordance was comparable between the HIV-1 subtypes, with 2 (7.4%) individuals infected by HIV-1B and 3 (11%) infected by HIV-1C (p = 0.67) (Table 1). In the group with CSF WBC counts of >5 cells/mm^3, discordance was found in 5 (25%) individuals, whereas in the group with CSF WBC counts of ≤5 cells/mm^3, discordance was not found (Table 1). The odds of CSF HIV viral load discordance were 18.36 times higher for a person with a CSF WBC count of >5 cells/mm^3 than for a person with a CSF WBC count within the normal range (≤5 cells/mm^3) [odds ratio (OR) = 18.36 (95% CI: 2.05–164.34), p = 0.009]. Two of the participants with CSF discordance and increased CSF WBC counts (20 and 382 cells/mm^3) were further studied by next-generation sequencing, with results showing HIV genetic compartmentalization in the CNS, both cases were HIV-1C, described previously (de Almeida et al. 2017; 2018A).

We also analyzed CSF discordance in the individuals with CSF viral loads higher than those of the plasma, independent of the HIV RNA values in both compartments. In the HIV group

as a whole, there were 12 (17.65%) such individuals, with the number being comparable between those with HIV-1B and those with HIV-1C (n = 5, 18.52% and n = 5, 19.23%, respectively; p = 1.0). The CSF WBC count in the group with higher HIV RNA levels in CSF than in the plasma was 15.5 (3; 38.5), whereas that in the group with lower HIV RNA levels in CSF than in the plasma was 1.7 (0.6; 3.8) (p = 0.003). The Log_{10} CSF HIV RNA level was 3.0 (2.3; 4.1) in the group with higher HIV RNA in the CSF than in the plasma, whereas it was 1.7 (1.7; 2.3) in the group with lower HIV RNA in the CSF than in the plasma (p < 0.0001). The median (IQR) plasma HIV RNA levels of these two groups were 1.97 (1.7; 3.5) and 1.7 (1.7; 3.5), respectively (p = 0.84).

In the group with CSF WBC counts of >5 cells/mm^3, the CSF viral load was higher than the plasma viral load in 8 (40%) individuals, whereas in the group with CSF WBC counts of ≤5 cells/mm^3, the same was true for 4 (8.3%) individuals (p = 0.004) (Table 1). The odds of the HIV RNA level being higher in the CSF than in the plasma were 8.8 times higher for a PWH with a CSF WBC count of >5 cells/mm^3 than for a person with a CSF WBC count within the normal range [OR = 8.76 (CI 95%: 2.21–34.8), p = 0.002].

Group with CSF HIV escape

CVE (Peluso et al., 2012) in the HIV group as a whole was found in 7 (10.29%) individuals (Table 1). The frequency of CVE was higher in the individuals with HIV-1B than in those with HIV-1C, although the numbers did not reach significance [n = 5 (14.81%) and n = 1 (3.85%), respectively, p = 0.61]. CVE was found in 3 (15%) individuals in the group with CSF WBC counts of >5 cells/mm^3, and in 4 (8%) individuals in the group with CSF WBC counts of ≤5 cells/mm^3 (p = 0.411) (Table 1). CNS penetration ARV regimens (Letendre et al. 2010) were comparable between groups described (Table 2)

Performance characteristics of the increased CSF WBC counts

The optimal cut-off point of CSF WBC count in predicting CSF discordance (Canestri et al. 2010) was >15 cells/mm^3 (Figure 1B). However, CSF WBC counts with a cut-off of >3.8 cells/mm^3 were efficient in predicting CSF discordance independent of the HIV RNA values in both compartments, with an AUC value of 0.78 (95% CI: 0.597−0.963). The performance characteristics of the increased CSF WBC count in predicting CSF discordance are indicated in Table 3 and Figure 1B.

Longitudinal CSF and HIV RNA analysis

In three participants for whom serial CSF and plasma samples were collected, CVE was classified in the following forms: for two individuals with HIV-1C infection, as CSF slow suppression and CSF and plasma discordance (this case was not on CART) (de Almeida et al. 2017; 2018A); and for one individual with HIV-1B as persistent CVE.

Neuropsychological impairment

The frequency of global neuropsychological impairment based on a GDS of ≥0.5 in the escape, discordance, HIV RNA in plasma higher than in CSF, CSF and plasma aviremic, and HIV seronegative groups was 83%, 80%, 61%, 55%, and 21%, respectively (Figure 2). The HIV seropositive group performed significantly worse than the seronegative group in

pairwise comparisons of the escape, discordance, or the CSF and plasma aviremia subgroups with those of the HIV− control group (p = 0.005, 0.014, 0.003 and 0.003, respectively). The three HIV seropositive subgroups were not different from each other when compared pairwise (p > 0.05). The odds of cognitive impairment for a participant with escape, discordance, HIV RNA in plasma higher than in CSF, and CSF and plasma aviremic were 19.00, 15.20, 5.97, and 4.68 times higher, respectively, than those for a person without HIV infection (p = 0.011, 0.020, 0.003 and 0.003, respectively).

DISCUSSION

In this cross-sectional study of individuals chronically infected with HIV, the overall frequency of CSF discordance or CVE was low, similar to the frequencies described previously by other authors, which ranged from 4.5 to 21% (Eden et al. 2010; Peluso et al. 2012; Rawson et al. 2012; Nightingale et al. 2016; Anderson et al. 2017; Pérez-Valero et al. 2019). We demonstrated that CSF discordance or CVE occurred at comparable frequencies between HIV-1 subtypes C and B infections. To the best of our knowledge, this has not been described before, as previous studies included only HIV-1 subtype B (Spudich et al. 2006; Garvey et al. 2009; Canestri et al. 2010; Eden et al. 2010; Nightingale et al. 2016; Pérez-Valero et al. 2019). Our group had previously described two individuals infected by HIV-1C, who demonstrated CSF discordance and inflammatory reaction in the CSF with increase of the CSF WBC count, and an extensive biomarker panel study was carried out. The phylogenetic analyses of paired peripheral blood and CSF samples from both individuals revealed distinct CSF compartmentalization of the viruses suggesting that CSF escape and discordance are not simply due to trafficking of blood virus into CSF, but rather reflect and independent source of CSF virus from the CNS (de Almeida et al. 2017; 2018A). HIV genetic compartmentalization is defined when there is genetic differences (characterizing quasispecies) are seen between HIV in the compartments (Harrington et al. 2009; Schnell et al. 2010). These reports showed that independent and isolated HIV replication occurs in the CNS in individuals living with HIV-1C.

The findings of the present study will provide additional support for previously published studies that investigated differences between HIV-1B and HIV-1C; which found no difference in the frequency of HIV-associated neurocognitive disorders (de Almeida et al. 2013) or major depression (de Almeida et al. 2016A). Moreover, there was no molecular evidence to support the hypothesis of reduced intrathecal chemotaxis with HIV-1C relative to that with HIV-1B. β-chemokines, including monocyte chemoattractant protein 1 (MCP-1), and interleukins were elevated in the CSF in comparable proportions of the HIV-1B- and HIV-1C-infected participants (de Almeida et al. 2016). However, the same research group found subtype-dependent differences in amyloid pathway impairment (de Almeida et al. 2018B; 2018C; 2019).

Our study found comparable CSF WBC counts and frequencies of CSF pleocytosis between HIV-1B and HIV-1C infections, corroborating a previous study (Abdulle et al. 2008). These authors also found no subtype-dependent difference in CSF HIV RNA levels between HIV-1B and HIV-1C infections (Abdulle et al. 2008).

CSF and plasma HIV RNA were comparable in the group with CSF pleocytosis; although in the group with CSF WBC count between normal ranges HIV RNA was higher in plasma than CSF, suggesting the traffic of WBC as well as virus particles from blood to CSF. Participants with pleocytosis showed higher quotient of albumin indicating blood CSF barrier dysfunction, which can be interpreted as cause, or consequence or both of the increase of CSF WBC and HIV RNA levels. There is an extensive literature showing a positive correlation between the CSF WBC counts and CSF HIV RNA levels (Spector et al. 1993; Conrad et al. 1995; Pratt et al. 1996; Ellis et al. 1997; Price et al. 2001; Shacklett et al. 2004; Spudich et al. 2005; Nightingale et al. 2016; Anderson et al. 2017). It raises the question of whether the CSF WBC count contributes to the rising CSF HIV-1 RNA levels or whether it only represents a response to high CSF HIV-1 RNA levels (Spudich et al. 2005). Our results are in accordance with those of previous literature, but we went further by investigating CSF WBC counts and HIV RNA levels in individuals infected with HIV-1C. Increased CSF WBC count was found in 43% of participants showing CVE and in 100% of participants showing CSF discordance, similar to the results of another publication (Cusini et al. 2013). The CSF WBC count cut-off of 15 cells/mm^3 showed very high performance characteristics as a predictive biomarker for CSF discordance. The LR+ value was high (i.e., 21). A LR+ value of ≥10.0 indicates that a positive test almost confirmed the diagnosis (McGee 2002; Akobeng 2007A).

There was a small number of participants with mild CSF pleocytosis and undetectable CSF HIV viral load; this can represent the persistence of latent HIV-1 in brain cellular reservoirs, as microglia or oligodendrocytes, despite the control of HIV in CSF (Wallet et al. 2019).

In this study patients with or without CSF HIV RNA discordance showed similar rates of neurocognitive impairment, similar to previously published (Pérez-Valero et al. 2019). Although, the odds of cognitive impairment for a PWH were higher, than those for a person without HIV infection. In the present study, neuropsychological impairment was present in 83% and 80% of the participants with CVE and CSF discordance, respectively. These results must be viewed with care, as the number of participants was low in each group. CVE has been described in association with and without symptoms (Eden et al. 2010; Ferretti et al. 2015), though symptomatic CVE comprises a range of symptoms rather than a single coherent entity. Asymptomatic CVE was reported in approximately 10% of a group of ART-treated patients with suppressed systemic viremia.

The present study was not free of limitations, such as the small number of samples with CVE and CSF discordance, mainly when categorized according to HIV-1 subtypes and the patterns of HIV RNA in paired CSF and plasma samples. However, the study was able to show the frequency of CVE or CSF discordance was comparable between the subtypes studied. The study was also limited by its cross-sectional design. A longitudinal study might be better in helping to gain an understanding of CVE and to classify the diverse forms of escape.

The positive points include the fact that this was the first study on CVE and CSF discordance to include participants infected with HIV-1C and not only HIV-1B, from the same geographical area. This was also the first study to evaluate the performance

characteristics of the increased CSF WBC count in predicting CSF discordance. Consequently, this study will contribute to our understanding of the pathophysiology of HIV infection and the impact of HIV-1 genetic diversity in CNS HIV infection as well as the treatment. Moreover, to establish a biomarker that could predict CSF discordance as well as CVE is of great importance, especially in low- and middle-income countries where CSF HIV-1 viral loads are not routinely available.

In conclusion, viral discordance or escape in the CNS occurs at a comparable frequency with HIV-1C and HIV-1B infections. The CSF WBC count with a cut-off 15 cells/mm^3 appears to be associated with high effectiveness as a predictive biomarker for CSF discordance. It should be useful for screening patients who will benefit from the CSF HIV RNA quantification. More studies are necessary, this cut-off needs to be tested in other populations and its performance should be confirmed. Our findings in conjunction with previous evidence of compartmentalization during CVE suggest that PWH with cognitive impairment may benefit from switching to an antiretroviral regimen with greater CNS penetration and with a resistance profile better matching CSF virus.

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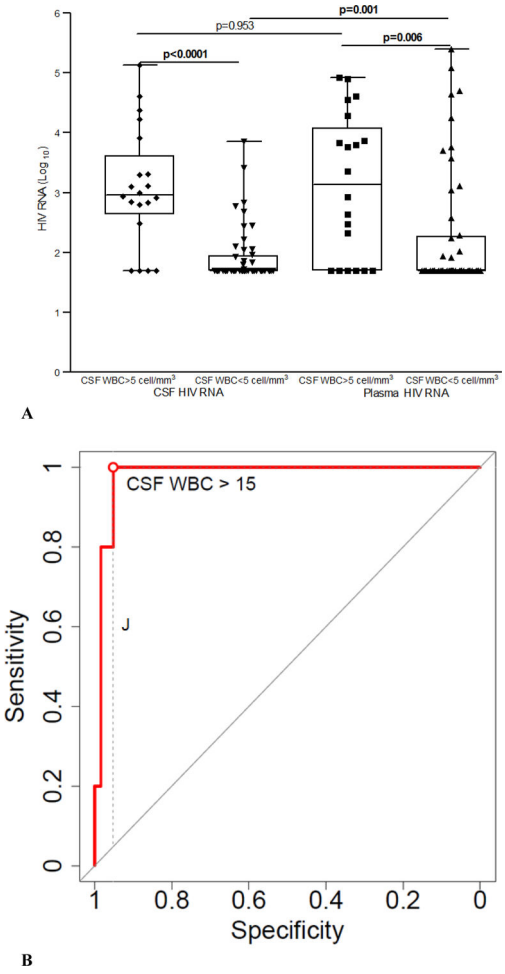

Figure 1.

A. Cerebrospinal fluid (CSF) and plasma HIV RNA levels (Log_{10}) in the groups with cerebrospinal fluid (CSF) white blood cell (WBC) count increased (>5 cell/mm^3) and on normal range (≤5 cell/mm^3).

The line in the center of the box represents median; the superior and inferior borders of the box represent interquartiles (IQRs); the whiskers represent the least and greatest values; the number of cases in each group are indicated by the dots.

B. Receiver operating characteristic (ROC) curve to evaluate predictive characteristics of CSF WBC in characterizing subjects with CSF HIV RNA discordance.

CSF HIV RNA discordance was defined as CSF HIV RNA greater than $1log_{10}$ of the plasma HIV RNA (Canestri et al. 2010); optimal cut-off point 15 cells/mm^3; area under the curve (AUC) = 0.981; 95% CI = 0.951−1. The AUC acts as a single measure, independent of prevalence, which summarizes the discriminative ability of a test across the full range of cut-offs, where the higher the AUC is, the better the test will be. The higher the AUC, the better the test. A perfect test would have an AUC of 1.0, while a completely ineffective test (where the curve falls on diagonal line) has an AUC of 0.5. Youden's index (J) is the difference between the true positive rate and the false positive rate. According to its definition, "J" is the vertical distance between the ROC curve and the first bisector (or chance line), dashed

line. Maximizing this index allows to find, from the ROC curve, an optimal cut-off point independently from the prevalence.

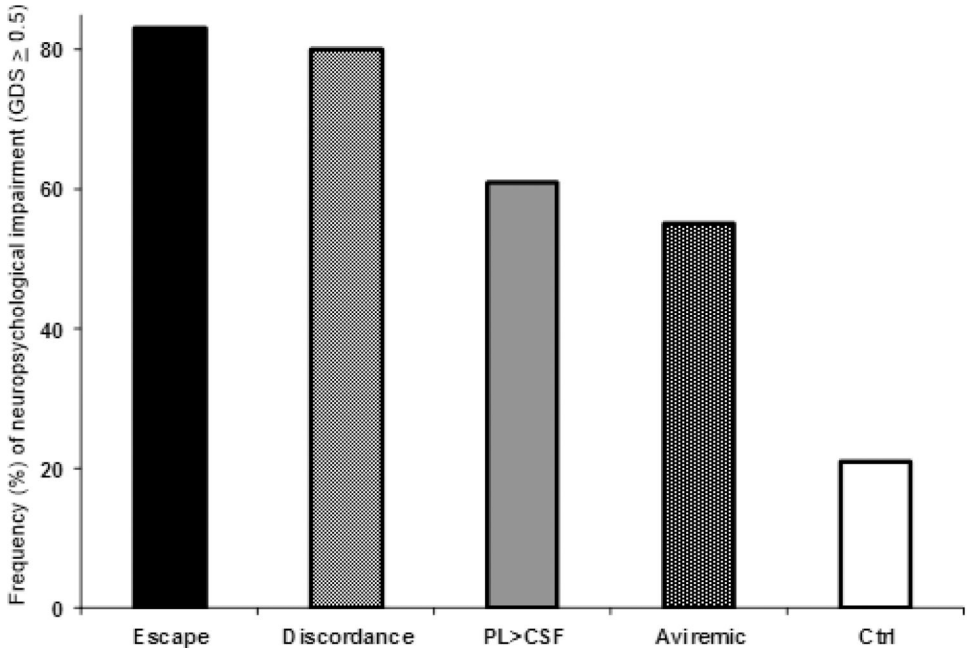

Figure 2. Frequency of global neuropsychological impairment based on global deficit score (GDS) ≥ 0.5 in the escape, discordance, HIV viral load higher in plasma than cerebrospinal fluid (CSF), aviremic in CSF and plasma, and HIV seronegative control groups.

All HIV seropositive groups were significantly worse than the seronegative group, but not different from one another. Comparison of all four groups p=0.0004, pairwise comparisons of the groups escape, discordance, HIV RNA in plasma higher than in CSF and aviremic in CSF and plasma with the HIV− control group were p= 0.005, 0.014, 0.003, 0.003 respectively. The other comparisons were not significant (p>0.05). The odds of cognitive impairment for a participant with escape, discordance, HIV RNA in plasma higher than in CSF, and CSF and plasma aviremic were 19.0 (95 % CI: 2.0 to 181.6), 15.2 (95 % CI:1.6 to 151.5), 6.0 (95 % CI: 1.8 to 19.4) and 4.7 (95 % CI: 1.7 to 12.8) times higher, respectively, than those for a person without HIV infection (p = 0.011, 0.020, 0.003 and 0.003, respectively).

Table 1.

Demographics, AIDS clinical characteristics and treatment in HIV+ participants as a whole, categorized by HIV-1 subtypes B and C participants, and CSF WBC > 5cell/mm^3 and on normal range

	HIV + (n= 68)	HIV1-B (n=27)	HIV1-C (n=26)	p	WBC> 5cell/mm^3 (n=20)	WBC≤ 5cell/mm^3 (n=48)	p
Demographics							
Age, years	43 (35; 48)	44(36.5; 50)	43 (34.5; 47.5)	0.450	42 (34; 46)	43.5(35; 49)	0.310
Education, years	8 (5; 11)	8(5; 12)	7 (5; 11.5)	0.550	9.5 (6; 13)	7 (5; 11)	0.140
Sex - male, n (%)	33 (49.0%)	14 (51.9%)	11 (42.3%)	0.590	12(60.0%)	21(44.0%)	0.290
Disease and Treatment							
Duration of infection (mths)	89 (31; 135)	91.03 (61.63; 144)	81.37(27.82; 132)	0.450	51 (15; 131)	93(46; 136)	0.172
AIDS	55 (80.9%)	22 (81.5%)	19 (73.1%)	0.526	13(65.0%)	42 (87.5%)	0.045
GDS	0.65(0.30;105)	0.95 (0.275; 1.725)	0.50 (0.225; 0.875)	0.126	0.58(0.25;0.97)	0.72(0.3;1.10)	0.567
B/C, n	27/26	27	26	-	6/8	21/18	0.544
Current CD4 cells/mm^3	369 (201; 534)	457 (255; 614)	359.5(176.5; 472.5)	0.200	360 (241; 543)	369 (193; 527)	0.540
Nadir CD4 cells/mm^3	90 (33; 266)	82 (26; 253.5)	159 (16.5; 359.5)	0.290	107 (11; 368)	85 (43; 240)	0.620
CART, n(%)	55 (80.9%)	24 (88.9%)	18 (69.2%)	0.099	12 (60.0%)	43 (89.6%)	0.014
CPE	8 (6; 9)	8 (6; 9)	6 (5.5; 9)	0.339	8.0 (6.5; 9.5)	8.0 (5.5; 9.0)	0.279
Adherence, n(%)	51/54 (94.4%)	21/23(91.3%)	18/18(100%)	0.495	11/12 (91.7%)	40/42 (95.2%)	1.00
Plasma HIV RNA (Log$_{10}$)	1.7 (1.7; 3.5)	1.7(1.7; 1.97)	2.8 (1.7; 3.8)	0.012	3.14 (1.7; 4.1)	1.7 (1.7; 2.3)	0.006
Plasma HIV RNA < 50 copies/mL	38 (55.9%)	20 (74.1%)	9 (34.6%)	0.006	6 (30.0%)	32 (66.7%)	0.008
CSF							
HIV RNA (Log$_{10}$)	1.7 (1.7; 2.8)	1.7 (1.7; 2.2)	2.2 (1.7; 2.9)	0.084	2.96 (2.3; 3.6)	1.7 (1.7; 1.9)	<0.0001
HIV RNA <50 copies/mL, n(%)	35 (51.5%)	16 (59.3%)	10 (38.5%)	0.173	4 (20.0%)	31(64.5%)	0.0012
HIV RNA CSF >plasma, n(%) [*1*]	12 (17.7%)	5 (18.5%)	5 (19.2%)	1.00	8 (40.0%)	4 (8.3%)	0.004
CSF escape [*2*]	7 (10.3%)	5(14.8%)	1(3.9%)	0.351	3(15.0%)	4 (8.3%)	0.411
Discordance [*3*]	5(7.4%)	2(7.4%)	3(11.5%)	0.670	5(25.0%)	0 (0.0%)	-
WBC cells/mm^3	2.1 (0.6; 7.2)	1.6 (0.30; 4.85)	2.65 (0.60; 11)	0.160	13 (9.5;31.5)	0.9 (0.5;2.4)	<0.0001
Glucose mg/dL	57 (53; 62)	63 (54; 66)	56(51.5; 59)	0.007	59 (48; 61)	56 (54; 63)	0.396
Total protein mg/dL	40 (32; 46)	42(35; 47.5)	40(28.5;47)	0.551	42.5 (35.5; 56.5)	36.5 (28.5; 45.5)	0.011
Total protein >45 mg/dL, n(%)	20 (29.4%)	10 (37.0%)	8 (30.8%)	0.773	8 (40.0%)	12 (25.0%)	0.251

	HIV + (n= 68)	HIV1-B (n=27)	HIV1-C (n=26)	p	WBC> 5cell/mm^3 (n=20)	WBC≤ 5cell/mm^3 (n=48)	p
Albumin mg/L	223.5 (164; 288.5)	248.0(189; 309)	218(138.5; 300)	0.328	226.5 (188.5; 360)	222.5 (145.5; 284.5)	0.104
Q. Albumin	0.0064 (0.0049; 0.0097)	0.0082(0.0061; 0.0108)	0.0060(0.0044; 0.0097)	0.52 [4]	0.0079 (0.0063; 0.0116)	0.0061 (0.0045; 0.0092)	0.014
Lactic acid mmol/L	1.6 (1.5; 1.8)	1.65(1.35; 1.9)	1.7(1.6; 1.8)	0.640	1.7 (1.5; 1.9)	1.6 (1.5; 1.8)	0.580
RBC cells/mm^3	0.5 (0; 7.5)	1.0(0; 24)	0.8(0; 36.5)	0.900	1.3 (0.2; 6.8)	0.3 (0; 8.0)	0.387

Data are median (IQR) or number of cases (%).

*[1]*any value of CSF or blood HIV RNA.

*[2]*CSF escape is defined as any HIV RNA levels in the CSF above the limit of detection of the assay used (usually 50 copies/mL) when the plasma HIV RNA levels is undetectable by the same assay.

*[3]*CSF discordance is defined as CSF viral load (VL) greater than 1$\log_{10}$ of the plasma HIV RNA levels (independent of the number).

*[4]*adjusted for plasma HIV VL suppression, nadir CD4.

CART, combination antiretroviral therapy

CNS Penetration-Effectiveness (CPE) rank (Letendre et al. 2010)

Table 2.

Demographics, AIDS clinical characteristics and treatment in the groups with HIV central nervous system escape, discordance, and aviremic in CSF and plasma

	A-Escape n=7 [1]	B-Discordance n= 5 [2, 3]	C-CSF/Pl Aviremic n=31	D-HIV RNA Pl > CSF, n=23	AxC p	BxC p	AxB p	CxD p
Demographics								
Age, years	39(36; 49)	39(27; 51)	42(35; 49.5)	46(37; 48)	0.598	0.552	1.0	0.807
Education, years	6 (4.5; 14.5)	7(4; 13)	8 (5.5; 11.5)	6(5; 11)	0.429	0.631	0.876	0.412
Sex - male, n (%)	2 (28.6%)	3 (60.0%)	18 (58.1%)	9 (39.1%)	0.222	1.0	0.558	0.271
Disease and Treatment								
Duration of infection (mths)	66.77(19.50; 145.4)	5.57(3.17; 127.6)	91 (43.28; 136)	94(47.38; 143.2)	0.429	0.032	0.268	1.0
GDS	1.353 (0.543; 2.710)	1.11(0.32; 3.47)	0.5(0.23; 1.0)	0.65(0.28; 1.10)	0.076	0.109	0.931	0.726
GDS≥0.5, n (%)	5/6 (83.3%)	4/5 (80.0%)	16/29 (55.2%)	11/18 (61.1%)	0.366	0.379	1.0	1.0
B/C, n	5/1	2/3	15/8	6/13	0.633	0.353	0.242	0.062
Current CD4 cells/mm^3	457(265; 747)	239(98; 692)	372(215; 626)	347(159; 443)	0.925	0.522	0.639	0.349
Nadir CD4 cells/mm^3	51(6; 293)	6(1; 692)	54(16.5; 193)	266(85; 368)	0.749	0.082	0.530	0.001
CART, n(%)	7(100.0%)	4(80.0%)	31(100.0%)	14 (61.9%)	-	-	-	-
CPE	8(6.5; 10)	9 (6; 10)	8(6; 9)	6(5; 9)	0.674	0.224	0.527	0.907
Adherence, n(%)	7 (100.0%)	4/4(100.0%)	26/28(92.9%)	14/14(100.0)	-	-	-	-
Time current regimen (mths)	1.87(0.26; 64) [3]	32(0; 64)	24.64(9.92; 44.53)	24.87(4.89; 47.21)	0.265	0.817	0.800	0.736
Pl HIV RNA (Log_{10})	1.7	1.7(1.7; 3.76)	1.7	3.77(2.81; 4.68)	1.0	0.149	0.268	<0.0001
plasma HIV RNA < 50 copies/mL	7 (100.0%)	3 (60.0%)	31 (100.0%)	0 (0%)	-	-	-	-
CSF								
HIV RNA (Log_{10})	2.05(1.85; 3.11)	3.112(2.85; 5.13)	1.7	2.69(1.94; 3.14)	<0.0001	0.0004	0.030	<0.0001
HIV RNA <50 copies/mL, n(%)	0 (0%)	0 (0%)	31(100%)	4 (17.39%)	-	-	-	-
WBC cells/mm^3	2.5 (1.1; 38.5)	37 (20; 382)	0.90 (0.30; 2.95)	3.10(0.95; 10.35)	0.113	0.001	0.106	0.010
Glucose mg/dL	54 (49; 59)	44 (38; 61)	60(55.5; 65.5)	56(52; 59)	0.010	0.007	0.268	0.011
Total protein mg/dL	42 (36; 123)	91 (42; 339)	40(30.5; 46.5)	36(30.5; 45)	0.397	0.003	0.073	0.529
Total protein >45 mg/dL, n(%)	2 (28.6%)	4 (80.0%)	10 (32.3%)	4 (17.4%)	1.0	0.064	0.242	0.347
Albumin mg/L	248 (211; 611)	501 (311; 1770)	236 (155; 292)	195(144; 266.5)	0.328	0.003	0.048	0.340
Q. Albumin	0.0082 (0.0067; 0.0224)	0.0164 (0.0096; 0.0917)	0.0062 (0.0045; 0.0101)	0.0060 (0.0047; 0.0090)	0.153	0.003	0.048	0.861
Lactic acid mmol/L	1.7 (1.55; 2.34)	1.9 (1.7; 2.8)	1.6(1.45; 1.85)	1.7 (1.6; 1.8)	0.412	0.027	0.268	0.489

	A-Escape n=7 [1]	B-Discordance n= 5 [2, 3]	C-CSF/Pl Aviremic n=31	D-HIV RNA Pl > CSF, n=23	AxC p	BxC p	AxB p	CxD p
RBC cells/mm^3	2 (0.15; 19)	30 (2; 92)	0.6 (0; 24)	0.3 (0.0; 8.0)	1.0	0.030	0.048	0.257

Data are median (IQR), number of cases (%)

[1] CSF escape is defined as any VL in the CSF above the limit of detection of the assay used (usually 50 copies/mL) when the VL in the plasma is undetectable by the same assay. All cases were on CART.

[2] CSF discordance is defined as CSF viral load (VL) greater than 1log$_{10}$ of the plasma VL (independent of the number).

[3] Data are median (min, max).

CART, combination antiretroviral therapy; Pl, Plasma

CSF, cerebrospinal fluid.

CNS Penetration-Effectiveness (CPE) rank (Letendre et al. 2010)

Table 3.

Performance characteristics of cerebrospinal fluid white blood cell count for predicting CSF and plasma HIV RNA discordance diagnosis

HIV RNA CSF WBC [3]	CSF>plasma [1] >3.8 cell/mm^3	CSF discordance [2] >15.0 cell/mm^3
Sensitivity	75.00	100.00
Specificity	77.00	95.00
PPV	41.00	62.50
NPV	94.00	100.00
Efficiency (test score)	77.00	96.00
Youden Index	0.52	0.95
LR+	3.23	21.00
LR−	0.33	0.00
CUI+	0.31	0.63
CUI−	0.72	0.95
AUC	0.78	0.98

PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood; LR−, negative likelihood; CUI+, Clinical utility index positive; CUI−, Clinical utility index negative; AUC, area under the receiver operating characteristic (ROC) curve.

[1] HIV RNA *higher than plasma, independent of the* value of CSF or plasma HIV RNA.

[2] CSF HIV RNA greater than 1log$_{10}$ of the plasma viral load (VL) (Canestri et al. 2010)

[3] Cut off points chosen by the ROC curve.