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HIV-1 RNA LEVELS IN CEREBROSPINAL FLUID AND PLASMA AND THEIR CORRELATION WITH OPPORTUNISTIC NEUROLOGICAL DISEASES IN A BRAZILIAN AIDS REFERENCE HOSPITAL

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ABSTRACT - *Background*: Plasma HIV RNA levels reflect systemic viral replication but in CNS it may occur relatively independent of systemic infection, yet clinical application of CSF HIV-1 RNA levels is less clear. *Objective*: to compare CSF and plasma HIV-1 RNA levels of patients with different opportunistic neurological diseases to those without neurological disease, as well as to correlate these levels with the outcome of the disease and use of HAART. *Method*: 97 patients who had lumbar puncture for routine work up of suspected neurological diseases, were divided in 2 groups: without neurological disease (23) and with neurological disease (74). NASBA was used for plasma and CSF HIV RNA. *Results*: Median CSF viral load was higher in toxoplasmic encephalitis, cryptococcal meningitis, HIV dementia and neurological diseases without a defined etiology when compared to patients without neurological disease. There was no difference between plasma viral load in patients with and without neurological diseases. Median viral load was higher in plasma and CSF among patients who died when compared to those successfully treated. CSF and plasma viral load were lower in patients with opportunistic diseases on HAART than without HAART. *Conclusion*: CSF viral load was higher in patients with any neurological disease, but this difference was not present in plasma viral load, suggesting that neurological disease influences more the CSF than plasma compartments. Notwithstanding different neurological diseases were not possible to be diferentiated by the levels of CSF HIV-1.

KEY WORDS: AIDS, HIV, cerebrospinal fluid, HIV-1 RNA, opportunistic infections, viral load, neurological disease.

Níveis de RNA do HIV-1 no líquido cefalorraqueano e plasma e sua correlação com doença neurológica oportunística em um hospital referência em AIDS

RESUMO - *Introdução*: Os níveis de RNA do HIV-1 no plasma refletem a replicação viral sistêmica e a replicação no sistema nervoso central pode ocorrer independentemente da infecção sistêmica, mas a utilidade da medida destes níveis no líquido cefalorraqueano (LCR) permanece indefinida. *Objetivo*: Comparar os níveis de RNA do HIV-1 no LCR e plasma de pacientes sem doenças neurológicas e com diferentes doenças neurológicas, bem como correlacionar estes níveis com a sua evolução e o uso de antiretrovirais. *Método*: Foram avaliados 97 pacientes com suspeita de doença neurológica que realizaram punção lombar e que foram divididos em dois grupos: sem doenças neurológicas (23) e com doenças neurológicas (74). Metodologia NASBA foi usada para quantificação do RNA do HIV-1. *Resultados*: A mediana da carga viral do LCR foi maior em pacientes com neurotoxoplasmose, neurocriptococose, demência pelo HIV e doença neurológica sem etiologia definida quando comparada aos pacientes sem doenças neurológicas. Não houve diferença da carga viral do plasma entre os pacientes com e sem doença neurológica. A mediana da carga viral do plasma e LCR foi maior nos pacientes que faleceram em relação aos tratados com sucesso. A carga viral do LCR e plasma foi menor nos pacientes com doenças oportunísticas que usavam HAART em relação aos que não a usavam. *Conclusão*: A carga viral no LRC foi maior nos pacientes com qualquer doença neurológica em relação aos sem doenças neurológicas, mas isto não ocorreu no plasma, sugerindo que doença neurológica influencia mais o compartimento do LCR que o do plasma, mas não foi possível diferenciar as doenças neurológicas pelos níveis de RNA do HIV-1 do LCR

PALAVRAS-CHAVE: AIDS, HIV, líquido cefalorraqueano, infecções oportunísticas, carga viral, doença neurológica.

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Opportunistic neurological diseases and neurological diseases primarily related to HIV-1 are common manifestations announcing the onset of AIDS and also during the course of the infection[1]. The introduction of highly active antiretroviral therapy (HAART) has led to immunological improvement of patients and a consequent reduction in morbidity and mortality and in the incidence of opportunistic diseases[2-8]. However, these diseases, including those affecting the central nervous system (CNS), continue to occur, especially in underdeveloped countries where the acquisition of antiretroviral (ARV) drugs is difficult. In Brazil, despite a public health system that provides drugs free of charge, opportunistic infections, especially highly incapacitating neurological diseases, continue to occur due to irregular immunovirological monitoring and late diagnosis of the infection.

The virus enters the CNS during primary HIV infection[9,10] and can be present during all stages of the disease, with replication in the CNS occurring in a manner relatively independent of systemic infection[11-15]. There is evidence of intrathecal replication of HIV-1 in patients with opportunistic and HIV-related neurological diseases, with the prompt diagnosis and treatment of these patients becoming important[16,17]. Plasma HIV-1 RNA levels reflect the status of systemic viral replication and represent the best predictive marker of HIV disease progression, being an important tool in monitoring and in studies regarding aspects of the viral dynamics of HIV infection[18-20]. In contrast, the clinical usefulness of HIV-1 RNA levels in the cerebrospinal fluid (CSF) of patients with opportunistic neurological diseases, or the effect of opportunistic diseases on CSF HIV levels in patients under HAART has not been well defined[21,22].

The objective of the present study was to compare HIV-1 RNA levels in CSF and plasma of patients with different opportunistic neurological diseases to those without neurological disease, as well as to correlate these levels with the evolution of the disease, CD4+ T lymphocyte count, and use of HAART.

METHOD

Patients – We prospectively evaluated 97 HIV-infected patients who were submitted to lumbar puncture between May 2001 and May 2002 as part of the work-up for a suspicion of neurological disease at a public AIDS Reference Hospital (Eduardo Menezes Hospital, FHEMIG), Belo Horizonte, Brazil. The study was approved by the Research Ethics Committee of the institution and written informed consent was obtained from all participants.

Data collected from each patient included gender, age, CD4+ lymphocyte count, use and type of ARV therapy, duration of ARV therapy, presence and type of neurological disease, and clinical outcome.

The patients were divided into groups with and without neurological diseases. Seventy-four (76.6%) of the 97 patients presented with neurological diseases, including 46 (62%) with opportunistic diseases, 17 (23%) with neurological diseases of undetermined etiology and 7 (9%) with HIV-related neurological diseases, and the remaining 23 patients (23.4%) did not have active opportunistic or HIV-related neurological diseases. In the case of patients without neurological diseases the clinical diagnoses were primary headache, metabolic/toxic disorders, and fever with a non-neurological focus. Patients submitted to lumbar puncture to exclude neurosyphilis or to assess clearance of cryptococcal meningitis after correct medication for 8 weeks were also studied. No clinical or laboratory evidence of active infection was observed in these patients. The diagnoses are summarized in Table 1.

Neurological diseases were diagnosed based on the following criteria: criteria of the Working Group of the American Academy of Neurology Task Force[23] for the diagnosis of HIV-associated dementia (HIV-D) and vacuolar myelopathy, a suggestive image on a skull computed tomography scan and a clinical and tomographic image response to specific drug treatment for the diagnosis of cerebral toxoplasmosis, and a positive India ink result, a specific cryptococcal antigen test or positive CSF culture for the diagnosis of meningitis. Tuberculous meningitis was diagnosed based on clinical-neurological signs of lymphocytic meningitis and the presence of alcohol-acid resistant bacilli or a positive CSF culture.

Table 1. Diagnosis obtained for the 97 patients studied.

Neurologically asymptomatic	23
Primary headache	9
Metabolic/toxic disorders	6
Fever (no neurologic focus)	2
Cryptococcal meningitis (treated)	3
Positive serum VDRL	3
Neurologically symptomatic (n)	74
HIV-Dementia	6
Vacuolar myelopathy	1
Opportunistic CNS infections	46
Toxoplasmosis	19
Cryptococcal meningitis	16
Tuberculosis meningitis	3
PML	2
Associations	6
Cryptococcal and tuberculous	3
Cryptococcal and toxoplasmosis	2
Cryptococcal and vacuolar myelopathy	1
Other (stroke, brain abscess, neuropathy)	4
Indeterminated neurological diagnosis	17

CNS, central nervous system; PML, progressive multifocal leukoencephalopaty.

Table 2. Clinical and laboratory characteristics of the patients with and without neurological disease.

	With disease (N=74)	Without disease (N=23)	p*
Mean age (years)	36.5	35.8	0.547
Male gender (%)	55	14	0.214
Mean time since diagnosis (months)	5	48	0.014
Pleocytosis in CSF (%)	60.8	4.3	0.000
Median CSF WBC	8.0	2.0	0.000
Median CSF protein (mg/dL)	61.0	43	0.083
HAART use (%)	50	65.2	0.223
Median CD4+ count (cells/mm^3)	96.5	89	0.504
Death (%)	36.5	13	0.034
Detectable CSF viral load (%) **	63.4	26.3	0.004
Detectable plasma viral load (%) **	82.8	60	0.058
Median CSF viral load (log_{10})	3.15	<1.9	0.002
Median plasma viral load (log_{10})	4.35	2.18	0.071

*nonparametric Mann-Whitney test and chi-square test; **higher than the detection limit of 80 copies per ml; WBC, white blood cells.

Stereotactic biopsies were obtained for the diagnosis of progressive multifocal leukoencephalopathy and bacterial abscess. An undetermined diagnosis was considered when the patients showed neurological syndromes characterized by meningitis, encephalitis or expansive focal brain lesions and when no etiology could be established after work-up.

Laboratory analysis – CSF and plasma samples were collected within an interval of 48 hours between each other and stored at - 70^{0}C until the time of processing within a maximum period of 6 months. HIV-1 RNA was quantified by the nucleic acid sequence based amplification (NASBA) technique using centrifuged plasma and not centrifuged CSF according to manufacturer instructions (Nuclisens HIV-1 QT, Organon TeKniKa, Boxtel, Netherlands), with a sensitivity of 80 copies/ml (1.90 log_{10} copies/ml). CSF samples containing more than 10 red cells/ml were excluded from the analysis. In addition, the number of cells and protein concentration were determined in CSF samples.

Among the samples collected, HIV-1 RNA was quantified in 90 CSF and 73 plasma samples. Seventy samples of 70 patients had valid quantification in both plasma and CSF.

Statistical analysis – The nonparametric Mann-Whitney test was used to determine the effect of the presence of neurological disease and use of ARV therapy on CSF and plasma viral load. Viral load and the characteristics of the different groups of patients with and without neurological diseases were compared by the nonparametric Mann-Whitney test and chi-square test. The correlation between viral load and CD4 count was determined using nonparametric Spearman's rank correlation. p values of < 0.05 were considered to indicate statistical significance.

CSF and plasma viral loads were log10 transformed since the HIV-1 RNA levels showed no normal distribution. For patients with undetectable HIV-1 RNA, a log-scale value of 0 was assigned to avoid the problem of expressing zero logarithmically. All statistical analyses were performed with the SPSS 8.0 program (SPSS Inc.).

RESULTS

Patient characteristics – The mean CD4+ T lymphocyte count was 137 cells/mm3 and no differences were observed in mean or median CD4 counts between patients with different types of neurological diseases and between those undergoing ARV therapy or not. Fifty-two (54%) of the 97 patients used ARV drugs, all in the HAART regimen. Seven (13.5%) patients used one drug that crossed the blood-brain barrier, 24 (46.2%) used two drugs, 20 (38.5%) used three drugs, and one patient used four drugs. The mean duration of ARV therapy was 10.4 months.

Comparison between the groups with and without active neurological diseases showed no significant difference in gender, CD4 count, use of ARV therapy or mean CSF protein content. In contrast, a significant difference was observed in terms of the number of cells in CSF, presence of pleocytosis in CSF, duration of HIV infection, and death during hospitalization (Table 2).

Association between CSF and plasma viral load and neurological disease – Median HIV-1 RNA levels in CSF were higher in patients with neurological diseases than in neurologically asymptomatic subjects, as was the median plasma viral load but the latter was not statistically significant (Table 2).

The CSF and plasma HIV-1 RNA means were, respectively, 0.84 and 2.39 log_{10} copies/mL in patients without neurological disease, while they were 2.44 and 3.68 log_{10} copies/mL in patients with neurological disease. The ratio of mean CSF viral

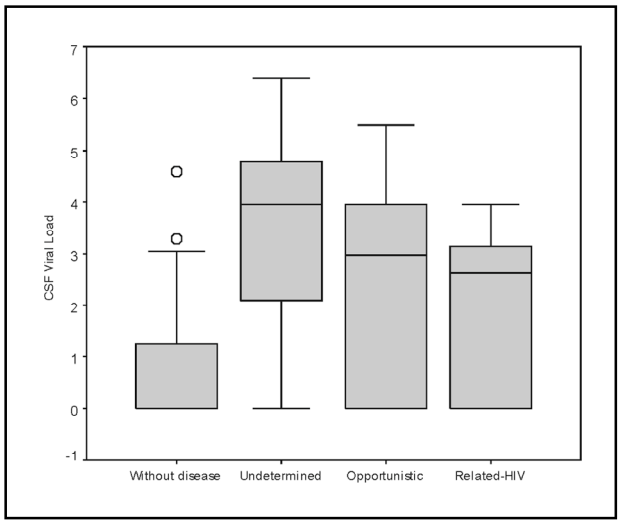

Fig 1. Median viral load in cerebrospinal fluid (CSF) according to disease group.

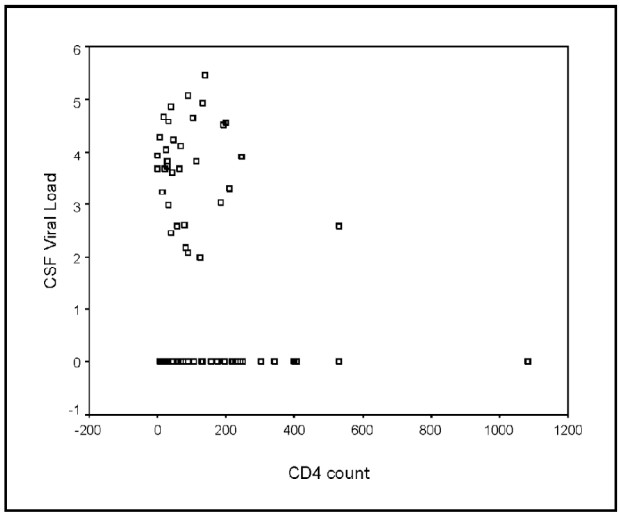

Fig 2. Correlation between cerebrospinal fluid (CSF) viral load and CD4 count.

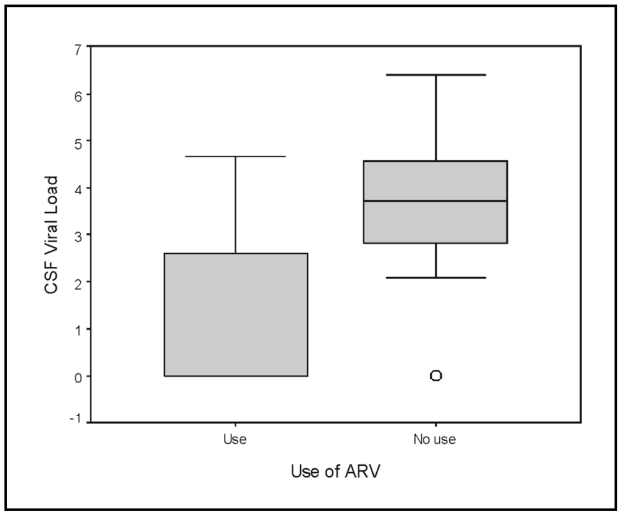

Fig 3. Correlation between cerebrospinal fluid (CSF) viral load and use of ARV therapy.

load of patients with and without neurological disease was 2.9 and the ratio of mean plasma viral load of patients with and without neurological disease was 1.5, showing that CSF viral load was almost 3 times higher in patients with neurological disease compared to those without neurological disease, whereas plasma viral load was 1.5 times higher in patients with neurological disease.

The median of CSF HIV-1 RNA was <1.9 $\log_{10}$ copies/mL in patients without neurological disease, 2.99 $\log_{10}$ in patients with general opportunistic disease, 2.62 $\log_{10}$ in patients with diseases directly related to HIV, 2.99 $\log_{10}$ in patients with cerebral toxoplasmosis, 3.69 $\log_{10}$ in patients with cryptococcal meningitis, and 3.96 $\log_{10}$ in patients with neurological disease of undetermined etiology. HIV-1 RNA levels in CSF were higher in all different groups of patients with neurological diseases compared to those without neurological diseases, but no difference in median HIV RNA levels in CSF was observed between these groups of diseases (Fig 1).

In plasma, differences in median viral load were only observed between patients with undetermined neurological diseases (3.96 $\log_{10}$ copies/mL) and those without neurological diseases (<1.9 $\log_{10}$ copies/mL) and between patients with undetermined neurological diseases and those with opportunistic diseases (2.99 $\log_{10}$ copies/mL).

Correlation between CSF viral load and immunity – A negative correlation was observed between CSF viral load and CD4 cell count (r = -0.307, p = 0.012) (Figure 2) and also between plasma viral load and CD4 T cell count (r = -0.283, p = 0.038). When analyzing patients with and without neurological disease, a correlation between CSF viral load and CD4 count was only observed in patients with neurological disease (r = -0.307, p = 0.03), while CSF viral load was not correlated with the number of CD4 cell in patients without neurological disease (r = -0.341, p = 0.901).

Association between CSF viral load and outcome – A difference in CSF viral load was observed between patients who were discharged and those who died, with the median HIV-1 RNA levels in CSF being higher in patients who died (3.26 versus <1.9 $\log_{10}$; p = 0.027). The same was observed for plasma viral load (4.70 versus 3.26 $\log_{10}$; p = 0.033).

Association between CSF viral load and use of ARV therapy. There was a significant effect of the use of ARV therapy on CSF viral load – Patients

who did not use ARV therapy showed a significantly higher median CSF viral load than those who did (3.30 versus 0,82 log_{10}/ml; $p < 0.001$). The same was observed for plasma viral load (4.47 versus 1.87 log_{10}/ml; $p < 0.001$).

Of the 43 patients with opportunistic neurological diseases submitted to HIV quantification in CSF, 26 received ARV therapy (median of <1.9 log_{10}) and 17 did not (median of 3.82 log_{10}). Of the 35 patients with opportunistic diseases submitted to HIV-1 quantification in plasma, 20 used ARV drugs (median of 2.82 log_{10}) and 15 did not (median of 4.52 log_{10}). Median HIV RNA levels in CSF and plasma were significantly higher in patients not taking ARV drugs ($p < 0.001$) (Fig 3).

DISCUSSION

Association between CSF viral load and neurological disease. In the present study, we determined HIV-1 RNA levels in CSF and plasma of patients without and with different HIV-associated neurological diseases – A correlation was observed between CSF viral load and neurological disease. The median viral load in CSF was higher in patients with neurological disease compared to those without neurological disease, and the same was also observed for plasma viral load, although in this compartment the difference was not statistically significant.

The ratio of mean viral load in patients with and without neurological disease was higher in CSF than in plasma, i.e., CSF viral load was almost 3 times higher in patients with neurological disease compared to those without neurological disease, whereas plasma viral load was only 1.5 times higher in patients with neurological disease, demonstrating a greater influence of neurological disease on CSF viral load than on plasma load, i.e., neurological disease caused a proportionally greater increase in CSF than in plasma. This finding may have been due to intrathecal replication or increased passage of the virus from the plasma to the CSF compartment, since patients with neurological diseases showed a higher median cell count in CSF than those without neurological disease.

Differences in median CSF viral load were observed between patients with diseases primarily related to HIV and those without neurological diseases and between patients with opportunistic neurological diseases and those without neurological diseases, but not between groups with HIV-related disorders and opportunistic diseases. This cross-sectional analysis did not permit the discrimination between subjects with opportunistic infections and those with HIV-related disorders in terms of CSF viral load or between patients with cerebral toxoplasmosis and cryptococcal meningitis. The absence of any significant difference in terms of HIV levels between patients with HIV-D and opportunistic diseases suggests that HIV-1 RNA levels in CSF are not a good marker for the diagnosis of this disease[24,25].

CSF viral load has been shown to be higher in patients with HIV-D than in those with mild or no neurological symptoms[25-27]. However, higher CSF HIV loads have been detected in patients with HIV-D, but significant concentrations have also been found in patients without any HIV-related neurological disease. On the other hand, low HIV RNA levels have been detected in both patients with and without HIV-induced neurological disease[28]. The association between HIV RNA levels in CSF and neurological status in HAART-treated individuals seems to be weaker now than in the pre-HAART era[29].

Viral load in CSF was higher in patients with opportunistic diseases than in individuals without neurdogical diseases. No significant difference between these two groups was observed for plasma HIV levels. Certain opportunistic diseases of the CNS affect HIV RNA levels in CSF, with elevated concentrations being observed in patients with lymphocytic meningitis such as cryptococcal and tuberculous meningitis[16,25]. These conditions are characterized by a meningeal inflammatory infiltration and also by a correlation between the number of lymphocytes in CSF and viral load[28]. High HIV RNA levels in CSF may not be simply due to transport of the virus from plasma to CSF, since some studies were unable to show a correlation between CSF viral load and integrity of the blood-brain barrier[16,25].

The quantity of HIV RNA in the CSF of patients with neurological diseases is more complex compared to asymptomatic individuals and other sources might contribute to its presence in CSF. The chronic presence of a lymphocytic inflammatory process may represent an ideal opportunity for the uncontrolled replication of HIV-1, particularly at an immunologically privileged site like the CNS[16] and infiltrative lymphocytes may harbor HIV-1, thus representing an exogenous source that also contributes to CSF viral load. In vitro studies[30,31] have shown that cryptococcosis and tuberculosis or their antigens can also directly increase viral replication.

Mean and median viral loads in CSF and plasma were higher in patients showing poor outcome and

who died during hospitalization than in those who improved and were discharged, suggesting that the determination of CSF viral load might be a prognostic factor in patients with active neurological disease, especially opportunistic diseases.

Correlation between CSF viral load and CD4 T lymphocyte count – In the present study, a negative correlation was observed between CSF and plasma viral load and CD4+ T lymphocyte count, with an increase in viral load thus being associated with a reduction in the number of CD4 lymphocytes.

In plasma, the correlation between viral load and the degree of immunity has been well established, with plasma viral load increasing as the CD4 cell count decreases[18]. With respect to CSF, this association is not as well established and there are reports in the literature indicating a correlation between CSF viral load and the degree of immunity determined on the basis of CD4 lymphocyte count[26], but most studies suggest that no such correlation exists[24,27,32-36], or even that the CSF viral load is lower in patients with a CD4 count below 200 cells/mm^3[3,37].

When the patients were stratified into two groups, with and without neurological disease, a correlation between CD4 count and CSF viral load was only observed in the group of patients with neurological disease in which opportunistic diseases predominated. These patients showed a higher CSF viral load and also a higher median number of cells in CSF, possibly as a result of intrathecal viral replication or passage of the virus from plasma to CSF.

No correlation between CSF viral load and CD4 T lymphocyte count was observed in the group of patients without neurological diseases. This group presented a median CSF viral load lower than that observed for patients with neurological diseases and an equally low CD4 count. This finding suggests that a control of viral load is achieved with the use of ARV therapy in these patients but in the absence of immune recovery.

The influence of CD4 count on CSF viral load seems to depend on other factors since previous studies have reported discordant results regarding its correlation, with these reports varying in terms of the characteristics of the population evaluated such as use of ARV therapy, number of cells in CSF and the presence of neurological diseases.

Association between CSF viral load and use of ARV therapy – An association was observed between the use of ARV therapy and CSF and plasma viral load. Patients not receiving ARV therapy had a significantly higher viral load than those who did. Comparison of CSF and plasma viral load between patients with opportunistic diseases who received ARV therapy and those who did not also showed higher HIV RNA levels in those not undergoing ARV therapy. This finding demonstrates the importance and efficacy of ARV therapy in the control of HIV RNA levels both in plasma and CSF, even in patients with a potentially higher viral load in CSF such as those with opportunistic diseases.

Control of the CSF compartment may result from the control of the plasma compartment, with suppression of the virus at the periphery and a consequent reduction in the amount of virus that enters the CNS, or even from penetration of the drug through the blood-brain barrier into the CNS and its consequent action on HIV in CSF, since 87% of the patients who received ARV therapy used at least two drugs known to cross the blood-brain barrier. The most frequently used ARV drugs were those that cross the blood-brain barrier such as lamivudine, zidovudine, efavirenz and stavudine[38-40]. The CNS serves as a reservoir, permitting escape cases to be identified more frequently[21]. It has been suggested that drugs crossing the blood-brain barrier need to be included in the ARV regimen[41]. CSF suppression is correlated with predicted CNS antiretroviral drug penetrance[42].

Several studies have evaluated the response of HIV in CSF to ARV therapy. Studies conducted before the advent of HAART did not find significant differences in the detection rate of HIV RNA or mean HIV levels in CSF with the use or duration of ARV therapy[26]. However, since the introduction of HAART several studies have documented a rapid decline in CSF viral load in patients with different stages of the disease, including neurologically asymptomatic individuals, patients with mild or severe HIV-related symptoms, and patients with opportunistic cerebral diseases[43-47], with the decline being more important in naive individuals[21]. Most HIV-infected individuals show a short-term fall in CSF viral load similar to that observed in plasma[47], although prolonged therapy may be required to suppress HIV levels within the CNS[36].

In conclusion, CSF viral load was higher in patients with some kind of neurological disease compared to those without active neurological disorders, while the same was not observed for plasma

HIV levels, suggesting that the presence of neurological diseases has a greater influence on the CSF than on the plasma compartment. It was not possible to differentiate patients with cerebral toxoplasmosis, cryptococcosis or HIV-D based on HIV-1 RNA levels in CSF. HIV-1 RNA levels in CSF were lower in patients undergoing ARV therapy, even in those with an opportunistic neurological disease.

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