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Intrathecal viral replication and cerebral deficits in different stages of human immunodeficiency virus disease

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The objectives of this study is to clarify whether there are phases critical for the infection of the central nervous system (CNS) as defined by active viral replication in the cerebrospinal fluid (CSF) in human immunodeficiency virus (HIV) infection. One hundred and nine HIV-1–positive homo- and bisexual patients in early and late disease stages with or without highly active antiretroviral therapy (HAART) were included in the cross-sectional, diagnostic (phase I) multicenter study. No patients had any overt neurological deficits; all underwent venous and lumbar puncture as well as neuropsychological testing. In untreated early-stage patients, cerebrospinal fluid (CSF) viral load correlated with inflammatory parameters, but not significantly with neuropsychological abnormalities. CSF viral load and inflammatory reactions were suppressed in HAART-treated early-stage patients. In HAART-treated late-stage patients, there was a weak correlation between CSF viral load and CSF cell count as well as a moderate correlation with immune activation markers and with distinct cerebral deficits independent of CSF viral load. Seventeen of the 109 patients had higher CSF than plasma viral loads and marked inflammatory reactions and immune activation. In patients with greater plasma than CSF viral loads, the factors contributing to cerebral deficits still need to be identified. The results suggest not only that there is an early "set point" for CSF/central nervous system (CNS) infection, but also that there is a subgroup of patients in whom intrathecal viral replication correlates with cerebral deficits. Lumbar puncture should be performed in all positive patients to identify members of this subgroup and to ascertain what characteristic factors they have in common in order to improve therapy. *Journal of NeuroVirology* (2007) **13,** 225–232.

Keywords: CSF; HIV; neurology; neuropsychological impairment

Introduction

The significance of cerebrospinal fluid (CSF) abnormalities (elevated cell count, protein content, and positive oligoclonal bands) in patients infected with the human immunodeficiency virus (HIV) has been a subject of discussion since the pandemic began (Marshall *et al*, 1988). It soon became clear that a high

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percentage of HIV carriers—although they show no neurological signs or symptoms and have no severe immunosuppression—do have inflammatory CSF abnormalities. In the study by Marshall and coworkers this percentage reached about 60. Unfortunately, no reliable predictor of impending central nervous system (CNS) complications has yet been found. After it became possible to analyze HIV viral load (VL) in different body compartments, several studies found correlations between CSF VL and acquired immunodeficiency syndrome (AIDS) dementia stage and neurocognitive impairment (Brew *et al*, 1997; Ellis *et al*, 1997; von Giesen *et al*, 2005). McArthur *et al* (1997), as well as Sevigny *et al* (2004), found no correlation between CSF VL and pleocytosis or antiretroviral therapy, but there was a correlation of CSF VL with VL in the brain of patients with HIV-associated dementia. The same McArthur study detected a relationship between plasma/CSF VL and levels of CSF β2-microglobulin, suggesting that both VL and the CNS itself are perhaps responsible for immune activation in neurological disease. By contrast, Gisslén *et al* (1998) found no significant differences between CSF VL of untreated patients with or without neurological complications. These authors detected a correlation between monocyte cell counts and CSF VL in patients without neurological deficits, but no significant correlation was seen between immune activation, CSF VL, and systemic immunosuppression in patients with neurological complications (Gisslén *et al*, 1999). In the earlier study, these authors had found an increase in CSF VL over time in a longitudinal comparison of patients without antiretroviral therapy that correlated with an increase in CSF levels of neopterin. This correlation reflected an intrathecal immune activation triggered by the virus (Gisslén *et al*, 1998). Although results on the significance of CSF VL for neurological disease are still contradictory, most authors agree that the CNS is a viral reservoir (Lambotte *et al*, 2003). Thus, the CSF constitutes a "window" to brain infection (Price and Staprans, 1997). Price and associates (1999) as well as others (Harrington *et al*, 2005) have postulated that HIV infection is compartmentalized. This idea is consistent with the model of two prototypes of CSF infection: a short-lived, transitory infection early in the course of the disease and a longer-lived autonomous type predominating in later stages. CSF cell counts remain the best predictor for CSF VL in both types (Staprans *et al*, 1999; Neuenburg *et al*, 2004; Spudich *et al*, 2005). Other groups have found that the virus evolves divergently in plasma and CSF, leading to the emergence of therapy-resistant mutants in the CSF, which escape routine diagnostic procedures. The CSF is thus not only a virus reservoir, but also a compartment that produces pharmaco-resistant variants (Stingele *et al*, 2001). Ellis and coworkers examined a small group of clinically asymptomatic HIV carriers without antiretroviral treatment. They analyzed longitudinal trends in series of CSF samples and found a "set point" for CSF VL, perhaps reflecting the reservoir of infected cells in the CNS. The number of infected cells might increase with advancing systemic immunosuppression (Ellis *et al*, 2003). Other authors found HIV RNA in the CSF of neurologically asymptomatic early-stage patients, including individuals with an intact blood-brain barrier, and an independent association of CSF abnormalities and CSF VL. These findings support the hypothesis that viruses are produced locally (Garcia *et al*, 1999). The results are in good agreement with those of Ellis and colleagues (2000) and provide evidence that HIV dynamics in the CNS become increasingly independent of the systemic disease in advanced stages. The greatest discrepancy between plasma and CSF parameters is found among patients with HIV-associated dementia (Ellis *et al*, 2000). An Italian group found CSF viral replication to be positively influenced by highly active antiretroviral therapy (HAART) and underlined the usefulness of the drugs' ability to penetrate into the CSF, especially in patients with high CSF viral loads, advanced systemic disease, or neurological disorder associated with significant macrophage activation (De Luca *et al*, 2002). However, studies comparing early- and late-stage patients with and without therapy with respect to CSF viral replication are still lacking. This also holds true for analyses of correlations between other inflammatory CSF and immune activation markers.

The present study investigates patients in different stages of HIV infection with and without therapy in order to determine whether CSF VL correlates with CSF routine parameters, inflammatory and systemic disease markers, as well as with cognitive and motor tests. Potential differences between early- and late-stage disease are of special interest, in order to identify which stages tend to be critical for CSF infection and CNS function.

Results

Figure 1 shows plasma and CSF VL copy rates for the patient groups and the status of the blood-brain barrier (BBB). Almost all of the untreated early-stage patients had detectable HIV RNA in the CSF, but only 8/27 showed disruption of the BBB. Most of the late-stage untreated patients also had an intact BBB, but they had relatively high CSF VL. Early-stage patients on HAART had a suppressed CSF VL in the majority of cases, whereas 17 of the 49 late-stage patients on HAART had detectable CSF VL. Only 10/39 revealed disrupture of the BBB.

Pleocytosis was common in untreated early-stage patients (group 1) and differed significantly from the other three groups. The other routine CSF parameters (protein, immunoglobulin G [IgG], and lactate) showed no statistically significant differences between groups after Bonferroni adjustment (Table 1).

Table 1 Demographics of patients and distribution of protein, IgG, and lactate levels in CSF in the different groups

	n	Age (years)	Duration of HIV-1 positivity	CD4+ cell count	Viral load Blood plasma	CSF		
						Protein (mg/dl)	IgG index	Lactate (mmol/L)
Early stage without HAART	27	42.41 (± 12.64)	4.76 (± 5.06)	508.04 (± 195.55)	23,151.61 (± 27,558.60)	46.74 (± 15.14)	0.76 (± 0.35)	1.53 (± 0.19)
Early stage with HAART	28	45.25 (± 9.00)	7.97 (± 5.37)	595.46 (± 320.53)	70.29 (± 234.04)	43.74 (± 10.82)	0.51 (± 0.13)	1.67 (± 0.31)
Late stage without HAART	5	42.39 (± 7.51)	3.73 (± 5.53)	456.80 (± 548.86)	32,896.20 (± 56,460.30)	39.58 (± 9.71)	0.64 (± 0.26)	1.64 (± 0.19)
Late stage with HAART	49	48.22 (± 9.01)	10.31 (± 6.03)	414.08 (± 268.82)	9356.44 (± 43,896.75)	46.18 (± 13.23)	0.59 (± 0.28)	1.85 (± 0.34)

Viral load in the CSF of untreated early-stage patients correlated positively with the duration of established HIV positivity (slight), CSF cell count (slight), and the IgG index (moderate). There was no correlation between CSF-VL and cognitive and/or motor tests at this stage (data not shown). However, 55% of these patients showed abnormal results in neuropsychological tests.

In early-stage patients on HAART, inflammatory CSF reactions (CSF cell count) were suppressed, whereas cognitive/motor abnormalities persisted in 16/28 individuals, demonstrating that functional damage is not positively influenced by therapy in every case.

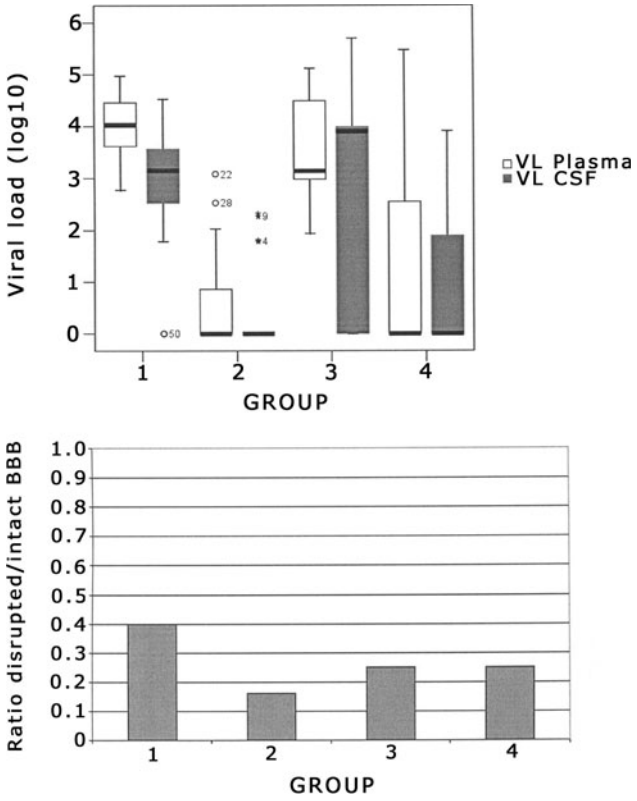

Figure 1 Plasma and CSF viral loads and number of patients with blood-brain barrier (BBB) disruption in the four groups (1: early stages without HAART; 2: early stages with HAART; 3: late stages without HAART; 4: late stages with HAART); almost all of the untreated patients show CSF viral replication, but also a high percentage of the treated late-stage patients (*upper panel*), most of them without having BBB disrupture (*lower panel*). The group mean differences are significant on a level of $P = .05$ (ANOVA; post hoc analysis: Scheffe); HAART comprised in most of the patient 2 NRTIs and 1 NNRTI/PI.

In the few late-stage patients without HAART, there was a positive correlation between CSF VL and CSF cell count, protein content, IgG index, immune activation parameters (monocyte chemoattractant protein [MCP]-1, galectin-3 [Gal3]), CSF lactate, and plasma VL (data not shown because of the low number of individuals in this group).

In late-stage patients on HAART, there was no correlation between CSF VL and any of the other CSF parameters and only a moderate correlation with plasma VL. However, results of some of the motor tests (motor test battery after Arendt *et al,* 1990) in this patient group revealed marked pathological conditions when compared to the results of HIV-negative controls and to those of early-stage carriers with and without HAART (Figure 2). Only on a group level were motor results similar to those of early-stage patients without HAART.

There was a subgroup of 17/109 (15.6%) patients spanning all four groups who had higher viral replication in CSF than in plasma (PL) ($VL_{CSF > PL}$; Figure 3). In fact, whenever viral replication was detectable in early-stage patients on HAART, VL was higher in the CSF than in plasma. In six patients with VL CSF > PL (HAART-treated, two early- and four late-stage patients), viral replication was suppressed by HAART below the detection limit in plasma, but could readily be detected in the CSF. The proportion of patients with reversed VL was similar in all groups except in the late-stage patients without HAART: two out of five of these patients had a higher VL in the CSF. Because of the small number of patients in this group, it is not yet clear whether this condition reflects the natural course of CNS infection. On the whole, plasma VL in patients with greater VL in the CSF than in the plasma was similar to that in patients with "normal" plasma/CSF ratios, but greater by more than one order of magnitude in CSF (data not shown). This applied to all groups except for the late-stage patients without HAART.

Correlation results of this subgroup (the 17 patients described above) are provided in Table 2. In contrast to patients with higher plasma than CSF VL,

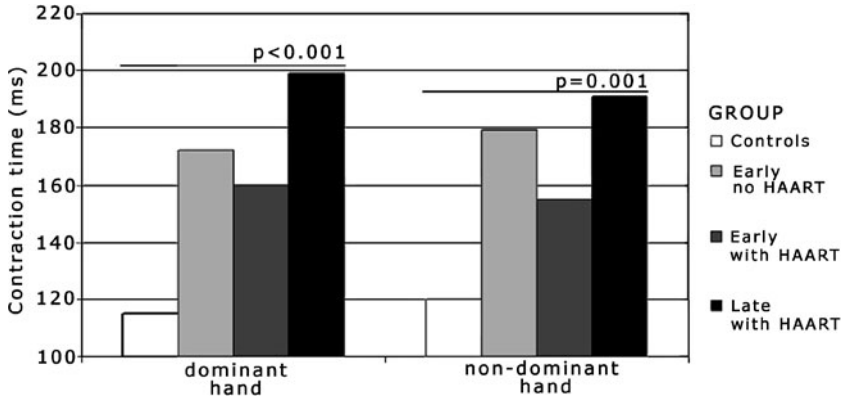

Figure 2 Comparison of motor test results across groups. There is statistically significant difference between the performance of late-stage patients on HAART and the other groups, with late-stage patients on HAART performing worst; the early-stage patients on HAART performed significantly worse than the HIV-negative control group, but better than early-stage patients without HAART (ANOVA).

significant correlations could be found between VL in CSF and inflammatory markers (CSF cell count, protein content, and IgG), immune activation markers (MCP-1, Gal3), and neuropsychological test results (Table 2).

Treatment failure was probably not the cause of higher CSF viral replication, because plasma VL was effectively suppressed in most of the patients with

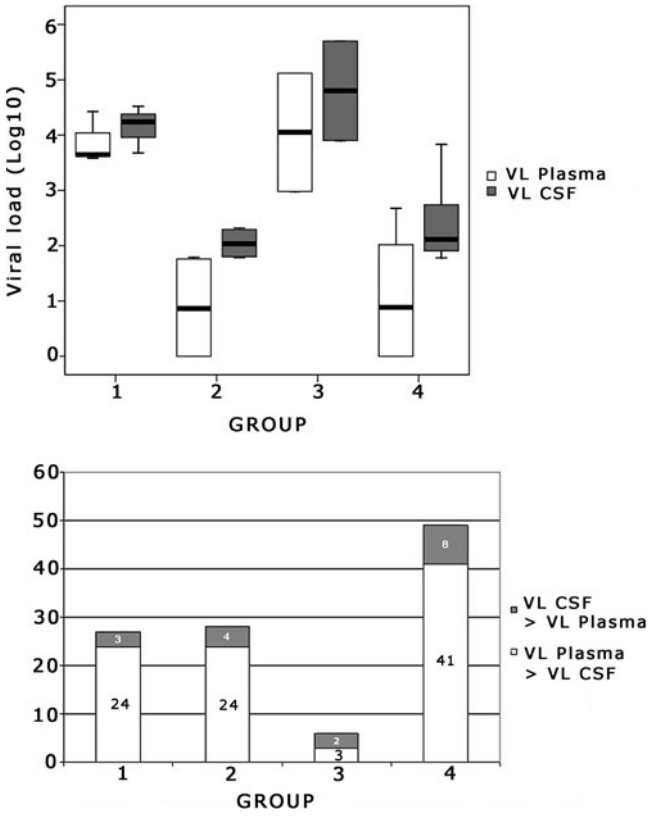

Figure 3 Comparison of blood and CSF viral loads of patients in the the four groups (1: early stages without HAART; 2: early stages with HAART; 3: late stages without HAART; 4: late stages with HAART). The bottom panel shows the number of patients with CSF viral load higher than blood viral load. Group mean differences are significant on a level of $P = .05$ (post hoc analysis: Scheffe).

$VL_{CSF > PL}$ on HAART. Inversely, there were three patients, one early-stage individual without therapy and two late-stage patients with HAART, who had high plasma VL but no detectable CSF VL and no disrupture of the BBB.

In summary, the longer an untreated early-stage patient is HIV positive, the higher is his CSF VL and the stronger the CSF inflammatory reaction and immune activation (see group results of untreated early-stage patients). Once treated, inflammation and immune activation are suppressed, but virus-induced functional brain damage is not lessened in every case. By contrast, inflammatory reactions were weak in late-stage patients on HAART, but immune activation and functional cerebral deficits strong. A subgroup of patients had a higher rate of viral replication in the CSF than in plasma; these patients showed marked inflammatory reactions and immune activation correlated with neuropsychological deficits.

Discussion

In our study CSF VL was examined as it relates to inflammatory and immune activation markers in patients with and without antiretroviral therapy in both early and late disease stages to ascertain its significance for HIV-associated brain disease. Similar studies have been undertaken in monkeys (Scheller *et al*, 2005).

In our clinically asymptomatic early- and late-stage patients without therapy, the vast majority showed CSF viral replication without BBB disruption, as reported earlier by other groups (Chiodi *et al*, 1992; Abdulle *et al*, 2005). White blood cell counts were elevated in patients without therapy, revealing an inflammatory reaction. Opportunistic cerebral infection as well as neurosyphilis had been excluded. An association between CSF pleocytosis and viral burden has been described by others (Martin *et al*, 1998), but those studies did not differentiate between early- and late-stage patients with and without therapy.

Table 2 Correlation of CSF markers and neuropsychological test results with viral load in patients with elevated viral load in CSF compared to patients with elevated viral load in blood

	Correlation of Log_{10} VL CSF with:							
	CSF cells	CSF protein	IgG index	CSF lactate	CD4 cell count	Log_{10} VL blood	CSF MCP1	CSF Gal3
				VL CSF > VL blood ($N=17$)				
Log_{10} VL CSF	0.695**	0.645**	0.587*	−0.375	0.312	0.703**	0.911**	0.681**
				VL blood ≥ VL CSF ($N=92$)				
Log_{10} VL CSF	0.393**	0.051	0.266*	−0.103	−0.212*	−0.063	0.792	0.003

	Correlation of Log_{10} VL CSF with:							
	GPT, dominant hand	GPT, nondominant hand	Digit Symbol Test	Rey Osterrieth Figure, Recall	TMT A	TMT B	CT, dominant hand	CT, nondominant hand
				VL CSF > VL blood ($N=17$)				
Log_{10} VL CSF	−0.152	−0.182	0.200	0.195	−0.196	−0.136	−0.060	0.047
				VL blood ≥ VL CSF ($N=92$)				
Log_{10} VL CSF	0.556*	0.521*	−0.336	−0.143	0.089	0.438*	−0.250	−0.279

Pearson test: * $P \leq .05$; ** $P \leq .005$.

Functional brain deficits, although spread over the entire spectrum of HIV carriers, did not correlate with CSF VL. Thus, the results presented here suggest that there are factors other than the virus itself provoking neurological symptoms in early-stage patients without therapy. On the other hand, correlations between CSF cell counts and viral burden in early stages support the results of Cinque *et al* (2000), who concluded from such correlations that infiltrating lymphocytes might be the main source of CSF VL. Marshall and colleagues (1988) found that CSF cell counts decrease without therapy in patients with more advanced systemic infection. Other groups (Ellis *et al*, 2000) described slower HIV decay in CSF than in plasma in all infected individuals. This finding could account for the persistence of cognitive/motor damage in early-stage patients under therapy, especially in those with persisting CSF viral burden. Price (2000) reported that in early stages a transitory CSF infection predominates and is then sustained by CD4+-cells migrating into the CSF, followed by an autonomous infection stemming from cells of the monocyte lineage. In the treated late-stage patients of our study, 8/49 had a higher CSF than plasma viral burden, most of them being HAART responders. They had fully suppressed plasma viral loads and relatively stable CD4+-cell counts, supporting the hypothesis of an independent HIV disease in the central nervous system. We agree with the findings of Price and colleagues (Spudich *et al*, 2006) that in general HAART successfully suppresses plasma and CSF VL, but we focused on two subgroups of patients in whom therapy is not effective in the CSF/CNS, i.e., on patients with $VL_{CSF > PL}$ and on early- and late-stage individuals with neurocognitive deficits despite HAART (>50%) whether or not these had a detectable CSF VL. This latter condition could be due to HAART combinations with low CSF penetration capacity or to genetic differences between blood- and CSF-derived virus types in the same individual (Korber *et al*, 1994; Peeters *et al*, 1995; Giancola *et al*, 2006). Most likely blood-derived sequences respond to therapy whereas CSF-derived types do not. Such differences would remain undiagnosed if lumbar puncture is not performed routinely in HIV carriers and if genotyping is not part of the diagnostic procedures.

Bestetti and coworkers (2004) found virological failure in both CSF and plasma associated with the emergence of new drug resistancies in both compartments. One subgroup of our treated late-stage patients (17/49) had persistent viral replication in the CSF and eight of them revealed higher CSF than plasma replication rates but not all of them showed therapy failure in plasma. In those patients showing HAART failure (in our study the minority), it needs to be clarified whether this failure is due to drug resistance in the CSF virus variants or to host-specific factors.

Spector and colleagues (1993) found neurocognitive deficits in patients with undetectable plasma VL and concluded that functional deficits cannot be dependent only on the presence of the virus in the CNS. We did not find any correlation between CSF VL and functional deficits in our early- and late-stage patients under therapy. This fact suggests that additional factors account for the high incidence of neurocognitive deficits in these patients. Furthermore, the role of antiretroviral drugs in CNS toxicity must also be carefully evaluated.

In untreated early-stage patients, there was a correlation between duration of known HIV positivity and CSF VL, i.e., the longer a patient is virus carrier, the higher is his CSF VL in early stages. In her review on HIV infection and dementia, Gartner discusses the hypothesis that "a virus threshold level" is probably reached in the brain before active damage occurs; there may be a limited range of host cells in the brain (Gartner, 2000). This hypothesis strongly suggests that the virus should not be allowed the time to reach this threshold level. Lumbar puncture should be integrated into routine patient care at the time of HIV diagnosis and in follow-ups based on the results of neuro-screen test batteries and because

the CNS functions as a long-term latent virus reservoir (Siliciano and Siliciano, 2004).

There were three patients with high plasma VL and BBB disrupture, but undetectable CSF VL. One of them was an early-stage patient without HAART; this individual might have been in a very early phase of viral replication in which the virus had not yet penetrated into the CSF. The other two HIV carriers were late-stage patients on HAART; they might now be in a phase of early therapy failure, a phase at which the brain is not yet involved but the BBB already disturbed.

Thus, in summary, HIV penetrates very early into the CSF, replicates there, and provokes an inflammatory reaction in untreated patients, correlating with the known duration of positivity. The inflammatory reaction is accompanied or followed by an immune activation and functional brain deficits. In some, but not in all, cases these deficits are reversed under HAART (Sacktor *et al*, 1999). In treated late-stage patients, VL is more likely to correlate with immune activation than with inflammatory markers and not at all with functional deficits.

In the subgroup of patients with higher CSF than plasma VL, the divergence from the usual condition of plasma greater than CSF VL cannot be due exclusively to therapy failure or to slower viral decay in CSF than in plasma. There might be compartment-specific virus quasispecies or host factors that still need to be identified. Among our patients there was a higher percentage of patients with $VL_{CSF>PL}$ (15.6%) than in the report of Spudich *et al* (2005) (10.0%), even though their study included only late-stage patients.

Furthermore, it is obvious that the group with higher viral replication rates in CSF than in plasma shows stronger correlations with respect to inflammatory values (Monteiro de Almeida *et al*, 2005), immune activation, and functional cerebral deficits, so that these patients must be identified, followed up carefully and perhaps put on HAART earlier than those with $VL_{PL>CSF}$. Thus, if these findings are confirmed in larger studies, it would be useful to perform lumbar puncture in every patient identified as HIV carrier. Furthermore, the virus- and/or host-specific factors leading to the constellation $VL_{CSF>PL}$, and thus to functional cerebral deficits, must be analyzed.

Patients with $VL_{PL>CSF}$ also reveal motor and cognitive deficits, most likely due to other factors than the virus itself. These patients must be identified in order to find therapeutic strategies other than HAART and help prevent these patients from developing brain disease.

Methods

Patients

In a cross-sectional multicenter study 109 HIV-1 positive homo- and bisexual Caucasian patients (all had at least a high school diploma) without neurological signs or symptoms were recruited consecutively. Patients were assigned to four groups: early-stage patients (revised CDC classification 1993 A1, A2, B1, B2; CDC, 1993) without (group 1) and with (group 2) HAART as well as late-stage patients (CDC A3, B3, C1–3) without (group 3) and with (group 4) HAART. Demographics are provided in Table 1.

Exclusion criteria were a history of opportunistic cerebral infections, bacterial or mycobacterial meningitis, primary cerebral lymphoma and psychiatric or neurological comorbidities. If a patient had CSF pleocytosis (>3 cells), plasma and CSF samples were tested for syphilis (TPPA [*Treponema pallidum* particle agglutination/assay], FTA-abs [fluorescent *Treponema* antigen–absorption], and VDRL [Venereal Disease Research Laboratory] test in case the FTA-abs test was positive).

All patients on medication had triple combination therapies, most of them two nucleoside inhibitors of the reverse transcriptase (NRTIs) plus a protease inhibitor (PI). Fewer patients had two NRTIs plus a non-nucleoside inhibitor of the reverse transcriptase (NNRTI). Only a small number of late-stage patients (8%) received a multidrug salvage therapy.

Venous blood samples and cerebrospinal fluid were taken from every patient classified as clinically asymptomatic. Each patient signed the informed consent papers approved by the local ethics committees.

Routine CSF, immunological, and virological analyses

Cell counts and analysis of total protein content and albumin were performed on CSF immediately after lumbar puncture. CSF/serum ratios for IgG, IgA, IgM, and albumin were calculated. Albumin was used to assess the integrity of the blood-brain barrier (Reiber's diagram). Aliquots of CSF were frozen and stored at -80°C for virological and immunological (MCP-1, Gal-3) analysis.

Viral load was quantified in plasma and CSF using the branched DNA assay (Quantiplex HIV-RNA 3.0; Bayer Diagnostics, Emeryville, CA). The detection threshold for HIV was 50 copies/ml. A log 10 transformation was performed on all RNA concentration values (copies/ml). If the number of RNA copies was below detection threshold, it was set at $0 = \log 1$ copy/ml.

Concentrations of the chemokine MCP-1 and the lectin galectin-3 (Gal-3) in serum and CSF were measured using commercially available sandwich ELISAs (Bender, Vienna, Austria) with a detection limit of 3.5 pg/ml for MCP-1 and 0.15 ng/ml for Gal-3. All tests were carried out according to the procedures recommended by the manufacturer.

Cognitive/motor testing
The following neuropsychological tests were used: the Grooved Pegboard test (GPT), the Digit Symbol and the Trail Making tests parts A and B (Klove, 1963; Spreen and Strauss, 1998; Reitan and Wolfson, 1985), as well as the motor test battery according to Arendt *et al* (1990).

The *Grooved Pegboard* is a fine motor function test. It requires rapid visual-motor coordination. The pegboard has 25 slotted holes orientated in different directions. Pegs with a flat and a rounded side must be rotated to match the hole before they can be successfully inserted.

In the *Digit Symbol* test (*HAWIE-R Handbook and Instructions*; Hans Huber Editions, 1991) patients have to attribute symbols to digits. The level of performance is time dependent.

The *Trail Making* tests A and B require rapid recognition of the symbolic significance of numbers and letters and the ability to identify numbers or letters in sequence. Parts A and B consist of 25 circles with numbers (A) and numbers and letters (B) distributed over the test sheet which have to be connected in the right order as fast as possible.

The *Motor Test Battery* was used for registering contraction times (CTs). Individual and group comparisons were made because contraction times have been shown to reliably identify patients with minor motor deficits (MMDs). CT is defined as the interval between the onset of a rapid extension of the index finger and the time at which the movement reaches its maximum (for details see Arendt *et al*, 1990; for normal values von Giesen *et al*, 1994).

Neuroimaging procedures
Cranial magnetic resonance imaging (MRI) scans (performed on every patient) with a 1.5-tesla machine were unremarkable with respect to HIV-specific pathology.

Statistics
For overall statistics, the SPSS software package version 12.0 was used. Pearson's and Spearman's correlation coefficients were used to determine correlations between VL and the other routine CSF and plasma values as well as cognitive/motor scores.

The Mann-Whitney *U* and the Kruskal-Wallis tests were used to reveal differences between group mean values and between group variables respectively. *P* values were corrected for multiple comparisons (Bonferroni adjustment).

References

Abdulle S, Hagberg L, Gisslén M (2005). Effects of antiretroviral treatment on blood-brain barrier integrity and intrathecal immunoglobulin production in neuroasymptomatic HIV-1-infected patients. *HIV Med* **6:** 164–169.

Arendt G, Hefter H, Elsing C, Strohmeyer G, Freund HJ (1990). Motor dysfunction in HIV-infected patients without clinically detectable central-nervous deficit. *J Neurol* **237:** 362–368.

Bestetti A, Presi S, Pienotti C, Bossolasco S, Sala S, Racca S, Carrera P, Lazzarin A, Cinque P (2004). Long-term virological effect of highly active antiretroviral therapy on cerebrospinal fluid and relationship with genotypic resistance. *J NeuroVirol* **10(Suppl 1):** 52–57.

Brew B, Pemberton L, Cunningham P, Law M (1997). Levels of human immunodeficiency virus type 1 RNA in cerebrospinal fluid correlate with AIDS dementia stage. *J Inf Dis* **175:** 963–966.

CDC (1993). Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *JAMA* **269(6):** 729–730.

Chiodi F, Keys B, Albert J, Hagberg L, Lundeberg J, Uhlen M, Fenyo EM, Norkrans G (1992). Human immunodeficiency virus type I is present in the cerebrospinal fluid of a majority of infected individuals. *J Clin Microbiol* **30:** 1768–1771.

Cinque P, Sestetti A, Morelli P, Presi S (2000). Molecular analysis of cerebrospinal fluid: Potential for the study of HIV-1 infection of the central nervous system. *J NeuroVirol* **6(Suppl 1):** 95–102.

De Luca A, Ciancio BC, Larussa D, Murri R, Cingolati A, Rizzo MG, Giancola ML, Ammassari A, Ortona L (2002). Correlates of independent HIV-1 replication in the CNS and of its control by antiretrovirals. *Neurology* **59:** 342–347.

Ellis RJ, Childers ME, Zimmerman JD, Frost SD, Deutsch R, McCutchan JA (2003). Human immunodeficiency virus-l RNA levels in cerebrospinal fluid exhibit a set point in clinically stable patients not receiving antiretroviral therapy. *J Infect Dis* **187:** 1818–1821.

Ellis R, Gamst A, Capparelli E, Spector S, Hisa K, Wolfson T, Abramson I, Grant I, McCutchan Hsic (2000). Cerebrospinal fluid HIV RNA originates from both local CNS and systemic sources. *Neurology* **54:** 927–936.

Ellis RJ, Hsia K, Spector SA, Nelson JA, Heaton RK, Wallace MR, Abramson I, Atkinson JH, Grant I, McCutchan JA (1997). Cerebrospinal fluid human immunodeficiency virus type 1 RNA levels are elevated in neurocognitively impaired individuals with acquired immunodeficiency syndrome. HIV Neurobehavioral Research Center Group [see comments]. *Ann Neurol* **42:** 679–688.

Garcia F, Niebla G, Romeu J, Vidal C, Plana M, Ortega M, Ruiz L, Gallart T, Clotet B, Miro JM (1999). Cerebrospinal fluid HIV-l RNA levels in asymptomatic patients with early stage chronic HIV-l infection: support for the hypothesis of local virus replication. *AIDS* **13:** 1491–1496.

Gartner S (2000). HIV infection and dementia. *Science* **287:** 602–604.

Giancola ML, Lorenzini P, Balestra P, Larussa D, Baldini F, Corpolongo A, Narciso P, Bellagamba R, Tozzi V, Antinori A (2006). Neuroactive antiretroviral drugs do not influence neurocognitive performance in less advanced HIV-infected patients responding to highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* **41:** 332–337.

von Giesen HJ, Adams O, Koller H, Arendt G (2005). Cerebrospinal fluid HIV viral load in different phases of HIV-associated brain disease. *J Neurol* **252:** 801–807.

von Giesen HJ, Hefter H, Roick H, Mauss S, Arendt G (1994). HIV-specific changes in the motor performance of HIV-positive intravenous drug abusers. *J Neurol* **242:** 20–25.

Gisslén M, Fuchs D, Svennerholm B, Hagberg L (1999). Cerebrospinal fluid viral load, intrathecal immunoactivation, and cerebrospinal fluid monocytic cell count in HIV-l infection. *J Acquir Immune Defic Syndr* **21:** 271–276.

Gisslén M, Hagberg L, Fuchs D, Norkrans G, Svennerholm B (1998). Cerebrospinal fluid viral load in HIV-1-infected patients without antiretroviral treatment: a longitudinal study. *J Acquir Immune Defic Syndr Hum Retrovirol* **17:** 291–295.

Harrington PR, Haas DW, Ritola K, Swanstrom R (2005). Compartmentalized human immunodeficiency virus type 1 present in cerebrospinal fluid is produced by short-lived cells. *J Virol* **79:** 7959–7966.

[1]Klove H (1963). *Clinical neuropsychology*. New York: Saunders.

Korber BT, Kunstmann KJ, Patterson BK, Furtado M, McEvilly MM, Levy R, Wolinsky SM (1994). Genetic differences between blood- and brain-derived viral sequences from human immunodeficiency virus type 1-infected patients: evidence of conserved elements in the V3 region of the envelope protein brain-derived sequences. *J Virol* **68:** 7467–7481.

Lambotte O, Deiva K, Tardieu M (2003). HIV-l persistence, viral reservoir, and the central nervous system in the HAART era. *Brain Pathol* **13:** 95–103.

Marshall D, Brey R, Cahill W, Houk R, Zajac R, Boswell R (1988). Spectrum of cerebrospinal fluid findings in various stages of human immunodeficiency virus infection. *Arch Neurol* **45:** 954–958.

Martin C, Albert J, Hansson P, Pehrsson P, Link H, Sonnerborg A (1998). Cerebrospinal fluid mononuclear cell counts influence CSF HIV-l RNA levels. *J Acquir Immun Def Syndr Human Retrovirol* **17:** 214–219.

McArthur JC, McClemon DR, Cronin MF, Nance-Sproson TE, Saah AJ, St Clair M, Lanier ER (1997). Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and brain [see comments]. *Ann Neurol* **42:** 689–698.

Monteiro de Almeida S, Letendre S, Zimmerman J, Lazzaretto D, McCutchan A, Ellis R (2005). Dynamics of monocyte chemoattractant protein type one (MCP-1) and HIV viral load in human cerebrospinal fluid and plasma. *J Neuroimmunol* **169:** 144–152.

Neuenburg JK, Sinclair E, Nilsson A, Kreis C, Bacchetti P, Price RW, Grant RM (2004). HIV-producing T cells in cerebrospinal fluid. *J Acquir Immune Defic Syndr* **37:** 1237–1244.

Peeters MF, Colebunders RL, Van den Abbeele K, Nys PC, Goeman J, Colans P, Geerts Y, Van den Groen G (1995). Comparison of human immunodeficiency virus biological phenotypes isolated from cerebrospinal fluid and peripheral blood. *J Med Virol* **47:** 92–96.

Price RW (2000). The two faces of HIV infection of cerebrospinal fluid. *Trends Microbiol* **8:** 387–391.

[2]Price RW, Staprans S (l997). Measuring the "viral load" in cerebrospinal fluid in human immunodeficiency virus infection: window into brain infection? *Ann Neurol* **42:** 675–678.

[1]Price RW, Yiannoutsos CT, Clifford DB, Zaborski L, Tselis A, Sidtis JJ, Cohen B, Hall CD, Erice A, Henry K (1999). Neurological outcomes in late HIV infection: Adverse impact of neurological impairment on survival and protective effect of antiviral therapy. AIDS Clinical Trial Group and Neurological AIDS Research Consortium study team. *AIDS* **13:** 1677–1685.

[2]Reitan RM, Wolfson D (1985).

Sacktor NC, Lyles RH, Skolasky RL, Anderson DE, McArthur JC, McFarlane G, Selnes OA, Becker JT, Cohen B, Wesch J, Miller EN (1999). Combination antiretroviral therapy improves psychomotor speed performance in HIV-seropositive homosexual men. Multicenter AIDS Cohort Study (MACS). *Neurology* **52:** 1640–1647.

Scheller C, Sopper S, Jenuwein M, Neuen-Jacob E, Tatschner T, Grunblatt E, ter Meulen V, Riederer P, Koutsilieri E (2005). Early impairment in dopaminergic neurotransmission in brains of SIV-infected rhesus monkeys due to microglia activation. *J Neurochem* **95:** 377–387.

Sevigny JJ, Albert SM, McDermott MP, McArthur JC, Sacktor N, Conant K, Schifitto G, Selnes OA, Stern Y, McClernon DR, Palumbo D, Kieburtz K, Riggs G, Cohen B, Epstein LG, Marder K (2004). Evaluation of HIV RNA and markers of immune activation as predictors of HIV-associated dementia. *Neurology* **63:** 2084–2090.

Siliciano JD, Siliciano RF (2004). A long-term latent reservoir for HIV-l: discovery and clinical implications. *J Antimicrob Chemother* **54:** 6–9.

Spector SA, Hsia K, Pratt D, Lathey J, McCutchan JA, Alcaraz JE; Atkinson JH, Gulevich S, Wallace M, Grant I (1993). Virological markers of human immunodeficiency virus type I in cerebrospinal fluid. The HIV Neurobehavioral Research Center Group. *J Infect Dis* **168:** 68–74.

Spreen O, Strauss E (1998). *A compendium of neuropsychological tests,* 2nd edition. New York: Oxford University Press.

Spudich SS, Lollo ND, Liegler T, Deeks StG, Price RW (2006). Treatment benefit on cerebrospinal fluid HIV-1 levels in the setting of systemic virological suppression and failure. *JID* **194:** 1686–1696.

Spudich SS, Nilsson AC, Lollo ND, Liegler TJ, Petropoulos CJ, Deeks SG, Paxinos EE, Price RW (2005). Cerebrospinal fluid HIV infection and pleocytosis: Relation to systemic infection and antiretroviral treatment. *BMC Infect Dis* **5:** 98.

Staprans S, Marlowe N, Glidden D, Novakovic-Agopian T, Grant RM, Heyes M, Aweeka F, Deeks S, Price RW (1999). Time course of cerebrospinal fluid responses to antiretroviral therapy: Evidence for variable compartmentalization of infection. *AIDS* **13:** 1051–1061.

Stingele K, Haas J, Zimmermann T, Stingele R, Hubsch-Muller C, Freitag M, Storch-Hagenlocher B, Hartmann M, Wildemann B (2001). Independent HIV replication in paired CSF and blood viral isolates during antiretroviral therapy. *Neurology* **56:** 355–361.

[1]HAWIE-R Handbook and Instructions. Hans Huber Editions, 1991.

[2]The Halstead-Reitan Neuropsychological Test Battery. Tucson, AZ, Neuropsychological Press.