Journal of NeuroVirology, 7: 542–547, 2001
 ISSN 1355–0284/01 $12.00+.00

Increased blood–brain barrier permeability in neuro-asymptomatic HIV-1-infected individuals—correlation with cerebrospinal fluid HIV-1 RNA and neopterin levels

LM Andersson,[1] L Hagberg,[1] D Fuchs,[3] B Svennerholm,[2] and M Gisslén[1]

[1]*Institute of Internal Medicine, Department of Infectious Diseases, Sahlgrenska University Hospital, Göteborg, Sweden;*
[2]*Department of Clinical Virology, Göteborg University, Sahlgrenska University Hospital, Göteborg, Sweden; and*
[3]*Institute of Medical Chemistry and Biochemistry, and Ludwig-Boltzman-Institute for AIDS Research, University of Innsbruck, Innsbruck, Austria*

The objective of this study was to assess the frequency of blood–brain barrier (BBB) impairment, as measured by the albumin ratio, in neuro-asymptomatic HIV-1-infected individuals without antiretroviral treatment and the correlation between BBB disruption and intrathecal immune activation and HIV-1 RNA levels. Serum and cerebrospinal fluid (CSF) albumin, neopterin, and HIV-1 RNA levels were analysed in 110 neuro-asymptomatic HIV-1-infected individuals at different stages of disease; 63 classified as CDC A, 25 as CDC B, and 22 as CDC C. Increased BBB permeability was found in 17 of 110 (15%) of HIV-1-infected individuals. This proportion was sustained throughout the CDC stages. The albumin ratio was correlated with the CSF neopterin levels ($r_s = 0.36$, $P < 0.001$), the serum neopterin levels ($r_s = 0.37$, $P < 0.001$), and the CSF HIV-1 RNA levels ($r_s = 0.26$, $P < 0.01$), but not with the plasma HIV-1 RNA levels. The correlations between the albumin ratio and the CSF and serum neopterin concentrations and the CSF HIV-1 RNA levels indicate that immune activation and, possibly, intrathecal HIV-1 virus replication are important factors associated with increased BBB permeability in HIV-1 infection. *Journal of NeuroVirology* (2001) **7,** 542–547.

Keywords: HIV-1; cerebrospinal fluid; neopterin; HIV-1 RNA; blood–brain barrier; albumin ratio

Introduction

Human immunodeficiency virus type 1 (HIV-1) infects the central nervous system (CNS) early in the course of infection and HIV-1 has been detected in brain tissue (Davis *et al*, 1992) and cerebrospinal fluid (CSF) (Chiodi *et al*, 1988; Schacker *et al*, 1996) from patients with neurological complications as well as from patients without such. Approximately 15–20% of HIV-1-infected individuals with acquired immunodeficiency syndrome (AIDS) without antiretroviral treatment develop a subcortical dementia, HIV-1-associated dementia (HAD) (Trujillo *et al*, 1995). Approximately 50% of patients with HAD have neuropathological changes consistent with HIV-1 encephalitis. HIV-1 encephalitis is characterized by multinucleated giant cells, microglial nodules, perivascular inflammation, astrocytosis, myelin pallor, and neuronal loss (Navia *et al*, 1986; Budka, 1991; Kure *et al*, 1991; Wiley *et al*, 1991). It is associated with increasing CNS viral burden (Wiley and Achim, 1994) and the presence of activated macrophages and microglia (Wiley *et al*, 1986).

The exact pathogenic pathways that lead to neuronal injury in HIV-1 infection have not been established, but the fact that productive infection

Address correspondence to Lars-Magnus Andersson, Department of Infectious Diseases, Sahlgrenska University Hospital, S-416 85 Göteborg, Sweden. E-mail: lars-magnus.andersson@medfak.gu.se

Received 5 June 2001; accepted 7 August 2001.

with HIV-1 within the CNS is restricted to cells of the monocyte/macrophage lineage, microglia, and macrophages, and, possibly, endothelial cells (Wiley *et al*, 1986; Takahashi *et al*, 1996; An *et al*, 1999) suggests an indirect mechanism of neural injury mediated by neurotoxic substances. Signs of immune activation in the CNS have been found both in neuro-asymptomatic HIV-1-infected individuals and in HIV-1-infected patients with neurological complications (Fuchs *et al*, 1989; Andersson *et al*, 1998; Gisslen *et al*, 1999). Neopterin is a sensitive marker for activation of the cellular immune system. Intrathecal immune activation, as measured by elevated CSF neopterin levels, has been found in HIV-1-infected individuals with neurological complications, especially HAD (Fuchs *et al*, 1989). In fact, intrathecal immune activation has been described as the best prognostic marker for HAD (Brew *et al*, 1996).

Impaired blood–brain barrier (BBB) integrity is a common finding in HIV-1 infection (Marshall *et al*, 1988; Marshall *et al*, 1991; McArthur *et al*, 1992). The factors influencing BBB integrity in HIV-1 infection are not known, although immune activation and monocyte infiltration are associated with impairment of the BBB in patients with HAD (Boven *et al*, 2000; Persidsky *et al*, 2000). The BBB breakdown in HIV-1 infection seems to be chronic and slowly progressive as compared to the acute and rapid breakdown associated with, for example, multiple sclerosis or cerebrovascular accidents. Nevertheless, disruption of the BBB has been suggested to be the priming event in the pathogenesis of HAD, either by promoting viral entry or entry of serum-derived factors into the CNS. Serum factors such as gp120 or TNFα could be directly toxic to neurons, or they could activate macrophages/microglia within the CNS that in turn could secret neurotoxic substances (Power *et al*, 1993). The proportion of HIV-1-infected individuals with impaired BBB, which seems to increase with more advanced disease, has, in previous studies, been found to be 2–22% in asymptomatic individuals, 50% in patients with AIDS, and 100% in patients with HIV-1 encephalitis (Marshall *et al*, 1988; Petito and Cash, 1992; Power *et al*, 1993; Martin *et al*, 1998; Dallasta *et al*, 1999).

In this study, we have analyzed the correlation between the integrity of the BBB as measured by the albumin ratio and CSF and plasma levels of neopterin and HIV-1 RNA in 110 neuro-asymptomatic HIV-1 infected individuals without antiretroviral treatment.

Results

An elevated albumin ratio was found in 17 of 110 patients (15%), 10 of 63 classified as CDC A (16%), 3 of 25 classified as CDC B (12%), and 4 of 22 classified as CDC C (18%). In the 110 patients, the albumin ratio was correlated to the CSF neopterin levels ($r_s = 0.36$, $P < 0.001$) (Figure 1), the CSF HIV-1 RNA levels ($r_s = 0.26$, $P < 0.01$) (Figure 2), the CSF mononuclear cell count ($r_s = 0.26$, $P < 0.01$), and the serum neopterin levels ($r_s = 0.37$, $P < 0.001$), but there was no correlation between the albumin ratio and the plasma HIV-1 RNA levels (Table 1).

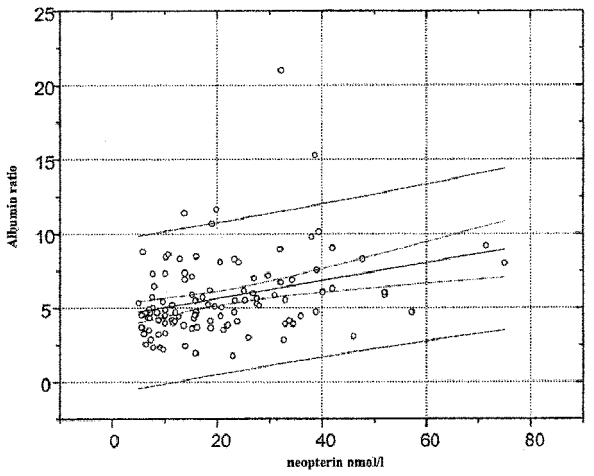

Figure 1 Correlation between the albumin ratio and the cerebrospinal fluid neopterin levels ($r_s = 0.36$, $P < 0.001$) (n = 110). 95% confidence intervals are indicated for data and line, respectively.

Plasma and CSF HIV-1 RNA levels were correlated ($r_s = 0.49$, $P < 0.001$) as were the CSF neopterin and CSF HIV-1 RNA levels ($r_s = 0.41$, $P < 0.001$), the CSF mononuclear cell count and CSF HIV-1 RNA levels ($r_s = 0.42$, $P < 0.001$), the CSF mononuclear cell count and CSF neopterin levels ($r_s = 0.23$, $P < 0.01$), and CSF and serum neopterin levels ($r_s = 0.62$, $P < 0.001$) (Table 1).

Patients with an elevated albumin ratio had a mean (±SD) serum neopterin concentration of 31 (±23) nmol/l, and CSF neopterin of 29 (±20) nmol/l

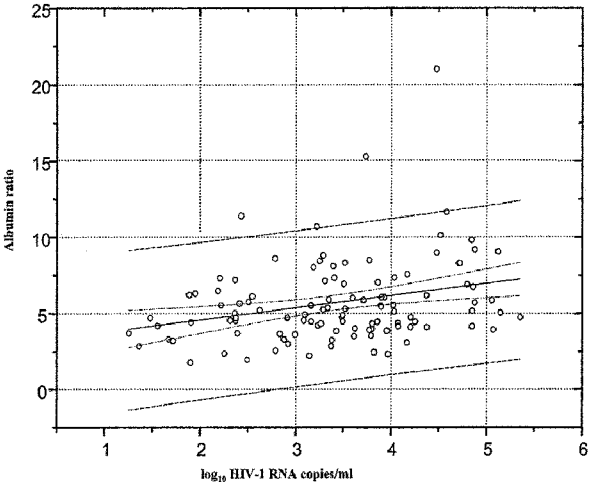

Figure 2 Correlation between the albumin ratio and the cerebrospinal fluid HIV-1 RNA levels ($r_s = 0.26$, $P < 0.01$) (n = 110). 95% confidence intervals are indicated for data and line, respectively.

Table 1 Correlations between the albumin ratio, cerebrospinal fluid (CSF) and plasma HIV-1 RNA levels, CSF and serum neopterin levels, and CSF mononuclear cell count

	CSF HIV-1 RNA	*Plasma HIV-1 RNA*	*CSF neopterin*	*Serum neopterin*	*CSF mononuclear count cell*	*Albumin ratio*
Albumin ratio	$r_s = 0.26$ $P < 0.01$ (n = 110)	No correlation (n = 104)	$r_s = 0.36$ $P < 0.001$ (n = 110)	$r_s = 0.37$ $P < 0.001$ (n = 101)	$r_s = 0.26$ $P < 0.01$ (n = 110)	$r_s = 1.0$ $P = 0.0$ (n = 110)
CSF HIV-1 RNA	$r_s = 1.0$ $P = 0.0$ (n = 110)	$r_s = 0.49$ $P < 0.001$ (n = 104)	$r_s = 0.41$ $P < 0.001$ (n = 110)	No correlation (n = 101)	$r_s = 0.42$ $P < 0.001$ (n = 110)	$r_s = 0.26$ $P < 0.01$ (n = 110)
CSF neopterin	$r_s = 0.41$ $P < 0.001$ (n = 110)	$r_s = 0.35$ $P < 0.001$ (n = 104)	$r_s = 1.0$ $P = 0.0$ (n = 110)	$r_s = 0.62$ $P < 0.001$ (n = 101)	$r_s = 0.23$ $P < 0.01$ (n = 110)	$r_s = 0.36$ $P < 0.001$ (n = 110)

in comparison to patients with a albumin ratio below the reference value who had a mean (±SD) serum neopterin concentration of 19 (±13) nmol/l ($P < 0.07$), and CSF neopterin of 19 (±13) nmol/l ($P < 0.1$). As shown, there were no significant differences between the two groups. No significant differences were found between the plasma or CSF HIV-1 RNA levels in the two groups. In patients with an elevated albumin ratio, CSF HIV-1 RNA and plasma RNA were correlated ($r_s = 0.64$, $P < 0.01$) [data not shown].

Discussion

BBB dysfunction, as measured by an elevated albumin ratio above the reference value according to age, was found in 17 of 110 (15%) neuroasymtomatic HIV-1-infected individuals without antiretroviral treatment. The proportion of patients with an elevated albumin ratio did not vary between CDC stages.

The relatively high proportion of asymptomatic HIV-1-infected individuals with an elevated albumin ratio is in contrast to the finding by Marshall *et al* (1988), who reported an elevated albumin ratio in 2% of asymptomatic HIV-1-infected individuals, but in concordance with the finding by Martin *et al* of an elevated albumin ratio in 22% of neuroasymptomatic HIV-1 infected individuals (Martin *et al*, 1998). Moreover, Marshall *et al* (1991) found a progressive elevation of the albumin ratio over time, and other investigators have found signs of increased BBB permeability in 50% of AIDS patients (Petito and Cash, 1992; Power *et al*, 1993). The low frequency of BBB impairment in asymptomatic HIV-1-infected individuals found by Marshall *et al* might thus be explained by the facts that patients were examined very early in the course of infection (Marshall *et al*, 1988, 1991) and that a more conservative definition of an elevated albumin ratio (>9) was used, although the study population had a mean age of less than 45 years, which is in contrast to the reference values established by Blennow *et al* (1993).

The albumin ratio was correlated with the CSF and serum neopterin and CSF HIV-1 RNA levels but not with the plasma HIV-1 RNA levels. Also, the CSF and serum neopterin levels were correlated. Patients with an elevated albumin ratio had higher mean CSF and serum neopterin concentrations than patients with an albumin ratio below the reference value. Although these differences did not reach statistical significance, they indicate that cellular immune activation is an important factor associated with BBB dysfunction in HIV-1 infection. A correlation between the albumin ratio and the CSF neopterin levels has been found in viral meningitis (Furukawa *et al*, 1992), but several investigators have failed to show such a correlation in HIV-1-infected patients at various stages of the disease (Sonnerborg *et al*, 1989; Brew *et al*, 1990). In fact, markedly elevated CSF neopterin levels have been found in HIV-1-infected individuals with neurological complications, and CSF neopterin has been proposed to be the best prognostic marker for HAD (Fuchs *et al*, 1989; Brew *et al*, 1996; Andersson *et al*, 1999). However, none of these studies included a large number of neuroasymtomatic HIV-1-infected individuals. Because the studies by Brew *et al* (1990) and Sönnerborg *et al* (1989) included large proportions of HIV-1-infected individuals with neurological complications, high CSF neopterin concentrations would be expected. Fuchs *et al* (1989) reported a correlation between CSF and serum neopterin, but there was also additional intrathecal production of neopterin in individuals with neurological complications.

The association is strong between neurological symptoms, especially HAD, in HIV-1-infected individuals and increased BBB permeability. In an autopsy study, Power *et al* found significant BBB impairment in 12 of 12 brains from patients with HAD and suggested that increased BBB permeability might be the priming event in the development of HAD (Power *et al*, 1993).

The CSF mononuclear cell count was, as previously observed (Hagberg *et al*, 1993; Martin *et al*, 1998; Gisslen *et al*, 1999), correlated to CSF HIV-1 RNA levels and CSF neopterin levels. The correlation

found between the albumin ratio and the CSF mononuclear cell count is in concordance with the finding by Persidsky *et al* (2000) of mononuclear cell infiltration in areas with disrupted BBB. As shown previously, there was a correlation between CSF neopterin and CSF HIV-1 RNA levels (Gisslen *et al*, 1999). We also found a correlation between CSF and plasma HIV-1 RNA levels. Data regarding the correlation between CSF and plasma HIV-1 RNA levels are conflicting. Some investigators have reported a correlation between CSF and plasma HIV-1 RNA, whereas others have failed to show any correlation (McArthur *et al*, 1997; Foudraine *et al*, 1998). We found, in an earlier study, CSF HIV-1 RNA levels exceeding plasma HIV-1 RNA levels in 20% of HIV-1-infected individuals not treated with antiretroviral therapy (Gisslen *et al*, 1999). The source of HIV-1 RNA in the CSF is not fully understood. The CSF viral load might originate from a productive infection within the brain parenchyma; a local production by meningeal macrophages; and/or a production by mononuclear cells trafficking into the CSF from blood. It is probable that combinations of these sources contribute and play different roles at different stages of the disease.

RNA is unstable and sensitive to storage at room temperature. Although the CSF samples used in this study in some cases were stored for up to 10 years before analysis with quantitative HIV-1 RNA PCR, the samples were centrifuged and stored immediately at -70°C until analyzed, which makes gradual degeneration of RNA during storage unlikely because the stability of cell-free virion RNA at -70°C is well documented (Coombs *et al*, 1993; Sebire *et al*, 1998), and RNA degradation is reduced by immediate centrifugation of samples (Katzenstein *et al*, 1996). In conclusion, we found an elevated albumin ratio in a relatively high proportion of neuro-asymptomatic HIV-1 infected individuals without antiretroviral treatment (15%). The albumin ratio was correlated with the CSF and serum neopterin concentrations and with the CSF HIV-1 RNA levels but not with the plasma HIV-1 RNA levels, indicating that cellular immune activation and, possibly, intrathecal HIV-1 virus replication are important factors associated with increased BBB permeability in HIV-1 infection.

Ethics

This study was approved by the research ethics committee at Göteborg University, Sweden, and all patients gave their informed consent.

Materials and methods

Patients

Between 1986 and 1999, paired blood and CSF samples were collected from 110 neuroasymtomatic HIV-1-infected individuals, 87 men and 23 women, without antiretroviral treatment. All patients were antiretroviral naïve and no patient had a history or signs/symtoms of neurological or major psychiatric complications. Mean age at the time of sampling was 38 years (range 18 to 71). The study population included 43 men and 17 women with heterosexually transmitted disease and 36 men with homosexual transmission. Three men and one woman had been infected through blood products, and five women and three men through intravenous drug use. The mode of transmission was unknown in two men. Sixty men and 14 women were of European, 19 men and 5 women of African, 7 men and 2 women of Asian, and 1 man and 2 women of South American origin. The mean CD4 cell count at the time of sampling was 302 (range 9 to 1664) $\times 10^6$/l. Sixty-three patients had an asymptomatic HIV-1 infection, Center for Disease Control and Prevention (CDC) stage A1-A3, 25 had a symptomatic infection not classified as AIDS (CDC B1-B3), and 22 had AIDS (CDC C2-C3) (MMWR, 1992). The mean CSF HIV-1 RNA level was 3.43 (range 1.26 to 5.35) $\log^{10}$ copies/ml, plasma HIV-1 RNA 4.21 (range 2.07 to 5.79) $\log^{10}$ copies/ml, CSF neopterin 21 (range 5 to 75) nmol/l, serum neopterin 21 (range 4 to 85) nmol/l, and the albumin ratio 5.7 (range 1.8 to 21) at the time of sampling.

Methods

Lumbar puncture was performed in a standardized manner whereby a total of 20–25 ml CSF was collected in portions. After cell counting, the first 12-ml portion was centrifuged and stored immediately in smaller fractions at -70°C until analyzed. Samples with a red blood cell count $>30 \times 10^9$/l were discarded. Paired blood samples were collected at the same time and stored in the same way until analyzed. HIV-1 RNA was analyzed in plasma and CSF by quantitative HIV-1 RNA PCR (Amplicor HIV-1 monitor, version 1.5, Roche Diagnostic Systems, Hoffman La Roche Inc., Basel, Switzerland) and run according to the protocol of the manufacturer. HIV-1 RNA levels were expressed as $\log^{10}$ copies of HIV-1 RNA/ml. Serum and CSF neopterin levels were analyzed by a commercially available radioimmunoassay (neopterin, BRAHMS, Berlin, Germany) and run according to the protocol of the manufacturer. The normal reference value at the study laboratory was <4.2 nmol/l in CSF and <8.8 nmol/l in serum. Quantitative determination of albumin in serum and CSF was performed by nephelometry (Behring Nelphelometer Analyzer, Behringwerke AG, Marburg, Germany). The CSF/serum albumin ratio was calculated as [CSF albumin (mg/l)/serum albumin (g/l)] and used as a measure of BBB function (Tibbling *et al*, 1977). The reference value at the study laboratory was <6.5 in individuals below the age of 45 years and <10.2 in individuals above the age of 45 years (Blennow *et al*, 1993).

The peripheral CD4 cell count was measured by direct immunoflourescence on a flow cytometer (FACS, Becton Dickinson, Mountain View, CA, USA).

Statistics

Statistical evaluations of differences between independent samples were analysed by means of Mann–Whitney *U*-test. Spearman's rank correlation coefficient was used to evaluate correlations. *P*-values <0.01 were regarded as significant.

Acknowledgements

This study was supported by grants from the Göteborg Society of Medicine, Göteborg, Sweden, from the Medical Faculty, Göteborg University, Göteborg, Sweden, and from the Austrian Ministry of Social Affairs and Generations, Austria.

References

An SF, Groves M, Giometto B, Beckett AA, Scaravilli F (1999). Detection and localisation of HIV-1 DNA and RNA in fixed adult AIDS brain by polymerase chain reaction/in situ hybridisation technique. *Acta Neuropathol (Berl)* **98:** 481–487.

Andersson L, Blennow K, Fuchs D, Svennerholm B, Gisslen M (1999). Increased cerebrospinal fluid protein tau concentration in neuro-AIDS. *J Neurol Sci* **171:** 92–96.

Andersson LM, Fredman P, Lekman A, Rosengren L, Gisslen M (1998). Increased cerebrospinal fluid ganglioside GD3 concentrations as a marker of microglial activation in HIV type 1 infection. *AIDS Res Hum Retroviruses* **14:** 1065–1069.

Blennow K, Fredman P, Wallin A, Gottfries CG, Karlsson I, Langstrom G, Skoog I, Svennerholm L, Wikkelso C (1993). Protein analysis in cerebrospinal fluid. II. Reference values derived from healthy individuals 18–88 years of age. *Eur Neurol* **33:** 129–133.

Boven LA, Middel J, Verhoef J, De Groot CJ, Nottet HS (2000). Monocyte infiltration is highly associated with loss of the tight junction protein zonula occludens in HIV-1-associated dementia.*Neuropathol Appl Neurobiol* **26:** 356–360.

Brew BJ, Dunbar N, Pemberton L, Kaldor J (1996). Predictive markers of AIDS dementia complex: CD4 cell count and cerebrospinal fluid concentrations of beta 2-microglobulin and neopterin. *J Infect Dis* **174:** 294–298.

Brew BJ, Bhalla RB, Paul M, Gallardo H, McArthur JC, Schwartz MK, Price RW (1990). Cerebrospinal fluid neopterin in human immunodeficiency virus type 1 infection. *Ann Neurol* **28:** 556–560.

Budka H (1991). Neuropathology of human immunodeficiency virus infection. *Brain Pathol* **1:** 163–175.

Chiodi F, Sonnerborg A, Albert J, Gaines H, Norkrans G, Hagberg L, Asjo B, Strannegard O, Fenyo EM (1988). Human immunodeficiency virus infection of the brain. I. Virus isolation and detection of HIV specific antibodies in the cerebrospinal fluid of patients with varying clinical conditions. *J Neurol Sci* **85:** 245–257.

Coombs RW, Henrard DR, Mehaffey WF, Gibson J, Eggert E, Quinn TC, Phillips J (1993). Cell-free plasma human immunodeficiency virus type 1 titer assessed by culture and immunocapture-reverse transcription-polymerase chain reaction. *J Clin Microbiol* **31:** 1980–1986.

Dallasta LM, Pisarov LA, Esplen JE, Werley JV, Moses AV, Nelson JA, Achim CL (1999). Blood–brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. *Am J Pathol* **155:** 1915–1927.

Davis LE, Hjelle BL, Miller VE, Palmer DL, Llewellyn AL, Merlin TL, Young SA, Mills RG, Wachsman W, Wiley CA (1992). Early viral brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology* **42:** 1736–1739.

Foudraine NA, Hoetelmans RM, Lange JM, de Wolf F, van Benthem BH, Maas JJ, Keet IP, Portegies P (1998). Cerebrospinal-fluid HIV-1 RNA and drug concentrations after treatment with lamivudine plus zidovudine or stavudine. *Lancet* **351:** 1547–1551.

Fuchs D, Chiodi F, Albert J, Asjo B, Hagberg L, Hausen A, Norkrans G, Reibnegger G, Werner ER, Wachter H (1989). Neopterin concentrations in cerebrospinal fluid and serum of individuals infected with HIV-1. *AIDS* **3:** 285–288.

Furukawa Y, Nishi K, Kondo T, Tanabe K, Mizuno Y (1992). Significance of CSF total neopterin and biopterin in inflammatory neurological diseases. *J Neurol Sci* **111:** 65–72.

Gisslen M, Fuchs D, Svennerholm B, Hagberg L (1999). Cerebrospinal fluid viral load, intrathecal immunoactivation, and cerebrospinal fluid monocytic cell count in HIV-1 infection. *J Acquir Immune Defic Syndr* **21:** 271–276.

Hagberg L, Dotevall L, Norkrans G, Larsson M, Wachter H, Fuchs D (1993). Cerebrospinal fluid neopterin concentrations in central nervous system infection. *J Infect Dis* **168:** 1285–1288.

Katzenstein TL, Pedersen C, Nielsen C, Lundgren JD, Jakobsen PH, Gerstoft J (1996). Longitudinal serum HIV RNA quantification: correlation to viral phenotype at seroconversion and clinical outcome. *AIDS* **10:** 167–173.

Kure K, Llena JF, Lyman WD, Soeiro R, Weidenheim KM, Hirano A, Dickson DW (1991). Human immunodeficiency virus-1 infection of the nervous system: an autopsy study of 268 adult, pediatric, and fetal brains. *Hum Pathol* **22:** 700–710.

Marshall DW, Brey RL, Butzin CA, Lucey DR, Abbadessa SM, Boswell RN (1991). CSF changes in a longitudinal study of 124 neurologically normal HIV-1-infected U.S. Air Force personnel. *J Acquir Immune Defic Syndr* **4:** 777–781.

Marshall DW, Brey RL, Cahill WT, Houk RW, Zajac RA, Boswell RN (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-1 RNA levels. *J Acquir Immune Defic Syndr Hum Retrovirol* **17:** 214–219.

McArthur JC, McClernon 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. *Ann Neurol* **42:** 689–698.

McArthur JC, Nance-Sproson TE, Griffin DE, Hoover D, Selnes OA, Miller EN, Margolick JB, Cohen BA, Farzadegan H, Saah A (1992). The diagnostic utility of elevation in cerebrospinal fluid beta 2-microglobulin in HIV-1 dementia. Multicenter AIDS Cohort Study. *Neurology* **42:** 1707–1712.

MMWR (1992). 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep* **41:** 1–19.

Navia BA, Cho ES, Petito CK, Price RW (1986). The AIDS dementia complex: II. Neuropathology. *Ann Neurol* **19:** 525–535.

Persidsky Y, Zheng J, Miller D, Gendelman HE (2000). Mononuclear phagocytes mediate blood-brain barrier compromise and neuronal injury during HIV-1-associated dementia. *J Leukoc Biol* **68:** 413–422.

Petito CK, Cash KS (1992). Blood-brain barrier abnormalities in the acquired immunodeficiency syndrome: immunohistochemical localization of serum proteins in postmortem brain. *Ann Neurol* **32:** 658–666.

Power C, Kong PA, Crawford TO, Wesselingh S, Glass JD, McArthur JC, Trapp BD (1993). Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alterations of the blood-brain barrier. *Ann Neurol* **34:** 339–350.

Schacker T, Collier AC, Hughes J, Shea T, Corey L (1996). Clinical and epidemiologic features of primary HIV infection. *Ann Intern Med* **125:** 257–264.

Sebire K, McGavin K, Land S, Middleton T, Birch C (1998). Stability of human immunodeficiency virus RNA in blood specimens as measured by a commercial PCR-based assay. *J Clin Microbiol* **36:** 493–498.

Sonnerborg AB, von Stedingk LV, Hansson LO, Strannegard OO (1989). Elevated neopterin and beta 2-microglobulin levels in blood and cerebrospinal fluid occur early in HIV-1 infection. *AIDS* **3:** 277–283.

Takahashi K, Wesselingh SL, Griffin DE, McArthur JC, Johnson RT, Glass JD (1996). Localization of HIV-1 in human brain using polymerase chain reaction/*in situ* hybridization and immunocytochemistry. *Ann Neurol* **39:** 705–711.

Tibbling G, Link H, Ohman S (1977). Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. *Scand J Clin Lab Invest* **37:** 385–390.

Trujillo JR, Garcia-Ramos G, Novak IS, Rivera VM, Huerta E, Essex M (1995). Neurologic manifestations of AIDS: a comparative study of two populations from Mexico and the United States. *J Acquir Immune Defic Syndr Hum Retrovirol* **8:** 23–29.

Wiley CA, Achim C (1994). Human immunodeficiency virus encephalitis is the pathological correlate of dementia in acquired immunodeficiency syndrome. *Ann Neurol* **36:** 673–676.

Wiley CA, Masliah E, Morey M, Lemere C, DeTeresa R, Grafe M, Hansen L, Terry R (1991). Neocortical damage during HIV infection. *Ann Neurol* **29:** 651–657.

Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MB (1986). Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci USA* **83:** 7089–7093.