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SHORT COMMUNICATION

Prevalence and predictors of blood-brain barrier damage in the HAART era

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Abstract Blood-brain barrier damage (BBBD) is prevalent in HIV-positive patients and may enhance cell trafficking to the central nervous system. A retrospective analysis in adult HIV-positive patients with no central nervous system disease was conducted in order to estimate the prevalence and risk factors of BBBD (according to cerebrospinal fluid to plasma albumin ratios). One hundred fifty-eight HIV-positive adult patients were included. BBBD impairment and intrathecal IgG synthesis were respectively observed in 45 (28.5 %) and 100 patients (63.3 %). Low CD4 nadir and high CSF HIV RNA were independently associated with both abnormalities. BBBD is common in HIV-positive patients, and its main determinants are advanced immune depression and compartmental viral replication.

Keywords Antiretroviral · Blood-brain barrier · CD4 nadir

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Introduction

Early in the course of HIV disease, the retrovirus invades the central nervous system and causes a chronic, progressive astrocytes and microglia infection eventually damaging neurons. Blood-brain barrier (BBB) impairment has been considered as a key event in the pathogenesis of AIDS dementia complex and other HIV-related neurological complications (Abdulle et al. 2005). BBB alterations were found in 2 to 22 % HIV-positive asymptomatic individuals, in about 50 % of patients with AIDS and in 100 % of patients with HIV-associated dementia (HAD) (Marshall et al. 1988; Petito and Cash 1992; Andersson et al. 2001). Several mechanisms have been involved including pro-inflammatory cytokines secretion, the effect of secreted viral proteins and direct infection of endothelial cells by HIV (Ivey et al. 2009).

It is not clear to what extent antiretroviral treatment can block these mechanisms and therefore reverse BBB impairment. Among untreated HIV-infected individuals, elevated BBB permeability, as reflected by higher CSF to serum albumin ratios (CSARs), could allow HIV and neurotoxins to access the CNS. Higher CSARs have been associated with longer duration and more severe HIV disease and can contribute to neurological symptoms in HIV-infected individuals. Among treated individuals, increased BBB permeability could allow higher concentrations of antiretroviral therapy (ART) to reach the CNS; this has been shown for raltegravir, tenofovir and emtricitabine (Yilmaz et al. 2009; Calcagno et al. 2011, 2014). Data from the CHARTER group suggested that age and lower CSAR are independent predictors of neurocognitive impairment (Letendre, CROI 2011, unpublished).

Patients/methods

Adult HIV-positive patients undergoing lumbar punctures for clinical reasons or in the context of longitudinal studies were included; patients with central nervous system opportunistic infections or neoplasms were excluded. Written informed consent was obtained from all study participants, and the study was approved by a local ethics committee. Demographic, immunovirological and therapeutic data were recorded.

Quantitative determination of albumin and IgG in serum and CSF was performed by nephelometry (Behring Nephelometer Analyser, Behringwerke AG, Marburg, Germany). The CSAR, calculated as CSF albumin (mg/L)/serum albumin (g/L), was used to evaluate BBB function. Blood-brain barrier damage definition was derived from age-adjusted Reibergrams (Reiber 1995). The IgG index is calculated through the following formula: $IgG_{CSF}/IgG_{serum} \times Albumin_{serum}/Albumin_{CSF}$. The reference value for the IgG index was <0.63 (Caroscio et al. 1986). The CNS IgG synthesis, evaluated according to the following formula: $[(IgG_{CSF} - IgG_{serum}/369) - (Albumin_{CSF} - Albumin_{serum}/230) \times (IgG_{serum}/Albumin_{serum}) \times 0.43] \times 5$, reported reference values for this parameter ranging from −9.9 to 3.3 mg/day (Tourtelotte and Ma 1978).

Cerebrospinal fluid penetration effectiveness (CPE) score was calculated by adding the individual drug CPE scores according to the work of Letendre and colleagues. (Letendre, CROI 2010, unpublished).

Data were analyzed using standard statistical methods; variables were described with medians (interquartile ranges (IQRs)), and they were compared using non-parametric tests (Mann-Whitney, chi-square and Spearman's test as specified in the text). A multivariate logistic regression was performed including all variables with a $p<0.20$ at univariate analysis. Data analysis was performed using SPSS software for Mac (version 20.0, IBM Corp.).

Results

One hundred fifty-eight subjects were included [mainly male (113, 71.5 %), Caucasian (121, 76.6 %), of median age of 45 years (IQR 39–52)]. Risk factors for HIV infections were reported as follows: 50 patients (31.6 %) reported intravenous drug use, while 38 (24.1 %) and 11 (7 %) declared respectively homo- or heterosexual intercourses. HCV antibodies were present in 36 patients (22.8 %), while chronic HBV infection was present in 14 subjects (8.9 %); 13 patients (8.2 %) had liver cirrhosis. A diagnosis of previous syphilis was performed in 22 patients (13.9 %), while psychiatric disorders were concomitant in 24 subjects (15.4 %).

Lumbar punctures were performed mainly in asymptomatic subjects enrolled in longitudinal studies [45 patients (28.5 %)], for HIV-associated neurocognitive disorders [36 patients (22.7 %)] and for late presentation with CD4 cell count below 100/μL and opportunistic non-CNS-involving infections [46 patients (29.1 %)], while in 26 patients (16.5 %), spinal tabs were performed for differential diagnosis of other clinical conditions (epilepsy, hepatic or vascular encephalopathy, sudden onset of psychiatric disorders).

The estimated median duration of HIV infection was 31.3 months (IQR, 0.6–184.4) with median current and CD4 nadir counts, respectively, of 165/μL (IQR, 47–439) and 64/μL (IQR, 24–169). Median plasma viral load was 1.67 Log_{10} copies/mL (IQR, 1.28–5.28) with CSF viral load of 1.81 Log_{10} copies/mL (IQR, 1.28–3.21). One hundred fourteen (72.2 %) patients were on highly active antiretroviral treatment (HAART) regimens including mostly three drugs [$n=83$, protease inhibitor (PI)-based 64 (77.1 %), NNRTI-based 10 (12 %) or raltegravir-based 7 (8.4 %)]; a few were receiving four ARVs [$n=13$, intensified with RAL (8) or MVC (2)] or dual PI-based regimens [$n=13$, plus raltegravir (8), etravirine (4) or maraviroc (1)].

In HAART-treated patients, plasma HIV RNA was below 50 copies/mL in 79 patients (69.2 %), while CSF HIV RNA was below 50 copies/mL in 75 subjects (65.2 %); median time on HAART was 12 months (IQR, 5.9–27). Eighty-three patients (52.5 %) had a CPE score above 6 [median of 7 (6–8)]. R5-tropic viruses were detected in the plasma in 47/65 patients (72.3 %) and in the CSF in 31/37 subjects (83.7 %); a detectable CSF EBV DNA was found in nine patients (5.7 %). CSF cellularity and biochemical abnormalities were observed in a few patients. CSF white blood cells were above 5/μL in 11 subjects [6.9 %, median 30 cells/μL (20–40)]. Median protein CSF level was 42 mg/dL (IQR, 34–54.5), and median glucose level was 54 mg/dL (IQR, 48–57).

Median CSF to serum albumin ratio was 5.2 (IQR, 3.8–6.8) as depicted in Fig. 1; an altered blood-brain barrier was found in 45 patients (28.5 %). BBB impairment was not significantly different in HAART-treated (28/114, 24.6 %) versus HAART-naïve subjects (17/44, 38.6 %; $p=0.08$).

Median IgG ratio was 4.5 (IQR, 2.9–6.8), and IgG index (Tibbling) was 0.7 (IQR, 0.6–1.1); the latter was altered in 100 patients (63.3 %). Local IgG synthesis was identified in 66 patients (41.8 %); in these subjects, 39 % (19–62) of IgGs were produced locally. Reibergram-defined inflammatory patterns were noted in 43 patients (27.2 %). Elevated IgG index prevalence was not significantly different in HAART-treated (69/114, 60.5 %) versus HAART-naïve subjects (31/44, 70.5 %; $p=0.27$).

Ten patients (6.3 %) presented with CD4 nadir above 200 cells/μL, plasma HIV RNA below 50 copies/mL since at least 12 months and current CD4 above 350 cells/μL: median CSAR and IgG index were 3.8 (3.6–5.4) and 0.35 (0.16–0.51), respectively. Blood-brain barrier damage (BBBD), elevated IgG index and CNS IgG synthesis were present in one (10 %), zero (0 %) and three patients (30 %).

Considering CSAR and IgG index as continuous variable, two multivariate logistic regression analyses were performed

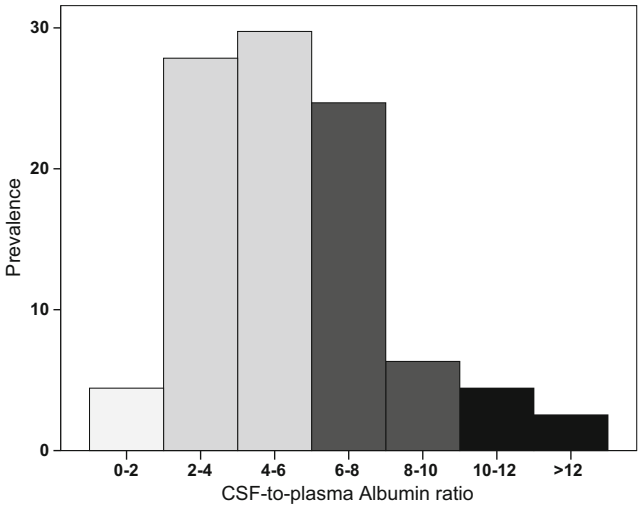

Fig. 1 Histogram of CSAR distribution

including age, CD4 nadir, years since HIV diagnosis, current CD4, plasma HIV RNA, CSF HIV RNA, HCV positivity and previous syphilis. CD4 nadir (p=0.012, aBeta −2.56, 95 % confidence interval (CI) −0.19 to −0.02) and CSF HIV RNA (p=0.018, Beta 2.41, 95 % CI 0.13–1.35) were independently associated with CSAR. CD4 nadir (p=0.011, Beta −2.59, 95 % CI −0.02 to 0.00), current CD4 (p=0.04, Beta 2.08, 95 % CI 0.00–0.02) and CSF HIV RNA (p<0.001, Beta 4.34, 95 % CI 0.06–0.18) were independently associated with IgG index.

Demographic, immunovirological and therapeutic factors according to BBB impairment are described in Table 1. At univariate analysis, CD4 nadir cell count below 200/μL (p=0.01), anti-HCV positivity (p=0.03), suppressed HIV viremia (p=0.02) and control of HIV viremia for more than 1 year (p=0.05) were associated with blood-brain barrier damage, while male gender (p=0.06) and detectable EBV DNA in the CSF (p=0.06) were borderline significant. At multivariate logistic regression analysis, CD4 nadir below 200 cells/μL was the only independent predictor of BBBD (p=0.036, Beta=9.02, 95 CI 1.15–70.87). Blood-brain barrier impairment was found in 56.1 % of patients with CD4 nadir <200/μL and in 12.4 % of patients with CD4 nadir >200/μL (Fig. 2). At univariate analysis, CD4 nadir cell count below 200/μL (p=0.004), CSF HIV RNA below 50 copies/mL (p=0.008) and plasma HIV RNA undetectability for more than 1 year (borderline, p=0.08) were associated with altered IgG index; nevertheless, the only independent predictor of abnormal IgG index was CD4 nadir below 200/μL (p=0.003, Beta 4.685, 95 CI 1.68–13.02).

Discussion

In our study, 24.6 and 38.6 % of treated and untreated patients, respectively, have a significant blood-brain barrier alteration. This finding has a possible double clinical implication. On one

Table 1 Demographic, immunovirological and therapeutic factors in patients with intact or damaged blood-brain barrier

	Intact BBB (n=113)	BBB damage (n=45)	p-values
Age (years)	45 (39–53)	45 (38–51)	0.64
Gender, male	76 (67.3 %)	37 (82.2 %)	0.06
Ethnicity, Caucasian	87 (77 %)	34 (75.6 %)	0.52
HCV antibody positive	31 (27.4 %)	5 (11.1 %)	0.03
Duration of HIV infection (months)	45.8 (1–188)	14 (1–183)	0.63
CD4 nadir (cell/μL)	81 (24–198)	42 (22.5–111)	0.03
Current CD4 (cell/μL)	194 (65–453)	104 (30–335)	0.08
On HAART	86 (76.1 %)	28 (62.2 %)	0.08
Plasma HIV RNA (Log_{10} copies/mL)	1.52 (<1.28–4.99)	2.25 (<1.28–5.51)	0.12
HIV RNA <50 copies/mL	63 (55.8 %)	16 (35.6 %)	0.02
HIV RNA <50 copies/mL for >12 months	23 (24.5 %)	3 (8.6 %)	0.05
CSF HIV RNA (Log_{10} copies/mL)	1.75 (<1.28–2.81)	2.11 (<1.28–3.93)	0.20
CSF HIV RNA <50 copies/mL	55 (48.7 %)	20 (44.4 %)	0.63
Detectable CSF EBV DNA	4 (3.5 %)	5 (11.1 %)	0.06
CPE score (in those on HAART)	7 (6–8)	7 (7–9)	0.28
CSF to plasma albumin ratio	4.4 (3.7–5.5)	7.9 (7–9.9)	<0.001
CSF to plasma IgG ratio	0.34 (0.17–0.50)	0.67 (0.46–0.86)	<0.001

Variables are described with number and percentage (categorical) or medians and interquartile ranges (numerical). Comparison were obtained by chi-square and Mann-Whitney tests

BBB blood-brain barrier, *CSF* cerebrospinal fluid, *HAART* highly active antiretroviral treatment, *CPE* CSF penetration effectiveness score, *EBV* Epstein-Barr virus

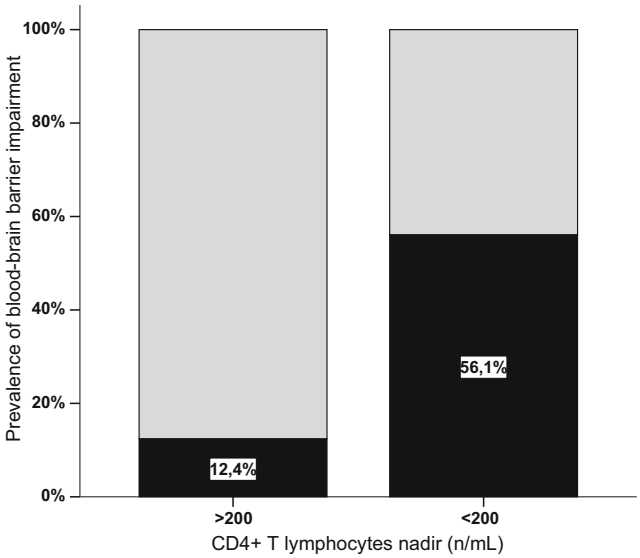

Fig. 2 BBB impairment according to CD4+ T lymphocyte nadir (above or below 200/mL)

hand, a correlation between BBB alteration and HIV-associated neurocognitive disorders has been recently proposed, where the underlying mechanism could be enhanced by cell trafficking or neurotoxins penetration. On the other hand impaired BBB can affect the pattern of antiretroviral penetration (as recently reported for some drugs), although total CSF concentrations may be increased in spite of reduced free concentrations (due to increased protein levels in the CSF). The alteration in BBB permeability is a common event after HIV infection, and three main mechanisms have been proposed: the effect of pro-inflammatory cytokines secretion, the effect of secreted viral proteins and the direct infection of endothelial cells by HIV (Ivey et al. 2009; Strazza et al. 2011). Even if antiretroviral combination treatment has been shown to effectively reduce CSF HIV-1 RNA levels in antiretroviral-naïve HIV-1-infected individuals, the exact effect on those mechanisms is poorly understood.

A significant proportion of patients had an elevated IgG index, suggesting intrathecal immunoglobulin synthesis, independent of antiretroviral treatment. This is in accordance with other findings of persisting intrathecal cell-mediated immune activation in patients receiving antiretroviral treatment (Abdulle et al. 2002, 2005). Although the targets of these antibodies are not known (and autoantibodies against neurones have been described), this can be linked to residual HIV replication within astrocytes or microglial cells. It would be interesting to measure IgG indexes in relation to neopterine or MCP-1 CSF levels; these data are not available, but they can address the question on clinical relevance of CSF immune activation markers. These results are somehow similar to those reported in patients with multiple sclerosis (MS) with 70 % of patients showing altered IgG index and mean values of 0.73 (as compared to 63.3 % and a median value of 0.7 in this report) (Lefvert and Link 1985; Nakashima et al. 1999); however, oligoclonal bands are still considered peculiar and useful in the diagnosis of MS (Comabella and Montalban 2014).

The CSAR values observed in this study are higher than those observed in healthy volunteer controls in several other reports (Blennow et al. 1993; Chalbot et al. 2011; Zetterberg et al. 2014) and similar to earlier data in untreated or unsuccessfully HIV-treated patients (Elovaara et al. 1987; Hall et al. 1992; Andersson et al. 2001). Even if we aimed at studying the factors associated to blood-brain barrier impairment, it should be highlighted that age-related cut-offs in CSAR have not been clearly validated and no study was performed in HIV-positive patients. However, some interesting factors were associated to this event at univariate analysis (such as current EBV replication), but only CD4 nadir was an independent predictor at multivariate regression test. This was not confirmed in the previously cited study where BBB impairment was independent of HIV stage or CD4 cell count. Nevertheless, the strong association between neurological symptoms (especially ADC) and increased BBB permeability (associated with perivascular inflammation) support these data. Patients exposed for longer time to HIV infection and with lower CD4 nadir are those at higher risk of HIV-associated neurological complications including HAND (Ellis et al. 2011); furthermore, immunological status was shown to affect the degree and progression of BBB alteration and CNS IgG production (Marshall et al. 1991).

The lowest CD4 value in a patient's history was the only predictor of increased IgG in the CSF despite effective HAART. This finding again suggests that the most fragile patients (the late and AIDS presenters) are at higher risk of continuous intrathecal immune activation, probably related to the increased burden of HIV in the reservoirs; it should be highlighted that 40–60 % of new HIV patients in Europe are diagnosed late (Mocroft et al. 2013). Despite effective antiretroviral treatment, BBB impairment may persist at least in a subset of patients (Abdulle et al. 2005); BBBD was observed in 10 % of patients with high CD4 nadir and good immunovirological response. Furthermore, CSF HIV RNA levels were associated to CSAR and IgG index in the multivariate linear regression analysis; complete control of HIV replication in the central nervous system may be beneficial in reducing inflammatory and neurotoxic cytokines (Kamat et al. 2012; Yilmaz et al. 2013).

In conclusion, this cross-sectional analysis showed a high prevalence of blood-brain barrier impairment in HIV-positive patients, related to the nadir of CD4+ cell count and to the control of CNS HIV replication. The long-term clinical implications of BBB damage deserve to be investigated in prospective longitudinal studies.

Conflict of interest All authors declare no potential conflict of interest.

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