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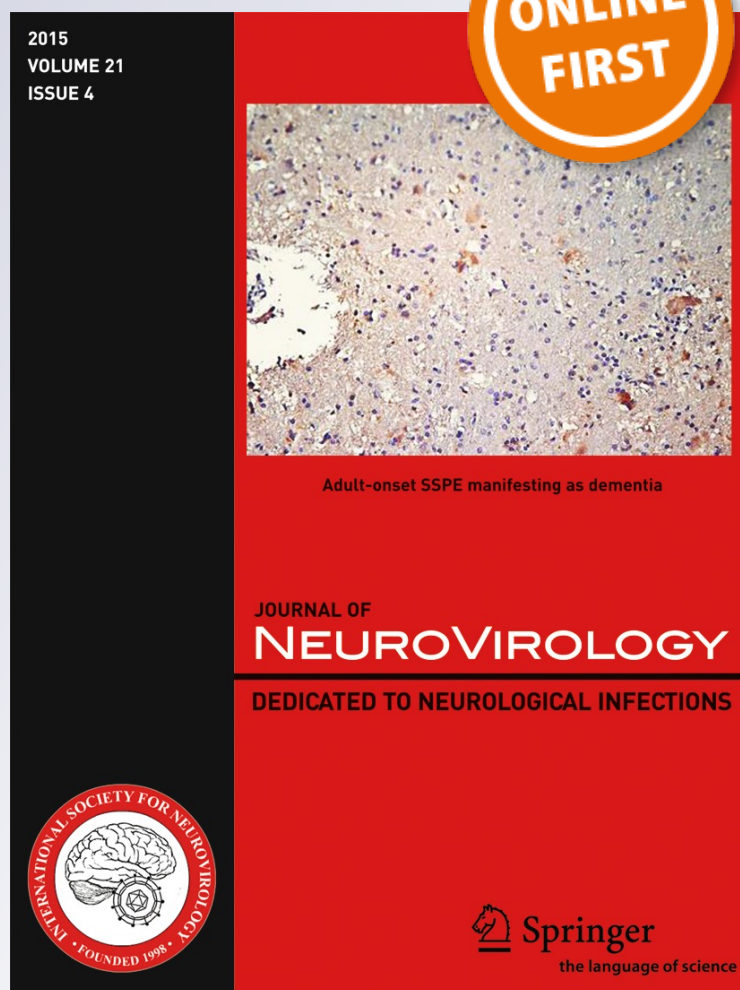
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# Blood brain barrier impairment is associated with cerebrospinal fluid markers of neuronal damage in HIV-positive patients

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**Abstract** Blood brain barrier impairment occurs early in the course of infection by HIV and it may persist in a subset of patients despite effective antiretroviral treatment. We tested the hypothesis that HIV-positive patients with dysfunctional blood brain barrier may have altered biomarkers of neuronal damage. In adult HIV-positive highly active antiretroviral treatment (HAART)-treated patients (without central nervous system infections and undergoing lumbar punctures for clinical reasons) cerebrospinal fluid albumin to serum ratios (CSAR), total tau, phosphorylated tau, 1–42 beta amyloid, and neopterin were measured. In 101 adult patients, cerebrospinal fluid-to-serum albumin ratios were 4.8 (3.7–6.1) with 12 patients (11.9 %) presenting age-defined impaired blood brain barrier. A significant correlation was observed between CSAR and total tau ( $p=0.005$ ), phosphorylated tau ( $p=0.008$ ), and 1–42 beta amyloid ( $p=0.040$ ). Patients with impaired blood brain barrier showed significantly higher total tau (201.6 vs. 87.3 pg/mL,  $p=0.010$ ), phosphorylated tau (35.3 vs. 32.1 ng/mL,  $p=0.035$ ), and 1–42 beta amyloid (1134 vs. 830 pg/mL,  $p=0.045$ ). Despite effective antiretroviral treatment, blood brain barrier impairment persists in some HIV-positive patients: it is associated with markers of neuronal damage and it was not associated with CSF neopterin concentrations.

**Keywords** Blood brain barrier · Albumin ratio · Tau · Phosphorylated tau · Neopterin

## Introduction

HIV-infected patients may develop symptomatic or chronic subclinical alterations in their neurocognitive function (Spudich 2013). Although the exact prevalence of neurocognitive disturbances (usually defined as HIV-associated neurocognitive disorders (HAND)) is still debated, several data suggest that neuronal damage might persist in adequately treated patients (Peluso and Spudich 2014; McDonnell et al. 2014). This has been shown with accurate neuropsychological tests, with advanced magnetic resonance or PET-scan techniques or with low-resolution brain electromagnetic tomography (Antinori et al. 2007; Ances and Hammoud 2014; Babiloni et al. 2014).

Perivascular macrophages, microglial cells and astrocytes are infected by HIV and, through the production of several cytokines and mediators, they induce indirect neuronal damage. Blood brain barrier (BBB) impairment occurs early in the course of infection, and it has been associated with enhanced cells trafficking into the central nervous system (CNS). BBB alterations were found in 2 to 22 % HIV-positive asymptomatic individuals and in 100 % of patients with HIV-associated dementia (HAD) (Abdulle et al. 2005; Andersson et al. 2001). Some recent data suggest that BBB impairment may persist in patients with controlled viral replication and that low CD4+ T lymphocyte count nadir may be a risk factor (Calcagno et al. 2014). One unpublished study reported that patients with altered BBB had a higher prevalence of neurocognitive deficits (Letendre, CROI 2011).

Here, we tested the hypothesis that HIV-positive patients with dysfunctional BBB may have altered biomarkers of

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neuronal damage. Here, we compared CSF biomarkers used in the diagnosis of Alzheimer's dementia with CSF-to-serum albumin ratios (CSAR, representing BBB permeability): total tau and phosphorylated tau (neuronal damage), 1–42 Beta amyloid (amyloid deposition) and neopterin (an inflammatory protein produced by activated macrophages).

## Patients and Methods

Adult HIV-positive patients on highly active antiretroviral treatment (HAART) for at least 6 months and 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 the local Ethics Committee ("Comitato Etico ASLTO2"). Demographic, immunovirological and therapeutic data were recorded.

Quantitative determination of albumin in serum and CSF was measured by Immunoturbidimetric methods (AU 5800, Beckman Coulter, Brea, CA, USA). CSAR, calculated as CSF albumin (mg/L)/serum albumin (g/L), was used to evaluate BBB function. BBB damage definition was derived from age-adjusted Reibergrams (normal if below 6.5 in patients aged <40 years and below 8 in patients >40 years) (Reiber 1995).

CSF total tau (t-tau), phosphorylated tau (p-tau), and  $\beta$ -amyloid1-42 (A $\beta$ 1-42) were measured by immunoenzymatic methods (Innogenetics) with limits of detection, respectively, of 87, 15, and 87 pg/ml. Neopterin was measured through validated ELISA methods (DRG Diagnostics). Reference values were as follows: t-tau [ $<300$  pg/mL (in patients aged 21–50),  $<450$  pg/mL (in patients aged 51–70), or  $<500$  pg/mL in older patients], p-tau ( $<61$  pg/mL), 1–42 beta amyloid ( $>500$  pg/mL), and neopterin ( $<1.5$  ng/mL).

HIV RNA was measured through the real-time polymerase chain reaction (PCR) assay CAP/CTM HIV-1 vs. 2.0 (CAP/CTM, Roche Molecular System, Branchburg, NJ; detection limit: 20 copies/mL of HIV-1 RNA). CSF escape was defined as CSF HIV RNA above 50 copies/mL in patients with plasma HIV RNA below 50 copies/mL or as CSF HIV RNA  $1 \log_{10}$  higher than plasma HIV RNA in patients with detectable plasma viral load.

HAND was diagnosed according to the Frascati criteria, and neurocognitive tests (testing eight different cognitive domains) were performed by a trained neuropsychologist: patients were categorized as having asymptomatic (ANI) or mild neurocognitive impairment (MND) or HIV-associated dementia (HAD) (Antinori et al. 2007). Concentration effectiveness scores (CPE) were derived from Hammond and Coll (Hammond et al. 2014).

Data were analysed using standard statistical methods: variables were described with medians [interquartile ranges (IQR) and ranges (minimum to maximum)], and they were compared using non-parametric tests (Mann-Whitney, chi-square, and Spearman's tests as specified in the text). Data analysis was performed using SPSS software for Mac (version 22.0, IBM Corp).

## Results

One hundred one patients were included. Patients' demographic and therapeutic characteristics are listed in Table 1. Median plasma viral load in those with HIV RNA above 50 copies/mL was 176 copies/mL (86–16,359) with 9 (8.9 %) patients showing values above 1000 copies/mL. Median CSF viral load in those with HIV RNA above 50 copies/mL was 135 copies/mL (88–496) with 6 (5.9 %) patients showing values above 1000 copies/mL; CSF escape was observed in 22 (21.8 %) patients.

Lumbar punctures were performed in patients with HAND (35, 34.7 %; 3 HAD, 3 MND, and 29 ANI), in asymptomatic subjects (30, 29.7 %), in JCV-negative white matter abnormalities (13, 12.9 %), or in patients presenting with other neurological conditions [peripheral neuropathies (6, 5.9 %), headache (3, 3 %), seizures (4, 4 %), systemic bacterial infections (3, 2.8 %), hepatic (3, 3 %), or vascular encephalopathies (3, 3 %)].

Cerebrospinal fluid biomarker results are shown in Table 2. T-tau, p-tau, 1–42 beta amyloid, and neopterin were outside reference ranges in 2 (2 %), 1 (1 %), 4 (4 %), and 10 (9.9 %) patients, respectively. A significant correlation was observed between CSAR and t-tau (Spearman's  $\rho=0.31$ ,  $p=0.005$ ) (Fig. 1a), p-tau ( $\rho=0.29$ ,  $p=0.008$ ) (Fig. 1b), 1–42 beta amyloid ( $\rho=0.23$ ,  $p=0.040$ ), but not between CSAR and neopterin concentrations ( $\rho=0.01$ ,  $p=0.396$ ) nor with CSF HIV RNA ( $\rho=0.06$ ,  $p=0.582$ ). Weak inverse correlations were noted between CSF markers and nadir CD4 (tau, p-tau, and CSAR with Spearman's  $\rho$ s  $>-0.5$  and  $p<0.01$ ), between p-tau and duration of viral suppression ( $\rho=-0.23$ ,  $p=0.03$ ) and between CSAR and duration of viral suppression ( $\rho=-0.31$ ,  $p=0.02$ ). No significant difference was observed among patients with HAND, asymptomatic patients, and those ones with other diagnosis ( $p>0.05$ , Kruskal-Wallis test). Conversely, CSAR were slightly higher in patients with HAND [5.5 vs. 4.5 (asymptomatic patients) vs. 4.7 (other neurological conditions),  $p=0.09$ ]; neopterin was lower in asymptomatic patients [0.5 ng/mL vs. 0.8 ng/mL (HAND) vs. 0.7 ng/mL (other neurological conditions),  $p=0.01$ ].

We compared patients with age-defined altered BBB (12, 11.9 %) with those with intact BBB. No difference was observed as for demographic or therapeutic variables with the exception of nadir CD4+ T lymphocyte

**Table 1** Demographic, immunovirological, and therapeutic factors

Characteristic	Median or number	IQR or percentage
Age (years)	48	41–55
Male gender	68	67.3 %
Ethnicity		
White	92	91.1 %
Black	8	7.9 %
Not available	1	1 %
Hepatitis C antibody positive	37	36.6 %
Duration of HIV infection (years)	1.8	1.4–2.5
CD4+ T lymphocytes (cells/mm <sup>3</sup> )	318	152–562
CD4+ T lymphocytes at nadir (cells/mm <sup>3</sup> )	81	27–203
Plasma HIV RNA >50 copies/mL	27	26.7 %
Cerebrospinal fluid HIV RNA >50 copies/mL	36	35.6 %
Type of HAART		
2 NRTIs+NNRTI	11	10.9 %
2 NRTIs+PI	54	53.4 %
2 NRTIs+INSTI	7	6.9 %
NRTI-sparing regimens	19	18.8 %
Complex regimens (more than 3 drugs)	10	9.9 %
CPE score	7	6–8

Variables are described with number and percentage (categorical) or medians and interquartile ranges (numerical)

*ARV* antiretroviral, *HAART* highly active antiretroviral treatment, *NRTI* nucleos(t)ide reverse transcriptase inhibitor, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor, *INSTI* integrase strand transfer inhibitor, *CPE* concentration penetration effectiveness

count: it was borderline lower in patients with impaired BBB (28 vs. 88 cell/mm<sup>3</sup>,  $p=0.07$ ). Patients with impaired BBB showed significantly higher t-tau (201.6 vs. 87.3 pg/mL, Mann-Whitney  $p=0.010$ , Fig. 1c), p-tau levels (35.3 pg/mL vs. 32.1 ng/mL,  $p=0.035$ , Fig. 1d) and 1–42 beta amyloid (1134 vs. 830 pg/mL,  $p=0.045$ ). No difference was observed in neopterin levels (0.8 vs. 0.6 ng/mL,  $p=0.71$ ) in the two groups.

**Table 2** Cerebrospinal fluid biomarkers

CSF biomarker	Median	IQR	range
CSAR	4.8	3.7–6.1	0.2–20.8
Total tau (pg/mL)	89.6	<75–167.9	<75–749.7
Phosphorylated tau (pg/mL)	32.5	23–37.2	17–72.5
142 beta amyloid (pg/mL)	836.7	672–943	113–1481
Neopterin (ng/mL)	0.6	0.5–1.1	0.1–3.6

Variables are described with median, interquartile ranges and ranges

*CSF* cerebrospinal fluid, *CSAR* CSF to plasma albumin ratio, *IQR* interquartile range

## Discussion

Our analysis shows that total tau and phosphorylated tau were higher in HIV-positive HAART-treated patients with impaired BBB compared to subjects with intact BBB.

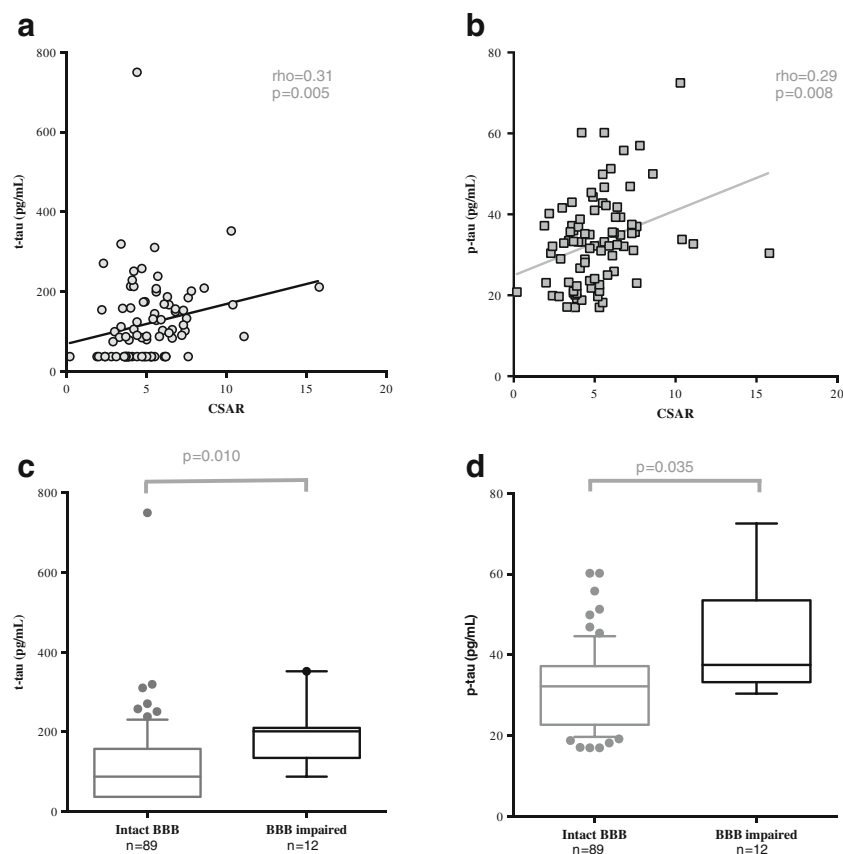
This may be relevant since BBB abnormalities may persist despite effective antiretroviral therapy; the association with markers of neuronal damage may support the role of BBB in HIV-associated neurodegenerative disease (Abdulle et al. 2002; Brown et al. 2014). The neurovascular unit (including cerebral endothelial cells and astrocytes) may be impaired in HIV-positive patients as thoroughly shown by a large cerebral tissue study using gene arrays: (Gelman et al. 2012) several adaptive mechanisms may affect astrocytes and microglia signaling leading to BBB impairment and neuronal damage. Enhanced cell trafficking (monocyte-derived cells) from the periphery can enhance CNS infection and neuroinflammation; recent data show that higher t-tau and p-tau are associated with worse neurocognitive performances in HIV-positive patients (Cysique et al. 2015).

In this study, BBB impairment, as defined by age-corrected albumin ratios, was present in 11.9 % of patients and it was borderline associated with low CD4+ T lymphocyte nadirs: this has already been reported, and late presentation is a recognized risk factor for the development of neurocognitive disorders (Ellis et al. 2011).

Two major limitations must be clearly highlighted: the cross-sectional design and the inclusion of patients with neurological or neurocognitive symptoms (although those with CNS infections or neoplasms were excluded): the design of the study (cross-sectional diagnostic vs. prospective interventional) does not allow for bias reduction. The weak observation that patients with longer duration of viral suppression and those ones without symptoms had lower markers of damage and immune activation needs to be further investigated: it might suggest that with longer follow-up, these abnormalities may resolve in the majority of patients, but it is somehow contradicted by the effect of low nadir CD4 cell count that is, unfortunately, an un-modifiable factor. The weak association between continuous variables is suggestive of a possible association rather than clear causation: ongoing longitudinal studies may show the evolution of both BBB alterations and neuronal damage with long-lasting viral suppression (Heaton et al. 2015).

CSF total tau, phosphorylated tau, and 1–42 Beta amyloid have been used as adjunctive markers for diagnosing Alzheimer's disease with sensitivity and specificity above 85 % (Ahmed et al. 2014). Increased total and phosphorylated tau may represent neuronal damage while decreased 1–42 beta amyloid levels may represent increased amyloid deposition. These CSF biomarkers have been studied in HIV-positive patients: higher total tau has been found in patients with CNS opportunistic

**Fig. 1** Total and phosphorylated tau according to CSF to plasma albumin ratios (**a** and **b**) or to age-defined blood brain barrier impairment (**c** and **d**). Trend lines are represented in the two graphs above; in the graphs below horizontal lines represent median values while *boxplot* interquartile ranges and whisker 10–90 percentile (*circles* are outliers)



infections, with low CD4 cell count and with HIV-associated dementia (Brown et al. 2014; Peterson et al. 2014). In this study, we observed that the majority of patients had tau and 1–42 beta amyloid levels within reference ranges (established in HIV-negative subjects); however, all these biomarkers were higher with higher CSARs. The role of blood-cerebrospinal fluid barrier dysfunction in Alzheimer's disease has been addressed but not yet established, and the association between CSAR and tau levels has not been observed in that setting (Hagberg et al. 2010). CSF 1–42 beta amyloid fraction is usually decreased in patients with Alzheimer's dementia as a result of amyloid deposition; here, we observed slightly higher levels in patients with impaired BBB. The relevance of this finding is unknown but it has been recently shown that HIV-positive cognitively impaired patients might have higher plasma levels of 1–42 beta amyloid (Mothapo et al. 2015).

Neopterin is a low-molecular weight protein predominantly produced by monocyte-derived cells, and it may be used as a sensitive marker of cellular activation; CSF neopterin is elevated throughout the course of HIV infection with the highest values observed in patients with HIV-associated dementia (HAD) (Yilmaz et al. 2013; Andersson et al. 2001). After HAART introduction,

CSF neopterin concentrations decay, but they soon reach a plateau and persist at higher levels than in HIV-negative matched controls; blood brain permeability was reported to be associated with neopterin levels in HIV-positive untreated patients (Eugenin et al. 2011). In this analysis, approximately 10 % of the included patients had CSF neopterin levels above 1.5 ng/mL supporting the persistence of compartmental immune activation; however, this biomarker was not associated with altered BBB permeability.

Taken together, these results may suggest that once effective antiretroviral treatment had dampened CNS viral replication, BBB impairment is reduced (although not completely normalized, as already shown by previous works) and it is not associated with CSF neopterin concentrations. They also support further investigations on factors associated with persistent damage to the neurovascular unit; astrocytes may be a possible target given their involvement in the BBB and their restricted infection by HIV (Borjabad et al. 2010).

In conclusion, cerebrospinal fluid total and phosphorylated tau were higher in HIV-positive patients with impaired blood brain barrier; further investigations are warranted studying the mechanisms supporting persistent neuronal damage and finding possible therapeutic targets.

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