No support for premature central nervous system aging in HIV-1 when measured by cerebrospinal fluid phosphorylated tau (p-tau)

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ABSTRACT

Background: The prevalence of neurocognitive deficits are reported to be high in HIV-1 positive patients, even with suppressive antiretroviral treatment, and it has been suggested that HIV can cause accelerated aging of the brain. In this study we measured phosphorylated tau (p-tau) in cerebrospinal fluid (CSF) as a potential marker for premature central nervous system (CNS) aging. P-tau increases with normal aging but is not affected by HIV-associated neurocognitive disorders.

Methods: With a cross-sectional retrospective design, p-tau, total tau (t-tau), neopterin and HIV-RNA were measured in CSF together with plasma HIV-RNA and blood CD4 T-cells of 225 HIV-infected patients <50 y of age, subdivided into 3 groups: untreated neuroasymptomatic (NA) (n = 145), on suppressive antiretroviral treatment (cART) (n = 49), and HIV-associated dementia (HAD) (n = 31). HIV-negative healthy subjects served as controls (n = 79). Results: P-tau was not significantly higher in any HIV-infected group compared to HIV-negative controls. Significant increases in t-tau were found as expected in patients with HAD compared to NA, cART, and control groups (p < 0.001). Conclusions: P-tau was not higher in HIV-infected patients compared to uninfected controls, thus failing to support a role for premature or accelerated brain aging in HIV infection.

KEYWORDS
biomarker analysis; cerebrospinal fluid; HIV; HIV-1 associated cognitive motor complex; tau protein

Introduction

HIV-infected patients are at increased risk for the premature development of age-associated comorbidities, including cardiovascular disease, osteoporosis, non-AIDS associated malignancies, and neurocognitive impairment. It has, therefore, been hypothesized that HIV infection actually accelerates aging processes. One of the speculated underlying mechanisms of this premature aging relates to the chronic inflammatory state characteristic of HIV infection.1 HIV-1 invades the brain at primary infection and continues to be detected in the CNS throughout the course of untreated chronic infection. Furthermore, this is accompanied by chronic neuroinflammation, which may continue at a low level even after suppressive antiretroviral treatment.2,3 In the pre-treatment years of the HIV pandemic, a common feature of late-stage AIDS was HIV-associated dementia (HAD), in which up to 20% of all patients developed severe cognitive and motor deficits.4,5 With combination antiretroviral treatment (cART), overt dementia is rare. However, studies indicate that a large proportion of HIV-infected patients on cART still exhibit milder cognitive disorders. These have been divided into minor cognitive disorder (MND) and asymptomatic neurocognitive impairment (ANI).6,7 Together with HAD, these conditions are summarized in the concept of HIV-associated neurocognitive disorders (HAND).8 Intrathecal immune activation is a general feature of HIV infection, and a persistent low-grade inflammation is often present in antiretroviral-treated patients despite several years of suppressed plasma viral loads.8,9 With an aging HIV-positive population on cART, there are rising concerns that the low-grade chronic inflammation in the CNS caused by HIV might lead to a premature decline of neuronal capacity.10,11 Since life expectancy in such patients is near normal, differential diagnostics in regard to other types of dementia, mainly Alzheimer dementia (AD) will be important.
A number of cerebrospinal fluid (CSF) biomarkers have been investigated as potential diagnostic tools in HAND, where neopterin and neurofilament light chain protein (NFL) are sensitive indicators for assessing intrathecal immune activation and neuronal damage respectively. NFL increases naturally with normal aging, but a significant increase in its concentration is seen in both treated and untreated asymptomatic HIV-positive patients, reflecting subclinical CNS injury. Increased CSF NFL is a general feature of HAD.

Another set of CSF biomarkers of interest in HIV infection is tau proteins and amyloid metabolites. These have become cornerstones in the clinical diagnosis of AD. It has been shown previously that the biomarker pattern of amyloid and tau differs in HAD, compared to AD and HIV-negative controls. Total tau (t-tau) is a constituent of neurons in the CNS, facilitating microtubule stability and transport of organelles. Like NFL, increased CSF t-tau is a signal of neuronal injury, though less sensitive than NFL. By contrast, phosphorylated tau (p-tau) is generally not increased in HIV-infected patients with neuronal injury and cognitive disease. Increased levels of t-tau reflect cortical axonal degeneration, whereas increased concentrations of p-tau in CSF indicate a pathological hyperphosphorylation of tau, where tau detaches from the microtubuli causing axonal instability and may end up as aggregates in neurofibrillary tangles. Mouse models suggest that inflammation might induce phosphorylation of tau, but the detailed characteristics of p-tau synthesis are not completely known. Increased concentrations of p-tau can be observed in the physiological aging process, but large increases are a sign of pathological processes in the CNS, commonly referred to as tauopathies. The hallmark disease associated with increased p-tau is AD, and analysis of tau proteins in CSF is one of the fundamentals in clinical diagnosis of AD. A concurrent increase of both p-tau and t-tau has also been seen in herpes simplex type-1 encephalitis. When looking at the ratio of p-tau to t-tau, it is largely decreased. Most likely, the increase of p-tau is due to a “spill-over” of high t-tau levels reflecting the inflammatory disruption of axons in the acute stage of infection.

As p-tau increases with normal aging, but typically not in HAND, we hypothesize that if HIV is associated with premature or accelerated aging, p-tau could be expected to be higher in HIV-infected patients compared to controls of same age. We, therefore, assessed CSF p-tau in untreated and antiretroviral-treated HIV-infected patients below the age of 50 without neurological disease, and compared them to HIV-negative controls of the same ages. For comparison, we also included patients who had HAD. Both CSF t-tau and neopterin were analyzed to assess possible neural injury and intrathecal immune activation.

Results

P-tau was lower in the cART group (p < 0.05) compared to controls, but not compared to the NA or HAD groups. No significant differences were seen when comparing the other groups (Fig. 1a and Table 1). When looking at a general linear model assessing dependency between p-tau and age, no significant differences in slopes or levels were found between the groups.

The HAD group clearly exhibited increases in t-tau compared to all other groups (p < 0.001 for all). Both NA and cART groups had somewhat lower concentrations of t-tau compared to controls (p = 0.01 and p < 0.01 respectively) (Table 1 and Fig. 1b).

T-tau was significantly correlated with CSF neopterin (p < 0.001, r = 0.24) and CSF HIV-RNA (p < 0.05, r = 0.16). P-tau did not correlate with CD4 cell counts, CSF neopterin, neither plasma nor CSF HIV RNA, but a relatively strong correlation was found between t- and p-tau (p < 0.0001, r = 0.55).

The ratio of p-tau to t-tau was significantly decreased in patients with HAD compared to all other groups (p < 0.001) (Table 1 and Fig. 1c).

Discussion

HIV-infected patients are at increased risk for developing neurocognitive disorders, probably due to a complex chronic inflammatory response to the virus in the CNS. It has been speculated that accelerated or premature CNS aging may be part of the pathogenesis of neurocognitive decline in HIV, mainly in untreated cases, but also in patients with suppressive cART.

We found no support for premature CNS aging in HIV-infected patients as measured by CSF p-tau concentrations. To the best of our knowledge, this is the first study using CSF p-tau as a potential marker for brain aging in HIV. Additionally, HIV-patients did not exhibit elevated levels of p-tau compared to HIV-negative controls. There were no differences in p-tau levels among HAD patients, characterized by severe neurocognitive decline and neuronal injury; neuroasymptomatic HIV-infected untreated patients; and patients on suppressive cART. Neither was any difference in p-tau elevations or slope found between groups when the relationship of p-tau to age was assessed with a general linear model. Patients on cART did have somewhat lower levels of p-tau than to controls. However, when looking at p-tau/ t-tau ratios, this difference disappeared, suggesting that the finding may be insignificant.
In accordance with previous studies, t-tau was largely increased in HAD, reflecting neuro-axonal damage; but no increase was seen in the neuroasymptomatic groups with and without treatment. Contradictory results regarding p-tau in HAD have previously been presented. Some have claimed that p-tau is increased in HAD,\textsuperscript{15,16,25} while others have not found such an association.\textsuperscript{15,17} In our study, no increase in p-tau was noted in HAD patients, and the p/t-tau ratio was accordingly significantly lower in those patients compared with the other groups.

There are several limitations to our study. First, even if the sample size was relatively large, it might still have been too low to detect small differences between groups. Second, the study was restricted to subjects below the age of 50, so that an incipient difference in older individuals could not be ruled out. Although our HIV-positive study patients are getting older with cART as a whole, a majority of the HIV-positive patients included in this study were below the age of 50. In future studies, it would be interesting to include older HIV-positive patients.

Third, p-tau is an unproved surrogate marker of brain aging and may not be a useful marker for neurological aging in HIV. However, as previously discussed, it is hypothetically an interesting biomarker as it increases with normal aging and does not interfere with HIV-related CNS injury. Conversely, it is not established whether well-treated HIV-positive patients are affected by premature brain aging. As this was a pilot study we did not include CSF neurofilament light (NFL) or other biomarkers associated with neuroinflammation or perturbed metabolism in HIV-associated neurocognitive disorder. Ideally, a larger biomarker panel, combined with neuropsychological testing on all patients, and possibly also neuroradiological methods such as functional MRI, should be performed to properly assess the value of tau proteins as a biomarker for premature brain aging. Fourth, and perhaps most importantly, this was a cross-sectional study. Ideally, a longitudinal study that followed patients and controls for a long period of time would have been ideal, although difficult to achieve.

In summary, whether premature brain aging is a real feature of HIV continues to be debated. We found no evidence for this in our present study. However, since p-tau may be an unreliable marker of CNS aging, the possibility of premature or accelerated CNS aging in HIV patients could not be precluded by this study.

Figure 1. (A) P-tau (y-axis) across group. Log10 transformed. P-tau significantly lower ($p < 0.05$) in cART patients compared to controls. No other significant differences noted. (B) T-tau (y-axis) across group. Log10 transformed. T-tau significantly higher in HAD patients compared to all other groups ($p < 0.001$). cART and NA significantly lower compared to controls ($p < 0.004$ and $p < 0.01$, respectively). (C) P-tau/t-tau ratio (y-axis) across group. Log10 transformed. P-tau/t-tau ratio significantly lower in HAD patients compared to all other groups ($p < 0.001$). No significant differences seen between other groups.
Table 1. Summary of biomarker concentrations across groups.

| Group         | P-tau (ng/l) Median (IQR) | T-tau (ng/l) Median (IQR) | P-tau/T-tau ratio (ng/l) Median (IQR) | CSF Neopterin (nmol/L) (Median (IQR)) |
|---------------|---------------------------|---------------------------|---------------------------------------|--------------------------------------|
| HIV+ NA       | 35 (26–45)                | 138 (103.8–207)          | 0.25 (0.17–0.33)                     | 17.1 (10.8–28.6)                     |
| HIV+ HAD      | 30.5 (23.8–42)            | 404 (273–690)            | 0.075 (0.04–0.11)                    | 50.5 (26.3–78.6)                     |
| HIV+ cART     | 31 (23–37)                | 132 (97–187.8)           | 0.26 (0.17–0.31)                     | 5.2 (4.2–6.9)                       |
| HIV- control  | 37 (28.3–49)              | 181.4 (124.2–265.7)      | 0.2 (0.17–0.23)                      | N/A                                  |

Notes. N/A= Not available

*"= 1 Patient missing
"**= 5 patients missing
"***= 3 patients missing

Methods

Study design and subjects

We used a retrospective, cross-sectional cohort design. Archived specimens of CSF and blood were collected between 1986 and 2014 at 3 academic centers; Sahlgrenska University Hospital in Gothenburg, Sweden, San Francisco General Hospital in California, USA and San Raffaele Hospital in Milan, Italy. There were 225 HIV-1 positive patients in the study, all under the age of 50, subdivided into 3 groups: neuroasymptomatics without antiretroviral treatment (NA); n = 145, neuroasymptomatics on suppressive antiretroviral treatment (cART) for at least 6 months; n = 49 and HIV-associated dementia (HAD); n = 31. The control group consisted of HIV-negative healthy volunteers, n = 79. Subject characteristics are shown in Table 2.

Lumbar puncture was performed on the HIV patients as part of the clinical evaluation, in conjunction with clinical studies of the other subjects. Patients were considered neuroasymptomatic if no signs or symptoms of cognitive deficits were found at clinical examination or follow-ups. Neuropsychological testing was only performed where there was suspicion of neurocognitive decline. HAD was defined by the Center for Disease Control and the American Academy of Neurology Task Force criteria using standard laboratory and clinical evaluations.6,26,27 Suppressive cART was defined as <50 HIV-RNA copies/mL in consecutive blood samples for at least 6 months. All subjects were studied under research protocols approved by the institutional review boards at each center and followed the guidelines of the Helsinki Declaration. All patients received written and verbal information about the study. Informed consent was obtained and documented in each patient file.

Analytical methods

CSF samples were collected in polypropylene tubes, subjected to low-speed centrifugation to remove cells, then aliquoted, and frozen to −70°C for storage until analysis within 1 hour of performing the lumbar puncture.

CSF p-tau and t-tau were measured using an enzyme-linked immunosorbent assay (ELISA) as described previously.28,29 Analyses of tau proteins were done at different time-points, and subsequently with different batches of ELISA kits.

Neopterin in CSF was analyzed using a commercially available immunoassay (BRAHMS, Berlin, Germany) with an upper normal reference value of 5.8 nmol/L.12

HIV RNA in CSF and plasma were measured using the Roche Amplicor Monitor version 1.5, Roche Taqman assay version 1 or 2 (Hoffman La-Roch e, Basel, Switzerland) or Abbott RealTime HIV-1 assay (Abbott Laboratories, Abbott Park, Illinois, USA). Other measurements, including blood CD4+ T-cell counts, CSF white blood cells

Table 2. Background characteristics.

| Group         | Gender | Age | Blood CD4+ cells |
|---------------|--------|-----|-----------------|
|               | Female (n) | Median years (IQR) | Median cells/μL (IQR) |
| HIV+ NA       | 37 | 36 (31–42) | N/A |
| HIV+ HAD      | 5 | 37 (33–44) | N/A |
| HIV+ cART     | 15 | 38 (32–43) | N/A |
| HIV- control  | 27 | 39 (31–44) | N/A |

Notes. N/A= Not available

HIV+ NA: HIV-positive neuroasymptomatic patients
HIV+ HAD: HIV-associated dementia
HIV+ cART: HIV-positive neuroasymptomatic patients on suppressive treatment

*5 patients missing
**2 patients missing
***4 patients missing
(WBC), and CSF: blood albumin ratio, were performed in local clinical laboratories at each study site.

**Statistical analysis**

Continuous variables were log_{10} transformed where appropriate for the tests used.

ANOVA, together with Tukey’s multiple comparisons post hoc test, was used to assess differences between multiple groups. A general linear model was used when comparing tau protein to age. Pearson correlation was used to examine dependency between neopterin, HIV-RNA, and tau proteins.

In cases of p- and t-tau levels below the detection limit, we used the lower reference divided in half in calculations (for p-tau 8 (<15) ng/l; for t-tau 36 (<72) ng/l).

General descriptive statistics are presented with the median and interquartile range (IQR). All statistical analyses were performed using IBM SPSS Statistics® version 20 or Prism® version 5 (Graphpad Software Inc., La Jolla, CA, USA).

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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**References**

[1] Deeks SG. HIV infection, inflammation, immunosenescence, and aging. Annu Rev Med 2011; 62:141-55; PMID:21090961; https://doi.org/10.1146/annurev-me-d-042909-093756

[2] Budka H. Neuropathology of human immunodeficiency virus infection. Brain Pathol 1991; 1:163-75; PMID:1669705; https://doi.org/10.1111/j.1750-3639.1991.tb00656.x

[3] Clifford DB, Ances BM. HIV-associated neurocognitive disorder. Lancet Infect Dis 2013; 13:976-86; PMID:24156898; https://doi.org/10.1016/S1473-3099(13)70269-X

[4] Portegies P, de Gans J, Lange JM, Derix MM, Speelman H, Bakker M, Danner SA, Goudsmit J. Declining incidence of AIDS dementia complex after introduction of zidovudine treatment. BMJ 1989; 299:819-21; PMID:25105843; https://doi.org/10.1136/bmj.299.6703.819

[5] Price RW, Brew B, Siddis J, Rosenblum M, Scheck AC, Cleary P. The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. Science 1988; 239:586-92; PMID:3277272; https://doi.org/10.1126/science.3277272

[6] Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, et al. Updated research nosology for HIV-associated neurocognitive disorders. Neurology 2007; 69:1789-99; PMID:17914061; https://doi.org/10.1212/01.WNL.0000287431.88658.8b

[7] Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, Ellis RJ, Letendre SL, Marcotte TD, Atkinson JH, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. Neurology 2010; 75:2087-96; PMID:21135382; https://doi.org/10.1212/WNL.0b013e318200d477

[8] Eden A, Price RW, Spudich S, Fuchs D, Hagberg L, Gisslen M. Immune activation of the central nervous system is still present after > 4 years of effective highly active antiretroviral therapy. J Infect Dis 2007; 196:1779-83; PMID:18190258; https://doi.org/10.1086/523648

[9] Yilmaz A, Yiannoutsos CT, Fuchs D, Price RW, Crozier K, Hagberg L, Spudich S, Gisslen M. Cerebrospinal fluid neopterin decay characteristics after initiation of antiretroviral therapy. J Neuroinflammation 2013; 10:62; PMID:23664008; https://doi.org/10.1186/1742-2094-10-62

[10] Ellis R, Langford D, Masliah E. HIV and antiretroviral therapy in the brain: neuronal injury and repair. Nat Rev Neurosci 2007; 8:33-44; PMID:17180161; https://doi.org/10.1038/nrn2040

[11] Kaul M, Lipton SA. Mechanisms of neuroimmunity and neurodegeneration associated with HIV-1 infection and AIDS. J Neuroimmune Pharmacol 2006; 1:138-51; PMID:18040780; https://doi.org/10.1007/s11481-006-9011-9

[12] Hagberg L, Cinque P, Gisslen M, Brew BJ, Spudich S, Bestetti A, Price RW, Fuchs D. Cerebrospinal fluid neopterin: an informative biomarker of central nervous system immune activation in HIV-1 infection. AIDS Res Ther 2010; 7:15; PMID:20525234; https://doi.org/10.1186/1742-6405-7-15

[13] Price RW, Peterson J, Fuchs D, Angel TE, Zetterberg H, Hagberg L, Spudich S, Smith RD, Jacobs JM, Brown JN, et al. Approach to cerebrospinal fluid (CSF) biomarker discovery and evaluation in HIV infection. J Neuroimmune Pharmacol 2013; 8:1147-58; PMID:23943280; https://doi.org/10.1186/11481-013-9491-3

[14] Jessen Krut J, Mellberg T, Price RW, Hagberg L, Fuchs D, Rosengren L, Nilsson S, Zetterberg H, Gisslen M. Biomarker evidence of axonal injury in neuro-asymptomatic HIV-1 patients. PLoS One 2014; 9: e88591; PMID:24523921; https://doi.org/10.1371/journal.pone.0088591

[15] Gisslen M, Krut J, Andresson U, Blennow K, Cinque P, Brew BJ, Spudich S, Bestetti A, Price RW, Hagberg L, Rosengren L, Nilsson S, Zetterberg H, Gisslen M. Biomarker evidence of axonal injury in neuro-asymptomatic HIV-1 patients. PLoS One 2014; 9: e88591; PMID:24523921; https://doi.org/10.1371/journal.pone.0088591
[18] Hampel H, Teipel SJ. Total and phosphorylated tau proteins: evaluation as core biomarker candidates in frontotemporal dementia. Dement Geriatr Cogn Disord 2004; 17:350-4; PMID:15178952; https://doi.org/10.1159/000077170

[19] Lee DC, Rizer J, Selenica ML, Reid P, Kraft C, Johnson A, Blair L, Gordon MN, Dickey CA, Morgan D. LPS-induced inflammation exacerbates phospho-tau pathology in rTg4510 mice. J Neuroinflammation 2010; 7:56; PMID:20846376; https://doi.org/10.1186/1742-2094-7-56

[20] Sy M, Kitazawa M, Medeiros R, Whitman L, Cheng D, Lane TE, Laferla FM. Inflammation induced by infection potentiates tau pathological features in transgenic mice. Am J Pathol 2011; 178:2811-22; PMID:21531375; https://doi.org/10.1016/j.ajpath.2011.02.012

[21] Sjogren M, Vanderstichele H, Agren H, Zachrisson O, Edsberg M, Wikkelso C, Skoog I, Wallin A, Wahlund LO, Marcusson J, et al. Tau and Abeta42 in cerebrospinal fluid from healthy adults 21-93 years of age: establishment of reference values. Clin Chem 2001; 47:1776-81; PMID:11568086

[22] Krut JJ, Zetterberg H, Blennow K, Cinque P, Hagberg L, Price RW, Studahl M, Gisslen M. Cerebrospinal fluid Alzheimer’s biomarker profiles in CNS infections. J Neurol 2012; 260:620-6; PMID:23052602

[23] Gartner S, Liu Y. Insights into the role of immune activation in HIV neuropathogenesis. J Neurovirol 2002; 8:69-75; PMID:11935459; https://doi.org/10.1080/13550280290049525

[24] Andersson L, Blennow K, Fuchs D, Svennerholm B, Gisslen M. Increased cerebrospinal fluid protein tau concentration in neuro-AIDS. J Neurol Sci 1999; 171:92-6; PMID:10581374; https://doi.org/10.1016/S0022-510X(99)00253-1

[25] Brew BJ, Pemberton L, Blennow K, Wallin A, Hagberg L. CSF amyloid beta42 and tau levels correlate with AIDS dementia complex. Neurology 2005; 65:1490-2; PMID:16275845; https://doi.org/10.1212/01.wnl.0000183293.95787.b7

[26] Castro KG, Ward JW, Slutsker L, Buehler JW, Jaffe HW, Berkelman RL, Curran JW. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. Clin Infect Dis 1993; 17:802-10; https://doi.org/10.1093/cid/17.4.802

[27] Janssen RS, Cornblath DR, Epstein LG, Foa RP, McArthur JC, Price RW, Beckett A, Benson DF, Bridge TP, et al. Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. Neurology 1991; 41:778-85; PMID:2046917; https://doi.org/10.1212/WNL.41.9.1355

[28] Blennow K, Wallin A, Agren H, Spenger C, Siegfried J, Vanmechelen E. Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? Mol Chem Neuropathol 1995; 26:231-45; PMID:8748926; https://doi.org/10.1016/S0304-3940(00)01036-3