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**No support for premature CNS aging in HIV when measured by cerebrospinal fluid
hyperphosphorylated tau (p-tau)**

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Abstract

Background: 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 or premature aging of the brain. In this study we measured hyperphosphorylated tau (p-tau) in CSF as a potential marker for premature CNS aging

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 below 50 years of age subdivided into three 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).

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Results: P-tau was not significantly higher in any HIV-infected group compared to HIV-negative controls. Significant increases in t-tau were as expected found in patients with HAD compared to NA, cART, and control groups ($p < 0.001$).

Conclusions: P-tau was not higher in HIV-infected compared to controls speaking against premature or accelerated aging of the brain in HIV.

Introduction:

HIV-infected patients are at increased risk for the premature development of age-associated comorbidities, such as cardiovascular disease, osteoporosis, non-AIDS associated malignancies and neurocognitive impairment. It has therefore been hypothesized that such individuals may be prone to accelerated aging.

HIV-1 invades the brain at primary infection and is present in the CNS throughout the course of chronic infection. It is well established that HIV in the CNS can cause a chronic, smoldering neuroinflammation, with axonal degeneration and increased prevalence of declining cognitive functions as an effect[1, 2].

In the pre-treatment years of the HIV pandemic, a common feature of late-stage AIDS was HIV-associated dementia (HAD), where up to 20% of patients developed severe cognitive and motor deficits[3, 4]. With combination antiretroviral treatment (cART), overt dementia is rare. However, studies indicate that still a large proportion of HIV-infected patients on cART exhibit milder cognitive disorders, divided into minor cognitive disorder (MND) and asymptomatic neurocognitive impairment (ANI)[5, 6]. Together with HAD, these conditions are summarized in the concept of HIV-associated neurocognitive disorders (HAND)[5].

Intrathecal immune activation is a general feature of HIV infection, and a persistent low-grade

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inflammation is often present also in antiretroviral treated patients despite several years of suppressed plasma viral loads[7, 8]. With an aging HIV-positive population on cART, there are rising concerns that the low-grade chronic inflammation caused by HIV in the CNS might lead to premature decline of neuronal capacity[9, 10]. Since the life expectancy is near to 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 HIV, where neopterin and neurofilament light chain protein (NFL) are sensitive markers to assess intrathecal immune activation and neuronal damage respectively.[11-13]. NFL increases naturally with normal aging, but a significant increase in concentration is seen in both treated and untreated asymptomatic HIV-positive patients reflecting subclinical CNS injury[13]. In HAD, increased CSF NFL is a general feature. Another set of CSF biomarkers of interest in HIV is tau proteins and amyloid metabolites. These are used as one of the cornerstones in the clinical diagnose of AD, and it has been previously shown that the biomarker pattern of amyloid and tau differ in HAD compared to AD and HIV-negative controls[14, 15]. 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[14, 16]. In contrast, hyperphosphorylated tau (p-tau) is generally not increased in HIV-patients with neuronal injury and cognitive disease. Increased levels of t-tau reflect cortical axonal degenerations, whereas increased concentrations of p-tau in CSF indicate a pathological hyperphosphorylation of tau, where tau detaches from the microtubuli causing axonal instability[17]. Mouse-models suggest that inflammation might induce phosphorylation of tau, but the detailed characteristics of p-tau synthesis are not completely known[18, 19]. Increased concentrations of p-tau can be observed in the physiological aging process[20], but

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large increases is a sign of pathological processes in the CNS, commonly referred to as tauopathies. The hallmark disease associated with increased p-tau is AD, where analysis of tau proteins in CSF is one of the cornerstones in clinical diagnosis.

We hypothesize that if HIV is associated with premature or accelerated aging, p-tau could be expected to be higher in HIV-infected compared to controls of same age. We therefore assessed CSF p-tau in untreated and antiretrovirally treated HIV-infected patients without neurological disease below the age of 50, and compared to HIV-negative controls in the same ages. For comparison we also included patients with HAD. CSF t-tau and neopterin were analyzed to assess possible neural injury and intrathecal immune activation.

Methods:

Study design and subjects

We used a retrospective, cross-sectional cohort design. Archived specimens of cerebrospinal fluid (CSF) and blood was collected between the years 1986-2014 from three academic centers; Sahlgrenska University hospital in Gothenburg, Sweden, San Francisco General Hospital, California, USA and San Raffaele Hospital, Milan, Italy. 225 HIV-1 positive patients, all under the age of 50 were included and subdivided into three 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 1.

Lumbar puncture was performed as part of the clinical routine on the HAD patients and under the auspices of clinical studies among the other subjects. Patients were defined as

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neuroasymptomatic if no signs or symptoms of cognitive deficits were found at clinical examination or follow-ups. Neuropsychological testing was only performed on suspicion of neurocognitive decline. HAD was defined using the CDC and American Academy of Neurology Task Force Criteria using standard laboratory and clinical evaluations[5, 21, 22]. 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 study information. Informed consent was obtained and documented in their respective patient file.

Analytical methods

CSF samples were submitted to low-speed centrifugation to remove cells, aliquoted, and frozen to -70°C within 1 hour of performing the lumbar puncture for storage until analysis.

CSF p-tau, and t-tau were measured using enzyme linked immunosorbent assay (ELISA) as described in previous publications[23, 24].

Neopterin was analyzed in CSF using a commercially available immunoassay (BRAHMS, Berlin, Germany) with an upper normal reference value of 5.8 nmol/L[11].

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-Roche, Basel, Switzerland) or Abbott RealTime HIV-1 assay (Abbott Laboratories, Abbott Park, Illinois, U.S.A.). Other measurements, including blood CD4⁺ T-cell counts, CSF WBC and CSF: blood albumin ratio, were performed in the local clinical laboratories at each study site.

Statistical analysis

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

ANOVA 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 establish 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 by two in calculations (for p-tau 8 (<15) ng/l, for t-tau 36 (<72) ng/l).

General descriptive statistics are presented as 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).

Results

P-tau was not significantly higher in any HIV-infected group compared to HIV-negative controls. In contrary, p-tau was lower in the cART group compared to controls ($p < 0.05$), but not when comparing to the NA and HAD groups. No significant differences were seen when comparing the other groups (Fig 1a and table 2). When looking at a general linear model assessing dependency between p-tau and age, no significant differences in slopes or levels were found between groups.

The HAD group exhibited clear 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 2 and fig 1b).

T-tau was significantly correlated with CSF neopterin ($p < 0.001$, $r = 0.24$) and CSF HIV-RNA ($p < 0.05$, $r = 0.16$). No such correlations were found for p-tau but a relatively strong correlation was found between t- and p-tau ($p < 0.0001$, $r = 0.55$).

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The ratio of P-tau to T-tau was significantly decreased in HAD compared to all other groups ($p < 0.001$) (table 2 and Fig 1c).

Discussion

HIV-infected patients are at increased risk for developing neurocognitive disorders, likely due to a complex chronic inflammatory response to the virus in CNS[7, 25]. Discrete neurocognitive impairments (ANI and MND) persist even with widespread access to suppressive cART[6] and it has been speculated that accelerated or premature CNS aging may be part of the pathogenesis of neurocognitive decline in HIV, mainly in untreated, but also in patients with suppressive cART.

To the best of our knowledge, this is the first study using CSF p-tau as a potential marker for brain aging in HIV. We found no support for premature CNS aging in HIV-infected patients as measured by CSF p-tau concentrations. HIV-patients did not exhibit elevated levels of p-tau compared to HIV-negative controls. There were no differences in p-tau levels between HAD, 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 relation of p-tau and age was assessed with a general linear model. Thus, we found no support for the hypothesis of premature brain aging in HIV by p-tau analyses.

Patients on cART had somewhat lower levels of p-tau compared to controls. When looking at p-tau/t-tau ratio, this difference disappeared, suggesting that this finding may have been coincidental.

In accordance with previous studies t-tau was largely increased in HAD, most likely reflecting prominent neuroinflammation and axonal damage, but no increase was seen in the

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neuroasymptomatic groups with and without treatment [14, 15, 26]. Contradicting results regarding p-tau in HAD have previously been presented, some have claimed that p-tau is increased in HAD[15, 27] while other have not found such association[14, 16]. In this study, no increase in p-tau was noted in HAD and the p/t-tau ratio was accordingly significantly lower in HAD compared to the other groups.

There are several limitations to our study. First, the sample size might be too low to detect small differences between groups. Second, the study was restricted to subjects below the age of 50 years and an incipient difference in older ages could not be ruled out. Third, although all patients were analyzed for p-tau and t-tau with the same commercial method, there could theoretically be inter-batch variations that influence the results since all samples were not analyzed at the same time points. Fourth, and maybe most important, this was a cross-sectional study. Ideally, a longitudinal study following patients and controls for a long period of time would have been ideal, but difficult to achieve.

In summary, it is debated and controversial if premature brain aging is a real feature of HIV. We did not find any evidence for this in our present study. However, p-tau may be an unreliable marker of CNS aging and the possibility of premature or accelerated CNS aging in HIV could not be precluded by this study.

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Table 1. Background characteristics

Group	N	Gender		Age	Plasma HIV RNA (log ₁₀)	CSF HIV RNA (log ₁₀)
		<i>Female</i>	<i>Male</i>	<i>Median years</i>	<i>Median copies/mL</i>	
		<i>(n)</i>	<i>(n)</i>	<i>(IQR)</i>	<i>(IQR)</i>	
HIV+ NA	145	37	108	36 (31-42)	4.76 (4.23-5.34)	3.64 (2.39-4.37)
HIV+ HAD	31	5	26	37 (33-44)	5.15(4.49-5.63)*	5.12 (3.95-5.41)**
HIV+ cART	49	15	34	38 (32-43)	0 (0-1.67)	0 (0-1.69)
HIV- control	79	27	52	39 (31-44)	N/A	N/A

N/A: Not available

*5 patients missing

**2 patients missing

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Table 2. Summary of biomarker concentrations across groups

Group	P-tau <i>(ng/l)</i> <i>Median (IQR)</i>	T-tau <i>(ng/l)</i> <i>Median (IQR)</i>	P-tau/T-tau ratio <i>(ng/l)</i> <i>Median (IQR)</i>	CSF Neopterin <i>(nmol/L)</i> <i>(Median (IQR))</i>
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

N/A = Not available

* = 1 Patient missing

** = 5 patients missing

*** = 3 patients missing

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Legends to figures

Fig 1a: P-tau (y-axis) across group. Log10 transformed. P-tau was significantly lower ($p < 0.05$) in cART compared to controls. No other significant differences were noted

Fig 1b: T-tau (y-axis) across group. Log10 transformed. T-tau was significantly higher in HAD compared to all other groups ($p < 0.001$). cART and NA was significantly lower compared to controls ($p < 0.004$ and $p < 0.01$ respectively)

Fig 1c: P-tau/t-tau ratio (y-axis) across group. Log10 transformed. P-tau/T-tau ratio was significantly lower in HAD compared to all other groups ($p < 0.001$). No significant differences were seen between other groups

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