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Cerebrospinal fluid biomarkers of central nervous system dysfunction in HIV-infected patients

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Table des matières

Introduction

In addition to opportunistic infections of the central nervous system (CNS), which are due to immunosuppression related to HIV, the latter virus, itself, can cause neuropathological abnormalities which are located mainly in the basal ganglia and are characterized by microglial giant cells, reactive astrocytosis and perivascular monocytes[1]. This HIV encephalopathy is characterized, clinically, by psycho-motor slowing, memory loss, difficulties in complex tasks requiring executive functions, as well as motor disorders [2]. These cognitive deficits are grouped under the acronym of HIV-associated neurocognitive disorders (HAND) [3].

In fact, HANDs are subdivided in three groups in accordance with the severity of the cognitive impairment: Asymptomatic Neurocognitive Impairment (ANI), Mild/moderate Neurocognitive Disorders (MND) and HIV Associated Dementia (HAD).
ANI may be considered as a presymptomatic form of HAND [4]. ANI is defined by a cognitive deficit in at least 2 cognitive areas (or domains), with at least one standard deviation below the values of age-matched healthy subjects. Typically, the deficits consist in impaired attention-concentration, mental slowing, decline of the memory, but, in ANI, there is no interference in everyday activities [2].
MND is described as cognitive impairment fulfilling the same criteria as ANI, but, in addition, there must be interference due to cognitive deficits in the daily activities.
HAD has the worse impact on the quality of life and survival [4]. It is characterized by at least two cognitive domains with values situated two standard deviations below age-matched healthy subjects and a severe functional impairment in daily activities. This dementia occurs usually, but not always, when the level of CD4+ T lymphocytes is under 200/ul [5]. The incidence of HAD has decreased significantly since the introduction of highly active anti-retroviral therapy (HAART) in 1996-1997.
However, the prevalence of mild cognitive complaints (MND) in HIV+ patients has paradoxically increased in the HAART era. There are many potential reasons to explain this state of facts.

First, since its introduction in 1996–1997, HAART has allowed many HIV+ patients to live longer [6]. Indeed the number of HIV+ patients older than 50 year-old is dramatically increasing [7]. However, this longer duration of HIV disease, together with the increasing age of HIV-infected persons may have a detrimental effect on cognitive function, possibly in relation with the development of several neurodegenerative diseases [7].
Another reason is the HAART penetration-effectiveness in the CNS. In fact, there could be limiting access of HAART within the brain through anatomical barriers such as the blood-brain barrier. Knowing that HIV has a strong neurotropism, its presence in the CNS may trigger and maintain a

moderately intense inflammatory response, which could be deleterious at term. Letendre et al. proposed a pharmacodynamic model to quantify the CNS penetration-effectiveness score (CPE) of different antiretroviral drugs [8]. Some validation of this CPE score has been obtained since the lower the CPE score, the higher the HIV viral load in the CSF [2]. However, the correlation between the CPE score and cognitive functions is missing [9] Therefore, prospective studies are needed to validate the CPE score as a clinical tool.

A third possibility is the putative neurotoxicity of HAART in the brain. Until now, there is no solid evidence supporting this hypothesis. However, Robertson and al. prospectively performed repeated neuro-psychological tests in a cohort of HIV + patients who had elected to discontinue antiretroviral therapy. Against all expectations, this study provided Class III evidence that discontinuing ART is associated with an improvement in 2 neuropsychological tests (Trail-Making Test A & B and the Wechsler Adult Intelligence Scale-Revised Digit Symbol subtest) for up to 96 weeks. However, Resuming ART was not associated with a decline in these scores for up to 45 weeks [10]. Nevertheless, there were several limitations to this study, one of them being the learning effect. Indeed, repeated neuropsychological assessment can result in practice or learning effects and falsely improvement in the neuropsychological tests [10].

Some new studies prove that there are similarities between HAND's and Alzheimer's disease (AD) [11].

Alzheimer’s disease (AD) is an age-related neurodegenerative disease characterized clinically by dementia and pathologically by the accumulation of amyloid plaques of neurofibrillary tangles. The toxic form of amyloid-β 1-42 is typically decreased in the CSF of HIV-negative patients with Alzheimer disease [12].

Interestingly, Clifford et al. (2009) found low concentration of amyloid-β 1-42 in the CSF of HIV patients with HAND, such as it is the case in AD patients. One of the reasons to explain this decrease of amyloid-beta in the CSF may be the protein Tat. Tat, a HIV-1 transactivator regulatory protein, is an endonuclease which inhibits neprilysin, this enzyme being responsible for amyloid-β breakdown [13]. Thus, through this mechanism, HIV infection may block the degradation of amyloid-β 1-42, which will lead to an accumulation of this compound in the brain and a decrease of its clearance in the CSF.

In addition to low concentrations of amyloid-β 1-42, the CSF of AD patients is also characterized by elevated levels of total Tau (t-Tau) as well as phosphorylated Tau (p-Tau) [7]. Tau is another protein involved in AD. The studies looking at the level of total Tau and p-Tau in the CSF of patients with HAND are contradictory. Some studies have shown that Tau is elevated in the CSF of HIV+ patients, such as it is found in AD [7] and others have described normal levels of Tau [14]. Recently, Gisslen et al. did a comprehensive study of several biomarkers and found that the combination in the CSF of a

low level of soluble amyloid precursor proteins (sAPP) alpha and beta and a normal level of phosphorylated Tau allowed to discriminate patients with HAD from AD patients, but not from those HIV+ patients with opportunistic infections. These authors concluded that parallel reductions of CSF sAPP in HAD and CNS opportunistic infections suggest an effect of CNS immune activation or inflammation on neuronal amyloid synthesis or processing [15].

However, other markers in the CSF of HIV+ patients may be of interest to try to understand the action of HIV in the CNS. One of them is the neopterin who is a product of guanosine triphosphate pathway [16]. Neopterin is produced by monocytes/macrophages activated mainly with IFN-γ. This marker seems to provide a stable indicator of the aggregate macrophage activation in the CNS compartment. One study [17] showed that high concentrations of neopterin are found in the CSF of patients with HAD. Actually, the more elevated is neopterin in the CSF, the more increased the risk of HAD [16].

S100-β is another potentially interesting biomarker. It is an acidic calcium-binding protein that is found in astrocytes. High concentration in CSF may be associated with neuronal apoptosis [16]. Elevated CSF concentration increases the risk of HAD. It has recently been shown that extensive astrocyte infection was prominent in patients with HIV-associated dementia, suggesting that astrocytes play a prominent role in HIV neuropathogenesis [18]. In another recent study, Eugenin and al. showed that, in cell culture, infection with HIV of only 5% of astrocytes was sufficient to cause a disruption of the blood-brain barrier. This disruption could increase the permeability to molecules that are normally absent in brain parenchyma, including large proteins, which would lead to apoptosis of endothelial cells [19].

An important question is to understand how soon the brain may be affected by HIV. Indeed, the fact that well-treated HIV+ patients with undetectable HIV viremia still develop HAND suggests that, in these patients, there is a low-grade dysfunction in the CNS that antedates the start of HAART. Since performing a biopsy in these patients is not an issue, the study of the CSF represents the best available way to look at putative biomarkers of inflammation/neurodegeneration in the CNS. Such as described above, such studies have been performed earlier. However, most of these studies compared HIV+ patients without HAND with HAND patients. Here, we decided to look very early, even before the onset of HAND, whether there would be early biomarkers indicating a sub-optimal control of HIV in the CNS.

To examine how sensitive are the CSF biomarkers to indicate CNS insults caused by HIV, we proposed to take advantage of the MOST (Monotherapy Switzerland/Thailand study) study, recently published in AIDS [20], and collaborated with Prof. Pietro Vernazza in St-Gall. Prof Vernazza was indeed the leading author of this trial, which was supported by the Swiss HIV Cohort Study.

In MOST study, monotherapy (MT) consisting in ritonavir-boosted lopinavir (LPV/r) was compared to continuous conventional antiretroviral therapy including several molecules, hereafter referred as CT. The rationale for testing a monotherapy was that it may be a potential method to reduce toxicity and costs while maintaining full viral suppression [20].

To be enrolled in MOST, patients needed to be on ART for at least 6 months with suppressed HIV RNA in the blood (< 50 copies/ml). At study termination, 60 patients had been enrolled, including 31 on CT and 29 on MT for 96 weeks, with an optional switch to monotherapy offered to all patients on continued treatment at week 48 [20]. At baseline (CSF#1), week 48 (CSF#2) and study termination (CSF#3), patients were supposed to have a lumbar puncture for quantitative HIV- RNA analysis. Since the study had to be stopped prematurely, some patients had only two LP's: CSF#1, corresponding to study entrance, and CSF#3, corresponding to study termination whereas the latter could occur before week 48.

The study had to be prematurely stopped because six patients under MT presented HIV-RNA failure in blood after around 12 weeks. All 60 patients had a lumbar puncture at baseline and 45 of them at study termination (25 MT with blood viral load < 400, 5 failing MT, 15 under CT with blood viral load < 50).

At study termination, the fraction of patients with detectable HIV-RNA in the CSF was significantly higher in patients on MT than on CT [20].

Whereas no study patient exhibited obvious neuropsychological impairment, the HIV viral load in the CSF was detectable in 15 patients. HIV VL was detectable in the CSF of only three patients at study entrance (BL), all the others CSF samples came from patients on MT. (see Table 1)

Table 2. Summary of all patients with treatment failure in blood or detection of elevated HIV-RNA in CSF at any time during the study.

Patient group and ID[1]	Sex	Pre-treatment	CD4 nadir (/µl)	Treatment arm[2]	Week on study/MT	log RNA blood	log RNA CSF	WBC count CSF (/µl)	Protein CSF (g/l)	CD4 cell at term[5]	Symptoms of acute HIV infection
Blood failure											
101	Male	ATV/r, TDF, 3TC	57	MT	12	4.3	5.1	124	0.6	680	Yes
108	Female	LPV/r, ZDV, 3TC	5	DS	60/12	4.2	3.1	10	0.4	361	Yes
126	Female	LPV/r, ABC, 3TC	149	MT	12	4.1	5.0	67	0.9	380	No
302	Male	EFV, ZDV, 3TC	7	MT	24	3.0	4.1	10	0.4	130	Yes
303	Male	LPV/r, TDF, 3TC	54	MT	6	5.0	nd[3]	nd	nd	250	No
713	Female	EFV, TDF, 3TC	160	MT	24	3.0	3.7	29	0.4	710	Yes
CNS +RNA MT											
107	Male	LPV/r, TDF, FTC	211	DS	96/48	<1.6	2.9	3	nd	nd	No
703	Male	ATV/r, TDF, 3TC	370	DS	66/18	2.2	3.4	56	0.7	1030	No
704	Female	LPV/r, ABC, 3TC	100	MT	63	2.3	4.3	47	0.7	380	Yes
707	Male	TDF, 3TC, ZDV, EFV	130	DS	68/20	2.1	3.4	15	0.4	780	No
702	Male	LPV/r, 3TC, ZDV	120	DS	72/24	<1.6	2.1	2	0.4	1050	No
709	Male	LPV/r, TDF, FTC	20	MT	37	<1.6	2.4	1	0.2	410	No
714	Female	ABC, ZDV, 3TC, LPV/r	220	MT	48	1.9	2.5	22	0.4	680	No
124	Female	LPV/r, ZDV, 3TC	17	MT	44	<1.6	1.9	2	0.2	474	No
CNS +RNA CT											
709	Male	LPV/r, TDF, FTC	20	BL	0	<1.6	1.6	1	0.5	360	No
309	Male	ATV/r, TDF, FTC	126	BL	0	<1.6	1.7	1	0.4	333	No
110	Male	TDF, 3TC, EFV	185	BL	0	<1.6	1.9	2	0.5	447	No
703	Male	ATV/r, TDF, 3TC	370	DS	48/0	<1.6	1.6	2	0.9	1010	No

ABC, abacavir; ATV/r, atazanavir, ritonavir-boosted; EFV, efavirenz; FTC, emtricitabine; LPV/r, lopinavir, ritonavir boosted; TDF, tenofovir; ZDV, zidovudine; 3TC, lamivudine. [1]Patient: ID Number (first digit for study center), Blood failure: HIV-RNA in blood plasma >400 cp/ml, CNS +RNA MT or CT: Patients with detectable HIV-RNA in CNS either in MT or CT arm, respectively. [2]Treatment arm: MT, Monotherapy; CT, continuation therapy; DS, delayed switch; BL, triple therapy at baseline. [3]nd: not done. [4]HIV-RNA values are given as $\log_{10}$ cp/ml, values shown in bold are above the predefined failing criteria (2.6 $\log_{10}$, i.e. 400 cp/ml). [5]term: termination visit.

Table 1. Summary of all patients with treatment failure in blood or detection of elevated HIV-RNA in CSF at any time during the study (From Gutman C and al., AIDS 2010, 24:2347-2354).

Therefore, this study represents a unique opportunity to examine the CSF of patients whose HIV is just beginning to escape therapy. The main goal of our study was thus to determine whether the CSF of patients on MT exhibit a different pattern of the one of patients on CT. In particular, we considered that it would be particularly interesting to see if any of our biomarkers could be an earlier indicator of treatment escape than detectable HIV VL in the CSF [20].

As discussed above, amyloid-β 1-42, Tau total (tTau), phosphorylated Tau (pTau), Neopterin and S100-β are interesting to study as they are representative biomarkers of the major cells of the CNS: neurons, macrophages/microglia and astrocytes. Thus, we decided to determine the levels of these five biomarkers in the CSF of MOST patients.

Material and Methods

Subjects

Gutmann et al. (2010) enrolled 60 patients in the MOST study. All patients were under continued anti-retroviral therapy (cART) for at least 6 month with suppressed HIV-RNA (<50 copies/ml) for at least 3 months. All patients had a lumbar puncture at baseline and 45 of them at study termination (25 MT with blood viral load < 400, 5 failing MT, 15 under CT with blood viral load < 50). Cerebrospinal fluid was collected and processed in polypropylenes tubes and

stored at -80°C until the time of the assay [20]. Not all CSF samples were available for our study, indeed, we obtained a total of 85 samples of CSF corresponding to 49 patients. All these 85 CSF samples were tested in our lab. Nevertheless, we ended up analyzing 65 CSF samples from these 49 patients (see explanations in the statistical analysis).

In 13 of the tested samples, HIV-RNA in the CSF was elevated at some point during the study. Two patients had elevated HIV-RNA in CSF at baseline and eleven later in the course of the study, all were on MT (see table 2).

Table 2. Summary of all patients with detection of elevated HIV-RNA in CSF at any time during the study

Patient ID	CSF#	Week on study/MT	Treatment	RNA CSF	CD4 nadir (/µl)
101,mo	3	12	MT	120'000	57
108,AG	3	60/12	DS	1250	5
124,VM	3	44	MT	84	17
126,JB	3	12	MT	96200	149
302	3	24	MT	14000	7
702,BB	3	72/24	DS	130	120
703,EC	3	66/18	DS	2330	370
704,KS	3	63	MT	20000	100
707,SP	3	68/20	DS	2500	130
709,dj/BL	1	0	CT	43	20
709,JD	2	37	MT	250	20
713 rb/BL	1	0	CT	4800	160
714,BN	2	48	MT	320	220

Table 2: **CSF#1** : Lumbar puncture at baseline, **CSF#2**: lumbar puncture at 48 weeks, **CSF#3** : lumbar puncture at study termination, **MT** : monotherapy, **CT:** conventional therapy, **DS:** Delayed switch (passage from CT to MT)

As controls, we used the CSF of 37 HIV-negative patients suffering from dementia (AD patients) that had been collected between 1999 and 2008 in the CSF/serum bank of the Division of Neurology of the CHUV. Thirty patients were diagnosed with Alzheimer's disease and 7 patients with frontotemporal dementia.CSF was collected and processed in polypropylenes tubes and stored at -80°C until the time of the assay.

Enzyme- Linked Immunosorbent Assay (ELISA)

To determine the presence of the five different biomarkers, we performed Enzyme- Linked Immunosorbent Assay (ELISA) according to manufacturer instructions. Principle of a solid phase sandwich is described in the figure.1.

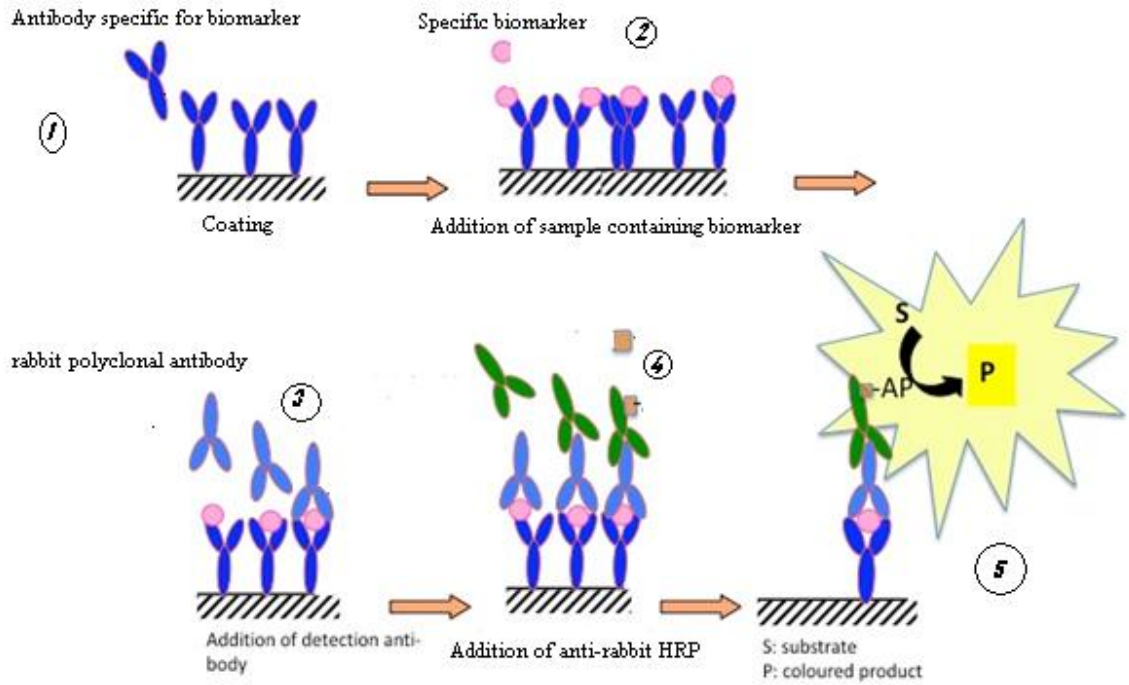

Figure 1 (1) Plate is coated with a capture antibody ; (2) sample is added, and if the studied antigen is present, it will bind to capture antibody ; (3) After washing, a rabbit polyclonal antibody specific for the given antigen is added and binds to it; (4) antibody against the detection antibody, such as an anti-rabbit, and coupled to HRP is added, and binds to detecting antibody ; (5) After a third incubation and washing to remove all the excess anti-rabbit HRP, a substrate solution is added, which will bind to the enzyme to produce color. The intensity of the color is proportional to the concentration of biomarkers. I thank Joana Le Boudec for this figure.

Amyloid-β 1-42 (Aβ42)

CSF concentrations of Amyloid-β 1-42 were determined with the Invitrogen Human Aβ42 US kit (Invitrogen, Camarillo, CA). This assay is specific for the COOH-terminus of the 1-42 Aβ sequence. This COOH-terminal sequence is created upon cleavage of the analyzed precursor. Absorbance was read with a spectrophotometer at 450 nm. The minimum detectable dose of Hu Aβ42 is 1.0 pg/mL. Samples were diluted for the assay and measured values were multiplied by the appropriate factor for the sample dilution. We analysed 85 samples of MOST study patients and 36 samples from AD patients.

Phosphorylated Tau (pTau)

CSF concentrations of phosphorylated tau (pTau) were determinded by an ELISA kit from Invitrogen (Camarillo, CA) and the Innotest Phospho-Tau (181p) ELISA from Innogenetics (Gent, Belgium). The minimum detectable dose of Hu Tau [pT181] is 10 pg/mL for Invitrogen ELISA kit and 15.6 pg/ml for Innotest Phospho-Tau ELISA. Samples were diluted for the assay and measured values were multiplied by the appropriate factor for the sample dilution. Forty-one samples from patients included in the MOST study were tested with the Invitrogen ELISA kit. Subsequently, Innotest Phospho-Tau

(181p) from Innogenetics was used for the remaining 44 MOST study patients and for 36 sample from AD patients

Total Tau (tTau)

CSF concentrations of tTau were determined by ELISA Invitrogen (Camarillo, CA, 2010). The minimum detectable dose of Hu Tau is 12 pg/mL. Samples were diluted for the assay and measured values were multiplied by the appropriate factor for the sample dilution.

S100-β

CSF concentrations of S 100-β were determined by Human S100B ELISA Kit (Abnova, Ville, Pays). Samples were diluted for the assay and measured values were multiplied by the appropriate factor for the sample dilution. This kit has a limit of detection of 15 pg/ml.

Neopterin

CSF concentrations of neopterin were determined by the ELISA kit (RE59321) of IBL (Hamburg, Germany,2008). For this ELISA, the intensity of the color developed after the substrate incubation is inversely proportional to the amount of antigen. Sample limit detection was 0.7 nmol/l .

Statistical analysis

In MOST study, each patient was supposed to undergo three LP's. One at study entrance (CSF#1), one at 48 weeks (CSF#2) and one at study termination (CSF#3), which was initially supposed to take place at week 96. However, this study was prematurely halted due to HIV VL failure in six patients. Therefore, only a minority of patients had all three CSF samples. Furthermore, we were not able to obtain all the CSF samples that had effectively been collected (loss of samples, insufficient CSF remaining, etc.). Thus, as we were interested in the effect of MT versus CT on CSF biomarkers, we decided to analyze the CSF corresponding to the longest time spent on CT, respectively on MT. This way of analysis allowed us to optimize the amount of samples at our disposal. For instance, if a patient was all along the study on CT, we analyzed only the last available CSF sample. However, if a patient was switched at week 48 from CT to MT, we analyzed the CSF at 48 week in the CT group, since it corresponded to the longest possible time spent on CT, and we analyzed the values of the CSF#3 (corresponding to study termination) in the MT group (whenever this third LP was performed between 48 weeks and 96 weeks). Finally, for patients who were put right away on MT, we used the entrance study CSF for CT (since it was an enrollment condition that all patients were on CT at study entrance) and, for MT, the CSF sample corresponding to the timepoint the patient had spent the longest time on MT.

Such as mentioned above, we enrolled patients with dementia (mostly AD) since several authors have drawn parallels between neurodegenerative diseases and CNS repercussion of HIV infection. Thus, we compared the CSF corresponding to the lengthiest time spent on CT or MT with the CSF of dementia patients.

Statistical analysis was performed with GraphPad Prism software (GraphPad Software, San Diego, CA, USA). The difference between two groups was tested using the non-parametric Mann–Whitney ranked test.

Results

The results are summarized in Table 3 and Figures 2 to 4.

Table 3 Median values +/- interquartile CSF concentration of the five biomarkers in all three categories of study patients (in pg/ml, median +/- interquartile ranges)

	Aβ42	pTau	tTau	Neopterin	S100-β
MT	391 ± 444	30 ± 36	199 ± 196	2.7 ± 4.6	499 ± 1125
CT	466 ± 489	35 ± 41	150 ± 153	1.2 ± 2.5	0 ± 532
AD	234 ± 328	46 ± 98	270 ± 414	1.1 ± 0.9	174 ± 214

Remark: for AD patients, S100-β was measured in the CSF of all 37 AD patients; however, the four other biomarkers were measured only in 36 CSF samples of AD patients due to the minimal amount of CSF at disposal in AD patient#37.

Group differences in CSF biomarkers

Figures 2, 3, and 4 show comparisons of the CSF levels for the five biomarkers between MT and CT, MT and AD, and CT and AD, respectively.

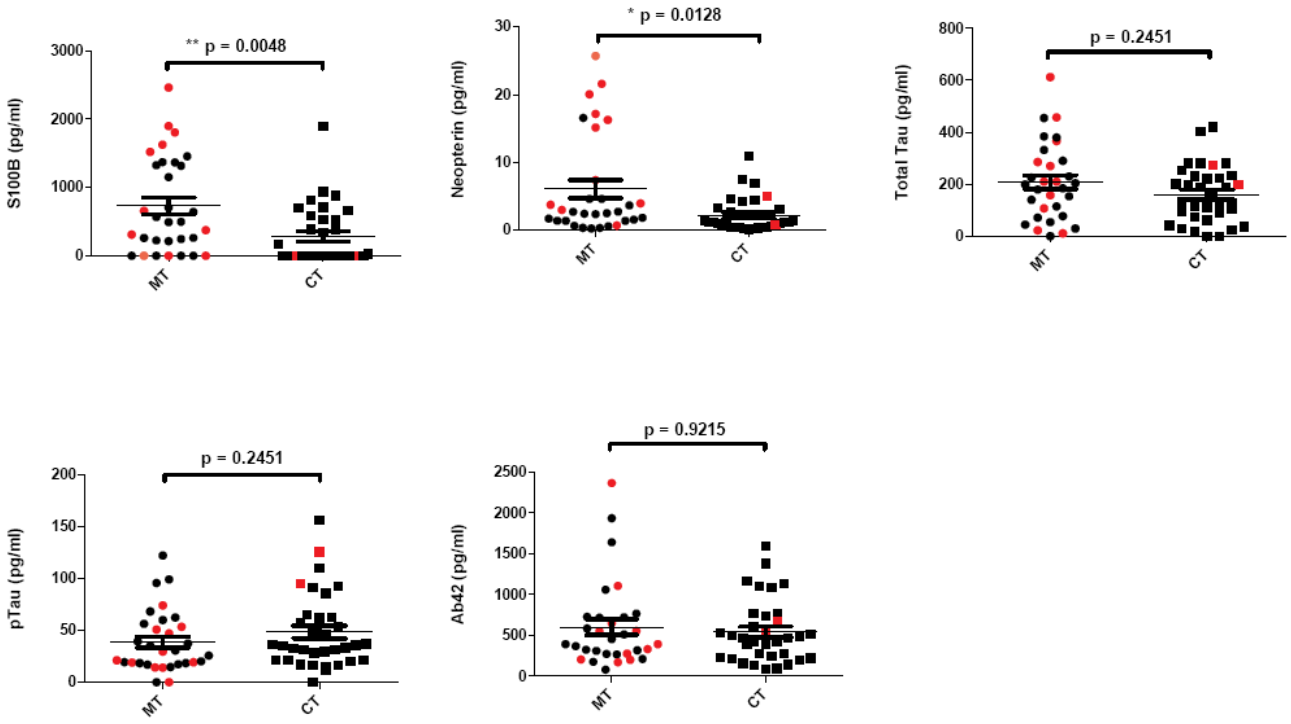

Figure 2 Comparison between MT and CT for the five CSF biomarkers.
S100β (p = 0.0048) and Neopterin (p = 0.0128) were significantly elevated in MT patients as compared to CT. We found no significant difference for the three other biomarkers between the two categories of study patients. Red points correspond to patients with detectable HIV RNA in the CSF at any time point during the study (see table 2)

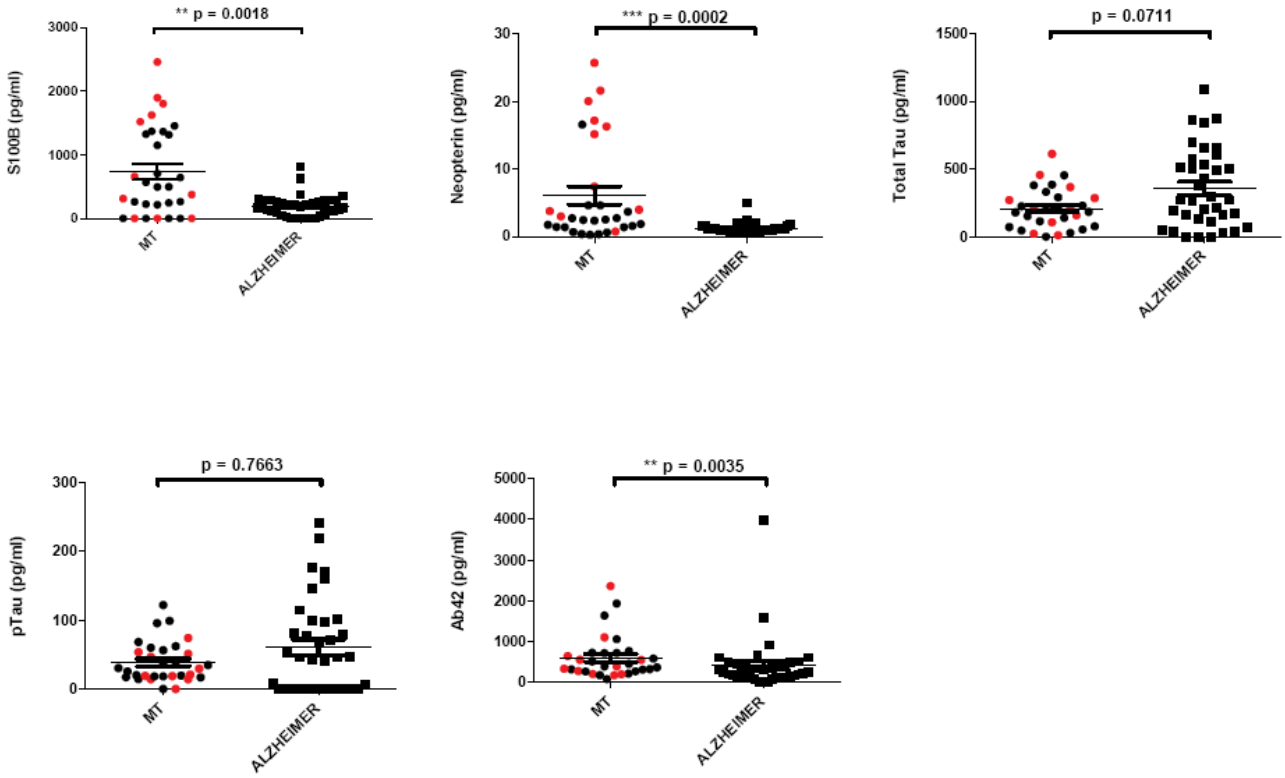

Figure 3 Comparison between MT and AD for the five CSF biomarkers
S100β (p = 0.0018) and Neopterin (p = 0.0002) were significantly elevated in MT patients as compared to AD. Aβ42 (p = 0.0035) was significantly lower in AD patients as compared to MT. We found no significant difference for the two other biomarkers between the two categories of study patients.

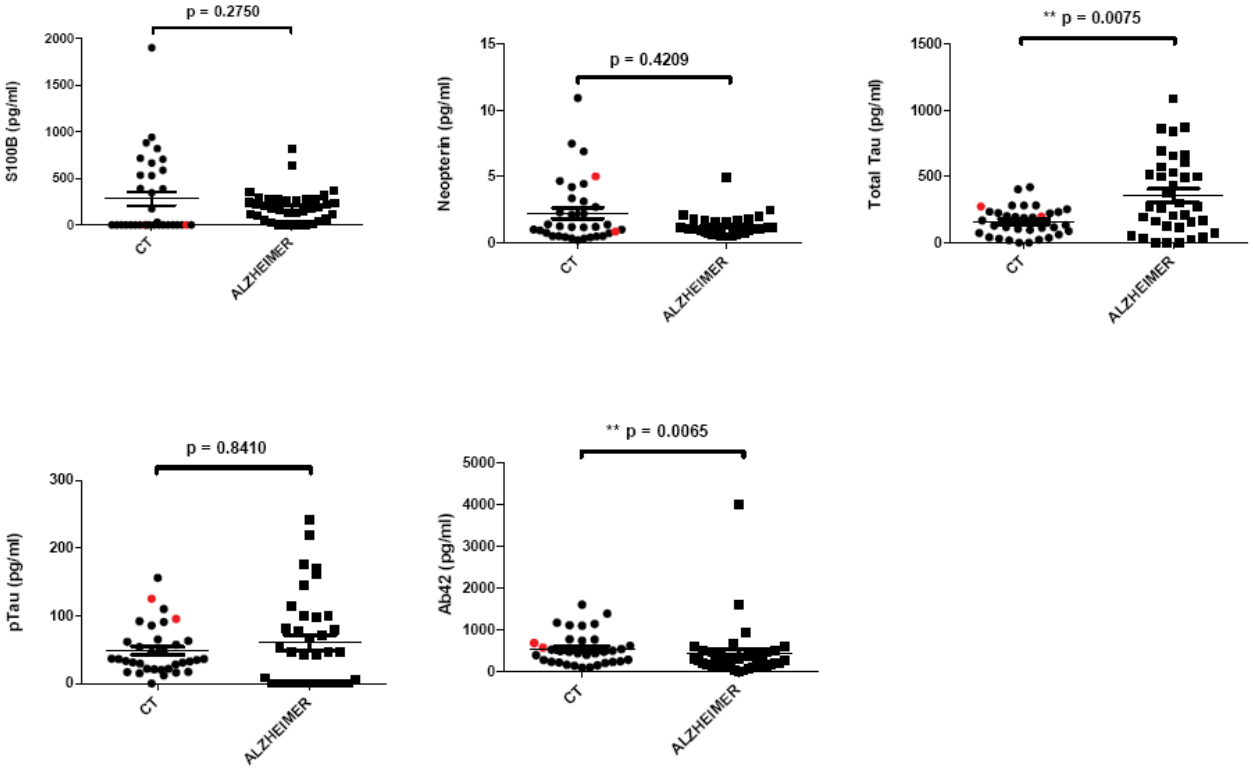

Figure 4 between CT and AD for the five CSF biomarkers

tTau (p = 0.0075) was significantly elevated in AD patients as compared to CT. Aβ42 (p = 0.0065) was significantly lower in AD patients as compared to CT. We found no significant difference for the other biomarkers.

Second step of analysis:

In order to determine how sensitive our biomarkers were, we removed from the analysis the patients with blood failure who led to study termination, i.e. subjects 101, 108, 126, 302, 303, and 713. Indeed, in these patients, the fact that the HIV VL became detectable on MT precluded the usefulness of CSF biomarkers. Thus we kept only those who had undetectable HIV VL in the blood at study termination . As we had not received CSF#2 or #3 from 303 and never received any CSF from 713, we ended up with four CSF samples to remove, corresponding to CSF#2 of 101, CSF#3 of 108, CSF#2 of 126, and CSF#2 of 302. However, we kept CSF#1 of 126 and 302 and CSF#2 of 108 since these CSF were taken while the patients were on CT. The results are summarized in figures 5 (MT vs CT) and 6 (MT vs AD). By definition, since the six patients with detectable HIV VL in the blood were all in the MT category, there was no change in the CT versus AD figure.

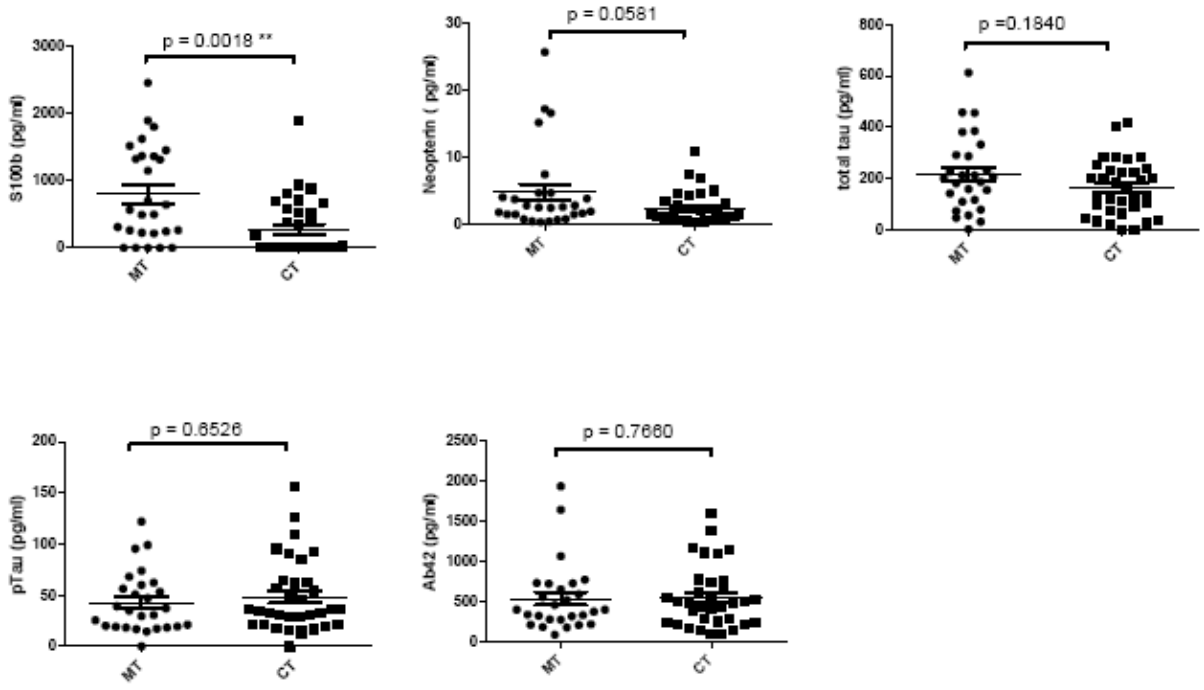

Figure 5 between MT and CT for the five CSF biomarkers
S100β (p = 0.0018) was significantly elevated in MT patients as compared to CT. We found no significant difference for the four other biomarkers between the two categories of study patients, although there was a strong trend for an increased level of neopterin in MT as compared to CT.

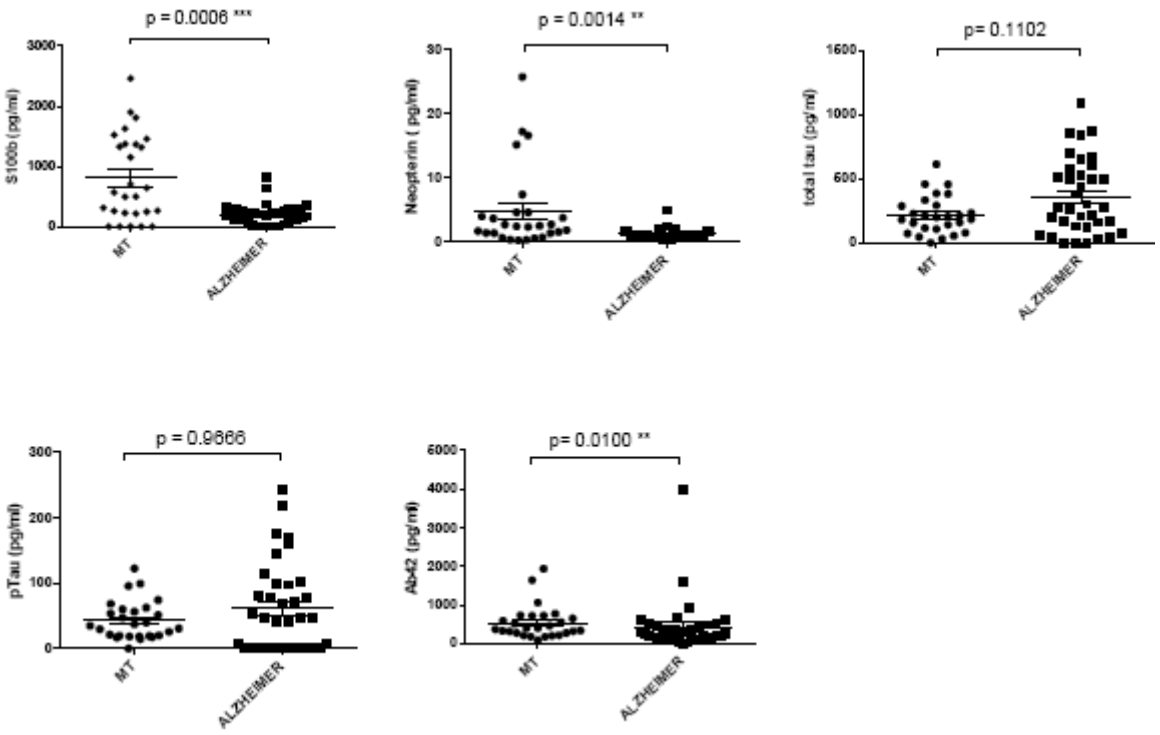

Figure 6 between MT and AD for the five CSF biomarkers
S100β (p = 0.0006) and Neopterin (p = 0.0014) were significantly elevated in MT patients as compared to AD. Aβ42 (p = 0.0035) was significantly lower in AD patients as compared to MT. We found no significant difference for the two other biomarkers between the two categories of study patients

Limitations

Limitation of this study could be the fact that we couldn't make ELISA with the same conditions for pTau. Actually, there were an interuption of the stock at the end of the first plate with ELISA kit from Invitrogen (Camarillo, CA). We continue the two last plates with another ELISA kit the Innotest Phospho-Tau (181p) ELISA from Innogenetics (Gent, Belgium). Forty-one samples from patients included in the MOST study (14 CT and 27 MT) were tested with the Invitrogen ELISA kit. Subsequently, Innotest Phospho-Tau (181p) from Innogenetics was used for the remaining 44 MOST study patients (20 CT and 24 MT) and for 37 AD patients.
However we found no difference between the method and the results of both ELISA, such as displayed in Figure 7.

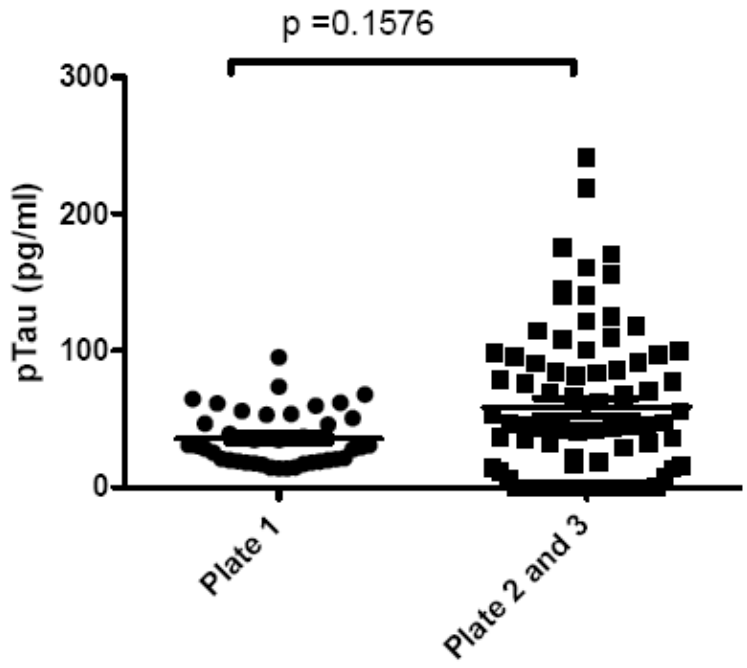

Figure 7 Comparison between two kits of pTau. There are no differences between the groups

Discussion

MT vs CT

First, we found that the CSF of patients on MT contained significantly higher levels of S100β than the CSF of CT or AD patients. These finding suggests that the astrocytes were damaged in patients on MT. Actually, Euginin and al., found that only few percentage of infected Astrocytes could cause a disruption of the blood-brain barrier, leading to an increased influx of cells and macro-molecules from the blood into the brain. Such an influx could contribute to the low-grade inflammation that is suspected to take place in the brain of patients under sub-optimal antiretroviral therapy. The MOST study had to be stopped due to blood HIV VL becoming detectable in six patients. When the CSF were analyzed, additional patients were found to have detectable HIV VL in the CSF (table 1). Thus, we know from MOST study that MT patients were sub-optimally treated, however, many of them had undetectable HIV VL in the blood and in the CSF (Figures 2 and 3). Yet, several of these patients were found to have elevated S100β. Therefore, we propose that S100β is a very early marker of suboptimal response to antiretroviral therapies. The promising value of S100β as a biomarker is further suggested by the fact that the difference between MT and CT or AD was maintained even when patients with detectable HIV VL in the blood (those who led to study cessation) were removed from the analysis (Figures 5 and 6). It also may explain why some patients on antiretroviral therapy and undetectable HIV VL in the CSF still develop cognitive impairment in long-term. Second, our data suggest that astrocytes may be crucial players in the pathogenesis of HIV encephalopathy, and thus possibly of HIV-associated neurocognitive disorders. Indeed, S100β at high doses causes neuronal apoptosis both via a direct action on neurons and via stimulation of nitric oxide (NO) release by astrocytes [21].

We also found that patients on MT had significantly higher CSF neopterin than CT or AD. Such as stated in the Introduction, neopterin is a marker of macrophages / microglia and thus suggests that inflammation goes on in the CNS of those patients with elevated CSF neopterin. With the notable difference, when compared to S100β, that when we removed the patients with detectable HIV VL in the blood, the difference between MT and CT disappeared (Figure 5), even if a strong trend remained. These data suggest that neopterin may be less sensitive than S100β to detect CNS inflammation in the brain of HIV+ patients. Nevertheless, neopterin has been much more studied that S100β as a biomarker of inflammation in the brain of HIV+ patients [22,23]. Another difference with S100β is that neopterin correlated better with the increased CSF HIV VL in patients. This feature suggests that the inflammation in the brain parallels the replication of HIV, which gives weight to neopterin as a good indicator of sub-

optimal therapy. However, it also means that neopterin is somewhat redundant with HIV VL in the CSF to identify patients who fail on anti-retroviral therapy.
Some study [17] shows that there is a correlation between concentration of neopterin and severity of the cognitive disorders. However, we cannot confirm this fact in the current study since MOST study patients had not been formally tested by a neuro-psychological battery.

Finally, we found no significant difference in terms of CSF Aβ42, Total tau, and pTau between patients on MT and patient on CT. In another study, authors [14, 15] have shown that there were difference in terms of CSF Aβ42, pTau and tTau between HIV+ patients with cognitive impairment and controls. However, in the MOST cohort, cognitive functions were not assessed. Based on our data, we hypothesize that the very first signs of sub-optimal response to anti-retroviral therapies are inflammation (neopterin) and astrocytic damage (S100β). Neuronal damages, such as reflected by elevated Tau, are a latter process.

AD vs CT

First, we found that patient on CT had lower Total Tau CSF than AD patients. Many studies [12] demonstrated that high total- and p-tau and low Aβ42 in the CSF are characteristic of AD. In our study, the CSF neuronal markers of patients on CT were at values similar to what is described in the literature for healthy subjects [12]. Indeed, CT had lower tTau and higher Aβ42 as compared to AD patients.

MT vs AD

First, we noticed that S100β and neopterin were significantly elevated in the CSF of MT as compared to AD patients. Of note, such differences were absent when CT was compared to AD. Thus these findings suggest that inflammation and astrocytic damages are restricted to MT patients and are not a feature of AD.
Such as for CT, the Aβ42 in the CSF of MT patients was significantly higher than in AD patients, suggesting that there is no dysregulation of the pathway of amyloid, even in sub-optimally treated HIV+ patients. However, contrasting with CT, there was no longer differences in terms of tTau between MT and AD patients. These data point to early development of neuronal damages in the brain of patients on MT, thus suggesting that the microglial and astrocytic damages are rapidly impacting on neurons. Nevertheless, the fact that tTau was not significantly higher in the CSF of MT as compared to CT ponders this observation. Thus, we cannot rule out that the observed difference between MT and AD may just be due to chance.
In conclusion, we consider that Neopterin and especially, S100β, are promising early biomarkers of sub-optimal response to antiretroviral therapy in the CNS of HIV+ patients.

In conclusion, we foreground two early biomarkers of HIV neurodegenerative process, S100β and neopterin. It will be very interesting to care of the evolution of these markers according to severity and differents stages of HIV-associated neurocognitive disorders (HAND). Better understand this neurodegenerative process is an important stake for the new search about HIV patients. Actually, results of those new studies could develop more effective therapy.

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