Title

Higher Cerebrospinal Fluid Soluble Urokinase-type Plasminogen Activator Receptor, But Not Interferon γ-inducible Protein 10, Correlate With Higher Working Memory Deficits

Journal

JAIDS Journal of Acquired Immune Deficiency Syndromes, 90(1)

ISSN

1525-4135

Authors

De Almeida, Sergio M
Rotta, Indianara
Tang, Bin
et al.

Publication Date

2022-05-01

DOI

10.1097/qai.0000000000002924

Peer reviewed

HHS Public Access

Author manuscript

J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2023 May 01.

Published in final edited form as:

J Acquir Immune Defic Syndr. 2022 May 01; 90(1): 106–114. doi:10.1097/QAI.0000000000002924.

Higher cerebrospinal fluid soluble urokinase-type plasminogen activator receptor, but not interferon γ-inducible protein 10, correlate with higher working memory deficits

Sergio M. De Almeida[1], **Indianara Rotta**[1], **Bin Tang**[2], **Anya Umlauf**[2], **Florin Vaida**[3], **Mariana Cherner, PhD**[2,4], **Donald Franklin**[4], **Scott Letendre**[4,5], **Ronald J. Ellis**[4,6], **HNRC Group**[4]

[1]Universidade Federal do Paraná, Curitiba, Paraná, Brazil

[2]Department of Psychiatry, University of California, San Diego, CA, USA

[3]Division of Biostatistics and Bioinformatics, Department of Family Medicine and Public Health, University of California, San Diego, CA, USA

[4]HIV Neurobehavioral Research Center, University of California, San Diego, CA, USA

[5]Division of Infectious Diseases, Department of Medicine, University of California, San Diego, CA, USA

[6]Department of Neurosciences, University of California, San Diego, CA, USA

Abstract

Background: We hypothesized that the induction of monocyte activation biomarkers, especially soluble urokinase-type plasminogen activator receptor (suPAR) and interferon γ-inducible protein 10 (IP-10), is lower in HIV-1C than HIV-1B owing to a defective Tat cysteine dimotif (C30S).

Methods: A total of 68 paired cerebrospinal fluid (CSF) and blood samples from persons with HIV (PWH), free of CNS opportunistic infections, from a Southern Brazil outpatient HIV clinic were evaluated; HIV-1B subtype (n= 27), HIV-1C (n=26), other (n=15), and 19 HIV-negative controls. The levels of SuPAR, IP-10, neopterin, and β_2 microglobulin (β_2m) in the CSF and serum were quantified using different immunoassays.

Results: Overall, in PWH, increases in CSF suPAR, CSF/serum suPAR, and CSF/serum β_2m correlated with worse working memory deficits (r= 0.303, 0.353, and 0.289, respectively, all

Corresponding Author: Sérgio Monteiro de Almeida, MD, PhD, Complexo Hospital de Clínicas–UFPR, Seção de Virologia, Setor Análises Clínicas, Rua Padre Camargo, 280, Curitiba, PR, Brasil, 80060-240, sergio.ma@ufpr.br, Telephone/Fax: +55 (41) 3360-7974.

Authors' contributions: SM de Almeida participated in the conception and design of the study; patient recruitment; acquisition, statistical analysis and interpretation of clinical and laboratorial data; and drafting, revision and finalization of the manuscript.
I Rotta participated in the patient recruitment; acquisition, laboratorial analysis, and interpretation of clinical and laboratorial data; revision and finalization of the manuscript.
B Tang participated in the statistical analysis, revision and finalization of the manuscript.
A Umlauf participated in the statistical analysis, revision and finalization of the manuscript.
F Vaida participated in the statistical analysis.
M Cherner participated in the conception and design of the study; revision and finalization of the manuscript.
D Franklin performed the analysis of the data, revision and finalization of the manuscript.
S Letendre participated in the conception and design of the study.
RJ Ellis participated in the conception and design of the study; revision and finalization of the manuscript.

The authors declare that there are no conflicts of interest regarding the publication of this article.

p<0.05). The medians of IP-10, suPAR, neopterin, and β_2m in CSF and serum, as well as the CSF/serum ratio and suPAR index were comparable between the HIV-1B and HIV-1C subtypes. CSF IP-10 and neopterin, and serum IP-10 and suPAR levels were higher in PWH than the HIV-negative controls (p=0.015, p=0.001, p<0.0001, and p<0.001, respectively). Serum β_2m level was higher in HIV-associated dementia (HAD) than neuropsychologically normal (NP-NML) or asymptomatic (ANI) (p= 0.024).

Discussion: We observed that higher levels of CSF suPAR and the suPAR quotient correlated with worse working memory deficit. Elevated levels of monocyte activation were similar in both HIV-1 B and C subtypes, providing no evidence of reduced neuropathogenicity of HIV-1 subtype C Tat compared to subtype B.

Keywords

HIV-1; subtypes; HIV-associated neurocognitive disorders; cerebrospinal fluid; SuPAR; IP-10; neopterin; β_2 microglobulin

1. INTRODUCTION

Persistent central nervous system (CNS) inflammation and chronic immune activation, play important roles in neuronal damage in HIV-associated neurocognitive disorders (HAND)[1]. Many studies on inflammatory biomarkers of HIV-1 infection have been carried out in settings where HIV-1B predominates[2,3,4,5,6,7]. As a result, little is known about these markers in the non-B HIV subtypes.

Soluble urokinase plasminogen activator receptor (suPAR), or cluster differentiation (CD)-87, is the soluble form of uPAR. Circulating suPAR originates from the shedding of uPAR. SuPAR is a marker of monocyte activation and chronic inflammation[8]. Levels of suPAR are higher in the cerebrospinal fluid (CSF) of people with HIV (PWH) and HIV-associated dementia (HAD) than neurologically normal people without HIV[3, 4, 6]. SuPAR is involved in cell adhesion, migration, and chemotaxis. UPAR is a membrane-bound receptor that is mainly expressed on immunologically active cells, such as monocytes, activated T cells, neutrophils, tumor cells, megakaryocytes, and endothelial cells[9, 10]. UPAR is a receptor for uPA, a matrix-degrading proteolytic enzyme that disrupts the BBB[11].

Previous studies have reported that human interferon γ-inducible protein-10 (IP-10 or CXCL10) levels are elevated in PWH compared to healthy control subjects[5], and IP-10 stimulates HIV-1 replication[12]. The main biological activity of chemotactic cytokines, such as IP-10, is the regulation and control of basal homeostatic and inflammatory leukocyte movement[13]. IP-10 attracts activated T lymphocytes and monocytes[14] and is induced by proinflammatory stimuli, such as interferon-γ (IFN-γ)[15], tumor necrosis factor-α (TNF-α), viruses, and microbial products[15, 16, 17], either directly or through activation of nuclear factor-kappa β (NF-kβ)[16, 18]. IP-10 is produced by astrocytes and hepatocytes[19, 20], and its increased production during HIV-1 infection has been partially attributed to HIV-1 proteins, including HIV-1 accessory protein transactivator of transcription (Tat)[21].

The Tat protein plays a pivotal role in the induction of chemokine secretion, mainly CC chemokines (β-chemokines) and IP-10 (α-chemokines)[13, 21]. Tat also upregulates the expression of several cytokines, including TNF-α[22], which is attributed to the C30C31 dicysteine motif[23]. In vitro studies suggested that HIV-1 subtype C was less neuropathogenic than subtype B, based on a defective Tat chemokine dimotif in the C30C31 position that might influence cellular trafficking and CNS inflammation[24, 25]. I*n vitro*, HIV Tat C (C30S) was less effective at upregulating markers, such as TNF-α, in monocytes[26, 27]. Further, HIV Tat C does not induce calcium influx, which results in lower levels of IL-10 in monocytes[28]. In a Brazilian population, the frequency of C31S substitution in HIV-1 Tat C was 82% vs. 10% in HIV-1B ($p<0.0001$)[29].

SuPAR and IP-10 are positively correlated with IFN-ɣ and TNF-α[30]. Further, IFN-ɣ and TNF-α stimulations were dependent of intact HIV-1 Tat C30C31 dimotif. Notably, IP-10 is directly stimulated by Tat[21]. Based on the above, we hypothesized that the stimulation of monocyte activation biomarkers, mainly suPAR and IP-10, would be lower in HIV-1C than HIV-1B due to a defective Tat chemokine dimotif (C30C31).

The aims of this study were to compare the effects of HIV-1 subtypes B and C on suPAR, IP-10, neopterin, and β_2 microglobulin (β_2m) in CSF and serum; compare these monocyte activation biomarkers levels in PWH and HIV-negative controls. Secondary exploratory comparisons were done to assess the relationships between these biomarkers and neurocognitive performance in the HIV groups.

2. METHODS

2.1 CSF and blood samples

In this study, 87 CSF and paired serum samples were collected from PWH (n=68) and HIV-negative controls (n=19).

PWH were recruited from Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR), Brazil. Exclusion criteria included opportunistic CNS infections, loss of consciousness for greater than 30 minutes, non-HIV-related neurologic injury or disorder (e.g., epilepsy, stroke, and developmental delay), psychotic disorder, and significant levels of current substance use defined as more than two alcoholic drinks per day over the past 30 days, or use of any illegal drugs in the past 30 days.

All volunteers underwent serological testing to confirm HIV status before enrollment. For participants with a clinically resistant infection, the infecting HIV subtype was genotyped using *pol* sequences, whereas *env* sequences were used for all other participants. Genotyping revealed that 27 individuals were infected with HIV-1B while 40 were infected with non-B HIV-1 subtypes (C, n = 26; BF, n = 10; BC, n = 1; CF, n = 1; and F, n = 2). The subtype of one participant could not be determined.

Nineteen age-matched subjects were recruited at the HIV Neurobehavioral Research Center, University of California, San Diego (HNRC-UCSD) for the HIV-negative control group, under a protocol approved by the IRB at the UCSD. As, according with the

National Commission of Ethics in Research (CONEP, Brazil), lumbar punctures could not be performed in HIV-negative subjects in Brazil. These subjects had no neurological comorbidities or cognitive complaints and had negative serological tests for HIV, hepatitis C, and syphilis. The CSF inclusion criteria for this group were white blood cell (WBC) count ≤ 5 cells/mm^3, total protein ≤ 45 mg/dL, and glucose ≥ 55 mg/dL.

2.2 Laboratory methods

2.2.1 Quantification of Inflammatory biomarkers—SuPAR was quantified in the CSF and serum by high-sensitivity enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN); IP-10 (CXCL10) was quantified in CSF and serum by multiplex bead suspension array immunoassays (EMD Millipore, Billerica, MA); neopterin was quantified in CSF by high-sensitivity ELISA (Thermo Fisher Scientific, Hennigsdorf, Germany); and human β_2m was quantified in CSF and serum using the nephelometric method (Dade Behring BNII, Deerfield, IL) with reagent, N Latex β_2 microglobulin (Siemens Healthcare Diagnostics Products GmbH, Marbug, Germany).

All samples were assayed concurrently in duplicate according to the manufacturers' instructions. The acceptable coefficient of variation between duplicates was <20%. Where the results fell below the minimum detection limit set by the kit manufacturers, the low-detection-limit value was used in the statistical analysis.

The reference values for suPAR, IP-10, and neopterin were calculated as mean ± 2 standard deviations in the HIV-negative control group. The reference values for β_2m were 1.3±0.5 mg/L in the CSF and 0.9–3.0 mg/L in the blood[31].

To enhance the specificity of suPAR as an inflammatory biomarker, the suPAR index was calculated as: $\text{suPAR}_{\textbf{Index}} = (\text{suPAR}_{\textbf{CSF}} \times \text{Alb}_{\textbf{serum}}) / (\text{suPAR}_{\textbf{serum}} \times \text{Alb}_{\textbf{CSF}})$. Albumin levels in the CSF and serum were quantified using nephelometry (Dade Behring BNII, Deerfield, IL) with antiserum N human albumin (Dade Behring BNII, Deerfield, IL). The index was not calculated for IP-10 and β_2m as they are small molecules (MW 8.6 and 17 kDa, respectively) that can cross the blood-CSF barrier independent of its integrity; this is because diffusion through an intact barrier may occur along a concentration gradient[32]. However, such diffusion may be affected by changes in the CSF flow rate. The index was not calculated for neopterin, as its level in serum was not quantified.

2.2.2 Quantification of HIV RNA in plasma and CSF—HIV RNA levels in plasma and CSF were measured using a branched DNA assay (VERSANT® HIV-1 RNA 3.0 bDNA Kit, Siemens Healthcare Diagnostics, Tarrytown, NY, USA) with 1 mL of CSF or plasma. Assays were performed immediately following sample collection. Samples with HIV RNA levels <50 copies/mL were recorded as being below the detection limit.

2.2.3 Clinical laboratory measures—CD4 counts were quantified using flow cytometry (FACSCalibur-Multitest). Nadir CD4 levels were retrieved from medical records.

CSF samples were collected purely for research purposes through lumbar puncture, performed using atraumatic spinal needles under aseptic techniques. Total CSF protein, glucose, and WBC counts were quantified using standard laboratory methods.

2.3 Neurobehavioral assessments, HAND diagnosis, and categorization

All PWH underwent neuropsychological (NP) assessments, as previously described[33]. The NP test battery assessed seven domains (executive function, motor performance, verbal fluency, recall, working memory, learning, and speed of information processing) and was composed of 18 individual NP measures widely used to study HIV infection in previous studies in English- and non-English speaking countries (Supplementary Table 1)[33]. Instruments not already validated for use in Brazil were translated into Brazilian Portuguese, back translated into English, and reviewed by several Brazilian native Portuguese speakers to ensure cultural and linguistic appropriateness[33].

Demographic corrections for each NP test were developed using data from Brazilian 48 HIV− controls. Norms were statistically derived by methods previously described[33, 34]. Briefly, raw scores for the individual tests were converted to normally distributed scaled scores, which have a mean of 10 and a standard deviation of 3. The scaled scores were then converted to T-scores (mean 50, SD 10), with regression-based adjustments for the effects of age, years of education, and sex.

Subjective neurocognitive difficulty was assessed using the Patient Assessment of Own Functioning Inventory[35]. HAND diagnoses were assigned based on the Frascati criteria[36]. Additionally, the global deficit score (GDS) method was used to classify the overall NP impairment status, as previously described. The GDS summarizes the number and severity of neurobehavioral deficits across the entire test battery. A GDS cutoff of <0.50 was used to classify overall NP impairment[37]. To assess the specific neurocognitive performance domains the domain deficit scores were calculated based on individual test deficit scores; it is the mean of the individual test deficit scores that make up each domain.

2.4 Data analyses

The results are presented as median and interquartile range (IQR) or number and percentage (%), as appropriate. Demographic data, HIV disease characteristics, and CSF biochemical, cytological, and virology measures were compared between individuals with subtypes B and C infections using the independent samples *t*-test for continuous variables and Fisher's exact test for binary and categorical variables (sex, AIDS diagnosis, ART, and HIV RNA in plasma and CSF). Demographic data and CSF biochemical and cytological measures were compared between PWH (including subtypes B, C, BF, BC, CF, and F) and HIV-negative controls using the same methods.

CSF and serum biomarker values were $\log_{10}$-transformed to improve the normality of data distribution. Using linear regression (adjusted analysis), the four biomarker levels were compared primarily between 1) PWH and HIV-negative controls controlling for age; and 2) subtype B and C groups controlling for plasma HIV viral load suppression and nadir CD4 count. Correlation analyses between the biomarkers and neurocognitive performance (GDS and domain scores), CSF WBC, CD4 (nadir, current, and recovery), infection

duration, age (current and at beginning of infection), CNS penetration effectiveness (CPE) HIV RNA (plasma and CSF), Q Alb, and the seven specific cognitive domains assessed, were carried out. Correlation coefficients (ρ) were estimated using Spearman's rank-order method. In addition, secondary exploratory comparisons were done to assess the differences in inflammatory biomarkers in the PWH groups categorized by the HAND classification were explored using the Kruskal–Wallis or Mann–Whitney nonparametric tests (unadjusted analysis).

Results were considered statistically significant at the 5% alpha level. Cohen's d effect sizes (and 95% confidence intervals [CIs]) were reported for between-group differences.

2.5 Standard protocol approvals, registrations, and patient consents

This study, a cross-sectional survey using stored blood and CSF samples, was approved by the UCSD (San Diego, CA, USA) institutional review board (IRB), Hospital de Clínicas–Universidade Federal do Paraná (HC-UFPR, Curitiba, Paraná, Brazil) IRB, and CONEP (Brazil). All participants signed informed consent forms approved by the IRBs in the United States and Brazil. CSF samples were collected through an NIMH-funded study (R21 MH076651–01).

2.6 Data Availability

Anonymized data from the current study will be made available at the request of qualified investigators if approved by our Research Ethics Board.

3. RESULTS

Demographic, clinical, and laboratory characteristics were compared between PWH and HIV-negative controls, and individuals infected with HIV subtypes B and C (Table 1). Subtype B- and C-infected individuals were similar in age, sex, and years of education. Subtype B-infected participants had non-significantly lower nadir CD4 counts (median, 82 cell/mm^3 vs. 159 cell/mm^3, p=0.29) and were non-significantly more likely to be on ART (89% vs. 69%; p=0.099). ART included non-nucleoside reverse transcriptase inhibitors for 9 of the 21 (43%) patients with subtype B infections vs. 4 of the 15 (27%) patients with subtype C infections (p=0.48) (Table 1).

3.1 PWH versus HIV-negative controls and HIV-1 subtype B versus subtype C

The median CSF IP-10 and neopterin levels and serum IP-10 and suPAR levels were higher in PWH than the HIV-negative controls (p=0.015, p=0.001, p<0.0001, and p<0.001, respectively); however, CSF suPAR values were comparable between PWH and the HIV-negative controls (p>0.05) (Table 2). The medians of IP-10, suPAR, neopterin, and β_2m in CSF and serum, as well as the CSF/serum ratio and suPAR index were found to be comparable between HIV-1B and HIV-1C.

The medians of suPAR, IP-10, neopterin, and β_2m in CSF and serum, as well as the CSF/serum ratio and suPAR index were comparable in the GDS ≥0.5 and normal GDS groups.

3.2 Correlations with GDS and specific cognitive domains

Overall PWH, none of the four biomarkers studied in CSF or serum, nor the CSF/serum ratio or suPAR index correlated with GDS. However, higher CSF suPAR, CSF/serum suPAR, and CSF/serum β_2m correlated with worse working memory deficits ([ρ = 0.303 (0.043; 0.524), p= 0.020]; [ρ = 0.353 (0.099; 0.564), p= 0.006], and [ρ = 0.289 (0.028; 0.514), p= 0.026], respectively; Figure 1). Lower serum β_2m levels correlated with worse motor performance deficit (ρ = −0.271[−0.497; −0.011], p=0.036). CSF neopterin, serum suPAR, and IP-10 (CSF, serum, or CSF/serum ratio) were not correlated with the cognitive domains studied (p>0.05).

3.3 HAND

The median (IQR) serum β_2m levels across the HAND groups categorized based on the Frascati criteria increased from neuropsychologically normal (NP-NML) to HAD (p= 0.024, Figure 2A). However, the levels of the other inflammatory biomarkers were comparable (Figure 2B, Table 3). Pairwise comparisons between the HIV-negative control group (Table 2) and PWH categorized based on the Frascati criteria (Table 3) showed higher values in PWH than HIV-negative controls for serum suPAR and IP-10, and CSF neopterin (all ps<0.05); however, the values of CSF suPAR and IP-10 were comparable between the two groups (all p>0.05).

The suPAR index was elevated for both the PWH NP-NML and ANI groups versus HIV-negative controls (p<0.0001 both); comparison of HAD group with HIV-negative control group (p=0.248) (Table 2 and 3).

3.4 Correlations with Inflammatory biomarkers and other CSF measures

Higher levels of suPAR, IP-10, neopterin, and β_2m in CSF or serum, as well as their CSF/serum ratios were associated with higher CSF WBC, higher HIV RNA in CSF or plasma, and higher QAlb, but they were correlated with lower current CD4 and lower CD4 recovery (Supplementary Table 2). Higher suPAR index was associated with lower QAlb (ρ = −0.705 [−0.810; −0.555], p<0.0001), but had no correlation with cognitive domains. No correlations were found with the other variables studied.

4. DISCUSSION

The present study evaluated the monocyte activation biomarkers, suPAR, IP-10, and β_2m, in CSF and serum, and neopterin, in serum, in PWH with chronic infection. IP-10 and neopterin in CSF samples, and IP-10 and suPAR in serum samples were higher in PWH than the HIV-negative controls. The increase in these biomarkers in the CSF and serum of PWH was subtype-independent; therefore, our findings did not confirm the original hypothesis of the study. The increases in CSF suPAR, Q suPAR, and Q β_2m were correlated with worse working memory deficits, while the other biomarkers were not correlated with specific cognitive domains. These findings indicated that an increase on CNS monocyte activation and chronic inflammation decreases the cognitive skill to hold in mind and mentally manipulate information over a short period of time when faced with distractions. Further, working memory is important for reasoning and decision-making and behavior[38].

The levels of the four biomarkers in CSF and serum were comparable between the groups with neurocognitive impairment and normal cognition as measured by the GDS; however, none was found to be correlated with GDS.

When study participants were categorized based on the Frascati criteria[36], serum β_2m, but not CSF β_2m, levels were increased in the HAD group, despite the few participants in this group. Contrary to the findings of our study, high levels of β_2m were previously detected in the CSF of PWH with HAND[39,40]. The other inflammatory biomarkers studied were comparable in the CSF and serum, in contrast to previous studies[2, 3, 4, 5, 6], though many of these studies included high proportions of PWH without viral suppression on ART. However, another study revealed that CSF suPAR levels were decreased in PWH[7].

The findings of the present study provide additional support for our previously published studies that found no differences in the frequency of HAND between HIV-1B and HIV-1C[33]. Moreover, CSF inflammation, investigated based on the increase in CSF WBC, β-chemokine, interleukin levels, IgG intrathecal synthesis, and the presence of oligoclonal bands, as well as CSF discordance or CSF viral escape, occurred at comparable frequencies between HIV-1 subtypes C and B[41, 42, 43]. However, previously, we found subtype-dependent differences in amyloid pathway impairment[44, 45].

In this study, the increase in CSF and serum suPAR correlated with an increase in CSF WBC, while the increase in serum suPAR correlated with an increase in CSF and plasma HIV RNA levels. In animal studies, uPAR is expressed in resident microglial cells in the normal brain and in both acute and chronic inflammation[46]. Monocytes/macrophages are important migratory cells and uPAR is expressed by cells of the monocyte lineage[9] and is induced by cytokines, in particular, transforming growth factor β1 (TGFβ1)[47]. Under normal conditions, uPAR expression is negligible. However, under challenge, they display defective recruitment and migration of neutrophils and lymphocytes[48]. Low levels of uPAR expression was observed in cultured microglial cells, although expression increased upon lipopolysaccharide (LPS) stimulation. However, uPAR was undetected in microglia immediately *ex vivo*[49]. Binding of uPA to uPAR facilitates the activation of proteases at the cell surface and in the extracellular spaces, and also activates cell signaling pathways directed by proximal transmembrane co-receptors, promoting cell migration. uPAR promotes cell migration by activating proteases and through its effects on cell signaling[50].

In this study, the increase in CSF and serum IP-10 correlated with the increase in CSF WBC, CSF, and plasma HIV RNA levels. Further, the increase in CSF and serum IP-10 levels correlated with poor CD4 recovery, and the increase in serum IP-10 was correlated with a decrease in current CD4. The ability of IP-10 to enhance its own inducer, IFN-ɣ, supports the hypothesis that a positive amplification loop exists between IP-10 and IFN-ɣ *in vivo*[51]. This chemokine has been proposed to be involved in the recruitment and potentiation of Th1 responses. Tat and cytokines, IFN-γ and TNF-α, also synergistically increase the expression of IP-10 in human astrocytes, providing an important reservoir for the generation of inflammatory mediators. For instance, IP-10 acts as a neurotoxin and a chemoattractant[52]. Further, HIV-1 Tat induces IP-10 expression in astrocytes[21].

We also evaluated neopterin and β_2m, which are traditional inflammatory biomarkers for HAND diagnosis[39, 40]. These markers were found to be elevated in the CSF of patients with HAD, suggesting that increased immune activation is related to more severe damage[2]. Our study revealed a positive correlation between β_2m levels in CSF and serum in the group with cognitive impairment measured using GDS (≥0.5) and the cognitively normal group (GDS <0.5). In a previous report, HIV-infected individuals without dementia showed a strong correlation in the levels of β_2m between plasma and CSF. However in patients with dementia, β_2m levels were independently higher in CSF than in plasma, suggesting intrathecal β_2m production[2]. Contrary to these findings, our study showed that β_2m levels were lower in CSF than serum across all groups.

CSF molecules can be derived exclusively from the blood, brain parenchyma, or from the leptomeninges[32]. Our data indicate that IP-10 and suPAR are derived from intrathecal synthesis, either because the concentration was higher in the CSF than serum, or because the coefficient of variation was smaller in the CSF than serum in the control group, respectively. The latter result suggests that inter-individual variations in CSF biomarkers do not depend on serum concentrations[32].

The main strength of this study was the fact that it was the first to examine inflammatory stimulation by quantifying suPAR, IP-10, neopterin, and β_2m in CSF and serum of PWH infected with the HIV-1 subtype C. All previous studies analyzed only HIV-1 subtype B. Additionally, the participants with HIV-1 subtypes B and C were from the same geographical region in southern Brazil and were similar in age and sex. PWH and HIV-negative samples were analyzed concurrently. The suPAR index was calculated to quantify intrathecal suPAR; suPAR molecular weight is similar to that of albumin (67 kDa; and Rh 3.51 nm). As a result, its diffusion does not occur through a functional blood-CSF barrier (BCSFB). The sample size was sufficient for power analysis of the comparisons of the PWH and HIV-negative control groups, as well as HIV-1B and HIV-1C, as the absolute values of Cohen’s d effect sizes were medium to large. However, when the PWH group was categorized based on HAND diagnosis, the number of cases was small, especially within the symptomatic HAND subgroups (HAD and MND), thereby limiting the conclusion on the association between monocyte activation biomarkers and neurocognitive impairment.

This study had the following limitations: (a) both PWH who were on antiretroviral treatment or untreated were enrolled, with most of the participants being on ART. We tried to overcome this limitation by considering the plasma HIV viral load in the multivariate analysis. Nonetheless, HIV-1B and HIV-1C were found to be comparable in the CSF to plasma HIV RNA ratio; and (b) the cross-sectional design limited the study. A longitudinal study might be able to predict the development of HAND in patients without apparent symptoms. (c) The method we used to quantify HIV RNA had a limit of detection of 50 copies/mL, which is less sensitive than some other assays currently available. Single-molecule assays, which quantifies plasma HIV-1 RNA down to a single copy, has enhanced the understanding of the source and dynamics of persistent HIV-1 in the plasma, cells as well reservoirs of PWH[53]. The application of single cell sequencing (scRNA-seq) technologies has emerged as an essential tool for studying perturbations to host immune cells during systemic HIV infection, and to understanding HIV reservoirs[54, 55]. Future CSF

studies will benefit from the utilization of these methodologies, as it was demonstrated viremia persists in the plasma and CSF after years of effective therapy[53].

5. CONCLUSION

To our knowledge, this is the first study to evaluate monocyte activation stimulation in both CSF and serum of the HIV-1 subtype C, thereby contributing to the understanding of the pathophysiology of HIV infection in the CNS and the impact of HIV-1 genetic diversity on monocyte activation biomarkers. The impact of HIV-1 on the studied monocyte stimulation biomarkers was not found to be subtype-dependent. Further, higher CSF suPAR, and suPAR quotient correlated with a higher working memory deficit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Role of funding source:

Funding organizations did not contribute to the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflicts of Interest and Source of Funding

This research was supported by NIH R21 MH76651 (principal investigators: R. Ellis, S. de Almeida), Ministério *da* Ciência e Tecnologia/Conselho Nacional de Desenvolvimento Científico e Tecnológico, MCT/CNPq-Universal 014/2008, Brazil (Almeida, Sergio M).

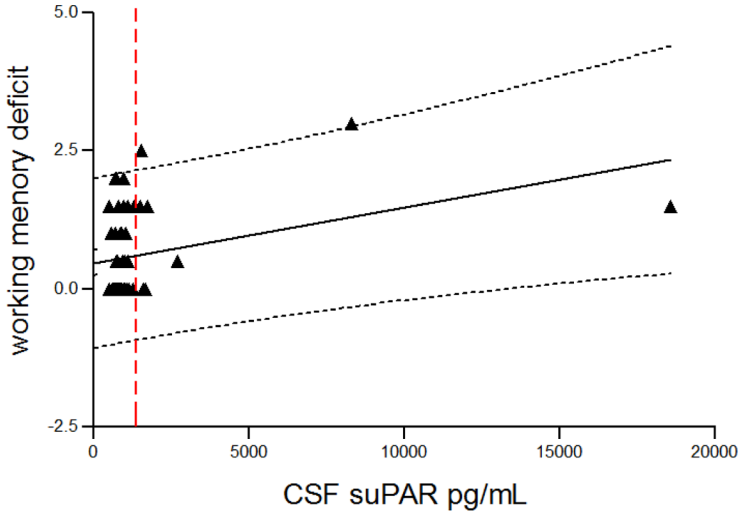
REFERENCES

1. Ruhanya V, Jacobs GB, Naidoo S, et al. Impact of Plasma IP-10/CXCL10 and RANTES/CCL5 Levels on Neurocognitive Function in HIV Treatment-Naive Patients. AIDS Res Hum Retroviruses 2021; 37:657–665. [PubMed: 33472520]
2. Enting RH, Foudraine NA, Lange JM, et al. Cerebrospinal fluid beta2-microglobulin, monocyte chemotactic protein-1, and soluble tumour necrosis factor alpha receptors before and after treatment with lamivudine plus zidovudine or stavudine. J Neuroimmunol. 2000; 102:216–221. [PubMed: 10636491]
3. Sidenius N, Nebuloni M, Sala S, et al. Expression of the urokinase plasminogen activator and its receptor in HIV-1- associated central nervous system disease. J Neuroimmunol. 2004; 157:133–139. [PubMed: 15579290]
4. Cinque P, Nebuloni M, Santovito ML, et al. The urokinase receptor is overexpressed in the AIDS dementia complex and other neurological manifestations. Ann Neurol. 2004; 55:687–694. [PubMed: 15122709]
5. Cinque P, Bestetti A, Marenzi R, et al. Cerebrospinal fluid interferon-gamma-inducible protein 10 (IP-10, CXCL10) in HIV-1 infection. J Neuroimmunol. 2005;168:154–163. [PubMed: 16091292]
6. Gianella S, Letendre SL, Iudicello J, et al. Plasma (1→3)-β-D-glucan and suPAR levels correlate with neurocognitive performance in people living with HIV on antiretroviral therapy: a CHARTER analysis. J Neurovirol. 2019; 25:837–843. [PubMed: 31297727]
7. Meeker RB, Poulton W, Markovic-Plese S, et al. Protein changes in CSF of HIV-infected patients: evidence for loss of neuroprotection. J Neurovirol. 2011;17:258–73. [PubMed: 21556959]
8. Desmedt S, Desmedt V, Delanghe JR, et al. The Intriguing Role of Soluble Urokinase Receptor in Inflammatory Diseases. Crit Rev Clin Lab Sc. 2017; 54: 117–133. [PubMed: 28084848]

9. Vassalli JD, Wohlwend A, Belin D. Urokinase-catalyzed plasminogen activation at the monocyte/macrophage cell surface: A localized and regulated proteolytic system. Curr Top Microbiol Immunol. 1992; 181: 65–86. [PubMed: 1330446]
10. Nykjaer A, Moller B, Todd RF 3rd, et al. Urokinase receptor. An activation antigen in human T lymphocytes. J Immunol. 1994;152:505–16. [PubMed: 8283034]
11. Marcos-Contreras OA, Lizarrondo SM, Bardou I, et al. Hyperfibrinolysis increases blood-brain barrier permeability by a plasmin- and bradykinin-dependent mechanism. Blood. 2016; 128: 2423–2434. [PubMed: 27531677]
12. Lane BR, King SR, Bock PJ, et al. The C-X-C chemokine IP-10 stimulates HIV-1 replication. Virology. 2003; 307:122–134. [PubMed: 12667820]
13. Neville LF, Mathiak G, Bagasra O. The immunobiology of interferon-gamma inducible protein 10 kD (IP-10): A novel, pleiotropic member of the C-X-C chemokine superfamily. Cytokine Growth Factor Rev. 1997; 8:207–219. [PubMed: 9462486]
14. Taub DD, Lloyd AR, Conlon K, et al. Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. J Exp Med. 1993; 177:1809–1814. [PubMed: 8496693]
15. Vanguri P, Farber JM. IFN and virus-inducible expression of an immediate early gene, crg-2/IP-10, and a delayed gene,I-A alpha in astrocytes and microglia. J Immunol. 1994;152:1411–1418. [PubMed: 8301141]
16. Cheng G, Nazar AS, Shin HS, et al. IP-10gene transcription by virus in astrocytes requires coopera-tion of ISRE with adjacent kappa B site but not IRF-1 or viral transcription. J Interferon Cytokine Res. 1998; 18:987–997. [PubMed: 9858321]
17. Shen Q, Zhang R, Bhat NR. MAP kinase regulation ofIP10/CXCL10 chemokine gene expression in microglial cells. Brain Res. 2006; 1086:9–16. [PubMed: 16635481]
18. Park C, Lee S, Cho IH, et al. TLR3-mediated signal induces proinflammatory cytokine and chemokine gene expression in astrocytes: Differential signaling mechanisms of TLR3-induced IP-10 and IL-8 gene expression. Glia 2006;53:248–256. [PubMed: 16265667]
19. Hua LL, Lee SC. Distinct patterns of stimulus-inducible chemokine mRNA accumulation in human fetal astrocytes and microglia. Glia. 2000;30:74–81. [PubMed: 10696146]
20. Harvey CE, Post JJ, Palladinetti P, et al. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation. J Leukoc Biol. 2003;74:360–369. [PubMed: 12949239]
21. Kutsch O, Oh J, Nath A, Benveniste EN. Induction of the chemokines interleukin-8 and IP-10 by human immunodeficiency virus type 1 tat in astrocytes. J Virol. 2000;74: 9214–9221. [PubMed: 10982368]
22. Chen P, Mayne M, Power C, et al. The Tat protein of HIV-1 induces tumor necrosis factor-alpha production. Implications for HIV-1-associated neurological diseases. J Biol Chem. 1997; 272: 22385–22388. [PubMed: 9278385]
23. Albini A, Benelli R, Giunciuglio D, et al. Identification of a novel domain of HIV tat involved in monocyte chemotaxis. J Biol Chem. 1998; 273: 15895–15900. [PubMed: 9632634]
24. Ranga U, Shankarappa R, Siddappa NB, et al. Tat protein of human immunodeficiency virus type 1 subtype C strains is a defective chemokine. J Virol. 2004;78: 2586–2590. [PubMed: 14963162]
25. Williams ME, Zulu SS, Stein DJ, et al. Signatures of HIV-1 subtype B and C Tat proteins and their effects in the neuropathogenesis of HIV-associated neurocognitive impairments. Neurobiol Dis. 2020; 136: 104701. [PubMed: 31837421]
26. Campbell GR, Watkins JD, Singh KK, et al. Human immunodeficiency virus type 1 subtype C tat fails to induce intracellular calcium flux and induces reduced tumor necrosis factor production from monocytes. J Virol. 2007; 81: 5919–5928. [PubMed: 17376903]
27. Gandhi N, Saiyed Z, Thangavel S, et al. Differential effects of HIV type 1 clade B and clade C Tat protein on expression of proinflammatory and antiinflammatory cytokines by primary monocytes. AIDS Res Hum Retrovir. 2009; 25: 691–699. [PubMed: 19621989]
28. Wong JK, Campbell GR, Spector SA. Differential induction of interleukin-10 in monocytes by HIV-1 clade B and clade C Tat proteins. J Biol Chem. 2010; 285: 18319–18325. [PubMed: 20378550]

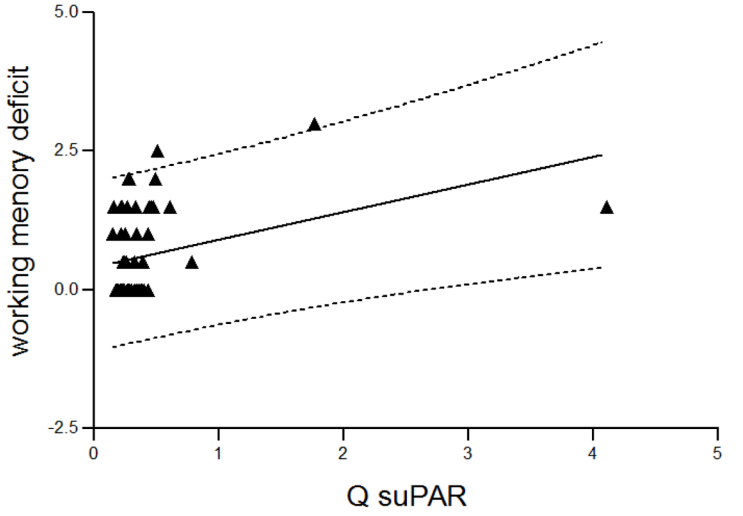
29. de Almeida SM, Rotta I, Vidal LRR, et al. HIV-1C and HIV-1B Tat protein polymorphism in Southern Brazil. J. Neurovirol 2021; 27: 126–136. [PubMed: 33462791]
30. Andersen O, Eugen-Olsen J, Kofoed K, et al. Soluble urokinase plasminogen activator receptor is a marker of dysmetabolism in HIV-infected patients receiving highly active antiretroviral therapy. J Med Virol. 2008;80:209–216. [PubMed: 18098145]
31. Caudie C, Bancel J, Dupont M, et al. CSF levels and diagnostic utility of cerebrospinal fluid beta2-microglobulin. Ann Biol Clin. 2005; 63: 631–637.
32. Reiber H, Padilla-Docal B, Jensenius JC, et al. Mannan-binding lectin in cerebrospinal fluid: a leptomeningeal protein. Fluids Barriers CNS 2012; 9: 17. [PubMed: 22889364]
33. de Almeida SM, Ribeiro CE, de Pereira AP, et al. Neurocognitive impairment in HIV-1 clade C- versus B-infected individuals in Southern Brazil. J Neurovirol. 2013; 19: 550–556. [PubMed: 24277437]
34. Heaton RK, Miller SW, Taylor MJ, et al. Revised Comprehensive Norms for an Expanded halstead-Reitan Battery: Demographically Adjusted Neuropsychological Norms for African American and Caucasian Adults. Psychological Assessment Resources, Inc; Lutz: 2004.
35. Chelune GJ, Heaton RK, Lehman RA. Neuropsychological and personality correlates of patients complaints of disability. In: Goldstein GJ, Tarter RE, editors. Advances in clinical neuropsychology.Plenum. 1986; New York. pp. 95–126.
36. Antinori A, Arendt G, Becker JT, et al. Updated research nosology for HIV-associated neurocognitive disorders. Neurology 2007; 69:1789–1799. [PubMed: 17914061]
37. Carey CL, Woods SP, Rippeth JD, et al. Initial validation of a screening battery for the detection of HIV-associated cognitive impairment. Clin Neuropsychol. 2004; 18: 234–248. [PubMed: 15587671]
38. Cowan N What are the differences between long-term, short-term, and working memory? Prog Brain Res. 2008;169: 323–338. [PubMed: 18394484]
39. Brew BJ, Bhalla RB, Fleisher M, et al. Cerebrospinal fluid beta 2 microglobulin in patients infected with human immunodeficiency virus. Neurology 1989; 39:830–834. [PubMed: 2657485]
40. Mcarthur JC, Nance-Sproson TE, Griffin DE, et al. The diagnostic utility of elevation in cerebrospinal fluid beta 2-microglobulin in HIV-1 dementia. Multicenter AIDS cohort study. Neurology 1992; 42:1707–1712. [PubMed: 1355286]
41. de Almeida SM, Rotta I, Jiang Y. et al. Biomarkers of Chemotaxis and Inflammation in Cerebrospinal Fluid and Serum in Individuals with HIV-1 Subtype C versus B. J Neurovirol. 2016; 22: 715–724. [PubMed: 27400932]
42. de Almeida SM, Rotta I, de Pereira AP, et al. Cerebrospinal fluid pleocytosis as a predictive factor for CSF and plasma HIV RNA discordance and escape. J Neurovirol. 2020; 26: 241–251. [PubMed: 32002817]
43. de Almeida SM, Rotta I, Tang B, et al. IgG intrathecal synthesis in HIV-associated neurocognitive disorder (HAND) according to the HIV-1 subtypes and pattern of HIV RNA in CNS and plasma compartments. J Neuroimmunol. 2021;355: 577542 [PubMed: 33845284]
44. de Almeida SM, Ribeiro CE, Rotta I, et al. Biomarkers of neuronal injury and amyloid metabolism in the cerebrospinal fluid of patients infected with HIV-1 subtypes B and C. J Neurovirol. 2018. 24: 28–40. [PubMed: 29063514]
45. de Almeida SM, Ribeiro CE, Rotta I, et al. Blood amyloid-β protein isoforms are affected by HIV-1 in a subtype-dependent pattern. J Neurovirol. 2020; 26: 3–13. [PubMed: 31281948]
46. Cunningham O, Campion S, Perry VH, et al. Microglia and the urokinase plasminogen activator receptor/uPA system in innate brain inflammation. Glia. 2009; 57:1802–1814. [PubMed: 19459212]
47. Lund LR, Romer J, Ronne E, et al. Urokinasereceptor biosynthesis, mRNA level and gene transcription are increased by transforming growth factor b 1 in human A549 lung carcinoma cells. EMBO J. 1991; 10:3399–3407. [PubMed: 1655420]
48. Gyetko MR, Sud S, Kendall T, et al. Urokinase receptor-deficient mice have impaired neutrophil recruitment in response to pulmonary Pseudomonas aeruginosa infection. J Immunol. 2000; 165:1513–1519. [PubMed: 10903758]

49. Washington RA, Becher B, Balabanov R, et al. Expression of the activation marker urokinase plasminogen-activator receptor in cultured human central nervous system microglia. J Neurosci Res. 1996; 45:392–399. [PubMed: 8872899]
50. Blasi F, Carmeliet P. uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol. 2002; 3: 932–943. [PubMed: 12461559]
51. Gangur V, Simons FE, Hayglass KT. Human IP-10 selectively promotes dominance of polyclonally activatedand environmental antigen-driven IFN-gamma over IL-4 responses. FASEB J. 1998;12:705–713. [PubMed: 9619449]
52. Williams R, Yao H, Dhillon NK, et al. HIV-1 Tat co-operates with IFN-γ and TNF-α to increase CXCL10 in human astrocytes. PLoS ONE 2009; 4: e5709. [PubMed: 19479051]
53. Wang XQ, Palmer S. Single-molecule techniques to quantify and genetically characterise persistent HIV. Retrovirol. 2018; 15: 10.1186/s12977-017-0386-x.
54. Gantner P, Pagliuzza A, Pardons M, et al. Single-cell TCR sequencing reveals phenotypically diverse clonally expanded cells harboring inducible HIV proviruses during ART. Nat Commun. 2020; 11:4089. [PubMed: 32796830]
55. Corley MJ, Farhadian SF. Emerging Single-cell Approaches to Understand HIV in the Central Nervous System. Curr HIV/AIDS Rep. 2021; 10.1007/s11904-021-00586-7



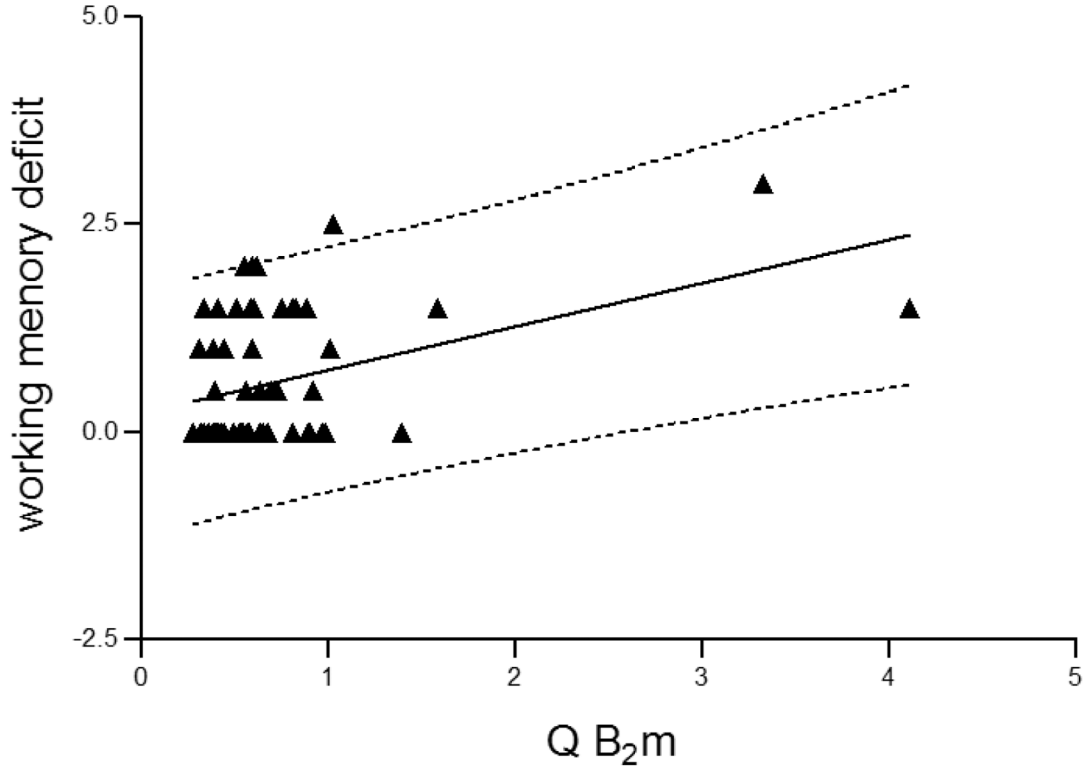
ρ = 0.303 (0.043-0.524), p= 0.020

A



ρ = 0.353 (0.099- 0.564), p= 0.006

B



ρ = 0.289 (0.028; 0.514), p= 0.026

C

Figure 1. Correlations of working memory deficit with inflammatory biomarkers

A. Correlation of working memory deficit and CSF suPAR (pg/mL), red dotted line indicates CSF suPAR reference value (1154 pg/mL).

B. Correlation of working memory deficit and Q suPAR (CSF/serum).

C. Correlation of working memory deficit and Q β2 microglobulin ($Q\beta_2m$, CSF/serum).

Correlation coefficients (ρ) were estimated using Spearman's rank-order method; results were shown in ρ (95% confidence interval [CI]).

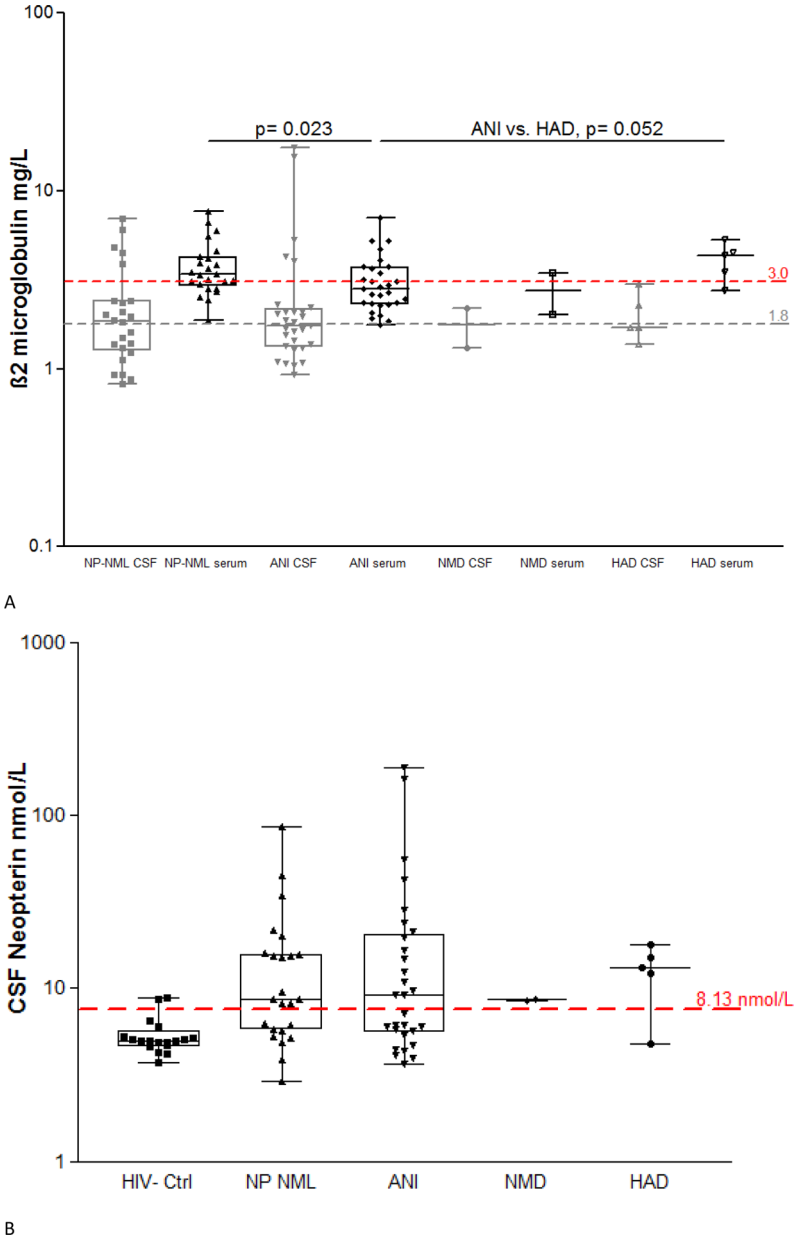


Figure 2. Inflammatory biomarkers in the HIV-1 groups categorized by the HAND classification by the Frascati criteria.

Boxes show median and interquartile range (IQR), and whiskers indicate maximum and minimum values. The dots indicate the number of individuals in each group.

HIV associated neurocognitive disorder (HAND, Antinori et al., 2007): HIV-associated dementia (HAD); minor neurocognitive disorder (MND); asymptomatic neurocognitive impairment (ANI); PWH neuropsychologically normal (NP NML). HIV-negative controls (Ctrl).

A. β2 microglobulin (β2m, mg/L) in CSF (gray graphic) and serum (black graphic), gray dotted line indicates reference value of CSF β2m (1.8 mg/L), red dotted line indicates reference value of serum β2m (3.0 mg/L) (Caudie et al, 2005). Pairwise comparisons of CSF β2m in HAND groups all p>0.05.

B. CSF neopterin (nmol/L), red dotted line indicates reference value (8.13 nmol/L). The values of the CSF neopterin median numerically increased from the Ctrl group to the HAD group. Pairwise comparisons of HIV negative control group (HIV- ctrl) with HAND categorized according to the Frascati criteria (HIV- ctrl vs. NP NML, p= 0.0006; vs. ANI, p= 0.0027; vs. HAD, p=0.019). Pairwise comparisons between the groups with HAND, all p>0.05.

Table 1.

Demographics and clinical characteristics of AIDS in PWH, HIV-negative controls, and individuals with HIV-1 subtypes B and C

	HIV NEGATIVE (n= 19)	PWH (n= 68)	p	HIV1-B (n=27)	HIV1-C (n=26)	p
Demographics						
Age, years	41 (38, 50)	43 (35; 48)	0.91	44 (36.5; 50)	43 (34.5; 47.5)	0.450
Education, years	12 (11, 15)	8 (5; 11)	0.0001	8 (5; 12)	7 (5; 11.5)	0.550
Sex - male, n (%)	14 (74)	33 (49.0)	0.07	14 (51.9)	11 (42.3)	0.590
Disease and Treatment						
Duration of infection (mths)	-	89 (31; 135)	-	91.03 (61.63; 144)	81.37 (27.82; 132)	0.450
AIDS, n (%)	0	55 (80.9)		22 (81.5)	19 (73.1)	0.526
GDS	-	0.65(0.30;105)	-	0.95 (0.275; 1.725)	0.50 (0.225; 0.875)	0.126
Current CD4 cells/mm^3	-	369 (201; 534)	-	457 (255; 614)	359.5 (176.5; 472.5)	0.200
Nadir CD4 cells/mm^3	-	90 (33; 266)	-	82 (26; 253.5)	159 (16.5; 359.5)	0.290
CART, n (%)	-	55 (80.9)	-	24 (88.9)	18 (69.2)	0.099
CPE	-	8 (6; 9)	-	8 (6; 9)	6 (5.5; 9)	0.339
Adherence, n (%)	-	51/54 (94.4)	-	21/23 (91.3)	18/18 (100)	0.495
Plasma HIV RNA (Log$_{10}$)	-	1.7 (1.7; 3.5)	-	1.7 (1.7; 1.97)	2.8 (1.7; 3.8)	0.012
Pl HIV RNA<50copies/mL, n (%)	-	38 (55.9)	-	20 (74.1)	9 (34.6)	0.006
CSF						
HIV RNA (Log$_{10}$)	-	1.7 (1.7; 2.8)	-	1.7 (1.7; 2.2)	2.2 (1.7; 2.9)	0.084
HIV RNA <50 copies/mL, n (%)	-	35 (51.5)	-	16 (59.3)	10 (38.5)	0.173
WBC cells/mm^3	2 (1.0, 2.5)	2.1 (0.6; 7.2)	0.402	1.6 (0.30; 4.85)	2.65 (0.60; 11)	0.160
Glucose mg/dL	63 (59, 71)	57 (53; 62)	0.0003	63 (54; 66)	56(51.5; 59)	0.007
Total protein mg/dL	30 (26, 38)	40 (32; 46)	0.003	42(35; 47.5)	40(28.5;47)	0.551
Total protein >45 mg/dL, n(%)	0	20 (29.4)	0.005	10 (37.0)	8 (30.8)	0.773
Albumin mg/L	180 (150.0; 240.0)	223.5 (164; 288.5)	0.019	248.0(189; 309)	218(138.5; 300)	0.328
Albumin quotient, Q.Alb	0.005(0.004; 0.006)	0.0064 (0.0049; 0.0097)	0.002	0.0082(0.0061; 0.0108)	0.0060(0.0044; 0.0097)	0.52 [1]
RBC cells/mm^3	2.0 (1.0, 4.0)	0.5 (0; 7.5)	0.399	1.0(0; 24)	0.8(0; 36.5)	0.900

Data represent the median (IQR) or number of cases (%). Comparisons were performed using independent samples t-test for continuous variables and Fisher's exact test for binary and categorical variables. [1] Adjusted for plasma HIV VL suppression and nadir CD4 count. CART, combination antiretroviral therapy; CPE, CNS Penetration-Effectiveness rank. B/C, HIV-1 subtypes B / C; pl, plasma; GDS, global deficit score; RBC, red blood cells; WBC, white blood cells.

Table 2.

Inflammatory biomarkers in the CSF and serum of the PWH, HIV subtypes B and C, and HIV-negative control groups

	PWH (n= 68)	HIV NEGATIVE (n= 19)	Coheńs d (95%CI)	p	HIV1-B (n=27)	HIV1-C (n=26)	Coheńs d (95%CI)	p
CSF								
suPAR, pg/mL	812.1(703.4; 1018)	823.8(722.1; 996.5)	−0.3 (−0.82; 0.23)	0.260	778.6(709.5; 1040)	863.3(665.8; 1032)	−0.14 (−0.7,0.42)	0.98
IP-10, pg/mL	**1277(616.5; 2685)**	**974.0(699.0; 1291)**	**0.43 (0.08; 0.77)**	**0.015**	1062(616.5; 2490)	1911 (515.5; 3327)	−0.06(−0.68,0.56)	0.86
Neopterin, nmol/L	**8.695(5.719; 15.81)**	**4.985(4.618; 5.641)**	**−0.9 (−1.45; −0.35)**	**0.001**	6.248(5.012; 16.53)	11.25 (5.889; 16.29)	−0.29 (−0.85, 0.28)	0.91
β_2m, mg/L	1.730(1.320; 2.325)	-	-	-	1.450(1.255; 2.150)	1.925(1.355; 2.385)	−0.19 (−0.76; 0.37)	0.80
Serum								
suPAR, pg/mL	**3134 (2684; 3617)**	**2115(1987; 2495)**	**−1.56 (−2.14; −0.99)**	**<0.001**	3063(2639; 3551)	3106(2662; 3617)	0.18 (−0.39,0.74)	0.13
IP-10, pg/mL	**1034 (518.0; 1471)**	**400.0 (283.5; 553.0)**	**1.42 (0.98; 1.87)**	**<0.0001**	754.0(467.0; 1349)	1101 (645.0; 1591)	−0.06(−0.68,0.56)	0.85
β_2m, mg/L	3.115(2.505; 4.020)	-	-	-	2.880 (2.400; 4.080)	3.125(2.575; 3.965)	−0.07 (−0.63; 0.5)	0.17
CSF/serum								
suPAR	0.269(0.219; 0.344)	0.392(0.301; 0.476)	0.46 (−0.06;0.99)	0.078	0.266(0.217; 0.327)	0.323 (0.210; 0.387)	−0.23 (−0.80,0.33)	0.61
IP-10	1.651(0.839; 2.599)	2.359(1.786; 3.975)	−0.21 (− 0.52;0.1)	0.190	1.837(1.029; 2.599)	1.440(0.747; 3.177)	−0.04 (−0.66,0.58)	0.90
β_2m	0.572 (0.412; 0.741)	-	-	-	0.555(0.391; 0.781)	0.582(0.463; 0.676)	−0.2 (−0.77,0.36)	0.63
suPAR index	40.02 (29.59; 55.20)	79.78 (62.35; 110.7)	−0.31 (−0.21;−0.42)	<0.0001	33.76 (27.21; 51.34)	43.06 (32.16; 66.60)	0.1(0.23, −0.04)	0.16

Values represent the median (IQR); PWH vs. HIV negative, p value adjusted for age; HIV-1B vs. HIV-1C, p-value adjusted for plasma HIV viral load suppression and nadir CD4 count. Group differences are presented as Cohen's d; CI: confidence interval.

soluble urokinase-type plasminogen activator receptor (suPAR), interferon γ-inducible protein 10 (IP-10), β_2 microglobulin (β_2m);

$suPAR_{Index} = (suPAR_{CSF} \times Alb_{serum}) / (suPAR_{serum} \times Alb_{CSF})$.

Table 3.

Inflammatory biomarkers in the CSF and serum of the groups according with global deficit score or with HIV-associated neurocognitive disorder (HAND) classified by the Frascati criteria

	A-GDS ≥ 0.5 (n= 37)	B-GDS <0.5 (n= 23)	A vs. B p	C-NP-NML n= 24	D-ANI n= 29	E- MND n= 2 [1]	F-HAD n= 5 [1]	C vs. D vs. F p
CSF								
suPAR, pg/mL	942.4 (697.1; 1032)	810.9 (709.5; 1191)	0.855	821.7 (709.5; 1100)	960.8(691.5; 1080)	695.1(611.4; 778.8)	778.6 (743.2; 2709)	0.964
IP-10, pg/mL	1303 (559.0; 2639)	1211 (616.5; 3327)	0.909	1482 (616.5; 3102)	1251 (559.0; 3297)	1210 (1117; 1303)	2236 (97.22; 2684)	0.836
Neopterin, nmol/L	9.133 (5.730; 17.29)	8.645 (5.755; 18.07)	0.927	8.637 (5.755; 15.81)	9.133 (5.532; 20.57)	8.595 (8.445; 8.744)	13.15 (4.746; 17.84)	0.897
β_2m, mg/L	1.750 (1.360; 2.210)	1.870 (1.270; 3.145)	0.909	1.855 (1.270; 2.420)	1.750 (1.335; 2.160)	1.760 (1.320; 2.200)	1.710 (1.370; 2.990)	0.912
Serum								
suPAR, pg/mL	3001 (2684; 3529)	3297 (2975; 3714)	0.153	3345 (2975; 3714)	2943 (2605; 3477)	3038 (2837; 3238)	3435 (2578; 3965)	0.190
IP-10, pg/mL	968.0(385.0; 1621)	1043 (578.0; 1595)	0.808	1060 (578.0; 1595)	871.0 (354.5; 1621)	901.5 (762.0; 1041)	1414 (532.0; 1846)	0.476
β_2m, mg/L	2.960 (2.340; 3.935)	3.370 (2.915; 4.230)	0.155	3.395 (2.915; 4.230)	2.810 (2.310; 3.705)	2.745 (2.020; 3.470)	4.320 (2.750; 5.340)	**0.024**
CSF/serum								
suPAR	0.288 (0.225; 0.372)	0.237 (0.217; 0.383)	0.294	0.235 (0.217; 0.359)	0.325(0.227; 0.392)	0.228 (0.216; 0.241)	0.264(0.196; 0.789)	0.388
IP-10	1.746 (0.839; 3.700)	1.837 (0.979; 2.958)	0.831	1.763 (0.843; 2.515)	2.010 (0.918;5.074)	1.391 (1.073; 1.710)	1.211 (0.066; 2.568)	0.333
β_2m	0.591 (0.419; 0.858)	0.567 (0.397; 0.741)	0.280	0.557(0.391; 0.661)	0.591(0.485; 0.906)	0.644 (0.634; 0.654)	0.425 (0.381; 0.692)	0.149
suPAR index	37.50(28.79; 55.32)	39.18 (31.02; 58.95)	1.000	40.02 (31.02; 54.39)	37.18 (28.79; 53.41)	48.32 (30.48; 66.15)	44.83 (17.51; 182.6)	0.900

Values represent the median (IQR); GDS- global deficit score. Comparisons were performed using Mann Whitney or Kruskal Wallis nonparametric tests (unadjusted analysis), as appropriate. Pairwise comparison (Mann Whitney test nonparametric tests, unadjusted analysis): CSF Neopterin, C vs. F, p= 0.840. Serum β_2m, D vs. F, p=0.052; C vs. F, p= 0.371. CSF IP-10, D vs. F, p= 0.662.

[1] median (min. max). CSF, cerebrospinal fluid; soluble urokinase-type plasminogen activator receptor (suPAR); interferon γ-inducible protein 10 (IP-10); β_2 microglobulin (β_2m).