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Neurocytoskeleton Proteins in Cerebrospinal Fluid of People With HIV-1 Subtypes B and C

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Abstract

Background: The objective was to compare the effect of HIV-1C and HIV-1B subtypes on neurofilament light (NfL) cerebrospinal fluid (CSF) levels and ratios of NfL to tau proteins. Additional comparisons were performed between people with HIV (PWH), participants with Alzheimer disease (AD), and HIV-negative controls (HIV–). We also calculated the diagnostic characteristics of CSF NfL and its ratios in HIV-associated neurocognitive disorder (HAND) diagnosis.

Methods: CSF NfL, T-tau, and P-tau$_{181}$ concentrations were measured using immunoassays in a total of 108 CSF samples, including PWH (n = 68), HIV– (n = 16), and participants with AD (n = 24). These proteins were compared between HIV-1B (n = 27) and HIV-1C (n = 26) using multiple linear regression adjusted for nadir CD4 and plasma viral load suppression. Comparisons between PWH, HIV–, and participants with AD were adjusted for gender and age.

Results: CSF neurocytoskeleton proteins and their ratios were comparable in HIV-1B and HIV-1C. However, the HIV-1C group had a higher proportion of samples of CSF NfL above the reference value (n = 14, 53.85%) than the HIV-1B group (n = 8, 29.63%), $P = 0.098$. The values of CSF NfL were higher in the AD group [2578 (1864; 3500) pg/mL] than those in PWH [683 (500; 1197) pg/mL, $P < 0.001$] and control [660 (539; 802) pg/mL, $P = 0.012$] groups. The value of CSF NfL and its ratios for HAND diagnosis were poor.

Conclusion: The effects of HIV-1B and HIV-1C on CSF NfL and tau ratios were comparable. The differences in CSF neurocytoskeleton proteins between PWH and individuals with AD suggested they might not share the same mechanisms of impairment. Further research is necessary to evaluate CSF NfL on the differential diagnoses of HAND with AD.

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Keywords

neurofilament; tau; HIV; cerebrospinal fluid; biomarkers; neuronal injury; subtype; central nervous system; dementia; diagnosis; Alzheimer disease

INTRODUCTION

Cerebrospinal fluid (CSF) neurofilament light (NfL; 68–70 kDa) is a sensitive marker of large-caliber myelinated axonal injury[1–3] in a variety of neurodegenerative conditions,[4–8] eg, HIV-associated neurocognitive disorder (HAND).[9–11] It was previously reported that immune activation and inflammation in people with HIV (PWH) correlate with biochemical and neuroimaging markers of neuronal injury, including elevated CSF NfL levels.[12,13]

The transactivator of transcription (Tat) protein is one of the key HIV regulatory proteins[14] and plays an important role in the pathogenesis of HAND. It is the only HIV protein actively secreted by infected primary immune cells in the central nervous system (CNS), even in patients with sustained blood and CSF virologic suppression.[15–17] Previous studies have shown that HIV-1B Tat reduces neurofilament (Nf) in neuron cell cultures.[14,18] In vivo, NfL is eliminated from the brain by the CSF, resulting in increased CSF levels in neuronal injury.[10,11]

The effect of HIV-1C on NfL is unknown. There is a C31S residue substitution in the cysteine-rich domain of Tat protein in HIV-1C, which leads, in animal and in vitro studies, to a reduced chemotaxis and consequently reduced neuroinflammation.[19–21]

The interactions of NfL and cytoskeletal protein, such as tau, have been previously demonstrated in neurons of patients with Alzheimer disease (AD).[22] Neurofibrillary tangles, which are a hallmark of AD, contain phosphorylated tau (P-tau$_{181}$) and other phosphorylated proteins, such as Nf.[22,23]

This study focuses on neurocytoskeletal proteins, chiefly CSF NfL, as well as its ratio with CSF T-tau and P-tau$_{181}$. The authors hypothesized that the CSF NfL level was lower in HIV-1C than that in HIV-1B, based on the reduced in vitro chemotaxis induced by HIV-1C Tat.

The study's objectives were to compare the effects of HIV-1 subtypes B and C on CSF NfL levels and the ratios of CSF NfL to tau proteins. In addition, we compared CSF NfL levels in PWH, participants with AD, and HIV− controls and calculated the diagnostic characteristics of CSF NfL and its ratios in HAND diagnosis. This was the first study to analyze CSF NfL in patients with HIV-1C.

METHODS

Subjects

A total of 108 CSF samples were included in the study by convenience and distributed into the following groups:

People With HIV

PWH (n = 68) were recruited at the Clinical Hospital of the Federal University of Paraná (HC-UFPR, Brazil). Individuals with opportunistic CNS infections were excluded. All volunteers underwent serological testing to confirm HIV status[24] before enrollment and provided blood and CSF samples after serum status confirmation. For participants with clinically resistant infection, the infecting HIV strain was genotyped with *pol* sequences, whereas *env* sequences were used for all other participants. Twenty-seven individuals were infected with HIV-1B, and 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). HIV-1 could not be genotyped in 1 participant.

Participants With Alzheimer Disease

Participants with AD (n = 24) were clinically diagnosed by the Dementia Investigative Team from the Cognitive Dysfunction Outpatient Clinic, HC-UFPR, Brazil. Diagnostic methods of the AD group were previously described in detail.[25–27] Participants with AD were classified, at the moment of CSF and serum collection, with probable AD; the Clinical Dementia Rating, median [interquartile range (IQR)], was 2 (2; 2.5), indicating moderate dementia with severe decrease of daily instrumental activity and no associated depression. The duration of symptoms [median (IQR)] was 36 (24; 60) months. The median (IQR) of CSF β-amyloid proteins was as follows: Aβ-38, 1653 (1274; 2589) pg/mL; Aβ-40, 3937 (3245; 5668) pg/mL; and Aβ-42, 276.0 (167; 444.5) pg/mL.[25]

Healthy HIV-Negative Control Group

We recruited a HIV-negative control group of 18 age-matched individuals at the HIV Neurobehavioral Research Center, University of California San Diego (HNRC-UCSD). They had no neurological comorbidities or cognitive complaints and tested negative on serological tests for HIV, hepatitis C, and syphilis. The CSF inclusion criteria in this group were white blood cell (WBC) count ≤5 cells/mm^3, total protein ≤45 mg/dL, and glucose ≥55 mg/dL.

Neurobehavioral Assessments, HAND Diagnosis, and Categorization

All participants underwent a neuropsychological (NP) evaluation by the same study team from Brazil, supervised by a neuropsychologist. The NP test battery was administered in Portuguese and assessed 7 ability domains and comprised 15 individual NP measures (see suppl Table 1, Supplemental Digital Content, http://links.lww.com/QAI/B468).[28] The NP tests have been widely used to study HIV infection in English-speaking and non–English-speaking countries. 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.[28] The subjective neurocognitive difficulty was assessed using the Patient's Assessment of Own Functioning Inventory.[29] HAND diagnoses were assigned according to the Frascati criteria.[30] In addition, the global deficit score (GDS) method was used to classify the overall NP impairment status. 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 NP impairment.[31,32]

Laboratory Methods

CSF Neurocytoskeleton Proteins—CSF NfL concentrations were quantified by high-sensitivity enzyme-linked immunosorbent assays using the commercial NF-light ELISA Kit (UmanDiagnostics AV, Umea, Sweden); the minimum level of detection value was 32 pg/mL. All samples were assayed concurrently in duplicate according to the manufacturer's instructions.

T-tau was quantified by electrochemiluminescence (MULTI-ARRAY, Meso Scale Diagnostics, LLC, Rockville, MD, USA); P-tau$_{181}$ (Thermo Fisher Scientific Inc., Waltham, MA, USA) was assayed by multiplex bead assays (FlexMAP 3D; Luminex Corporation, Austin, TX, USA). The CSF values of T-tau and P-tau$_{181}$ were published previously.[25] In this article, we calculated the ratios between CSF neurocytoskeletal proteins, NfL/T-tau, and NfL/P-tau$_{181}$.[33]

The acceptable coefficient of variation (CV) between duplicates was less than 20%. When the results were under the minimum level of detection determined by the manufacturer, the low-detection limit value was considered in the statistical analysis.

Specimen Collection and Storage—The CSF was collected by lumbar puncture. The samples were collected in polypropylene tubes to avoid the adherence of proteins to the tube walls and centrifuged immediately after lumbar puncture to separate cells and debris and avoid false increases in T-tau and P-tau$_{181}$. All PWH and AD samples were collected at the same time of the day to limit diurnal variability. CSF aliquots were frozen and stored at HC-UFPR, Brazil, and maintained at −80°C.

Data Analyses

Demographic variables (age, sex, and education) were compared among all groups with pairwise Student *t* tests for continuous variables and the Fisher exact test for binary and categorical variables. Demographic and HIV disease characteristics were compared between HIV-1B and HIV-1C individuals with similar methods. Values were $\log_{10}$ transformed before the statistical analyses if their distributions were not approximately normal.

First, CSF NfL levels and the ratios of CSF NfL to tau proteins were compared between the HIV-1B and HIV-1C groups. A multivariable model was applied to control for plasma HIV viral load (VL) suppression and nadir CD4 counts. The *P* values within each class of biomarkers (CSF or ratio) were then corrected for multiple testing with the Benjamini–Hochberg (BH) procedure.

Second, a hierarchy of comparisons was performed with the AD versus PWH groups as the primary comparison, HIV–versus AD groups as the secondary comparisons, and HIV–versus PWH groups as the exploratory comparison, without adjustment for multiple comparisons. Age and gender were included as covariates in multivariable linear regression models if they had *P* values less than 0.2 in the adjusted model. If the age effect was significantly nonlinear, a smooth age effect was used within a generalized additive model.[34] The *P* values for the biomarker effects were corrected for multiple testing.

The correlation coefficients (r_S) were estimated using the Spearman rank-order method. We tested the correlation of CSF NfL and protein ratios in the groups, PWH overall, and HIV-1 subtypes B and C, with the variables such as GDS, HIV infection characteristics (duration of infection, current age, age at the beginning of infection, plasma HIV RNA, and CSF HIV RNA), cell immunity characteristics (nadir CD4, current CD4, and CD4 recovery = current CD4 – nadir CD4), and CSF WBC count as a marker of inflammation.

Results were considered statistically significant at the 5% alpha level. Statistical analyses were implemented using R version 3.2.3, 2015 (The R Foundation, https://www.r-project.org/). Cohen's d effect sizes and 95% confidence intervals (CIs) were reported for differences between groups.

The diagnostic characteristics of the CSF NfL and NfL to tau protein ratios (index tests) for diagnosing HAND were calculated using clinical and NP evaluation as the reference method. The proportion of cognitive impairment (GDS ≥ 0.50) among PWH in this population was 62% [95% CI: 47% to 74%].[28]

The following diagnostic characteristics were calculated: sensitivity, specificity, accuracy (efficiency), positive predictive value (PPV), negative predictive value (NPV), Youden index,[35] positive and negative likelihood ratios (LRP and LRN),[36] clinical utility index positive, clinical utility index negative,[37] and diagnostic odds ratio (DOR = LRP/LRN). The receiver operating characteristic (ROC) curve was used to evaluate the ability of CSF NfL values and ratios to accurately classify impaired and normal participants. The ROC curve and area under the curve were determined, and an optimal cutoff value of CSF NfL to tau protein ratios was selected using the method of maximizing the sum of sensitivity and specificity.[38] The age-related upper reference value of CSF NfL, for each participant, was calculated by the equation: CSF NfL = 201.2×1.031^{age} [2].

Standard Protocol Approvals, Registrations, and Patient Consent

This study, which was a cross-sectional survey with stored CSF samples, was approved by the UCSD (San Diego, CA, USA) Institutional Review Board (IRB), Hospital de Clinicas-Universidade Federal do Paraná (HC-UFPR, Curitiba, Paraná, Brazil) IRB, and the National Commission of Ethics in Research (CONEP, Brazil). All participants signed informed consent forms approved by the IRBs in the United States and Brazil. The CSF samples were collected under an NIMH-funded protocol (R21 MH076651-01).

Data Availability Statement

Anonymized data from this study will be made available at the request of qualified investigators if approved by our research ethics board.

RESULTS

Demographic, clinical, and laboratory characteristics of the groups studied are summarized in Table 1.

CSF Neurocytoskeleton Proteins

CSF neurocytoskeleton protein values and ratios were comparable for HIV-1B and HIV-1C (Table 2 and Fig. 1A; *P* values > 0.05). The HIV-1C group showed the highest number of samples with the CSF NfL value above the reference value corrected by age for each participant (n = 14, 53.85%) than the HIV-1B group (n = 8, 29.63%), although the differences did not reach significance (*P* = 0.098). The odds of elevated CSF NfL in HIV-1C was 2.77 times higher than that of HIV-1B [95% CI: (0.895 to 8.578), *P* = 0.077].

The numerical values of all neurocytoskeleton proteins in the CSF studied were elevated in the AD group compared with the PWH and control groups (Table 3 and Fig. 1B). The frequency of samples with the CSF NfL value above the reference value was higher in the AD group (17 samples, 70.83%) than the PWH group (30 samples, 44.12%), *P* = 0.033. The other comparisons were not significant.

Across all participants (n = 108), higher CSF NfL levels correlated with a higher CSF T-tau and P-tau$_{181}$ (r_s = 0.563, 95% CI: [0.414 to 0.683], *P* < 0.0001; r_s = 0.413, 95% CI: [0.238 to 0.562], *P* < 0.0001, respectively).

The median (IQR) of CSF NfL levels across the groups with HAND categorized according to the Frascati criteria[30] was comparable: NP-NML 666.4 (456.0; 1422.0) pg/mL, asymptomatic neurocognitive (ANI) 669.5 (551.5; 1033.0) pg/mL, and HIV-associated dementia (HAD) + minor neurocognitive disorder (MND) 712.1 (683.9; 1379), univariable pairwise analysis, all *P* > 0.05. The number of cases with CSF NfL levels above the upper reference value adjusted by age were comparable (*P* > 0.05). Three participants (60%) with HAD showed CSF NfL levels within the normal range adjusted by age.

Overall, in the PWH samples, CSF NfL levels increased with the severity of previous (CD4 nadir) or current cell immunity impairment [r_s = −0.309, 95% CI: (−0.515 to −0.069), *P* = 0.010; r_s = −0.293, 95% CI: (−0.502 to −0.052), *P* = 0.015, respectively], and the level of CSF inflammation was measured by the elevated CSF WBC count [r_s = 0.260, 95% CI: (0.015 to 0.475), *P* = 0.033]. There was no correlation with other variables studied, including HIV RNA in CSF or plasma, HIV RNA CSF/plasma ratio, and GDS or CD4 recovery.

The ratios of CSF NfL/T-tau and NfL/P-tau$_{181}$ for the PWH, participants with AD, and HIV– healthy controls are shown in Table 3.

The ratio of CSF NfL/P-tau$_{181}$ of PWH categorized by HAND classification[30] was numerically higher in the HAD and MND groups than the NP-NML, ANI, and HIV– control groups, although they did not reach significance (pairwise analysis, all *P* > 0.05, Fig. 1C).

Higher NfL/T-tau ratios correlated with higher CSF WBC counts [r_s = 0.304, 95% CI: (0.063 to 0.511), *P* = 0.012] and the NfL/P-tau$_{181}$ ratio correlated with higher CSF HIV RNA [r_s = 0.313, 95% CI: (0.073 to 0.518), *P* = 0.009] but not with plasma HIV RNA.

In the PWH group with GDS ≥0.5 or <0.5, there was no correlation of GDS with the CSF NfL/P-tau$_{181}$ or NfL/T-tau ratios, although in the group with GDS ≥0.5 there was a trend

toward positive correlation between GDS and CSF NfL/P-tau$_{181}$ ratio (r_s = 0.306; 95% CI: [−0.036 to 0.583]; P = 0.070).

There was no difference in CSF NfL, NfL/P-tau$_{181}$ or NfL/T-tau ratios between groups of PWH with (n = 55) or without (n = 13) antiretrovirals (ARV) (all P > 0.05).

Diagnostic Characteristics of CSF NfL, NfL/T-tau, and NfL/P-tau$_{181}$ for Diagnosing HAND

The sensitivity of CSF NfL to diagnose symptomatic HAND [MND + HAD (n = 7)] compared with PWH NP asymptomatic [ANI + NP-NML (n = 52)] and healthy control (n = 16) groups was low, meaning a positive test does not often occur in those with symptomatic HAND. Specificity was also low, that is, a negative test does not often occur in those PWH NP asymptomatic or normal. The PPV of the test was extremely low, and the NPV was very high. Hence, the clinical utility of NfL for case finding (confirmation) was very poor, the clinical utility of NfL for screening (ruling out) was fair, and the overall value of the single test for combined screening and case finding was very poor (scoring 56%). The LRP was 1.3, a value of approximately 1.0 indicates that the test is not able to show whether the disease was present or not. Diagnostic characteristics of NfL/T-tau and NfL/P-tau$_{181}$ ratios for HAND diagnosis are described in Table 4. The clinical utility of NfL/P-tau$_{181}$ ratio for screening (ruling out) symptomatic HAND was excellent (0.81), although the overall values of this single test for combined screening and case finding were poor, scoring 83%.

DISCUSSION

This study adds to the contributions of previous reports[9–11,40] by further investigating the impact of HIV subtypes B and C on axonal injury through the quantification of CSF NfL. We showed that the impact of HIV-1 on NfL, P-tau$_{181}$, and T-tau is not subtype dependent.

Furthermore, the results showed that CSF NfL values increased with the severity of past or current cellular immunity impairment, with evidence of viral replication in the CSF (but not plasma) and with the level of CSF inflammation.

CSF concentrations of NfL provide a sensitive, but not specific, marker of CNS injury in several neurological diseases, including HAND.[7,41,42]

In this study, in the PWH group, the highest levels of CSF NfL were seen in patients with HAD. This agrees with previous research.[2,11,40,43] It has been demonstrated that increased CSF NfL correlates with HAND severity.[9,10] However, in our study, there was a limited number of participants with HAD, which limited the conclusions. It has been shown that patients with severe HAD have higher CSF NfL levels than patients with less severe HAD.[9] We described a high percentage of participants with HAD and CSF NfL levels within the normal range; this was described previously in a smaller proportion of 7%–12%.[9,40,43] This indicates that this group of patients, besides the neurocognitive impairment, did not have signs of active ongoing brain injury measured by NfL. Instead, they may have had inactive impairment related to earlier neuronal damage, before initiation of ARV therapy.[2]

In this study, CSF NfL, as well as its ratios with tau proteins, showed limited power to diagnose HAND, although it was a cross-sectional study. Longitudinal studies showed CSF NfL as a useful marker of ongoing CNS damage in HIV-infected individuals.[9]

This study is not free of limitations. It presented together PWH on ARV treatment and untreated; we tried to overcome this problem by taking into consideration the plasma HIV VL in the multivariate analysis. Validating our results, the groups of PWH with or without ARV were comparable on CSF NfL levels and NfL/P-tau$_{181}$ or NfL/T-tau ratios. Also, HIV-1B and HIV-1C were comparable on the CSF to plasma HIV RNA ratio. The comparison between groups with and without treatment and subtypes was compromised because of the small number of cases without treatment by subtype. Samples from the HIV– control group were from a different study site than those from PWH and AD groups. Although PWH and HIV– groups did not differ on race or ethnicity, the Brazilian population has a higher genetic heterogeneity than the population in the United States. This limitation did not apply to the comparison between HIV-1 subtypes B and C because all participants were from the same geographical region in Brazil and were similar in age and sex. The cross-sectional design limited the study. The study did not include a substantial number of older (>65 years) PWH, who are more vulnerable to AD than young PWH. The HIV– group was age- and gender-matched with the PWH group, and the consequence was that individuals were younger than those from the AD group. A longitudinal study might be able to predict the development of HAND in patients without apparent symptoms. The sample size was sufficient for power analysis because absolute values of Cohen's d effect sizes were medium to large; however, when the PWH group was categorized by HAND diagnosis,[30] the number of cases was small especially in symptomatic HAND subgroups (HAD and MND), limiting the conclusion on the association of CSF NfL with neurocognitive impairment.

The main strength of this study was the fact that it was the first study to investigate the HIV-1C subtype impact on CSF NfL and its ratio to other neurocytoskeleton proteins (T-tau and P-tau$_{181}$) in PWH. There was a positive correlation of CSF NfL and CSF T-tau or P-tau$_{181}$, corroborating the calculation of these ratios. Furthermore, the combination of these biomarkers in ratios enhances the specificity of neuronal injury proteins,[44] and it allows researchers to investigate the predominant type of CNS axonal injury. Besides this, it will add to the previously published studies by calculating, for the first time, the diagnostic characteristic of CSF NfL for HAND diagnosis. This study contributes to the understanding of the pathophysiology of HIV infection in the CNS and the impact of HIV-1 genetic diversity in HIV-related neurocytoskeleton changes. We concluded that the impact of HIV-1 on NfL, NfL/P-tau$_{181}$, or NfL/T-tau ratios was not subtype dependent. The differences between HIV and AD in the patterns of CSF NfL and ratios suggested different pathogenetic mechanisms. However, it must be considered that this difference might be due to clinical differences between groups. More research is necessary to test the usefulness of CSF NfL for the differential diagnoses of HAND with AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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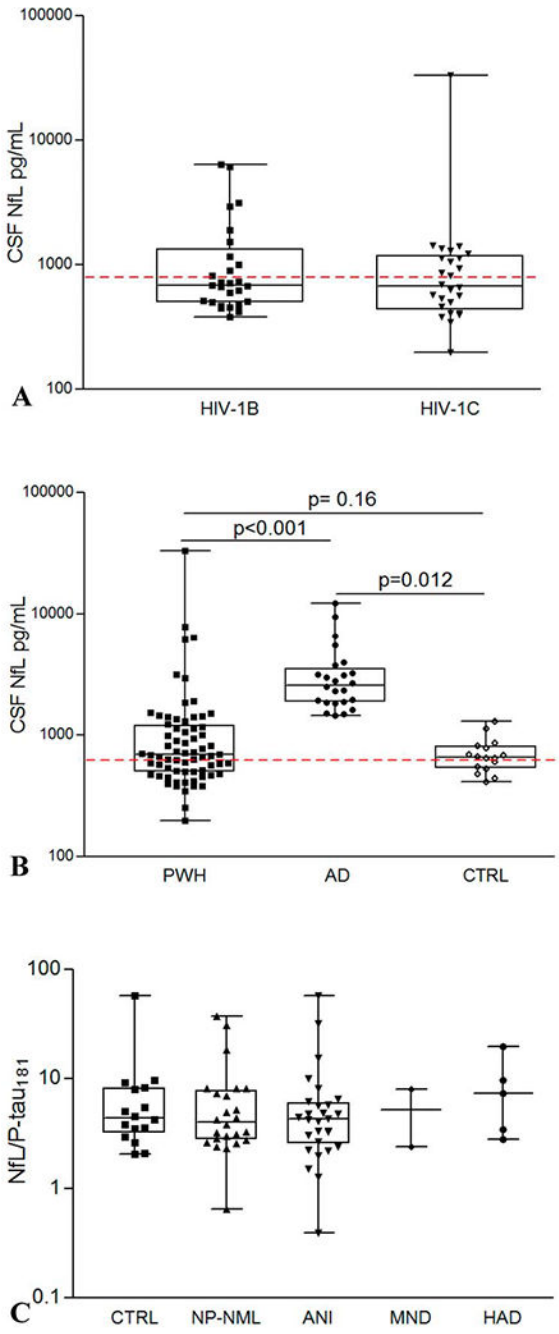

FIGURE 1.

A, The CSF light subunit of Nf protein (NfL) levels of PWH according to HIV subtypes, HIV-1B and HIV-1C. The dashed red line shows the upper reference value of CSF NfL for the median age of HIV-1B and HIV-1C (44 years, 770.92 pg/mL); *P* value was adjusted for plasma HIV VL suppression and nadir CD4 count in the adjusted model, and then corrected for multiple testing with the BH method, $P = 0.80$. B, The CSF light subunit of Nf protein levels of PWH, patients with AD, and healthy HIV-negative controls. All *P* values were adjusted for multiple testing with the BH method; PWH vs. AD and AD vs.

CTRL comparisons were additionally adjusted for gender or age. The dashed red line shows the upper reference value of CSF NfL for the median age of CTRL (38.50 years, 651.76 pg/mL). C, The CSF light subunit of Nf protein and P-tau$_{181}$ ratio in HAND diagnosis categorized according to the Frascati criteria[30] and the healthy HIV-negative controls. There was a numerical increase in the NfL/P-tau$_{181}$ ratio in MND and HAD, although it did not reach significance (pairwise comparisons, all $P > 0.05$). Boxes show median and IQR, and whiskers indicate maximum and minimum values. The dots indicate the number of individuals in each group. CTRL, healthy HIV-negative controls.

TABLE 1.

Demographic, Clinical, and Comorbidities Data of PWH, Patients With AD, and Healthy HIV-Negative Controls (CTRL)

	HIV-1B (n = 27)	HIV-1C (n = 26)	p*	PWH (n = 68)	AD (n = 24)	CTRL (n = 16)	p †
Age, yr	44 (36.5; 50)	43 (34.5; 47.5)	0.45	43 (35; 48)	76.5 (67; 79.5)	38.5 (33.5; 47.5)	<0.0001
Education, yr	8 (5; 12)	7 (5; 11.5)	0.55	8 (5; 11)	4 (2; 6)	12 (10.5; 15.5)	0.0001
Gender, n male (%)	14 (51.9)	11 (42.3)	0.59	33 (49)	8 (33)	12 (75)	0.035
Whites, n (%)	27 (100)	26 (100)	—	66 (97)	22 (92)	12 (75)	0.010
Duration of disease, mo‡	91.03 (61.63; 144.00)	81.37 (27.82; 131.7)	0.45	89 (31; 135)	36 (24; 60)	—	—
Cognitive impairment§	0.95 (0.275; 1.725)	0.50 (0.225; 0.875)	0.13	0.65 (0.30; 1.05)	14 (9.5; 20)	0.11 (0.0; 0.28)	
AIDS, n (%)	22 (81)	19 (73)	0.53	55 (81)	—	—	—
Current CD4 count, cells/mm^3	457 (255; 614)	359.5 (176,5; 472,5)	0.20	369 (201; 534)	—	—	—
Nadir CD4 count, cells/mm^3	82 (26; 253.5)	159 (16.5; 359.5)	0.29	92 (37; 267)	—	—	—
Log plasma HIV RNA	1.7 (1.7; 1.97)	2.8 (1.7; 3.8)	0.012	1.7 (1.7; 3.5)	—	—	—
Plasma HIV RNA <50 copies/mL, n (%)	20 (74.1)	9 (34.6)	0.006	38 (56)	—	—	—
On CART, n (%)‖	24 (88.9)	18 (69.2)	0.10	55 (81)	—	—	—
CPE¶	8 (6; 9)	6 (5.5; 9)	0.34	8 (6; 9)	—	—	—
HCV#, n (%)	6 (22.0)	2 (7.7)	0.126	12 (18)	0	0	—
CSF							
WBC, cells/mm^3	1.6 (0.3; 4.85)	2.65 (0.6; 11)	0.160	2.1 (0.6; 7.2)	0.6 (0.3; 1.4)	1.5 (1.0; 2.0)	0.0031
WBC count >5 cells/mm^3, n (%)	6 (22.22)	8 (30.77)	0.544	20 (29)	0	0	—
RBC, cells/mm^3	1 (0; 24)	0.8 (0.0; 36.5)	0.900	0.5 (0; 7.5)	3.4 (0.95; 53)	1.5 (1.0; 3.0)	0.0136
Glucose, mg/dL	63 (54; 66)	56 (51.5; 59)	0.007	57 (53; 62)	60.5 (54.0; 72.5)	63.5 (59.0; 71.0)	0.0016
Total protein, mg/dL	42 (35; 47.5)	40 (28.50; 47.00)	0.550	40 (32; 46)	37.35 (30.7; 49.0)	30.5 (25.5; 38.0)	0.0142
Total protein > 45 mg/dL, n (%)	10 (37.04)	8 (30.77)	0.773	20 (29)	7 (29)	0	0.0302
Log CSF HIV RNA	1.70 (1.70; 2.25)	2.16 (1.70; 2.88)	0.084	1.70 (1.70; 2.80)	—	—	—
VL < 50	16 (59.26)	10 (38.46)	0.173	35 (51.47)	—	—	—
HIV RNA CSF > blood, n (%)	6 (22.22)	5 (19.23)	1.00	12.00 (18.00)	—	—	—
HIV RNA (log) CSF/plasma	1.00 (1.00; 1.00)	0.96 (0.73; 1.00)	0.118	1.00 (0.85; 1.00)	—	—	—

Data are presented as median (IQR) or number of cases (%).

*HIV-1B vs. HIV-1C.

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[†] PWH vs. CTRL vs. AD.

[‡] Duration of disease: infection on HIV-positive or duration of symptoms on AD.

[§] Cognitive impairment evaluated by the GDS on PWH and HIV− controls or Mini-Mental State Examination (MMSE) on patients with AD.

[‖] CART, combination antiretroviral therapy.

[¶] CPE, antiretroviral CNS penetration effectiveness.[39]

[#] Hepatitis C virus (HCV) status was assessed by antibody testing (Abbott-Architect). Participants coinfected with HCV were not on treatment with interferon gamma.

TABLE 2.

CSF Neurocytoskeleton Protein Levels and Ratios According to HIV Subtypes, HIV-1B and HIV-1C

Biomarker	HIV-1B	HIV-1C	Diff (95% CI)	*P*
NfL, pg/mL	683.40 (503.40; 1342)	655.60 (435.10; 1134)	−0.23 (−0.85 to 0.43)	0.80
T-tau, pg/mL	298.10 (42.76; 600.40)	194.60 (97.10; 385.50)	−0.091 (−0.63 to 0.47)	0.82
P-tau$_{181}$, pg/mL	186.00 (120.50; 200.50)	146.00 (122.50; 202.00)	0.21 (−0.45 to 0.84)	0.80
NfL/T-tau	2.66 (1.80; 22.77)	3.15 (1.96; 10.01)	−0.069 (−0.61 to 0.49)	0.82
NfL/P-tau$_{181}$	4.33 (3.25; 9.93)	4.53 (2.77; 8.17)	−0.32 (−0.93 to 0.35)	0.80

Data are presented as median (IQR); Diff, group differences presented as Cohen's d. All *P* values were adjusted for plasma HIV VL suppression and nadir CD4 count in the adjusted model, and then corrected for multiple testing with the BH method.

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TABLE 3.

CSF Neurocytoskeleton Biomarker Levels and Ratios in the PWH, Patients With AD, and Healthy HIV-Negative Controls (CTRL)

Biomarkers	PWH	AD	CTRL	Diff (95% CI)*
NfL, pg/mL	683.40 (499.90; 1197)	2578 (1864; 3500)	659.60 (538.80; 801.60)	1.61 (0.97 to 2.21)
T-tau, pg/mL	198.50 (66.39; 513.80)	1201 (864.00; 1538)	633.70 (531.20; 764.60)	1.65 (0.96 to 2.34)
P-tau$_{181}$, pg/mL	164.00 (123.50; 241.00)	475.00 (302.50; 709.00)	132.00 (106.50; 179.50)	1.28 (0.71 to 1.82)
NfL/T-tau	3.33 (1.80; 13.50)	2.00 (1.65; 3.08)	1.02 (0.81; 1.44)	−0.59 (−1.09 to −0.095)
NfL/P-tau$_{181}$	4.35 (2.85; 8.14)	6.16 (3.13; 11.21)	4.40 (3.24; 8.19)	0.23 (−0.23 to 0.70)

Biomarkers	*P* *	Diff (95% CI)†	*P* †	Diff (95% CI)‡	*P* ‡
NfL, pg/mL	<0.001	0.42 (−0.16 to 0.99)	0.16	1.92 (0.19 to 1.58)	0.012
T-tau, pg/mL	<0.001	−1.03 (−1.67 to −0.39)	<0.001	1.66 (0.62 to 2.34)	<0.001
P-tau$_{181}$, pg/mL	<0.001	0.37 (−0.17 to 0.92)	0.16	1.40 (0.51 to 1.99)	<0.001
NfL/T-tau	0.018	1.23 (0.50 to 1.95)	<0.001	1.50 (0.64 to 2.36)	<0.001
NfL/P-tau$_{181}$	0.330	−0.40 (−0.94 to 0.16)	0.16	0.78 (0.069 to 1.33)	0.030

Data are presented as median (IQR); Diff, group differences presented as Cohen's d. All *P* values were adjusted for multiple testing with the BH method; * and ‡ additionally adjusted for gender or age.

*AD vs. PWH.

†PWH vs. CTRL.

‡AD vs. CTRL.

TABLE 4.

Diagnostic Characteristics of the Light Subunit of NfL and Its Ratios With Tau Proteins in the CSF for HAND Diagnosis

	NfL		NfL/T-tau		NfL/P-tau$_{181}$	
	HAND vs. NP-NML and CTRL*	**HAND Symptomatic vs. ANI, NP-NML, and CTRL**	**HAND vs. NP-NML and CTRL***	**HAND Symptomatic vs. ANI and NP-NML, and CTRL**	**HAND vs. NP-NML and CTRL***	**HAND Symptomatic vs. ANI and NP-NML, and CTRL**
TP	16	04	23	05	06	02
Sensitivity, %	45.71	57.14	65.71	71.43	17.14	28.57
Specificity, %	55.00	55.88	40.00	38.24	90.00	88.24
PPV, %	47.06	11.76	48.94	10.64	60.00	20.00
NPV, %	53.66	92.68	57.14	92.86	55.38	92.31
LRP	01.02	01.30	01.10	01.16	01.71	02.43
LRN	00.99	00.77	00.86	00.75	00.92	00.81
DOR	01.03	01.69	01.28	01.55	01.86	3.00
CUIP	00.22	00.07	00.32	00.08	00.10	00.06
CUIN	00.30	00.52	00.23	00.36	00.50	00.81
Youden index	00.01	00.13	00.06	00.10	00.07	00.17
Efficiency, %	50.67	56.00	52.00	41.33	56.00	82.67

HAD; PWH neuropsychologically normal (NP NML). HAND[30]: MND, HAD, and ANI (n = 35); HAND symptomatic: MND and HAD (n = 7); PWH cognitively asymptomatic: ANI and NP-NML (n = 28 + 24); healthy HIV-negative controls (CTRL, n = 16). Clinical utility index (CUI) was classified as follows: utility excellent, ≥ 0.81; good, ≥ 0.64; fair, ≥ 0.49; poor, ≤ 0.49; and very poor, ≤ 0.36.[37] The cutoff value of CSF NfL, for each participant, was calculated by the equation: CSF NfL = 201.2 × 1.031^{age} [2]; for the CSF NfL/tau protein ratios, the cutoff values were selected from the ROC curve (NfL/T-tau, 1.51; NfL/P-tau$_{181}$, 9.74).

*The area under the ROC curve for NfL was 0.56 (95% CV: 0.43–0.68), for NfL/T-tau was 0.57 (95% CV: 0.44–0.70), and for NfL/P-tau$_{181}$ was 0.48 (95% CV: 0.35–0.62). CUIN, clinical utility index negative; CUIP, clinical utility index positive; DOR, diagnostic odds ratio; TP, true positive.