Serum NFL and neuropsychological performance over ~8 years in women with and without HIV: a longitudinal repeated measures study

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Summary

Background Blood-based biomarkers of Alzheimer's disease (AD) and stroke, including serum neurofilament light chain (sNFL), are understudied in women living with and without HIV.

Methods We assessed cross-sectional and longitudinal change in sNFL between 2008 and 2019 associated with neuropsychological performance (NP) among women living with HIV (WLWH) and without HIV (WLWOH) age ≥40 years in the Women's Interagency HIV Study. Baseline and repeated ~8-year fasting sNFL levels were measured using Simoa. Sociodemographically-adjusted NP T-scores (attention, working memory, executive function, processing speed, learning, verbal fluency and global) were calculated. Multivariable linear regression analyses stratified by HIV serostatus examined cross-sectional baseline and follow-up associations, and ~8-year change in sNFL level related to global and domain-specific NP T-scores.

Findings 417 participants (290 WLWH, 127 WLWOH), African American/Black (55%), ≥high school education (69%), current/former smokers (79%), and overweight/obese (BMI ≥25.0 kg/m^2, 74%) were included. Compared to WLWOH at baseline, WLWH performed worse on memory and global NP. WLWH versus WLWOH had higher baseline ($p \leq 0.001$) and follow-up median ($p < 0.0001$) sNFL levels and ~8-year change (46.5% in WLWH versus 24.4% in WLWOH, $p < 0.0001$). Among WLWH, higher baseline sNFL was associated with poorer processing speed, learning, memory and verbal fluency. Among WLWOH, higher baseline sNFL was associated with poorer executive function, processing speed and verbal fluency. Among WLWH, higher follow-up sNFL was associated with poorer executive function. Among WLWOH, higher follow-up sNFL was associated with poorer executive function, processing speed, attention, memory, and global NP. ~8-year increase in sNFL occurred in both WLWH and WLWOH and was associated with poorer executive function, processing speed, memory, and global performance at follow-up among WLWOH, and poorer executive function in WLWH. Adjustment for multiple comparisons showed associations at cross-sectional follow-up and ~8-year increase in sNFL in WLWOH, only. Higher sNFL was associated with poorer baseline processing speed in WLWH only.

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Interpretation Higher levels and greater ~8-year increases in sNFL were associated with poorer NP by domain in WLWH and WLWOH differentially over time.

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Keywords: Cognition; Neuropsychological performance; Neurofilament light chain; HIV; Women

Research in context

Evidence before this study

There are a dearth of data on blood-based biomarkers and brain health in people living with HIV (PLWH), particularly women who are underrepresented in research. We searched PubMed using key words: HIV, HIV Associated Neurocognitive Disorder (HAND), cognition, neuropsychological performance, cognitive impairment, dementia, Alzheimer's Disease and Related Dementias (ADRD), multiple sclerosis, Amyotrophic Lateral Sclerosis, stroke and traumatic brain injury, neurofilament light chain, and serum NFL (sNFL). There were no exclusion criteria other than age ≥40 years. The search included all studies published through 10/01/2024. A potential bias is that most studies publishing on blood levels of NFL among people living with HIV were clinical, cross-sectional, or case–control studies and included men. There are no longitudinal data available to our knowledge on this blood-based biomarker in women with HIV.

Added value of this study

We present first ever longitudinal, observational data on change in blood levels of sNFL in relation to neuropsychological performance among underrepresented women in the United States. These women lived with HIV or are a similar group without HIV and were predominantly non-White race.

Implications of all the available evidence

Identifying blood-based biomarkers associated with brain health are imperative for under-resourced settings and among underrepresented people in research. Blood-based biomarkers may be useful for the screening, diagnosis and prognosis of cognitive impairment, dementia and ADRD. They are of interest for the establishment of public health and clinical care guidelines among aging people with and without HIV.

Introduction

Chronic HIV and its intersection with noncommunicable diseases (NCD) is a burgeoning global reality due to highly effective and available antiretroviral therapies (ART) in the 21st century. This has resulted in increasing life expectancies among PLWH.[1] As a result, there is a dire need to address the HIV-NCD intersection, particularly in relation to neurological and cerebrovascular outcomes among older PLWH, such as cognitive impairment, ADRD, and acquired brain injury.[2,3]

Stroke is the second and AD, the seventh leading cause of death globally.[4] There are 101.5 million people with stroke[5] and >50 million people with AD worldwide.[3] Certain populations may be more susceptible to both stroke and AD, notably, those with multimorbidities, vascular risk factors, African American

and Black people and people from Low and Middle Income Countries.[3] In addition, stroke increases the risk for ADRD and vascular cognitive impairment and dementia. WLWH and similar WLWOH may be at risk for stroke and ADRD with aging due to higher levels of vascular risk factors such as hypertension, obesity and cigarette smoking as well as substance abuse, lower education, lower income, and inadequate healthcare access, pointing to a syndemics approach.[6,7] Easily attained biomarkers that can be used to assess risk for central and peripheral neurological outcomes, as well as monitoring disease course and treatment efficacy over time, are essential.

One biomarker of neurodegeneration and neuronal damage in people without HIV and elevated in AD, multiple sclerosis, Amyotrophic Lateral Sclerosis (ALS), and acquired brain injury, such as stroke and traumatic brain injury in both blood and cerebrospinal fluid, is sNFL.[2,8–10] In PLWH, sNFL levels may also be elevated with peripheral neuronal damage and neuropathies that are associated with HIV.[11] In addition, WLWH more often experience comorbid psychiatric and cardiovascular disorders, which are also associated with higher NFL levels.[6,10] Therefore, it is often difficult to know if sNFL levels are a marker of acute neuronal injury, cerebrovascular or cardiovascular disease, or chronic neurodegenerative or neuroaxonal processes over time.[10] Studies suggest that blood levels of NFL may confirm neurodegeneration in patients with an ADRD diagnosis based on the Amyloid-Tau-Neurodegeneration (ATN) criteria[12]; and reflect the contribution of neuronal injury to overall health over time with aging. Exploration of sNFL levels within the context of the HIV-NCD intersection is paramount.

There are few observational, cross-sectional or longitudinal studies on sNFL levels in WLWH. This study is needed to fill the knowledge gap related to key neurological fluid-based biomarkers and brain health in underrepresented populations. Therefore, we explored the hypothesis that cross-sectional or ~8-year change in sNFL may be associated with NP among WLWH and WLWOH age ≥40 years at baseline enrolled in the Women's Interagency HIV Study (WIHS).

Methods

Study population and setting

The WIHS was the largest prospective study of HIV in women, defined as sex at birth and gender, in six clinical research sites in the USA–San Francisco, Los Angeles, Chicago, Washington DC, and the Brooklyn and Bronx boroughs in New York City.[6] WIHS began in 1994 and initially enrolled 2054 WLWH and 1712 WLWOH who were similar sociodemographically and regarding HIV-related risk factors (e.g., number of sexual partners, intravenous drug use). Two additional enrollment waves occurred in 2001–2002 and 2011–2014. In 2012, one site was dropped (Los Angeles). Semiannual WIHS core visits included sociodemographic, behavioral, and clinical measures. A standardized comprehensive NP battery was administered every two years starting in 2009. In relation to this analysis, a subset of WIHS participants completed one in-person baseline NP assessment between June 2008 to October 2011 and one follow-up in-person NP assessment between November 2014 to September 2019 (N = 417; 290 WLWH, 127 WLWOH) and had batch testing of fasting sNFL levels at those visits. Serum was collected using Becton Dickenson Vacutainer® CPT™ (Cell Preparation Tubes) with Sodium Heparin. Serum was collected and processed in a Clinical Laboratory Improvement Amendments of 1988 (CLIA)-certified laboratory. Subsequently, serum was stored at Precision for Medicine, which is the National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases (NIAID) Specimen Repository at –70 °C to –80 °C over time.

Ethics

The institutional review boards (IRBs) of each clinical research site approved the WIHS research protocol and all participants provided written informed consent. The IRBs included: SUNY Downstate Institutional Review Board & Privacy Board Protocol #266921; Georgetown-MedStar IRB System #1993-077; Cook County Health IRB #13-184; Albert Einstein College of Medicine IRB #03-07-174; and Human Research Protection Program IRB, University of California, San Francisco #10-02621.

This report meets the STROBE reporting guidelines for cohort studies. Dr. Gustafson, Dr. Yucel and Mr. Li had full access to all data in the study and take responsibility for the integrity and the accuracy of the data analysis. In addition, the majority of co-authors are WIHS investigators and have contributed to data collection and interpretation over the course of this data collection and analysis period.

Procedures

Sociodemographic factors were self-reported and included: date of birth, race (African American or Black, White, and other (American Indian or Alaskan, Asian, Native Hawaiian or Pacific Islander, and Multi-Racial), and ethnicity (Hispanic or non-Hispanic), highest educational level attained, annual income, use of tobacco, alcohol, marijuana, and other drugs (crack, cocaine or heroin). Medical history of hypertension, use of hypertensive medications; diabetes and use of diabetes medications; and cognitive impairment and dementia, and dementia medications, was queried. Current pregnancy status was also queried.

All data collected were collected at two study visits when venipuncture was performed. Body weight and body height measures were measured with participants wearing undergarments. Body mass index (BMI) was calculated as kilograms per meter squared (kg/m^2) and categorized as:

underweight, <18.5 kg/m^2; 'normal' or healthy weight, 18.5–24.9 kg/m^2; overweight, 25.0–29.9 kg/m^2; and obese, ≥30 kg/m^2.[13]

Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation.[14]

A clinically-relevant depressive symptom burden was defined as Center for Epidemiological Studies - Depression (CES-D) score ≥16 (out of 60).[15]

Laboratory-confirmed HIV status, HIV viral load, and CD4 count and self-reported ART use and Acquired Immune Deficiency Syndrome (AIDS) diagnosis were conducted in WLWH.

Outcomes

Neuropsychological performance

The NP battery included the Letter-Number Sequencing (LNS), Trail Making Test Part B (Trails B), Stroop Test (color word, word reading), Hopkins Verbal Learning Test-Revised (HVLT-R), Symbol Digit Modalities Test (SDMT), Controlled Oral Word Association Test (COWAT), Category Fluency Test (Animals), and Grooved Pegboard (GPEG). Performance on these assessments was used to assess seven NP domains: 1) attention/working memory (outcomes: total correct on LNS control and experimental conditions); 2) executive function (outcomes: time to completion on Trails B and Stroop color-word [interference] trial); 3) processing speed (outcomes: total correct on SDMT, time to completion on Stroop word-reading trial); 4) memory (outcome: HVLT-R delayed recall); 5) learning (outcome: total learning across HVLT-R trials); 6) verbal fluency (outcomes: total correct on COWAT and Animals); and 7) fine motor skills (outcomes: total time to completion for each hand on the GPEG).[16,17]

All timed outcomes were natural log (ln)-transformed and reverse scored so higher scores represented better performance. Cohort-derived sociodemographically-adjusted T-scores were derived for each outcome based on WLWOH.[17] Sociodemographic factors included age, education, Wide Range Achievement Test reading subtest (WRAT-3) score, race (African American versus not), and ethnicity (Hispanic versus non-Hispanic). T-scores were calculated to create domain-specific and global NP scores.[16,17] Global NP score was the calculated average of the 7 NP domains.

Primary exposure

Baseline and ~8-year fasted sNFL concentrations were measured in batch from stored serum samples using Single molecule array (Simoa) technology on an HD-X Analyzer (Quanterix, Billerica, MA). sNFL concentrations were measured using the Neuro 4-Plex E kit according to instructions from the manufacturer (Quanterix, Billerica, MA).[18] Standards and controls were tested in duplicate. Paired longitudinal samples were run side-by-side on the same plates. The measurements were performed in one round of experiments using one batch of kits. The intra-assay coefficient of variation was <5%. sNFL levels were also categorized according to published criteria.[19]

Statistics

Participants were characterized overall and by HIV serostatus. Covariates of interest included sociodemographic, behavioral, and clinical factors. Among WLWH, HIV viral load, CD4 count and history of AIDS were characterized. T-tests for continuous variables and Chi–Square tests for categorical variables were used to assess differences by HIV serostatus, BMI category, and published plasma NFL cut-offs[19] at baseline. Partial Pearson correlation analyses were performed between sNFL and key covariates noted to be associated with sNFL, such as age, eGFR and BMI. We also examined the correlation between sNFL and HIV-related variables including HIV viral load and CD4 count and the association with history of AIDS via ANOVA. Data missingness was explored by HIV serostatus. To assess the validity of our inferences using the complete-cases only, we assessed the factors influencing the missingness of the response variables by fitting logistic regression models on the missingness indicator of the response variables.

Primary independent variables were baseline and follow-up sNFL levels and ~8-year change in sNFL levels calculated as baseline sNFL level subtracted from follow-up sNFL level. The primary outcomes were NP domains and global NP. Multivariable linear regression analyses were performed to examine cross-sectional baseline and follow-up associations, as well as ~8-year change in sNFL level in association with the outcome, individual domain and global NP T-scores, among all women (adjusted for HIV serostatus); and stratified by HIV serostatus. In analyses of WLWH only, we additionally adjusted for baseline HIV viral load, CD4 count category, ART use, and history of AIDS.

Covariates were selected based on previous WIHS analyses of NP outcomes and included annual income, BMI, CES-D score, history of diabetes, use of hypertensive medication, alcohol, tobacco, marijuana, and other drug use (crack, cocaine, or heroin).[16,20] We also adjusted for eGFR since kidney function may influence sNFL level, and age.[21] In the sNFL change model, we adjusted for baseline NP. Race, ethnicity, WRAT score, and education were not included as covariates since they were included in the demographically-adjusted NP T-scores, however we additionally adjusted for chronological age given the association between blood NFL levels and age.[22]

Missing data patterns were assessed among WLWH and WLWOH. Among WLWH data, the Little's Missing Completely at Random (MCAR) test statistic[23] was 106 ($p = 0.856$, degrees of freedom, $df = 123$); and in WLWOH data, the Little's MCAR test statistic was 41.1 ($p = 0.337$, $df = 37$)). Supplementary Table S1 describes

missingness patterns by NP domain. As supplementary analyses, we also investigated logistic regression models that revealed that none of the covariates used in the models had any statistically discernible association with the missingness of the response variables. There were no patterns of missingness in the data that would imply bias in our statistical inferences. Of note, participants with missing data were not included in multivariable regression analyses as can be seen from the N provided for each regression analysis. Results were considered significant at $p < 0.05$ (alpha = 0.05). We also adjusted for multiple comparisons using a Bonferroni adjustment. This adjustment would mean that a statistically significant result would require a p-value less than the adjusted alpha of 0.05/7 or $p = 0.007$.

Data analyses were conducted using R software (R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

Role of funding source

The funding source had no role in the study design, data collection, data analyses, interpretation, or writing of this report.

Results

Baseline survey and clinical characteristics of participants are presented in Table 1. The majority (93.3%) of 417 participants (290 WLWH, 127 WLWOH) were aged 40–59 years; 6.7% (N = 28) aged ≥60 years, of whom four were aged ≥70 years. At baseline the average age of WLWH was 51.6 years and among WLWOH, 48.7 years. Approximately half of the women (55%) self-identified as African American or Black. Most women had a high school education or higher (69%); were current or former smokers (79%); or were overweight or obese (74%). No participant was pregnant at the time of the sNFL measurements. Compared to WLWOH at baseline, WLWH performed worse on memory and global NP. No one in this sample had a self-reported diagnosis of AD, other type of dementia, or other neurological disorder, nor use of medications for a neurological disorder. Of note, there was a positive correlation between sNFL and age ($r = 0.18$ at baseline and $r = 0.15$ at follow-up, $p < 0.05$). The average length of time between baseline and follow-up was 7.9 years referred to as ~8-years. Baseline, follow-up, and ~8-year change in sNFL characteristics by HIV serostatus are presented in Table 2. Baseline ($p < 0.001$) and follow-up median ($p < 0.0001$) sNFL levels and ~8-year change in sNFL ($p < 0.0001$) were higher among WLWH versus WLWOH. Among WLWH, there was an unadjusted 46.5% increase in NFL, and among WLWOH, a 24.3% increase over ~8 years. A diagram illustrating trajectories of sNFL over time in the entire sample and by HIV serostatus is provided as Supplementary Figure S1.

Variable	All women mean (SD) or N (%)	WLWH mean (SD) or N (%)	WLWOH mean (SD) or N (%)	p
Age, median years (IQR)	50 (8)	51 (7)	47 (9)	<0.0001
Age group, years, N (%)				0.000
40–49	190 (45.6)	114 (39)	76 (60)	
50–59	199 (47.7)	156 (54)	43 (34)	
≥60	28 (6.7)	20 (7)	8 (6)	
Race, N (%)				0.022
Black/African American	228 (55)	156 (54)	72 (57)	
White	50 (12)	43 (15)	7 (6)	
Other	139 (33)	91 (31)	48 (38)	
Ethnicity, N (%)				0.888
Non-Hispanic	335 (80)	234 (81)	101 (80)	
Hispanic	82 (20)	56 (19)	26 (20)	
Education, N (%)				0.713
<High school	131 (31)	89 (31)	42 (33)	
≥High school	286 (69)	201 (69)	85 (67)	
Annual income, N (%)				0.800
≤$12,000	179 (43)	126 (43)	53 (42)	
>$12,000	227 (54)	156 (54)	71 (56)	
Tobacco use, N (%)				0.389
Never smoker	88 (21)	65 (22)	23 (18)	
Current/Former smoker	329 (79)	225 (78)	104 (82)	
Marijuana use, N (%)				0.034
Never smoker	362 (87)	259 (89)	103 (81)	
Current/Former smoker	55 (13)	31 (11)	24 (19)	
Drinking history, N (%)				0.149
Abstainer/None	257 (62)	187 (64)	70 (55)	
Low/Moderate	132 (32)	86 (30)	46 (36)	
High	27 (6)	16 (6)	11 (9)	
Other drug use (crack, cocaine or heroin), N (%)				0.035
Yes	28 (7)	14 (5)	14 (11)	
No	389 (93)	276 (95)	113 (89)	
Hypertension, N (%)				0.498
Yes	59 (14)	48 (17)	11 (9)	
No	93 (22)	70 (24)	23 (18)	
Type II diabetes, N (%)				0.518
Yes	92 (22)	67 (23)	25 (20)	
No	325 (78)	223 (77)	102 (80)	
BMI, kg/m^2, N (%)				0.010
Underweight (BMI<18.5)	4 (1)	3 (1)	1 (0)	
Healthy (BMI 18.5.24.9)	98 (24)	78 (27)	20 (16)	
Healthy (BMI<25.0)	102 (24)	81 (28)	21 (17)	
Overweight (25.0 ≤BMI <30)	115 (28)	84 (29)	31 (24)	
Obese (BMI ≥30)	192 (46)	121 (42)	71 (56)	
eGFR, mL/min, N (%)				0.516
<30	8 (2)	5 (2)	3 (2)	
30–44.9	8 (2)	7 (2)	1 (1)	
45–59.9	26 (6)	20 (7)	6 (5)	
≥60	375 (90)	258 (89)	117 (92)	
CES-D, N (%)				0.108
<16	299 (72)	215 (74)	84 (66)	
≥16	114 (27)	72 (25)	42 (33)	

(Table 1 continues on next page)

Variable	All women mean (SD) or N (%)	WLWH mean (SD) or N (%)	WLWOH mean (SD) or N (%)	p
(Continued from previous page)				
HIV variables				
CD4 count, cells/mL, N (%)			–	
<200		33 (11)	–	
200–499		96 (33)	–	
≥500		160 (55)	–	
HIV RNA viral load, copies/mL			–	
Viral load undetectable, <200 copies/mL, N (%)		207 (71)	–	
Viral load detectable, copies/mL, median (IQR)		62,391 (13,803)	–	
On art, N (%)				
Yes		244 (84)	–	
No		46 (16)	–	
History of AIDS, N (%)				
Yes		138 (48)	–	
No		152 (52)	–	
NP assessments, T-score, mean (SD)*				
Executive	48.9 (9.3)	48.3 (9.2)	50.5 (9.4)	0.088
Processing speed	48.7 (10.0)	48.5 (9.5)	49.1 (11.1)	0.675
Attention	52.7 (9.9)	52.2 (10.3)	53.9 (8.5)	0.228
Learning	52.4 (9.4)	51.9 (9.4)	53.6 (9.4)	0.215
Memory	51.0 (9.8)	51.0 (9.4)	54.2 (10.6)	0.021
Verbal fluency	49.9 (8.2)	49.7 (8.4)	50.2 (7.7)	0.625
Global	51.5 (6.1)	51.0 (6.0)	52.9 (6.0)	0.039

Variables reported as N (%) were analyzed with Chi-Square tests. Variables reported as Mean (SD) were analyzed using independent sample t-tests. T-scores are demographically adjusted for age, education, Wide Range Achievement Test reading subtest (WRAT-3) score, race (African American versus not), and ethnicity (Hispanic versus not). The Women's Interagency HIV Study.

Table 1: **Baseline demographic, clinical and neuropsychological performance characteristics for all women and by HIV serostatus (N = 417; 290 WLWH, 127 WLWOH).**

The proportion of participants stratified by HIV serostatus by published sNFL categories[19] is shown in Table 3. WLWH predominated in the 10–14.9 pg/mL category compared to WLWOH (p = 0.031). There were no associations between sNFL by traditional BMI category with age adjustment (Table 4). We also observed an inverse age-adjusted correlation between sNFL and eGFR (r = –0.30, p < 0.001). In terms of HIV variables, sNFL was not related to HIV viral load (r = 0.02, P > 0.05). However, CD4+ was inversely (r = –0.08, p = 0.03) and history of AIDS, positively associated with sNFL (p = 0.009) at baseline.

Without adjustment for multiple comparisons, baseline cross-sectional analyses associating NP by sNFL level showed that a higher sNFL level among WLWH was associated with poorer processing speed, attention, and learning. Among WLWOH, higher sNFL was associated with poorer processing speed and attention (Table 5). Cross-sectionally at follow-up among WLWH, higher sNFL was associated with poorer executive function. Among WLWOH, sNFL was associated with poorer executive function, processing speed, attention, memory, and global NP (Table 6). Among WLWH, ~8-year increase in sNFL was associated with worse executive function. Among WLWOH, greater sNFL increase was associated with worse executive function, processing speed, memory and global performance (Table 7).

With adjustment for multiple comparisons, at baseline higher sNFL among WLWH was associated with poorer processing speed. At follow-up, higher sNFL was associated with poorer processing speed and global NP among WLWOH only. Greater sNFL increase over time was associated with poorer processing speed and global NP among WLWOH only.

Variable	All women median (IQR) N = 417	WLWH N = 290	WLWOH N = 127	Crude p-value	Age-adjusted p-value
Baseline					
sNFL (pg/mL)	9.08 (6.16)	9.69 (6.41)	8.20 (4.52)	<0.001	<0.001
Follow-up					
sNFL (pg/mL)	13.00 (9.76)	14.20 (9.35)	10.20 (7.04)	<0.001	<0.001
~8-year change					
sNFL (pg/mL)	3.20 (5.90)	3.64 (6.16)	2.26 (4.31)	<0.001	0.002

Variables reported as Median (IQR) were analyzed with the median test. p-values refer to differences by HIV serostatus. The Women's Interagency HIV Study.

Table 2: **Baseline, follow-up and ~8-year sNFL change for all women and by HIV serostatus.**

Discussion

Most associations between change in sNFL and NP after ~8-year (2008–2019) were observed among WLWOH. However, among WLWH, higher sNFL levels were observed consistently at baseline and follow-up, as well as greater ~8-year increase compared to WLWOH. Despite WLWH experiencing a worse neurological profile based on sNFL levels, poorer NP was more associated with higher levels and greater increase in sNFL among WLWOH. To our knowledge, there are no comparable published data among older WLWH and WLWOH.

sNFL has been evaluated for over 20 years among PLWH as a biomarker of neuronal damage and in relation to brain and overall health.[24] Initially, cerebrospinal fluid was the only accessible fluid available for NFL measurement, which limited its utility as a biomarker outside of clinical settings. Plasma NFL levels in PLWH were first reported in 2016,[25] which has allowed greater exploration of NFL's role as a biomarker of neuronal health including chronic neurodegeneration and acute neuronal injury. A published meta-analysis showed a correlation of 0.72 between cerebrospinal fluid (CSF) and blood NFL levels, supporting the use of blood versus CSF as a tissue of choice for measurement in larger community- or population-based studies.[26]

Of the few published studies on blood NFL in PLWH, most have been cross-sectional, with short-term follow-up, e.g., 12–24 weeks, and among men with HIV. Cross-sectionally NFL levels have been observed to be higher among PLWH who are ART-naïve, who have HIV-associated dementia, or who have HIV over many years in the ART era.[18,25] Among ART-naïve PLWH, ART initiation has been associated with decreases in NFL over time.[27,28] However, the data reported herein as well as our data from a different subsample of WIHS participants based on one-year repeated blood collection in 2018 and 2019, showed higher plasma NFL levels among well-controlled WLWH compared to WLWOH, and no difference ($p \geq 0.05$) in one-year change in the entire sample, albeit one-year change in NFL was relatively small, i.e., 3.6% in the entire sample.[18] In separate regression models stratified by serostatus, among WLWH a one-year increase in plasma NFL was associated with worse processing speed, and among WLWOH a one-year increase plasma NFL was associated with poorer motor performance. However, regression coefficients and p-values were modest.[18]

Perhaps over time and with older age, change in sNFL is more critically related to NP and clinical outcomes such as Mild Neurocognitive disorder, MCI, and ADRD. Aging brain research studies show that there are temporal differences in biomarker, clinical and risk factor relationships over the life course.[29] While blood NFL levels are associated with neurodegeneration, change in NFL measured in longitudinal samples may predict cognitive deterioration. NFL measures are not specific with regard to cause of axonal injury. For example, in older people with more cerebrovascular disease and other brain changes that increase in incidence and prevalence with age, NFL concentrations are higher. There may also be sex and acute and long-term syndemic differences causing varying neurological stressors in brain and periphery that alter sNFL levels.

Related to NP, sNFL reflects one of the ATN criteria for AD, that of Neurodegeneration (N).[12] Blood-based AD biomarkers reflecting central and peripheral neurodegeneration and/or neuronal damage may be keys to early diagnosis of AD and underlie the National Institute on Aging (NIA)/Alzheimer's Association (AA) 2018 research framework[12] to better understand the mechanisms underlying AD. As aforementioned, higher NFL levels are also associated with stroke, cerebrovascular disease, cardiovascular disease and psychiatric disorders, which are also related to AD.[10,30] Unfortunately, in our cohort of women, we do not have adequate numbers of outcomes between 2008 and 2019 and/or measures to explore all of these potential comorbidities, although we did consider clinically-relevant depressive symptoms based on a commonly-used depression screener.

That we observed fewer associations between higher sNFL levels and lower NP among WLWH over time, despite WLWH having consistently higher and greater increase in sNFL levels may not be surprising. Data published from the WIHS and Multicenter AIDS Cohort Study (MACS) since 2005 show that differences between PLWH and PLWOH related to aging health outcomes are decreasing, and that associations between certain non-HIV risk factors and outcomes among older PLWH and PLWOH are becoming more similar. In addition, different levels of risk factors, including biomarkers, may indicate different etiopathogenic mechanisms among PLWH on ART and PLWOH.[16,18,20,31,32] Survivorship among WLWH with optimal ART, as well as more recent HIV diagnosis at older ages with ready access to optimal ART is making a difference in the lives of WLWH versus WLWOH. Thus, higher sNFL levels may not be informative related to NP in WLWH. Age at exposure measurements, age at outcomes, temporality of exposures and outcomes, the evolution of treatments, and years of follow-up are important considerations.[29]

Greater increase in sNFL among Black people without HIV has been associated with development of AD and other underlying brain events[33]; and greater sNFL increase over ~6 years was associated with chronic racial discrimination in the US.[34] However studies among PLWH and people without HIV are conflicting, especially with age and BMI adjustments[9,10]; and there are a dearth of data on race/ethnic differences in NFL

	All women N (%)	WLWH N (%)	WLWOH N (%)	Crude p-value	Age-adjusted p-value
Baseline sNFL Cut-off (pg/mL)				Overall 0.001	Overall 0.069
<10	242 (58.0)	152 (52.4)	90 (70.9)	0.036	0.100
10–14.9	107 (25.7)	80 (27.6)	27 (21.3)	0.022	0.031
15–19.9	28 (6.7)	25 (8.6)	3 (2.4)	0.297	0.238
≥20	40 (9.6)	33 (11.4)	7 (5.5)	0.388	0.499

p-values refer to the difference in proportions by HIV serostatus. The Women's Interagency HIV Study.

Table 3: **Baseline sNFL cut-offs by HIV status.**

	All women sNFL (pg/mL), median (IQR)	WLWH sNFL (pg/mL), median (IQR)	WLWOH sNFL (pg/mL), median (IQR)	Crude p-value	Age-adjusted p-value
Baseline BMI, kg/m^2				Overall 0.001	Overall 0.069
Underweight (BMI<18.5)[a]	25.6 (59.4)	34.0 (88.0)	6.1 (0.0)	N/A	N/A
Healthy (BMI 18.5–24.9)	12.1 (8.2)	13.1 (8.6)	8.9 (4.4)	<0.001	0.103
Overweight (BMI 25.0–29.9)	9.1 (5.8)	9.9 (5.6)	7.2 (3.9)	0.002	0.194
Obese (BMI≥30)	8.1 (4.7)	8.1 (4.8)	8.2 (4.7)	0.782	0.591

Variables reported as Median (IQR) were analyzed with the median test. The Women's Interagency HIV Study.
[a]BMI<18.5 kg/m^2 was observed among N = 3 WLWH and N = 1 WLWOH, so the p-value is not available (N/A).

Table 4: **Median sNFL by baseline BMI Category by HIV Status.**

Model[a]	All			WLWH			WLWOH		
	N	β (95% CI)	p	N	β (95% CI)	p	N	β (95% CI)	p
Executive function									
sNFL	262	−0.05 (−0.14, 0.05)	0.325	173	0.05 (−0.12, 0.21)	0.558	76	−0.24 (−0.48, 0.00)	0.052
Processing speed									
sNFL	269	−0.17 (−0.27, −0.08)	0.001	176	−0.22 (−0.37, −0.08)	0.003	80	−0.35 (−0.63, −0.07)	0.014
Attention									
sNFL	223	0.00 (−0.12, 0.11)	0.945	149	−0.05 (−0.22, 0.12)	0.585	62	−0.68 (−1.22, −0.15)	0.013
Learning									
sNFL	267	−0.08 (−0.18, 0.02)	0.101	175	−0.18 (−0.33, −0.03)	0.018	79	−0.05 (−0.32, 0.21)	0.694
Memory									
sNFL	267	−0.13 (−0.23, −0.03)	0.014	175	−0.15 (−0.31, 0.00)	0.055	79	−0.26 (−0.55, 0.04)	0.085
Verbal fluency									
sNFL	268	−0.10 (−0.18, −0.01)	0.024	176	−0.13 (−0.27, 0.01)	0.078	79	−0.31 (−0.50, −0.13)	0.001
Global									
sNFL	204	−0.04 (−0.11, 0.03)	0.256	138	−0.06 (−0.17, 0.05)	0.290	60	−0.30 (−0.67, 0.08)	0.116

The Women's Interagency HIV Study. [a]All models are adjusted for baseline age, HIV status, income, BMI, CES-D score, eGFR, diabetes, tobacco use, marijuana use, alcohol use, and other drug use (crack, cocaine or heroin). β represents an adjusted, unstandardized coefficient. NP assessments are reverse scored and a higher β indicates better NP. WLWH models are additionally adjusted for baseline CD4 count, HIV viral load, ART use, and history of AIDS. p-values are not adjusted for multiple comparisons. The adjusted p-value for determining statistical significance is p < 0.007.

Table 5: **Linear regression models associating cross-sectional baseline sNFL levels with neuropsychological performance by domain in all women and stratified by HIV serostatus.**

levels among people with and without HIV.[10] Data on the direction of association between BMI and NFL are also conflicting. Some studies report an inverse association between BMI and blood NFL levels,[10] others report positive associations between BMI and CSF NFL levels.[35] Our data showed no association between BMI and sNFL. Therefore, additional measures may be important for interpretation of sNFL levels aside from underlying neurological disorders. These measures include race/ethnicity, social determinants of health, cardiovascular risk factors, and pregnancy status in underrepresented populations.

Our analyses have many strengths. First, the WIHS cohort was active with a consistent research protocol for

Model[a]	All			WLWH			WLWOH		
	N	β (95% CI)	p	N	β (95% CI)	P	N	β (95% CI)	p
Executive function									
sNFL	384	−0.03 (−0.07, 0.00)	0.054	251	−0.06 (−0.11, −0.01)	0.025	116	−0.15 (−0.28, −0.02)	0.021
Processing speed									
sNFL	390	−0.05 (−0.09, −0.02)	0.005	255	−0.02 (−0.08, 0.03)	0.445	118	−0.29 (−0.44, −0.14)	<0.001
Attention									
sNFL	362	0.00 (−0.03, 0.03)	0.937	234	−0.01 (−0.06, 0.03)	0.579	111	−0.21 (−0.38, −0.04)	0.018
Learning									
sNFL	390	0.00 (−0.04, 0.04)	0.983	256	0.00 (−0.06, 0.05)	0.902	117	−0.13 (−0.28, 0.02)	0.078
Memory									
sNFL	388	−0.01 (−0.05, 0.03)	0.624	256	−0.01 (−0.07, 0.05)	0.852	115	−0.18 (−0.33, −0.03)	0.022
Verbal fluency									
sNFL	389	−0.02 (−0.05, 0.02)	0.338	256	−0.02 (−0.07, 0.02)	0.300	116	−0.11 (−0.23, 0.00)	0.055
Global									
sNFL	356	−0.01 (−0.04, 0.01)	0.281	231	−0.01 (−0.05, 0.02)	0.459	108	−0.23 (−0.36, −0.11)	<0.001

The Women's Interagency HIV Study. [a]All models are adjusted for follow-up age, baseline HIV status, income, BMI, CES-D score, eGFR, diabetes, tobacco use, marijuana use, alcohol use, and other drug use (crack, cocaine or heroin). β represents an adjusted, unstandardized coefficient. NP assessments are reverse scored and a higher β indicates better NP. WLWH models are additionally adjusted for baseline CD4 count, HIV viral load, ART use, and history of AIDS. p-values are not adjusted for multiple comparisons. The adjusted p-value for determining statistical significance is p < 0.007.

Table 6: **Linear regression models associating cross-sectional follow-up sNFL levels with neuropsychological performance by domain in all women and stratified by HIV serostatus.**

Model[a]	All			WLWH			WLWOH		
	N	β (95% CI)	p	N	β (95% CI)	p	N	β (95% CI)	p
Executive function									
sNFL	384	−0.03 (−0.06, 0.00)	0.089	251	−0.06 (−0.11, −0.01)	0.028	116	−0.14 (−0.28, −0.01)	0.038
Processing speed									
sNFL	390	−0.04 (−0.08, −0.01)	0.025	255	−0.02 (−0.07, 0.04)	0.563	118	−0.26 (−0.42, −0.09)	0.002
Attention									
sNFL	362	0.00 (−0.03, 0.04)	0.845	234	−0.02 (−0.07, 0.03)	0.488	111	−0.14 (−0.32, 0.05)	0.141
Learning									
sNFL	390	0.00 (−0.04, 0.04)	0.977	256	−0.01 (−0.07, 0.05)	0.797	117	−0.09 (−0.25, 0.06)	0.223
Memory									
sNFL	388	−0.01 (−0.05, 0.03)	0.589	256	−0.01 (−0.07, 0.05)	0.791	115	−0.18 (−0.34, −0.02)	0.027
Verbal fluency									
sNFL	389	−0.01 (−0.05, 0.02)	0.470	256	−0.03 (−0.07, 0.02)	0.302	116	−0.07 (−0.20, 0.05)	0.225
Global									
sNFL	356	−0.01 (−0.04, 0.01)	0.343	231	−0.02 (−0.05, 0.02)	0.415	108	−0.19 (−0.32, −0.06)	0.004

The Women's Interagency HIV Study. [a]All models are adjusted for baseline age, baseline NP, HIV status, baseline NFL, income, BMI, CES-D score, eGFR, diabetes, tobacco use, marijuana use, alcohol use, and other drug use (crack, cocaine or heroin). β represents an adjusted, unstandardized coefficient. NP assessments are reverse scored and a higher β indicates better NP. WLWH models are additionally adjusted for baseline CD4 count, HIV viral load, and history of AIDS. p-values are not adjusted for multiple comparisons. The adjusted p-value for determining statistical significance is $p < 0.007$.

***Table 7*: Linear regression models associating ~8-year change in sNFL levels with neuropsychological performance by domain in all women and by HIV serostatus.**

over 25 years. Second, the WIHS is comprised of WLWH and WLWOH who are underrepresented in research. They are predominantly non-White, have lower educational attainment, and lower annual income. Third, we used state-of-the-art Simoa technology to analyze sNFL. Single Molecule Protein Detection is based upon the isolation of individual immunocomplexes on paramagnetic beads using standard ELISA reagents and is 1000 times more sensitive than traditional ELISA assays. However, differences in laboratory methods and evolution in sensitivity and specificity over time in this research field may contribute to differences in published results. Fourth, to our knowledge there are no other publications presenting data based on a cohort of WLWH and comparison group of similar WLWOH assessing repeated sNFL levels over an ~8-year period in association with NP. Fifth, we evaluated one biomarker in association with NP by domain. Finally, due to extensive clinical data available in the WIHS, we were able to adjust for eGFR, however this adjustment did not influence the results in all essential aspects. Of note, there was an inverse association between sNFL and eGFR in age-adjusted analysis.

As with any epidemiologic observational study, there are limitations to our analyses. First, while the study demonstrated several associations between NP and sNFL, external validity may be compromised. WIHS participants in these analyses comprised a well-studied group of women who were followed for ~8 years, and there was no 'healthy' control group. Second, since WIHS was an observational study with active referral related to ART, these WLWH may be healthier than WLWH who do not participate in a research study or WLWOH who are similar in terms of HIV-related risk factors. At the time of the 2018/2019 sNFL measure, >90% of WLWH in the cohort reported being adherent to ART.[6] Yet, while ART has been associated with lower blood levels of NFL,[36] the WLWH in our study had higher sNFL levels compared to WLWOH from 2008 to 2019. Third, NFL measures are sensitive but not specific. Higher blood levels of NFL have been observed in several neurological and psychiatric conditions and accompany peripheral and central neurological injuries or events, including multiple sclerosis, ALS, and acquired brain injuries, such as stroke.[2,10] Reported clinical values and cut-offs may vary over time due to changing fluid biomarker technologies. In addition, based on self-report these conditions are underrepresented in our sample; our protocol did not include a clinical neurological or psychiatric exam. Therefore, adequate measures of clinically relevant neurological and psychiatric conditions, such as peripheral neuropathy, traumatic brain injury, which have been observed among PLWH, were not available. To our knowledge, there were also no participants with clinically diagnosed cognitive impairment, such as Mild Neurocognitive disorder, Mild Cognitive Impairment (MCI), or ADRD based on self-report or medication use; nor can we address Asymptomatic Neurocognitive Impairment. Fourth, blood NFL levels have a high degree of inter-individual variability even in healthy individuals and are influenced by a number of physiological and clinical factors that go beyond brain pathology that were not measured or directly accounted for (only adjusted for, as opposed

to structural equation modelling for example) in the current study.[10] Fifth, other blood-based biomarkers of inflammation (e.g., chemokines, interleukins, tissue inhibitor of metalloproteinases-1) and altered metabolism (e.g., leptin, adiponectin, ghrelin, amylin, gastric inhibitory peptide) have been associated with NP in WLWH in the WIHS at different times over the adult life course.[16,20,37] We cannot consider their contribution here. However other biomarkers study results have been consistent with HIV pathophysiology and associated comorbidities such as obesity, and are potential biomarkers that may be useful in the creation of a biomarker panel among WLWH with promising clinical applications. Sixth, our sample is comprised primarily of Black or Hispanic women predominantly living in urban environments, thus extrapolation to other race/ethnic groups, men, and living environments is limited. Most published data on NFL in PLWH have been in White and/or European ancestry men; and there are a dearth of data in underrepresented people without HIV.[10] Seventh, we used traditional BMI cut-offs for overweight and obesity, which may not be appropriate for this sample, but do allow comparability with other studies. Finally, given the lack of historical EDTA plasma samples in the cohort, we could not address change in other ADRD plasma biomarkers as previously published[18] since EDTA plasma (at least for Aβ) is required for measuring these ADRD biomarkers via Simoa in a reliable manner at the time of this publication.

Single timepoint sNFL levels and change in sNFL levels over time, are promising preclinical biomarkers of NP and brain health in underrepresented samples of WLWH and WLWOH. Our observations underscore the importance of longitudinal studies over the adult life course among case and comparison groups to better understand temporality between biomarker exposures and outcomes, chronological age at biomarker exposure and outcome measurements, as well as secular changes related to treatments for the conditions under study.[29] These data also importantly support the clinical relevance for measuring sNFL as an indicator of neurological morbidities and outcomes. The Global Burden of Disease Study 2021 reported that disorders of the nervous system were leading causes of overall disease burden and death from 1990 to 2021.[38] Correspondingly, serum and plasma NFL are used in clinical routine for several years in an increasing number of laboratories globally, including Low and Middle Income Countries. Other blood-based biomarkers for neurological outcomes such as acute ischemic stroke are also being evaluated.[39] Therefore, a blood-based biomarker such as sNFL could support effective prevention, treatment, and rehabilitation strategies for several disorders affecting the nervous system worldwide.

Contributors

Conceptualization (DRG), data curation (XL, JZ, RY), formal analysis (XL, RY), funding acquisition (DRG, AEB, HZ, KB, JZ, ABS, PM, AS, KW, RY), investigation (DRG, HZ, KB), methodology (DRG, XL, HZ, KB, RY), project administration (DRG, ABS, PM, AS, KW), supervision (DRG, RY), writing—original draft (DRG, XL, RY), and writing—review & editing (all authors contributed equally). All authors had access to the data. DRG verified the underlying data. All authors read and approved the final version of the manuscript.

Data sharing statement

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the first and corresponding author (DRG) on reasonable request. When granted, access to individual-level data from the MACS/WIHS Combined Cohort Study (MWCCS) may be obtained upon review and approval of an MWCCS concept sheet and contact with the first author (DRG). Links and instructions for online concept sheet submission are on the study website: https://statepi.jhsph.edu/mwccs/.

Declaration of interests

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Appendix A. Supplementary data

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