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Plasma Neurofilament Light Chain and Glial Fibrillary Acidic Protein as Biomarkers of Cognitive Decline in People With Human Immunodeficiency Virus

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Background. We examined the relationship between neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) and cognition in people with human immunodeficiency virus (HIV) at baseline and longitudinally.

Methods. Plasma and clinical data were available from virally suppressed people with HIV (PWH) aged ≥45 years in the AIDS Clinical Trials Group HAILO study. Four neuropsychological assessments standardized and averaged (NPZ-4) represented cognition. Plasma collection date marked baseline; slope summarized longitudinal NPZ-4 changes. Linear regressions examined biomarkers associations with baseline NPZ-4 and longitudinal change.

Results. The study included 503 participants with a median age of 52 (interquartile range [IQR, 48–57]) years and observation of 6 (IQR, 5–7) years, and 26% had baseline cognitive impairment defined by HAILO. Cross-sectionally, higher NfL ($\beta = -.76$, $P < .01$) and GFAP ($\beta = -.44$, $P = .02$) were associated with worse NPZ-4. Longitudinally, the median NPZ-4 slope was 0.003 (IQR, −0.06 to 0.06) units/year with 48% demonstrating cognitive decline. Higher NfL ($\beta = -.08$, $P < .01$), but not GFAP ($\beta = -.03$, $P = .08$), was associated with cognitive decline.

Conclusions. NfL and GFAP were associated with worse cognition cross-sectionally; only NfL was associated with cognitive decline. Their clinical utility remains uncertain given small effect sizes and should be studied in populations with more rapid decline.

Keywords. plasma biomarkers; cognitive decline; aging; cognition; NPZ-4 score.

People with human immunodeficiency virus (HIV) are aging; it is anticipated that >70% of people with HIV (PWH) in the United States (US) will be over the age of 50 by 2030 [1]. These demographic shifts heighten concerns regarding age-associated dementias affecting PWH, particularly among individuals with preexisting cognitive disorders [2–4]. Recent observational data estimated that dementia prevalence was 1.8 times higher among PWH compared with age- and sex-matched counterparts without HIV [5]. Additionally, model-based projections suggest that 16%–22% of 60-year-olds with HIV may develop age-associated dementia by age 80, in contrast to 13%–15% of the general population [6].

Blood-based biomarkers for neurodegeneration, including neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP), are being investigated in clinical trials and memory care clinics [7–10]. NfL, a biomarker of neuro-axonal injury, and GFAP, a biomarker of astrocyte activation, are elevated in neurodegenerative disorders [8, 10]. Studies on cerebrospinal fluid or plasma have shown higher NfL in PWH subgroups, including those with HIV-associated dementia, but these studies have included individuals who were not virally suppressed [11–17]. Their inclusion complicates interpretation of results for clinical care, particularly for people with viral suppression, who represent the more common scenario for using neurodegenerative biomarkers. Two cross-sectional studies in virally suppressed cohorts found higher plasma NfL to be associated with global cognitive impairment, supporting its use as a biomarker for

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PWH [18, 19]. However, associations between plasma NfL and global cognitive decline based on longitudinal neuropsychological (NP) test scores show mixed results among PWH [20, 21].

Unlike NfL, cross-sectional data for GFAP in PWH on antiretroviral therapy (ART) are limited and, thus far, have found no associations with global cognitive impairment or cognitive performance based on NP tests [15, 16, 18, 22]. No longitudinal studies have assessed the relationship between GFAP and cognitive decline in PWH on ART to date.

Because of this limited understanding of plasma NfL or GFAP and global cognitive performance or longitudinal cognitive decline, we investigated these relationships among virally suppressed PWH aged 45 years or older enrolled in the multisite AIDS Clinical Trials Group (ACTG) HIV Infection, Aging and Immune Function Long-term Observational (HAILO) study.

METHODS

Participant Selection

We analyzed clinical and cognitive data and obtained banked heparin plasma samples for NfL and GFAP quantification from participants enrolled in HAILO, a prospective multisite observational study of PWH aged 40 years and older who initiated ART through the ACTG [23, 24]. Data were collected through medical chart abstraction, questionnaires, NP assessments, and laboratory testing at semiannual follow-up visits [25]. Written informed consent was obtained from all participants before study enrollment. The study was approved by the institutional review board at each participating site.

Our aim was to focus on older PWH, emulating a clinical scenario where a provider might consider obtaining these blood biomarkers. To be selected for NfL and GFAP quantification, HAILO participants had to be aged ≥45 years, have an HIV RNA <200 copies/mL on ART at plasma collection with at least 2 NP assessments, and have an observation time of >1.5 years after plasma collection. We prioritized obtaining plasma samples from individuals with longer observation times to ensure adequate assessment of longitudinal cognitive performance. Plasma samples used for biomarker quantification were those closest to the first NP assessment that met the participant selection criteria.

Definition of Global Cognitive Performance

Cognitive performance in HAILO was assessed using the following standardized NP assessments: Trail Making Test Parts A and B, the Wechsler Adult Intelligence Scale–Revised Digit Symbol subtest, and the Hopkins Verbal Learning Test–Revised total learning trials 1 to 3, as previously described [26]. The raw score for each assessment was standardized using age-, sex-, race- and education years–adjusted normative means scaled such that lower scores indicate worse performance, and combined in a summary composite z-score (ie, NPZ-4 score), with normalization applied to account for practice effects as previously reported [27–29]. The NPZ-4 score at baseline was used in these analyses as an indicator of global cognitive function for cross-sectional analysis. The NPZ-4 slope for each participant was calculated by using the NPZ-4 scores over time since baseline for longitudinal analysis. Cognitive decline was defined as a negative change in the NPZ-4 slope (slope <0).

Definition of Cognitive Impairment

Participants were classified by HAILO investigators as cognitively impaired if the summary composite z-score was at least −2.0 standard deviations (SD) on 1 NP assessment or at least −1.0 SD on 2 NP assessments, as previously described [28].

NfL and GFAP Quantification

Plasma levels of NfL and GFAP were measured using the Simoa Neurology 2-plex B kit (Quanterix, Item 103520) on a fully automatic Quanterix HD-X analyzer in the Massachusetts General Hospital Clinical and Translational Research Unit Biomarker Core according to the manufacturer's specifications. Plasma samples were measured in duplicate and diluted 1:4 onboard the analyzer. The functional lower level of quantification was 0.8 pg/mL for NfL and 16.6 pg/mL for GFAP. The mean coefficient of variation (CV) for the $\log_{10}$-transformed values was 2.3% (SD, 2.5%) for NfL and 1.7% (SD, 1.6%) for GFAP. Samples were distributed over 8 plates, and 4 analytical controls were included on all plates to assess plate-to-plate variability. For $\log_{10}$-transformed NfL, the mean interplate CV for the 4 quality control samples was 4.9% (SD, 3.5%), and the intraplate CV was 2.6% (SD, 2.8%). For GFAP, the interplate CV was 2.5% (SD, 0.9%), and the intraplate CV was 1.3% (SD, 1.1%) for the controls. The standardized mean concentrations for all the analytical controls on the different plates fell within our prespecified acceptance range of 0.8–1.2 (NfL: 0.93–1.10; GFAP: 0.84–1.11), and no plate-to-plate normalization was considered necessary.

Covariates

Covariates obtained at the time of entry into the HAILO study included sex at birth, race, ethnicity, years of education, and injection drug use (current vs past/never). Baseline covariates at the time of plasma collection included age, insurance status, smoking (current vs past/never), chronic hepatitis C diagnosis (HCV), antidepressant and alcohol use, $CD4^+$ T-cell nadir count, plasma HIV RNA load, $CD4^+$ T-cell count, and ART duration and regimen, as previously reported [26, 30–32]. The baseline estimated glomerular filtration rate (eGFR) was calculated using the non-race-based Chronic Kidney Disease Epidemiology Collaboration creatinine equation [33]. Neuropathy was defined as the presence of bilateral hypoactive ankle reflexes or loss in bilateral vibration perception, measured by HAILO, closest to plasma collection [34].

Statistical Analyses

Cross-sectional Analyses

We calculated medians and interquartile ranges (IQRs) for continuous variables and counts and percentages for categorical variables. Demographics and plasma biomarkers were compared between cognitively impaired and unimpaired groups using χ^2 or Wilcoxon rank-sum tests, as appropriate. Plasma NfL and GFAP were $\log_{10}$-transformed for linear regressions, as done previously [18, 19]. Multivariable linear regression models were used to assess the relationship between baseline NPZ-4 score (defined as the assessment closest to plasma collection) and NfL and GFAP, adjusting for age, sex at birth, race, ethnicity, and years of education. Although NPZ-4 scores were adjusted for some of these variables, differences between the impaired and unimpaired groups remained, and as a result, included them as potential predictors to avoid residual confounding. Given the association between eGFR, alcohol use, depression, and NfL and the importance of $CD4^+$ T-cell nadir count, duration of ART and HCV coinfection in assessing cognition among PWH, a second model added related disease indicators as covariates [35–40]. Neuropathy could also influence NfL levels in PWH, so a third model adjusted for presence or absence of neuropathy [41]. NfL and GFAP concentrations were analyzed separately.

Longitudinal Analyses

We assessed the relationship between NPZ-4 slope and slopes for individual z-scores with baseline plasma $\log_{10}$-transformed biomarker values using multivariable linear regression models. These regression models were adjusted for age, sex at birth, race, ethnicity, years of education, and baseline NPZ-4 or respective individual z-scores, and in a secondary model, we additionally adjusted for $CD4^+$ T-cell nadir count, duration of ART, eGFR, HCV diagnosis, and antidepressant and alcohol use. Observations were weighted based on the frequency of NP assessments per participant. Statistical significance was set at $P < .05$. Analyses were performed using R software (version 4.3.2) [42].

RESULTS

A total of 550 participants met the selection criteria, and their plasma samples were used to run NfL and GFAP assays (Figure 1). Plasma samples used in this study were collected between 2013 and 2016. Among these participants, NPZ-4 score could not be calculated for 42 individuals lacking at least 1 NP score, and biomarker quantification could not be performed in 2 samples. Three participants with stage 4 or 5 chronic kidney disease (CKD; eGFR <30 mL/minute/1.73 m^2) were excluded due to extreme outlier data points and the association between poor kidney function and high plasma NfL levels [36]. The remaining 503 participants were included in these analyses.

The baseline demographic and clinical characteristics of the 503 participants are shown in Table 1 and Supplementary Table 1. The median age in the cohort was 52 (IQR, 48–57) years, with 17% (n = 85) aged ≥60 years; 48% (n = 239) reported having private insurance. Twenty percent (n = 101) were female sex at birth, 53% (n = 268) non-Hispanic White, 26% (n = 131) non-Hispanic Black, and 21% (n = 104) Hispanic. The median $CD4^+$ T-cell count was 661 (IQR, 500–865) cells/μL, and 49% percent (n = 246) had a $CD4^+$ T-cell nadir count <200 cells/μL. All participants had HIV RNA <200 copies/mL, with 96% (n = 482) having <50 copies/mL. The median duration of ART use was 8.6 (IQR, 5.5–12.2) years, with 41% (n = 205) on ART for at least a decade; nonnucleoside reverse transcriptase inhibitor (41%, n = 204) and protease inhibitor (36, n = 183) were the most common regimens.

Of 503 participants, 26% (n = 132) had cognitive impairment at baseline. The median NPZ-4 at baseline for the cohort was 0.10 (IQR, −0.48 to 0.75). In people with and without cognitive impairment, the median NPZ-4 was −0.88 (IQR, −1.40 to −0.58) and 0.42 (IQR, −0.04 to 0.98), respectively ($P < .01$; Table 1). The median interval between the neuropsychological assessment and plasma collection was 0 days, indicating that these assessments were typically conducted on the same day. Thirteen of 503 participants had an interval >14 days, with the longest gap being 86 days (Supplementary Table 1).

Cross-sectional Associations of Plasma Biomarkers and Baseline Cognitive Impairment

The median $\log_{10}$-transformed NfL and GFAP levels for the total cohort were 1.03 (IQR, 0.90–1.16) and 1.89 (IQR, 1.77–2.03), respectively (Table 1). When comparing NfL levels between participants with and without cognitive impairment, the median NfL levels were modestly higher in participants with cognitive impairment (1.07 [IQR, 0.95–1.20] vs 1.02 [IQR, 0.89–1.15]; $P < .01$; $\log_{10}$ NfL [pg/mL] difference of 0.05). The median GFAP levels were higher in individuals with cognitive impairment, although the difference was not statistically significant (1.92 [IQR, 1.78–2.09] vs 1.89 [IQR, 1.77–2.02]; $P = .11$; $\log_{10}$ GFAP [pg/mL] difference of 0.03); Supplementary Figure 1*A* and 1*B*). NfL correlated with GFAP ($r = 0.41$, $P < .01$; Supplementary Figure 1*C*).

Cross-sectional Associations Between Plasma Biomarkers and Baseline Global Cognitive Performance Based on NPZ-4 Score, Adjusted for Covariates

We performed linear regression models to assess the relationship between $\log_{10}$-transformed NfL and GFAP and cognitive performance using NPZ-4 score as the outcome variable and controlling for age, sex at birth, race, ethnicity, and years of education. Race and ethnicity covariates were included based on Table 1 findings, which indicated statistical differences between cognitively impaired and unimpaired participants. In these adjusted models, both higher NfL and GFAP were associated with lower

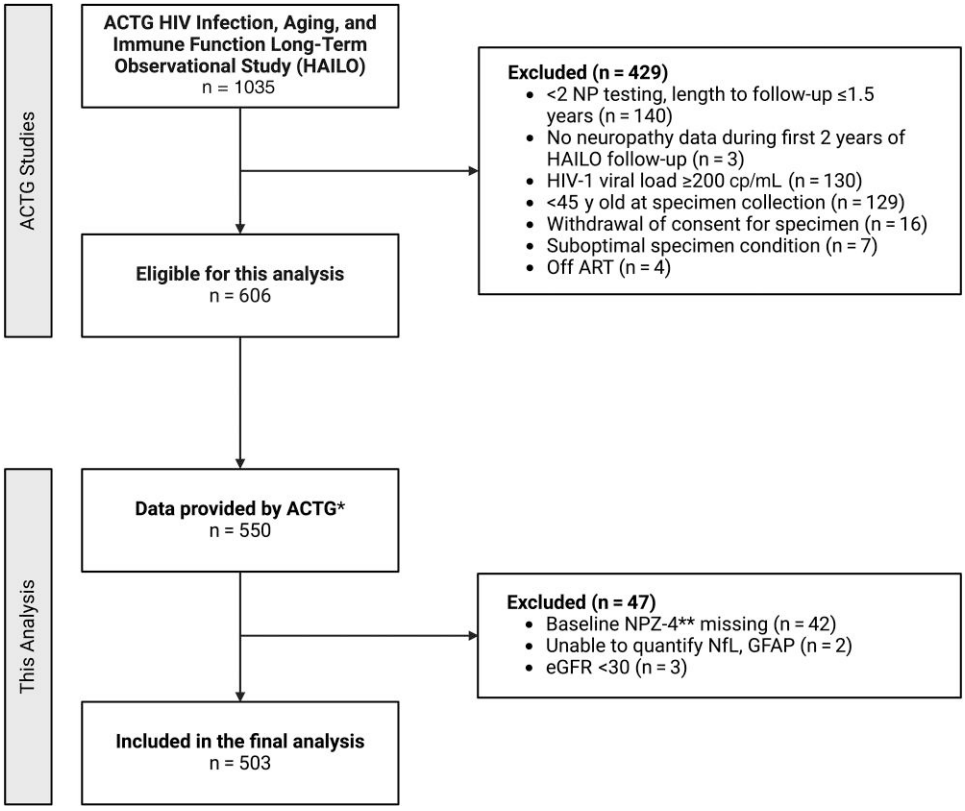

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram for study cohort selection. *Requested sample size. **Composite z-scores of neuropsychological tests. Abbreviations: ACTG, AIDS Clinical Trials Group; ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; GFAP, glial fibrillary acidic protein; HAILO, HIV Infection, Aging and Immune Function Long-term Observational study; HIV-1, human immunodeficiency virus type 1; NfL, neurofilament light chain; NP, neuropsychological; NPZ-4, 4 neuropsychological assessments, standardized to z-scores and averaged.

(worse) NPZ-4 scores. Specifically, for a 1-unit increase in the $\log_{10}$-transformed NfL value, the predicted NPZ-4 score decreased by −0.76 (Model I, $\beta = -.76$, $P < .01$). While GFAP also showed an association with lower NPZ-4 scores, its effect size was smaller than NfL (Model I, $\beta = -.44$, $P = .02$; Table 2).

In a second model, after additionally adjusting for $CD4^+$ T-cell nadir count, duration of ART, eGFR, HCV diagnosis, and antidepressant and alcohol use, the relationship between NfL or GFAP and NPZ-4 score remained significant (Table 2). A final adjustment for the presence or absence of neuropathy did not affect the relationship (data not shown). Unadjusted associations between the biomarkers and NPZ-4 score is shown in Supplementary Table 2.

Association of Plasma Biomarkers in Participants With and Without Cognitive Decline

The median number of visits with NP assessments was 7 (IQR, 6–8), and length of follow-up was 5.9 years (IQR, 5.4–7.3). The median NPZ-4 slope for the total cohort was 0.003 (IQR, −0.06 to 0.06) units/year. Forty-eight percent of the participants exhibited an annual decline in the NPZ-4 score (slope <0; n = 239), consistent with worsening longitudinal cognitive performance (Table 1).

When comparing NfL levels between participants with and without cognitive decline (ie, NPZ-4 slope <0 vs slope ≥0), the median NfL levels were modestly higher in participants with cognitive decline (1.05 [IQR, 0.91–1.20] vs 1.01 [IQR, 0.88–1.14]; $P = .01$; Supplementary Table 3). The median GFAP levels were similar between groups and not statistically significant (1.91 [IQR, 1.78–2.03] vs 1.88 [IQR, 1.76–2.03]; $P = .37$).

Associations Between Baseline Plasma Biomarkers and Longitudinal Cognitive Performance Based on NPZ-4 Slope, Adjusted for Covariates

In linear regression models to assess the relationship adjusting for age, sex at birth, race, ethnicity, years of education, and baseline NPZ-4, each 1-unit increase in baseline $\log_{10}$ NfL was associated with a 0.08 unit/year decline in the NPZ-4 slope ($P < .01$). A second model adjusting for HIV-associated covariates, eGFR, HCV diagnosis, and antidepressant and alcohol use did not substantially affect the magnitude or statistical significance of the association between NfL and NPZ-4 slope (Table 3).

In contrast to NfL, the association between baseline GFAP and lower NPZ-4 slope was not statistically significant ($\beta = -.03$,

Table 1. Demographic and Clinical Characteristics of the Study Population

Sample Characteristic[a]	Overall (n = 503)	Impaired (n = 132)	Not Impaired (n = 371)	*P* Value[b]
Sociodemographics				
Age, y				.90
45–50	195 (38.77)	49 (37.12)	146 (39.35)	
51–59	223 (44.33)	58 (43.94)	165 (44.47)	
60–69	72 (14.31)	21 (15.91)	51 (13.75)	
70–78	13 (2.58)	4 (3.03)	9 (2.43)	
Sex at birth				.08
Female	101 (20.08)	34 (25.76)	67 (18.06)	
Male	402 (79.92)	98 (74.24)	304 (81.94)	
Race/ethnicity				**<.01**
Non-Hispanic Black	131 (26.04)	20 (15.15)	111 (29.92)	
Non-Hispanic White	268 (53.28)	59 (44.70)	209 (56.33)	
Hispanic (regardless of race)	104 (20.68)	53 (40.15)	51 (13.75)	
Medical history				
CKD stage (eGFR, mL/min/1.73 m^2)				.21
CKD 1 (eGFR ≥90)	255 (50.70)	77 (58.33)	178 (47.98)	
CKD 2 (eGFR 60–89)	210 (41.75)	48 (36.36)	162 (43.67)	
CKD 3a (eGFR 45–59)	33 (6.56)	6 (4.55)	27 (7.28)	
CKD 3b (eGFR 30–44)	5 (0.99)	1 (0.76)	4 (1.08)	
Chronic hepatitis C				**.03**
Diagnosed	12 (2.39)	7 (5.30)	5 (1.35)	
Not diagnosed	491 (97.61)	125 (94.70)	366 (98.65)	
Antidepressant use[c]				**.01**
Yes	121 (24.20)	43 (33.08)	78 (21.08)	
No	379 (75.80)	87 (66.92)	292 (78.92)	
Alcohol use[c]				**.04**
Abstain	190 (39.42)	62 (50.00)	128 (35.75)	
Light	189 (39.21)	42 (33.87)	147 (41.06)	
Moderate	27 (5.60)	6 (4.84)	21 (5.87)	
Heavy	76 (15.77)	14 (11.29)	62 (17.32)	
Smoking status[c]				.92
Never	215 (43.00)	54 (41.54)	161 (43.51)	
Prior	166 (33.20)	44 (33.84)	122 (32.97)	
Current	119 (23.80)	32 (24.62)	87 (23.51)	
HIV laboratory values				
$CD4^+$ T-cell count, cells/μL	661.00 (499.50–864.75)	701.50 (528.25–929.50)	644.00 (485.25–846.00)	.07
$CD4^+$ T-cell nadir, cells/μL[c]				**.03**
≥350	89 (17.80)	32 (24.62)	57 (15.41)	
200–349	165 (33.00)	45 (34.61)	120 (32.43)	
<200	246 (49.20)	53 (40.77)	193 (52.16)	
HIV medications				
ART duration, y	8.60 (5.50–12.15)	8.10 (4.97–12.22)	8.70 (5.70–12.10)	.64
INSTI[c,d]				.22
Yes	142 (28.29)	43 (32.82)	99 (26.68)	
No	360 (71.71)	88 (67.18)	272 (73.32)	
NNRTI[c,d]				.10
Yes	204 (40.64)	53 (40.46)	151 (40.70)	
No	298 (59.36)	78 (59.54)	220 (59.30)	
PI[c,d]				.27
Yes	183 (36.45)	42 (32.06)	141 (38.01)	
No	319 (63.55)	89 (67.94)	230 (61.99)	
Neuropsychological testing				
Education, y	14.00 (12.00–16.00)	14.00 (12.00–16.00)	14.00 (12.00–16.00)	.87
NP testing visits, count	7.00 (6.00–8.00)	6.00 (5.00–8.00)	7.00 (6.00–8.00)	.12
Observation duration, y	5.91 (5.41–7.34)	5.72 (5.00–7.16)	5.97 (5.43–7.37)	**.05**
NPZ-4[e] at baseline	0.10 (−0.48, 0.75)	−0.88 (−1.40, −0.58)	0.42 (−0.04, 0.98)	**<.01**

Table 1. Continued

Sample Characteristic[a]	Overall (n = 503)	Impaired (n = 132)	Not Impaired (n = 371)	*P* Value[b]
NPZ-4 slope[f]	0.00 (−0.06, 0.06)	0.02 (−0.04, 0.08)	0.00 (−0.07, 0.05)	**<.01**
Cognitive decline[g]				**.01**
Yes	239 (47.51)	50 (37.88)	189 (50.94)	
No	264 (52.49)	82 (62.12)	182 (49.06)	
Blood-based biomarkers				
Log_{10} NfL, pg/mL	1.03 (0.90–1.16)	1.07 (0.95–1.20)	1.02 (0.89–1.15)	**<.01**
Log_{10} GFAP, pg/mL	1.89 (1.77–2.03)	1.92 (1.78–2.09)	1.89 (1.77–2.02)	.11

NOTES: Bold values indicate statistical significance set at $P < .05$.

Abbreviations: ART, antiretroviral therapy; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; GFAP, glial fibrillary acidic protein; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitor; NfL, neurofilament light chain; NNRTI, nonnucleoside reverse transcriptase inhibitor; NP, neuropsychological; NPZ-4, 4 neuropsychological assessments, standardized to z-scores and averaged; PI, protease inhibitor.

[a]Reported as No. (%) for categorical variables and median (interquartile range) for continuous variables.

[b]Differences between the "Impaired" and "Not impaired" groups were evaluated using the χ^2 or Fisher exact test for categorical variables and the Wilcoxon rank-sum text for continuous variables.

[c]Missing data were excluded from the analysis, and percentages were calculated based on known data. Unknown: antidepressant use, n = 3; alcohol use, n = 21; smoking status, n = 3; $CD4^+$ T-cell nadir, n = 3; INSTI, n = 1; NNRTI, n = 1; PI, n = 1.

[d]Some participants were on multiclass drug regimens.

[e]Composite z-scores of neuropsychological assessments (Trail Making Test Part A, Trail Making Test Part B, Wechsler Adult Intelligence Scale–Revised Digit Symbol, and Hopkins Verbal Learning Test–Revised).

[f]Annual change from baseline.

[g]Defined as NPZ-4 slope <0.

Table 2. Linear Regression Models Associating Cross-sectional Baseline Plasma Neurofilament Light Chain and Glial Fibrillary Acidic Protein Levels With NPZ-4 Scores

	Log_{10} NfL, pg/mL				Log_{10} GFAP, pg/mL			
Variable	Model I β (95% CI)	*P* Value	Model II β (95% CI)	*P* Value	Model I β (95% CI)	*P* Value	Model II β (95% CI)	*P* Value
Biomarker	−.76 (−1.20, −.32)	**<.01**	−.80 (−1.26, −.35)	**<.01**	−.44 (−.81, −.07)	**.02**	−.48 (−.85, −.10)	**.01**
Age	.00 (−.01, .02)	.63	.00 (−.01, .02)	.76	.00 (−.02, .01)	.72	.00 (−.02, .01)	.68
Sex at birth								
Female (ref)	…		…		…		…	
Male	.38 (.17–.58)	**<.01**	.35 (.14–.56)	**<.01**	.33 (.12–.54)	**<.01**	.29 (.08–.51)	**<.01**
Race								
Non-White (ref)	…		…		…		…	
White	−.06 (−.23, .12)	.54	−.06 (−.24, .12)	.52	−.09 (−.26, .09)	.31	−.10 (−.27, .08)	.28
Ethnicity								
Hispanic (ref)	…		…		…		…	
Non-Hispanic	.78 (.57–.99)	**<.01**	.77 (.54–.99)	**<.01**	.77 (.56–.98)	**<.01**	.76 (.54–.99)	**<.01**
Education, y	−.01 (−.04, .01)	.43	−.01 (−.04, .02)	.42	−.01 (−.04, .01)	**.38**	−.01 (−.04, .01)	.34
$CD4^+$ T-cell nadir, cells/μL	…		.00 (.00–.00)	.20	…		.00 (.00–.00)	.23
ART duration, y	…		.02 (.00–.05)	**.02**	…		.03 (.01–.05)	**.01**
eGFR, mL/min/1.73 m^2	…		.00 (−.01, .00)	.38	…		.00 (−.01, .00)	.52
Chronic hepatitis C								
Not diagnosed (ref)	…		…		…		…	
Diagnosed	…		−.60 (−1.11, −.10)	**.02**	…		−.63 (−1.13, −.12)	**.01**
Antidepressant use								
No (ref)	…		…		…		…	
Yes	…		−.16 (−.34, .02)	.09	…		−.16 (−.35, .02)	.08
Alcohol use								
Abstain (ref)	…		…		…		…	
Light	…		.19 (.00–.37)	**.05**	…		.19 (.01–.38)	**.04**
Moderate	…		.16 (−.20, .52)	.38	…		.18 (−.18, .53)	.33
Heavy	…		.08 (−.15, .32)	.49	…		.07 (−.17, .31)	.57

NOTES: Bold values indicate statistical significance set at $P < .05$.

Model I: Adjusted for age, sex at birth, race, ethnicity, and years of education. Model II: Adjusted for Model I variables, $CD4^+$ T-cell nadir, ART duration, eGFR, chronic hepatitis C diagnosis, antidepressant use, and alcohol use.

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; eGFR, estimated glomerular filtration rate; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; NPZ-4, 4 neuropsychological assessments, standardized to z-scores and averaged; ref, reference group.

Table 3. Linear Regression Models Associating Baseline Plasma Neurofilament Light Chain and Glial Fibrillary Acidic Protein With NPZ-4 Slope During Follow-up, Weighted by Number of Testing Visits

	Log_{10} NfL, pg/mL				Log_{10} GFAP, pg/mL			
Variable	Model I β (95% CI)	*P* Value	Model II β (95% CI)	*P* Value	Model I β (95% CI)	*P* Value	Model II β (95% CI)	*P* Value
Biomarker	−.08 (−.12, −.03)	**<.01**	−.08 (−.12, −.03)	**<.01**	−.03 (−.07, .00)	.08	−.04 (−.08, .00)	.07
Age	.00 (.00–.00)	.09	.00 (.00–.00)	**.02**	.00 (.00–.00)	**<.01**	.00 (.00–.00)	**<.01**
Sex at birth								
Female (ref)	…		…		…		…	
Male	.00 (−.02, .02)	.89	.00 (−.03, .02)	.80	−.01 (−.03, .02)	.62	−.01 (−.03, .02)	.51
Race								
Non-White (ref)	…		…		…		…	
White	−.01 (−.03, .01)	.30	−.01 (−.03, .01)	.24	−.01 (−.03, .00)	.15	−.02 (−.03, .00)	.11
Ethnicity								
Hispanic (ref)	…		…		…		…	
Non-Hispanic	−.02 (−.05, .00)	.06	−.01 (−.04, .01)	.33	−.03 (−.05, .00)	**.04**	−.01 (−.04, .01)	.29
Education, y	.00 (.00–.01)	**<.01**	.00 (.00–.01)	**<.01**	.00 (.00–.01)	**<.01**	.00 (.00–.01)	**<.01**
NPZ-4 at baseline	−.03 (−.04, −.02)	**<.01**	−.03 (−.04, −.02)	**<.01**	−.03 (−.04, −.02)	**<.01**	−.03 (−.04, −.02)	**<.01**
$CD4^+$ T-cell nadir, cells/μL	…		.00 (.00–.00)	.68	…		.00 (.00–.00)	.76
ART duration, y	…		.00 (.00–.00)	.69	…		.00 (.00–.00)	.61
eGFR, mL/min/1.73 m^2	…		.00 (.00–.00)	.71	…		.00 (.00–.00)	.53
Chronic hepatitis C								
Not diagnosed (ref)	…		…		…		…	
Diagnosed	…		−.03 (−.08, .03)	.32	…		−.03 (−.08, .03)	.28
Antidepressant use								
No (ref)	…		…		…		…	
Yes	…		−.03 (−.04, −.01)	**.01**	…		−.03 (−.05, −.01)	**<.01**
Alcohol use								
Abstain (ref)	…		…		…		…	
Light	…		−.01 (−.03, .01)	.22	…		−.01 (−.03, .01)	.21
Moderate	…		−.02 (−.05, .02)	.37	…		−.02 (−.05, .02)	.38
Heavy	…		−.01 (−.04, .01)	.25	…		−.02 (−.04, .01)	.20

NOTES: Bold values indicate statistical significance set at $P < .05$.

Model I: Adjusted for age, sex at birth, race, ethnicity, years of education, and NPZ-4 at baseline. Model II: Adjusted for Model I variables, $CD4^+$ T-cell nadir, ART duration, eGFR, chronic hepatitis C diagnosis, antidepressant use, and alcohol use.

Abbreviations: CI, confidence interval; ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; NPZ-4, 4 neuropsychological assessments, standardized to z-scores and averaged; ref, reference group.

$P = .08$) after adjusting for age, sex at birth, race, ethnicity, years of education, and NPZ-4 at baseline.

Associations Between Baseline Plasma Biomarkers and Longitudinal Cognitive Performance Based on Individual NPZ-4 Slopes, Adjusted for Covariates

We explored the association between baseline NfL and the decline in individual NPZ-4 component scores, adjusting for all covariates. Higher baseline NfL was significantly associated with decline in the slope for Trail Making Test Part A ($\beta = -.10$, $P = .01$), Digit Symbol ($\beta = -.10$, $P = .02$), and Hopkins Verbal Learning Test–Revised ($\beta = -.08$, $P = .04$). The association between baseline NfL and Trail Making Test Part B slope was not statistically significant ($P = .11$; Supplementary Table 4).

For associations with baseline GFAP, a decline in slope was observed for Digit Symbol ($\beta = -.11$, $P < .01$). Other domains were not statistically significant (Trail Making Test Part A, $P = .95$; Trail Making Test Part B, $P = .10$; Hopkins Verbal Learning Test–Revised, $P = .67$; Supplementary Table 5).

DISCUSSION

Among the 503 PWH from the multisite HAILO cohort assessed in this study, all of whom were virally suppressed on ART, we found a consistent inverse relationship between plasma NfL values and cognitive performance in both cross-sectional and longitudinal analyses. Higher NfL levels were associated with cognitive impairment, with levels correlating with worse NP scores cross-sectionally. Additionally, higher NfL was associated with a longitudinal cognitive decline. Plasma GFAP was also significantly associated with worse baseline NP scores, but there was little evidence of an association with longitudinal cognitive change. Despite being statistically significant, the effect sizes and confidence intervals across all models indicated only modest associations for both blood biomarkers with baseline cognitive function, and in the association between NfL and cognitive decline in PWH on ART. Given the objective of using neurodegenerative blood-based biomarkers in clinical decision-making, these novel data suggest that caution is warranted if relying solely on NfL or GFAP as single-use,

standalone biomarkers for cognitive decline in PWH on ART, especially among individuals 45–59 years old, the predominant age demographic investigated here [7].

While NfL has been a biomarker used to assess neuroaxonal injury in PWH with and without cognitive impairment for >15 years [11, 43, 44, 45, 46], many earlier cohort studies included viremic individuals off ART, potentially influencing group differences [11, 14, 16]. In contrast, in virally suppressed PWH, especially older adults on ART, the utility of using NfL or GFAP as biomarkers for cognitive impairment has not been fully addressed. Previous cross-sectional studies among PWH suggest that higher NfL levels are associated with worse cognitive performance [14, 15], including in 1 multisite study restricted to PWH with viral suppression [18]. When GFAP was assessed, higher levels were generally associated with worse performance, though these associations were not statistically significant [15, 18].

Our study involved individuals in their 50s, with 17% aged >60 years at baseline, all on ART with viral suppression. Cross-sectional analyses confirm that higher NfL levels are associated with lower NP scores, while showing that higher GFAP levels are also associated with worse cognitive performance when analyzed in a larger sample of PWH on ART. Investigating the use of these biomarkers to differentiate between cognitive groups, we observed small mean differences in $\log_{10}$-transformed NfL pg/mL (0.05, equivalent to about a 12% higher level of NfL) or $\log_{10}$-transformed GFAP pg/mL (0.03, equivalent to about a 7% higher level of GFAP) between cognitively impaired and unimpaired individuals. The median NfL levels observed here closely match a recent cross-sectional study using the same measurement platform, which also reported marginal differences between individuals with and those without cognitive impairment [19]. Similarly, 2 cross-sectional studies reported small differences in GFAP levels [16, 18]. Overall, the cross-sectional evidence suggests substantial overlap in NfL and GFAP levels between cognitively impaired and unimpaired PWH. The overlap complicates the use of either biomarker to identify individuals at risk for neurodegenerative cognitive disorders within the studied population of virally suppressed PWH on ART, most of whom are under age 60, underscoring the need for clear clinical definitions and thresholds for an "abnormal" test result in this group to justify further clinical evaluation.

Although 26% of the HAILO cohort was defined as cognitively impaired at study baseline, our longitudinal analysis indicated overall cognitive stability, with the median annual NPZ-4 slope remaining at zero. We also showed that cognitively impaired individuals exhibit a higher NPZ-4 slope (0.02 vs 0.00; Table 1) alongside significantly lower baseline NPZ-4 levels (−0.88 vs 0.42). Including baseline NPZ-4 in regression analyses (Table 3) adjusted the NPZ-4 slope by amounts that mirror observed slopes (+0.0264 in the impaired vs −0.0126 in the unimpaired group). We interpret this to mean that the impaired group has a slightly less steep decline due to their initially lower baseline NPZ-4 levels, which moderates the effect size. These results support a consistent relationship between baseline NPZ-4 levels and NPZ-4 slope, underscoring the robustness and internal consistency of models presented. Among individuals with cognitive decline (slope <0), the median NPZ-4 score decreased by 0.06 units annually, indicating stability in the analyzed age ranges. This stability is consistent with results from the CNS HIV Antiretroviral Therapy Effects Research study, a US cohort of PWH, where stable participants showed an average annual decrease of 0.01 units on a composite z-score of 15 NP tests, while those classified as declining had a greater average annual decline of 0.18 units, exceeding that observed in HAILO [47].

In our analysis, $\log_{10}$-transformed NfL overlapped between people who decline versus remained stable, and the effect sizes for the regression models were minimal, possibly because the declines in our population were small. Preliminary analyses suggest that higher baseline levels of NfL are modestly associated with declines in the slopes for the Trail Making Test Part A, Digit Symbol, and Hopkins Verbal Learning Test–Revised. However, validation in clinical cohorts is needed to confirm NfL's specificity as a marker for these domains. Baseline GFAP levels showed a modest association with a decline in the Digit Symbol slope, with no associations observed for other cognitive domains or the overall NPZ-4 slope. To enhance the clinical utility of blood biomarkers and establish meaningful threshold values for clinical decision-making, future studies should focus on PWH on ART who are undergoing more rapid cognitive decline, possibly at older ages than studied here.

Despite the strengths of this study, including its large sample size of virally suppressed PWH on ART and the longitudinal cognitive assessments, there are inherent limitations in observational data. External validation is needed to confirm observed associations. HAILO participants, potentially healthier due to their ACTG involvement and who had a median of 14 years of education, suggesting some college attainment, may differ from clinic patients with greater disease burden and cognitive decline. Additionally, the HAILO cohort's 48% private insurance rate contrasts with the 22% shown in the North American AIDS Cohort Collaboration on Research and Design cohort, which may pose limitations in generalizing results to clinical cohorts [48]. While we do not expect the timing variation between NP assessment and plasma collection to significantly impact findings, gaps exceeding 14 days may have introduced some variability. Finally, the absence of data on additional neurological symptoms, neurological events (eg, stroke, trauma, meningitis), and neuroradiographic findings between baseline and follow-up limits the comprehensiveness of the longitudinal NPZ-4 evaluation. Although cognitive performance in this cohort remained stable, suggesting minimal impact from neurological events, future

studies in clinical cohorts should account for confounding factors (eg, central nervous system events, ART changes, HIV viremia) and include time-varying covariates to better assess blood biomarker utility in HIV care.

CONCLUSIONS

Both NfL and GFAP showed cross-sectional correlations with worse cognitive function in virally suppressed PWH on ART; only NfL demonstrated an association with cognitive decline over time. Given the modest effect sizes observed, cautious interpretation is warranted before clinical application, especially among cognitively stable populations.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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