

Utility of Plasma Neurofilament Light in the 1Florida Alzheimer's Disease Research Center (ADRC)

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Abstract.

Background: Plasma NfL (pNfL) levels are elevated in many neurological disorders. However, the utility of pNfL in a clinical setting has not been established.

Objective: In a cohort of diverse older participants, we examined: 1) the association of pNfL to age, sex, Hispanic ethnicity, diagnosis, and structural and amyloid imaging biomarkers; and 2) its association to baseline and longitudinal cognitive and functional performance.

Methods: 309 subjects were classified at baseline as cognitively normal (CN) or with cognitive impairment. Most subjects had structural MRI and amyloid PET scans. The most frequent etiological diagnosis was Alzheimer's disease (AD), but other neurological and neuropsychiatric disorders were also represented. We assessed the relationship of pNfL to cognitive and functional status, primary etiology, imaging biomarkers, and to cognitive and functional decline.

Results: pNfL increased with age, degree of hippocampal atrophy, and amyloid load, and was higher in females among CN subjects, but was not associated with Hispanic ethnicity. Compared to CN subjects, pNfL was elevated among those with AD or FTLT, but not those with neuropsychiatric or other disorders. Hippocampal atrophy, amyloid positivity and higher pNfL levels each added unique variance in predicting greater functional impairment on the CDR-SB at baseline. Higher baseline pNfL levels also predicted greater cognitive and functional decline after accounting for hippocampal atrophy and memory scores at baseline.

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Conclusion: pNfL may have a complementary and supportive role to brain imaging and cognitive testing in a memory disorder evaluation, although its diagnostic sensitivity and specificity as a stand-alone measure is modest. In the absence of expensive neuroimaging tests, pNfL could be used for differentiating neurodegenerative disease from neuropsychiatric disorders.

Keywords: Alzheimer's disease, amyloid, diagnosis, hippocampal atrophy, magnetic resonance imaging, plasma neurofilament light, positron emission tomography

INTRODUCTION

Mixed pathologies are the most common cause of cognitive impairment, although AD is the most frequent pathology occurring in 65% of cases. On neuropathological examination, AD occurs in isolation only 9% of cases [1]. The NIA-AA Research Framework classification, summarized as ATN (Amyloid, Tau, Neurodegeneration), is independent of the clinical syndrome, and provides a template for research studies to link the clinical syndrome to biomarkers of AD pathology and neurodegeneration. To that end, molecular biomarkers in both cerebrospinal fluid (CSF) and blood have been developed to measure the pathological and neurodegenerative changes in the brain associated with AD. These biomarkers in CSF and plasma include $A\beta_{42}$ and $A\beta_{40}$ associated with the deposition of amyloid- β plaques, and total tau (T-tau) and phosphorylated tau (P-tau), associated with neurofibrillary tangles.

Neurofilament light (NfL) is a protein that can be measured in the CSF (cNfL) and blood (pNfL). It is a marker for subcortical large-caliber axonal degeneration [2]. CSF NfL and pNfL concentrations correlate with each other and are associated with cognitive decline and increased brain atrophy, including in the hippocampus [3–6]. Plasma NfL and cNfL concentrations are elevated in many neurological disorders, including AD, frontotemporal lobe degeneration (FTLD), vascular dementia, and dementia with Lewy bodies, as compared to cognitively normal (CN) older subjects [7]. Among CN subjects and in most neurological conditions, cNfL has been shown to increase with age [7]. Compared to women, men have been shown to have elevated cNfL among CN older subjects and those with AD [7].

While pNfL has been shown to have potential use as a clinical tool, several gaps in current knowledge exist. For example, it is unknown if elevated pNfL levels distinguish between those with evidence of neurodegeneration and those with cognitive impairment resulting from depression, anxiety and other

psychiatric conditions. It is also not clear whether factors such as age and Hispanic ethnicity have a bearing on the diagnostic utility of pNfL levels. The diagnostic utility of pNfL levels, relative to cognitive test performance and other biomarkers such as hippocampal atrophy and amyloid load in the brain is not known. Finally, there is insufficient research to determine whether pNfL levels provide complementary information, in the prediction of disease progression, to established predictors such as imaging measures and memory scores.

In the current study, we evaluated the utility of pNfL levels in the 1Florida Alzheimer's Disease Research Center (1Florida ADRC), a longitudinal study of aging sponsored by NIA as one of the 32 Alzheimer's Disease Research Centers. This cohort is bi-ethnic, with nearly equivalent numbers of Hispanic and non-Hispanic participants, including those who are cognitively normal or have various stages of mild cognitive impairment (MCI). In contrast to the Alzheimer's Disease Neuroimaging Initiative (ADNI), there were no inclusion criteria based on functional or memory scores, and few exclusion criteria. This resulted in a cohort having a continuous spectrum of cognitive levels, including those who are CN, the entire spectrum of amnesic and non-amnesic MCI and those in an intermediate stage of impairment between CN and MCI. This intermediate stage of impairment is categorized as pre-MCI by some [8, 9] and "cognitively impaired/not MCI" by others [10]. In addition, this cohort is diverse with respect to etiology, and includes many non-AD diagnoses such as FTLD, Lewy body disease, vascular cognitive impairment, and neuropsychiatric disorders. About half of the participants in this study were recruited from a memory disorder clinic, making the results more generalizable to a clinic setting. The primary goal of the current study was to determine the utility of pNfL as a clinical tool in the evaluation of AD and related disorders, as well as in predicting the rate of cognitive and functional decline among subjects with AD.

METHODS

Participants

Participants were enrolled in the 1Florida ADRC. The focus of the 1FloridaADRC is the study of older subjects with normal cognition, MCI, and mild dementia, and those in the gap between CN and MCI. This cohort is unique due to its ethnic and cultural diversity, as over 50% identify as Hispanic and report Spanish as their primary language. In addition, subjects were assessed using novel neuropsychological test measures, and most had structural brain MRI, amyloid PET, and apolipoprotein E (*APOE*) genotyping. About half of this cohort has been recruited from a large outpatient memory disorder clinic and the remainder from a free memory screening program and from outreach efforts in the community.

Participants in this study completed an extensive medical, neurological, psychiatric, and neuropsychological evaluation, including all elements required by the National Alzheimer's Coordinating Center (NACC) Uniform Data Set, version 3.0 [10]. The Clinical Dementia Rating Scale (CDR) [11] was administered by an experienced geriatric psychiatrist (MTG), who was blinded to the neuropsychological test results. The CDR Sum of Boxes (CDR-SB) score was used as a global cognitive/functional measure, since it has been shown to accurately stage the severity of AD in the spectrum from MCI to severe dementia [12]. Ethnicity of the participants was determined using self-report [13]. For the 309 participants included in this study, blood was drawn at baseline for DNA extraction for genetic studies, including *APOE* genotyping, and plasma biomarker studies. Two hundred and forty-four (244) participants had a 1st follow-up visit, and 164 a 2nd follow-up visit. All participants had a study partner who served as a collateral informant for the CDR. This study was approved by the Mount Sinai Medical Center IRB and was conducted in accordance with the declaration of Helsinki. All participants and their study partners provided informed consent

A neuropsychological battery was administered in the participant's preferred language (English or Spanish) by a psychometrician who was blinded to the clinical evaluation and the CDR score. The neuropsychological protocol included items from either Version 2 or Version 3 of the Alzheimer Disease Centers' Neuropsychological Test Battery in the Uniform Data Set (UDS), utilizing the Spanish version [10] as applicable. Additional tests were administered

including the Hopkins Verbal Learning Test-Revised (HVLT-R) [14] and the Mini-Mental State Examination (MMSE) [15]. The total recall score for HVLT-R was used in this study.

Diagnostic procedures

Cognitive diagnoses followed the NACC D1 classification protocol, which specifies the diagnosis of: Cognitively Normal (CN), cognitively impaired, not MCI (CInMCI); amnesic and non-amnesic Mild Cognitive Impairment (MCI), and Dementia (DEM) [10]. Etiological diagnoses for subjects with cognitive impairment were operationalized according to NACC D1 guidelines and consolidated into categories as shown in Table 1): cognitively normal (CN); Alzheimer [16]; neuropsychiatric (primarily anxiety and depression); vascular brain injury (VBI); FTLD; and Lewy body dementia (LBD). The remaining diagnoses were consolidated into "Dx-Other", which included less common causes of cognitive deficits, such as sleep disorder, amyloid angiopathy, chemo-brain, and ruptured aneurysm.

MR imaging

MRI scans were performed using a Siemens Skyra 3T MRI scanner at Mount Sinai Medical Center, Miami Beach. MRI scans were evaluated by visual inspection for assessment of medial temporal atrophy and classified as positive or negative for hippocampal atrophy (Hp+, Hp-) [17]. In addition, FreeSurfer Version 6.0 software was used to obtain parcellation of regional brain volumes and cortical thickness at 1.0 mm isotropic resolution (<http://surfer.nmr.mgh.harvard.edu>) and for co-registration with amyloid PET scans [18]. The ratio of hippocampal volume to intracranial volume was used as a continuous measure. Cerebrovascular disease on imaging was assessed using the NACC D1 binary categories for presence or absence of large vessel infarcts, lacunar infarcts, moderate white matter hyperintensities, and extensive white matter hyperintensities.

Amyloid PET imaging

Amyloid PET scans were performed using the tracer [¹⁸F] florbetaben for approximately 85% of the scans and [¹⁸F] Florbetapir for the remainder. The methodology for PET scanning has been previously described [19]. All amyloid PET scans were read initially by an independent, trained radiologist,

Table 1
Demographics and Clinical Characteristics

	CN	Alzheimer	Neuro-psychiatric*	VBI	FTLD	LBD	Dx-Other**	F or Chi-Square (p)
n (total = 309)	51	156	47	12	6	5	32	
Age (y)	70.8 ^a ± 5.9	74.8 ^b ± 8.2	70.5 ^a ± 6.7	74.9 ^a ± 4.8	71.6 ^a ± 4.5	70.7 ^a ± 12.9	69.9 ^a ± 6.9	4.6 (<0.0001)
Education (y)	16.0 ± 2.8	14.8 ± 3.3	14.4 ± 3.8	15.6 ± 2.5	14.5 ± 4.0	16.6 ± 2.2	14.6 ± 3.8	1.43 (0.20)
Sex (Female/Male)	40 / 11	88 / 68	36 / 11	4 / 8	2 / 4	0 / 5	21 / 11	24.8 (0.0004)
Race (white/black/other)	41/2/8	147/5/4	46/0/1	12/0/0	6/0/0	5/0/0	29/3/0	5.9 (0.44)
Hispanic Ethnicity (%)	52.9%	60.3%	66.0%	83.3%	50.0%	20%	56.2%	7.2 (0.20)
Follow-up Visits (number)								
Year 2 (total = 244)	46	118	37	11	5	1	26	
Year 3 (total = 164)	37	81	20	8	2	1	15	-
CDR-SB at Baseline	0.1 ^a ± 0.3	2.9 ^b ± 2.6	0.8 ^a ± 0.8	1.9 ^a ± 1.8	3.3 ^b ± 2.6	3.2 ^b ± 1.0	1.5 ^b ± 2.6	12.5 (<0.0001)
CDR-SB (change)								
Year 1 - Year 2	-0.04 ^a ± 0.3	-1.1 ^b ± 2.1	-0.1 ^a ± 0.6	0.1 ^a ± 0.4	-3.2 ^b ± 2.3		-0.4 ^a ± 0.9	6.46 (<0.0001)
Year 2 - Year 3 (only shown for n ≥ 5)	0.0 ^a ± 0.2	-1.2 ^b ± 2.2	0.2 ^a ± 0.7			-	0.0 ^a ± 0.6	5.75 (<0.0001)
MMSE Score	29.4 ^a ± 0.8	25.3 ^b ± 4.3	28.1 ^a ± 2.1	28.5 ^a ± 1.7	26.2 ^a ± 3.9	27.8 ^a ± 2.2	27.5 ^a ± 3.2	5.8 (0.02)
HVLT-R Total	26.0 ^a ± 4.6	17.03 ^b ± 6.0	21.8 ^b ± 5.0	21.3 ^a ± 5.8	11.2 ^b ± 9.4	18.2 ^b ± 4.6	20.6 ^b ± 6.2	17.5 (<0.0001)

* Anxiety (n = 16), Apathy (n = 1), Bipolar (n = 1), Depression (n = 27), Lack of Motivation (n = 1), PseudoDementia (n = 1); **Other includes: Amyloid Angiopathy (n = 2), Attention Deficit Disorder (n = 1), Chemobrain (n = 1), Cognitive Impairment NOS (n = 1), Essential tremor (n = 1), Herpes Simplex Encephalitis (n = 1), Hydrocephalus (n = 1), mild behavioral impairment (n = 1), Medication (n = 1), Multifactor Etiology (n = 1), Neurodegenerative Disease (n = 5), Personality Disorder (n = 1), Ruptured MCA Aneurysm (n = 1), Schizophrenia (n = 1), Sleep Disorder (n = 5), Space Occupying Lesion (n = 2), Subjective Memory Disorder (n = 1), Unknown 5. CN, cognitively normal; VBI, vascular brain injury; FTLD, Frontotemporal lobar degeneration; LBD, Lewy Body Disease; MMSE, Mini-mental State Examination score; CDR-SB, Clinical Dementia Rating Scale Sum of Boxes; HVLT-R, Hopkins Verbal Learning Test-Revised. F values are adjusted for age, where appropriate. Means with different alphabetic superscripts are statistically significant at p = 0.05 from CN at p = 0.05 by Dunnett's *post-hoc* procedure.

who was not otherwise involved in the study, and a trained and experienced reader (RD), both of whom were blinded to the cognitive and clinical diagnosis, using a methodology similar to that described by Sabri et al. [20]. Images were displayed using a reader-adjustable gray scale to provide optimal discrimination of the cerebellar gray matter from white matter. A final dichotomous classification of elevated amyloid (A+ versus A-) was rendered. Duara et al. [19] found high agreement between raters in reading these scans.

Quantitative assessment of PET scans was performed using a composite standard uptake value ratio (SUVR) calculated by the ratio of the mean tracer uptake from 6 cortical regions (frontal, temporal, parietal, precuneus, anterior and posterior cingulate cortex regions, each region summed from left and right hemispheres) to the whole cerebellum. A centiloid score for each participant was calculated for Florbetaben [21] and Florbetapir [22] PET scans.

APOE genotyping

All samples for APOE genotyping were performed in Dr. Nilüfer Ertekin-Taner's laboratory (Mayo Clinic, Jacksonville, FL, USA); the APOE ε2, ε3, and ε4 alleles used predesigned TaqMan SNP Genotyping

Assays for SNPs rs7412 and rs429358 (Thermo Fisher Scientific, MA, USA) on the QuantStudio 7 Flex Real-Time PCR system (Applied Biosystems, CA, USA) following the manufacturer's protocol.

Plasma biomarker analysis (using Quanterix single molecule array – SIMOA technology)

Blood samples were drawn at the baseline visit and centrifuged to obtain plasma. Aliquots were stored at -80°C until the samples were shipped to Quanterix Corporation (113 Hartwell Avenue, Lexington, MA, USA, E-mail: techsupport@quanterix.com.) for analysis of pNfL utilizing the Simoa NF-light kit on the Quanterix Simoa HD-1 Analyzer. Concentrations of pNfL were measured, in units of pg/ml, in duplicate from each sample, that were blinded to demographic and clinical data. All samples tested were within the assay dynamic range with an average coefficient of variation of 3.9% (range from 0 to 15% for measurement).

Data analysis

All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Plasma NfL had a skewed distribution (based on visual inspection

of histograms and Kolmogorov-Smirnov statistic in the entire cohort and in the two largest subgroups, CN and AD), so a natural log transformation was applied for analyses that required normal distribution or normal residuals. Comparisons of means between diagnostic groups, for demographic and clinical variables, were performed using general linear models (GLM). *Post-hoc* tests were conducted using Dunnett procedure, with the CN subjects as the *post-hoc* control group. Differences between groups for categorical variables were evaluated using chi-square tests; *post-hoc* comparisons were conducted with chi-square tests (or Fisher's exact for cell sizes less than 5) using the CN subjects as the control group and Bonferroni correction.

The relationship between demographic factors (age, sex, and Hispanic ethnicity) and pNfL was assessed with Pearson correlations or GLM. These relationships were assessed separately in larger etiological subgroups (i.e., CN and AD). The relationship of pNfL to binary and continuous measures of hippocampal atrophy and amyloid load was assessed using Pearson correlations.

To determine differences in pNfL between CN subjects and those in the 6 diagnostic groups, we performed a GLM, using age as a covariate, and etiological diagnosis as independent variable. The Dunnett *post-hoc* procedure was used to compare the CN group to all other groups. Similar analyses were done with binary and continuous variables for hippocampal atrophy and elevated brain amyloid, using GLM or Chi-Square analyses, as a way to compare their utility with pNfL for making an etiological diagnosis.

The association of pNfL to cognitive-functional status was assessed with a GLM using CDR-SB as a dependent measure, and pNfL, presence of amyloid, hippocampal atrophy, and *APOE* genotype as independent predictor variables. A second GLM was performed using binary and continuous measures for hippocampal atrophy and amyloid.

Diagnostic accuracy of pNfL for distinguishing CN from AD (and other etiologies with elevated pNfL) was assessed using logistic regression after controlling for age and sex. Area under the receiver operating characteristic (AUROC) curve was obtained and Youden's criteria was used to determine the optimal sensitivity and specificity and cut-point.

The utility of pNfL in predicting cognitive-functional decline among subjects with cognitive impairment at baseline was assessed using CDR-SB as a repeated measure in a GLM. CDR-SB has been shown to be a good outcome measure for cognitive

decline in longitudinal studies [12]. Plasma NfL, presence of amyloid, hippocampal atrophy, score on the HVLT-R, Immediate, and the number of *APOE4* alleles (0, 1, 2) served as independent predictor variables, with age as a covariate. To assess the ability of pNfL to predict clinical progression, logistic regression analyses were performed to determine how pNfL and other biomarkers would predict a decline of two or more points on the CDR-SB over 2 years among subjects with cognitive impairment at baseline. A decline of 2 points was based on the typical decline in AD subjects with MCI or mild dementia [23]. All tests were 2-sided. Statistical significance was set at $p < 0.05$.

RESULTS

Demographics and clinical characteristics by disease etiology

Age

Compared with the CN group, only subjects in the AD group were older (Table 1). There was a difference in the distribution of sex between the 7 diagnostic groups. The percentage of females in the CN subjects (78.4%) was greater than in those with AD (57%), VBI (33%), or LBD (0%). There were no differences between the groups in education, race, or the percentage of subjects reporting Hispanic ethnicity. The CDR-SB score was higher in the AD, FTLD, Dx-Other, and LBD groups than in the CN group. Subjects in the AD group had a lower MMSE score than those in the CN group. On the HVLT-R test, the CN subjects scored significantly better than subjects in the other groups, except for the VBI group.

Association of pNfL and imaging biomarkers with age, sex, and Hispanic ethnicity

There was a positive association between pNfL and age among the CN and AD subjects (Table 2). Age and pNfL were also significantly correlated among the 29 CN subjects who were negative for elevated amyloid and hippocampal atrophy ($r = 0.58$; $p = 0.0009$). Among AD subjects, pNfL and age were significantly correlated; this remained significant after controlling for CDR-SB ($r = 0.42$, $p < 0.0001$). Normalized hippocampal volume was correlated with age in both the CN and AD groups, but the dichotomous measure for hippocampal atrophy was associated with age only in the AD subjects. Amyloid measures

Table 2

Relationship between age and biomarkers in Cognitively Normal and AD Subject

Measure	Pearson Correlation Coefficient	<i>p</i>
Cognitively Normal Subjects		
pNfL*(pg/ml) (all CN, <i>n</i> = 51)	0.44	<0.0001
Amyloid (<i>n</i> = 41)	-0.02	NS
Elevated (N/Y)		
Centiloids	0.22	NS
Hippocampal Atrophy (<i>n</i> = 45)	0.24	NS
Present (N/Y)		
HV/ICV	-0.39	0.007
Alzheimer's Disease Subjects		
pNfL*(pg/ml) (all AD, <i>n</i> = 156)	0.40	<0.0001
Amyloid Positivity (<i>n</i> = 132)		
Elevated (N/Y)	-0.08	NS
Centiloids	-0.03	NS
Hippocampal Atrophy (<i>n</i> = 151)		
Present(N/Y)	0.18	0.02
HV/ICV	-0.35	<0.0001

CN, cognitively normal; HV, hippocampal volume; ICV, intracranial volume *pNfL was log transformed for analyses in this table.

were not correlated with age in either group of subjects (Table 2).

CN women had a higher pNfL level than men after adjusting for age (pNfL: 15.0 ± 13.0 versus 11.1 ± 4.2 ; $F(2,48)=4.7$; $p=0.03$), but there were no sex differences for pNfL in any of the three largest diagnostic subgroups: AD, MD, or Dx-Other groups. There was no difference in pNfL between Hispanic and non-Hispanic subjects in either the CN, AD, neuropsychiatric, or Dx-Other diagnostic groups, adjusting for age.

Association of pNfL with imaging biomarkers (Table 3)

In the entire cohort of subjects with cognitive impairment, pNfL was associated with binary and continuous measures of amyloid and hippocampal atrophy. In the subgroup with AD, only normalized hippocampal volume was associated with pNfL, while in the CN subjects, pNfL was not associated with any of the imaging measures.

Biomarkers by etiological diagnosis (Table 4)

pNfL

Among all subjects, after adjusting for age, pNfL was higher in the AD and FTLD groups compared to the CN group, using a *post-hoc* Dunnett Hsu comparison with CN as the control group (Table 4 and Fig. 1). An exploratory analysis revealed that subjects in the FTLD group had higher pNfL than those

Table 3

Relationship between pNfL and biomarkers for Atrophy and Amyloid Load

Measure	Pearson Correlation Coefficient	<i>p</i>
All Subjects with Cognitive Impairment		
Amyloid (<i>n</i> = 197)		
Elevated (N/Y)	0.27	<0.0001
Centiloids	0.18	0.01
Hippocampal Atrophy (<i>n</i> = 245)	0.32	<0.0001
Present (N/Y)		
HV/ICV	-0.35	<0.0001
Cognitively Normal Subjects		
Amyloid (<i>n</i> = 41)		
Elevated (N/Y)	-0.19	NS
Centiloids	0.11	NS
Hippocampal Atrophy (<i>n</i> = 45)		
Present (N/Y)	-0.10	NS
HV/ICV	0.02	NS
Alzheimer's Disease Subjects		
Amyloid Positivity (<i>n</i> = 132)		
Elevated (N/Y)	0.20	NS
Centiloids	0.14	NS
Hippocampal Atrophy (<i>n</i> = 151)		
Present(N/Y)	0.04	NS
HV/ICV	-0.36	<0.0001

CN, cognitively normal; HV, hippocampal volume; ICV, intracranial volume *pNfL was log transformed for analyses in this table.

in the AD group after controlling for age ($F=9.4$, $df=1$; $p=0.003$).

Using logistic regression, pNfL was a predictive marker for differentiating CN from AD subjects ($\chi^2=24.8$, $df=1$; $p>0.0001$) with an AUROC curve of 0.798 (95% CI: 0.737–0.860). Using Youden's procedure and high pNfL levels as test positive for AD, the optimal sensitivity/specificity was 76%/73%, demonstrating fair levels of discrimination. For the differentiation of CN from FTLD subjects, pNfL was predictive by logistic regression ($\chi^2=6.7$, $df=1$; $p=0.01$) with an AUROC curve of 0.955 (95% CI: 0.899–1.000). The optimal sensitivity/specificity was 100%/84%.

Amyloid

The frequency of positive amyloid PET was different between the groups ($\chi^2=67.5$, $df=6$; $p<0.0001$). *Post-hoc* comparisons with CN subjects (14.6% A+) showed that there was a higher frequency of elevated amyloid only in the AD group (60.9%). Using a continuous measure for amyloid load, i.e., centiloid units, a difference between groups was found to be present, after controlling for age, and a measure of functional severity, i.e., the CDR-SB ($F(8,216)=8.76$, $p<0.0001$), with *post-hoc* Dunnett Hsu procedure

Table 4
Plasma NFL and other Biomarker Values by Diagnosis

	CN	Alzheimer	Neuro-psychiatric	VBI	FTLD	LBD	Dx-Other	F or Chi-Square (p)
n (total = 309)	51	156	47	12	6	5	32	
Plasma NFL	14.2 ^a ± 6.1	21.9 ^b ± 13.1	14.8 ^a ± 9.0	16.1 ^a ± 5.7	46.9 ^b ± 48.8	16.4 ^a ± 9.2	14.6 ^a ± 5.5	18.6 (<0.0001)
Amyloid Measures								
Binary Read (% with visually rated Amyloid positivity/# scans)	14.6% ^a / 41	60.9% ^b / 132	3.1% ^a / 32	22.2% ^a / 9	0% ^a / 6	20% ^a / 5	0% ^a / 13	68.9 (<0.0001)
Centiloid Values	42.9 ^a ± 32.5	82.6 ^b ± 53.1	33.9 ^a ± 20.3	70.3 ^a ± 51.7	16.1 ^a ± 29.1	29.4 ^a ± 9.8	53.4 ^a ± 43.1	8.09 (<0.0009)
Hippocampal Atrophy								
Binary Rating (% with Visually rated atrophy / # scans)	13.3% ^a / 45	90.1% ^b / 151	13.9% ^a / 43	33.3% ^a / 12	83.3% ^b / 6	40% ^a / 5	25.0% ^a / 28	150.6 (<0.0001)
Hippocampal Volume / ICV (x 10 ⁻³)	5.2 ^a ± 0.5	4.4 ^b ± 0.7	5.1 ^a ± 0.5	4.6 ^a ± 0.6	4.4 ^b ± 0.3	4.4 ^b ± 0.5	4.7 ^b ± 0.6	10.3 (<0.0001)
APOE Genotype								
APOE4 positive % / # genotyped)	13.3% ^a / 49	25.0% ^b / 150	8.9% ^a / 45	12.5% ^a / 12	16.7% ^a / 6	10.0% ^a / 5	8.3% ^a / 30	15.5 (0.02)

CN, cognitively normal; VBI, vascular brain injury; FTLD, frontotemporal lobar degeneration; LBD, Lewy body disease F values are adjusted for age. Means with different alphabetic superscripts are statistically different from CN at p = 0.05 by Dunnett's *post-hoc* procedure. Percentages with different superscripts are statistically different from CN at p = 0.05 using Bonferroni correction. pNFL was log transformed for the above analyses due to non-normal residuals. F values and *post-hoc* tests were adjusted for age, but means shown are un-adjusted.

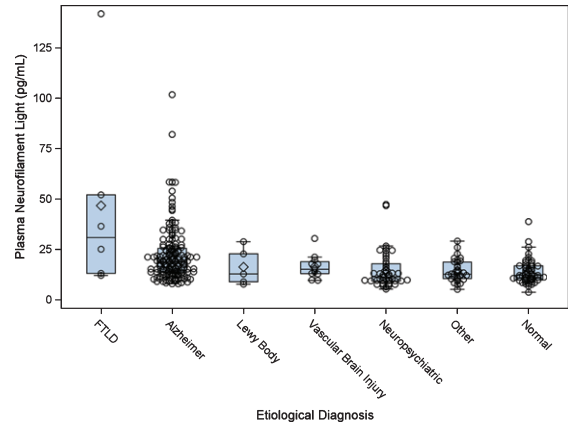


Fig. 1. Plasma neurofilament light by etiological diagnosis. Box-plots show the distribution of plasma neurofilament light by diagnostic category. FTLD, frontotemporal lobar degeneration.

showing that the AD group had higher centiloid values than the CN groups ($p = 0.03$) (Table 3).

Hippocampal atrophy

The presence of hippocampal atrophy ($\chi^2 = 152.6$, $df = 6$; $p < 0.0001$) was also different between the groups, with the *post-hoc* comparisons showing that the CN group had less atrophy than the AD group (90.1% versus 13.3%, $\chi^2 = 102.2$, $df = 1$; $p < 0.0001$) and the FTLD group (83.3% versus 13.3%, $\chi^2 = 12.4$, $df = 1$; $p < 0.0001$). Using continuous measures for hippocampal volume, there was a difference between groups after controlling for age ($F(6,271) = 10.3$, $p < 0.0001$). The AD ($p < .0001$), FTLD ($p = 0.03$), LBD ($p = 0.01$), and Dx-Other ($p = 0.002$) groups had lower hippocampal volume compared to the CN group using a Dunnett Hsu procedure with the CN as the control group (Table 4).

APOE

The frequency of *APOE4* positive subjects was different across diagnostic groups ($\chi^2 = 15.6$, $df = 6$; $p = 0.02$). *Post-hoc* comparisons showing that subjects in the CN group had a lower frequency of *APOE4* positivity (13.3%) than those in the AD group (90.1%).

Table 5 shows the results of a GLM, with CDR-SB score at baseline as the dependent variable, age as a covariate, and pNFL concentration, binary status for both elevated amyloid and hippocampal atrophy, and the number of *APOE* $\epsilon 4$ alleles as independent predictor variables. In that model, all biomarker variables were significant predictors of severity of impairment, after controlling for the other variables.

Table 5
Prediction of CDR sum of boxes at baseline, using pNfL, Imaging Biomarkers, and APOE genotype

Variable	Models with one biomarker			Model with Imaging Biomarkers			Model with all Biomarkers		
	df	Type III Mean Square	F	df	Type III Mean Square	F	df	Type III Mean Square	F
Amyloid Status (A + versus A-)	1,225	152.8	32.0***	1,224	97.2	22.5***	1,223	74.8	18.7***
Hippocampal Atrophy (HP+versus Hp-)	1,225	164.0	34.7***	1,224	108.4	25.2***	1,223	84.5	21.1***
Plasma NfL	1,224	138.2	28.5***				1,223	42.9	18.5***
Age				1,224	0.0	0.0	1,223	11.6	2.9
APOE (# of ε4 alleles)				1,224	7.1	1.6	1,223	9.1	2.3
Model R ²		14.5 (pNfL)		24.1			29.9		
		16.5 (Hippocampal atrophy)							
		15.6 (Amyloid status)							

All models include age and APOE as covariates. Only subjects with all imaging biomarkers are included in these models. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; A + versus A-: elevated versus not elevated amyloid; Hp + versus Hp-: positive versus negative for hippocampal atrophy.

Table 6

Prediction of change in CDR sum of boxes from baseline value to year 2, using pNfL and imaging biomarkers in cognitively impaired subjects

Variable	Model with pNfL, imaging biomarkers, memory score, age and APOE genotype			Model with pNfL, MRI imaging biomarker, memory score, age and APOE genotype		
	df	Type III Mean Square	F for interaction with time	df	Type III Mean Square	F for interaction with time
Amyloid Status (A + versus A-)	2,178	16.4	7.6**			
Hippocampal Atrophy (HP+versus Hp-)	2,178	3.8	1.8	2,178	5.4	2.3
Plasma NfL	2,178	4.4	2.0	2,178	8.7	3.8*
Age	2,178	2.4	1.1	2,178	2.7	1.2
APOE (# of ε4 alleles)	2,178	4.2	1.9	2,178	2.7	1.2
HVLT-R	2,178	19.9	9.2***	2,178	32.4	14.0***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Only subjects with all imaging biomarkers are included in these models. A + versus A-: elevated versus not elevated amyloid; Hp + versus Hp-: positive versus negative for hippocampal atrophy; HVLT-R, Hopkins Verbal Learning Test-Revised.

The total variance explained was 29.9% with pNfL in the model. When pNfL was removed from the model, the variance explained was 24.1%. When these analyses were conducted with continuous measures for amyloid load (centroid) and hippocampal atrophy (volume normalized to ICV), the model with imaging biomarkers had a R² of 27.0%; when pNfL was added to the model, R² increased to 33.9 and pNfL was a significant predictor of CDR-SB after controlling for other variables ($F = 22.2$; $p < 0.0001$).

Prediction of cognitive/functional decline: Added value of pNfL

Change in the CDR-SB scores over a mean follow-up period of 2.3 ± 0.3 years was correlated with the baseline value of all three biomarkers: amyloid status ($r = -0.53$, $p < 0.0001$), hippocampal atrophy status ($r = -0.43$, $p < 0.0001$) and log transformed

pNfL ($r = -0.44$, $p < 0.0001$), as well as with the score on the HVLT-R ($r = 0.58$, $p < 0.0001$). In a repeated measures GLM among 96 cognitively impaired subjects who had at least 2 follow-up visits, score on HVLT-R and amyloid status, but not pNfL or hippocampal atrophy status, were predictors of decline on the CDR-SB from baseline to visit 2, independent of the other variables in the model, including age and APOE (Table 6). In the same group of subjects, when amyloid status was dropped from the model, HVLT-immediate and pNfL were predictive of decline, independent of the other variables. Figure 2 shows three ROC curves for the prediction of a decline of two or more points on the CDR-SB over 2 follow-up visits. In three separate models, using baseline measures of 1) pNfL, 2) HVLT-R, and 3) both pNfL and HVLT-R as predictors, the AUROC curves were 0.821 (95% CI: 0.734–0.909), 0.825 (95% CI: 0.741–0.910), and 0.866 (95% CI:

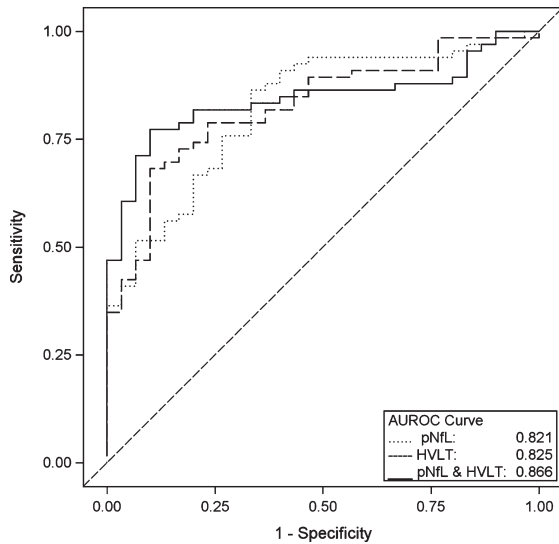


Fig. 2. ROC Curves for Prediction of Decline on the CDR. Area under the receiver operating characteristic (AUROC) curve for prediction of a decline of 2 or more points on the CDR sum of boxes in cognitively impaired subjects over 2 follow-up visits (an average of 2.3 years), using baseline measures of Hopkins Verbal Learning Test-Revised (HVLt-R) total recall, plasma neurofilament Light (pNfL) and the combination of both HVLt-R and pNfL. The logistic regression models were adjusted for age and *APOE* $\epsilon 4$ genotype.

0.792–0.940). The corresponding optimal sensitivities/specificities at a minimum 80% sensitivity were 80.3%/66.6%, 80.3%/63.3%, and 80.3%/80.0%.

DISCUSSION

Within the ethnically diverse IFlorida ADRC cohort, we investigated the relationship between pNfL and both demographic and clinical characteristics. Consistent with prior research, we found that pNfL increases with age, amyloid load, and hippocampal atrophy, and is elevated in those with neurodegenerative diseases including AD and FTLT (Tables 2–4). In our cohort, pNfL was not related to Hispanic ethnicity and was related to sex only in CN subjects, where women had higher levels. We also showed that pNfL may have a complementary and supportive role to brain imaging and cognitive testing in a memory disorder evaluation, although its diagnostic sensitivity and specificity appears to be modest as a stand-alone measure.

The association of pNfL with Hispanic ethnicity was explored because we have found differences between older Hispanic and non-Hispanic adults in the Miami area on other biological measures. For

example, it was found that the association of *APOE* $\epsilon 4$ allele to amyloid load was weaker in Hispanics as compared to non-Hispanic whites [19]. We also showed that the *APOE* dose effect on risk for AD may be greater in non-Hispanics compared to Hispanics [24]. In the current study, we found no effect of Hispanic ethnicity on pNfL in the entire IFlorida ADRC cohort or any diagnostic subgroup.

Increased age was associated with greater pNfL in cognitively normal subjects, a finding that remained even among those normal subjects without AD pathology on brain imaging. Among AD subjects, there was also an age-related increase in pNfL (Table 2). A recent meta-analysis showed that CSF NfL increased with age in CN subjects and in many neurological conditions, including AD [7]. The findings in this study also suggest that the association between age and biomarkers may be greater for NfL than for MRI and amyloid PET biomarkers. As a result of age-related increases in pNfL and cNfL across CN and AD subjects, reference ranges for NfL may have to be age-specific for clinical use. In addition, CN subjects with elevated NfL may score lower on cognitive tests, which could have an effect on the calculation of cutoffs for normative data for neuropsychological tests [6]. It has also been suggested that better normative data could be obtained by excluding “cognitively normal” subjects with elevated amyloid [25]. We found that CN women had higher pNfL than men, contrary to a recent meta-analysis which found higher CSF NfL for men than women in both CN and AD subjects [7]. The role of sex in NfL should be further studied.

Consistent with other studies, pNfL was associated with level of cognitive and functional impairment as measured with CDR-SB [5]. Additionally, in this study we showed that impairment was associated with pNfL after controlling for imaging biomarkers, which themselves are predictive measures of severity of impairment (Table 5). This implies that the pathology associated with pNfL is additive to that associated with neurodegeneration measured on MRI and amyloid measured on PET, in regards to its effect on global cognition and function. The combination of reduced $A\beta_{42}$ and elevated pNfL in plasma was shown to be a strong risk factor for subsequent AD and all-cause dementia [26] in a large epidemiological study of non-demented subjects. Using the ATN framework, subjects with combined biomarkers have been shown to be more cognitively impaired and at greater risk for cognitive decline, using imaging and blood-based biomarkers [27, 28].

However, there are no studies directly showing that subjects with elevated pNfL have additional risk for cognitive/functional impairment in the presence of both hippocampal atrophy and elevated amyloid. A neuropathology study showed that NfL may influence cognition through a non-AD pathway, namely, cerebral atherosclerosis [28]. An initial estimate of the utility of pNfL as a stand-alone measure was obtained by computing its ability to discriminate CN subjects from those subjects with a diagnosis of AD. The AUROC was 0.80 with a modest sensitivity/specificity of 75%/73%. This is consistent with an AUROC for ADNI of 0.79 for pNfL differentiating between CN and AD dementia [5].

Compared to cognitively normal control subjects, in this study, pNfL was elevated to the greatest degree in those with FTLD and to a lesser extent in those diagnosed with AD (Table 4), but not among those with cognitive impairment attributed to neuropsychiatric disorders, such as anxiety and depression, VBI, LBD, or a miscellaneous category, Dx-Other. Elevated NfL among subjects with FTLD and AD has been shown previously, as has increased NfL among subjects with many other neurological conditions, including vascular brain injury and LBD [7]. It should be noted however, that the sample sizes in this cohort may have been too small to find an effect for those subjects in this study.

Among subjects with cognitive impairment, baseline pNfL, elevated amyloid, hippocampal atrophy, and score on a memory test (HVLT-R) were correlated with cognitive and functional decline as measured by the CDR-SB over a 2.3-year period. However, in a model with all biomarkers and memory score as predictors, only amyloid status and memory score independently added to the prediction of decline. Since amyloid PET is rarely available outside of a research setting, another model without amyloid status was performed; in that model, both pNfL and memory score were independent predictors of decline, but hippocampal atrophy was not (Table 6). This underscores the well-known role of memory testing in clinical evaluation and the potential role of pNfL as an adjunct to imaging and to the ATN framework. In the ATN framework for a biological definition of AD, the “N” stands for neurodegeneration or neuronal injury [29]. The “N” is usually measured with structural MRI, FDG PET, or total tau levels in CSF. These measures, like NfL, are not specific for AD, but are used to stage disease severity and prediction of cognitive decline. Using those standards, the current study and prior

investigations [3, 5] support a role for NfL in the ATN classification. Other pathways outside the ATN framework which influence cognitive decline include cerebrovascular disease [30], limbic-predominant age-related TDP-43 encephalopathy (LATE) [31], and primary age-related tauopathy (PART) [32]. In addition, factors, such as cognitive reserve [33], genetic influences, immune dysfunction, and individual lifestyles may either enhance or suppress the association of cognitive status to the underlying neuropathology [34].

In addition to demonstrating that pNfL may have a complementary role to neuroimaging, another strength of the current study was a relatively large group of subjects ($n=47$) who were diagnosed with cognitive disorder with a primary neuropsychiatric etiology. Their mean pNfL level was not different from CN subjects, after controlling for age (Table 4). Further, their CDR-SB score at baseline and their change in CDR-SB at follow-up visits was not different from CN subjects, although they had lower scores on a list-learning test. In this group, the frequency of abnormal biomarkers is equivalent to that for CN subjects. In the absence of expensive neuroimaging tests, pNfL could be a valuable biomarker for differentiating those patients presenting with cognitive complaints who have neuropsychiatric symptoms versus those with neurodegenerative disease [35]. Similarly, pNfL may have diagnostic utility for subjects presenting with miscellaneous conditions such as sleep disorders and other non-neurodegenerative disorders, included in the Dx-Other group.

Limitations of the study include a small number of African American participants, the lack of serial pNfL measurements, the small number of participants with non-AD neurodegenerative disorders, and the relatively short longitudinal assessment interval. In addition, the Hispanics in this study were mostly from Cuba and South America and most self-reported as being white. Therefore, the results may not be generalizable to other Hispanic groups composed of people from Central America and other countries in the Caribbean.

Our findings suggest that pNfL provides a convenient marker of the severity of neurodegeneration and is associated with global cognitive and functional impairment, independent of hippocampal atrophy and abnormal amyloid load. An elevated pNfL concentration, similar to an elevated cholesterol, fasting glucose, or blood pressure measurement, could indicate the need for more detailed diagnostic evaluation and the institution of therapeutic and behavioral

interventions. Plasma NFL was also shown to have utility in the prediction of cognitive and functional decline, beyond that provided by current standard of care assessments, such as brain imaging and cognitive testing. The clinical utility of pNFL as a marker of neurodegeneration is limited by its lack of specificity for AD since disorders such as FTLN also evidence elevated levels.

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