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Cerebrospinal fluid and plasma neurofilament light relate to abnormal cognition

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Abstract

Introduction: Neuroaxonal damage may contribute to cognitive changes preceding clinical dementia. Accessible biomarkers are critical for detecting such damage.
Methods: Plasma and cerebrospinal fluid (CSF) neurofilament light (NFL) were related to neuropsychological performance among Vanderbilt Memory & Aging Project participants (plasma n = 333, 73 ± 7 years; CSF n = 149, 72 ± 6 years) ranging from normal cognition (NC) to mild cognitive impairment (MCI). Models adjusted for age, sex, race/ethnicity, education, apolipoprotein E ε4 carriership, and Framingham Stroke Risk Profile.
Results: Plasma NFL was related to all domains (P values $\leq$ .008) except processing speed (P values $\geq$ .09). CSF NFL was related to memory and language (P values $\leq$ .04). Interactions with cognitive diagnosis revealed widespread plasma associations, particularly in MCI participants, which were further supported in head-to-head comparison models.
Discussion: Plasma and CSF NFL (reflecting neuroaxonal injury) relate to cognition among non-demented older adults albeit with small to medium effects. Plasma NFL shows particular promise as an accessible biomarker with relevance to cognition in MCI.

Keywords: Neurofilament light; Cerebrospinal fluid; Plasma; Alzheimer's disease; Mild cognitive impairment; Cognition

1. Introduction

Neurofilament light (NFL) is a well-established protein biomarker for large-caliber neuroaxonal injury [1,2]. Elevated cerebrospinal fluid (CSF) NFL concentrations are found in disorders characterized by neuroaxonal damage in the cerebral white matter [3], including connections to white matter hyperintensities [4] and white matter changes observed on diffusion tensor imaging in older adults [5,6]. Elevated CSF NFL is found in patients with mild cognitive impairment (MCI) and clinical Alzheimer's disease (AD) [2] and relates to severity of memory impairment [2,7]. Furthermore, CSF NFL also correlates with hippocampal volume loss in older adults with normal cognition (NC) [8], suggesting a potential link between CSF NFL and memory functions before meeting the clinical threshold for MCI

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[9]. However, whether CSF NFL correlates with early memory changes preceding MCI remains unknown. Once MCI is clinically diagnosed, irreversible neurodegeneration [10] and white matter damage are commonly present [11]. Sensitive biomarkers that correlate with cognitive change are important for future drug development. Identification and validation of such biomarkers will support targeting pathological pathways associated with preclinical cognitive changes, making earlier, more personalized intervention possible.

Owing to recent immunoassay technological advances [12,13], NFL can now be measured in plasma, offering wider application. Plasma and CSF NFL are strongly correlated in clinical populations with prominent white matter disease (e.g., HIV [13], amyotrophic lateral sclerosis [14]), but are only moderately correlated in MCI and clinical AD [12,15]. Nevertheless, emerging research suggests plasma NFL is elevated in individuals with cognitive impairment, including MCI and AD [12]. However, as with CSF NFL, it remains unknown how plasma NFL relates to subclinical cognitive changes in NC older adults. Therefore, to elucidate whether neuroaxonal injury detected by plasma and CSF NFL corresponds to early cognitive changes preceding clinical dementia, this study relates plasma and CSF NFL to detailed neuropsychological performance among community-dwelling older adults with NC and MCI. The magnitude of association between each NFL measure and neuropsychological data will be compared to assess whether plasma and CSF NFL account for similar variance in cognition.

2. Methods

2.1. Participants

The Vanderbilt Memory & Aging Project is a longitudinal observational study of vascular health and brain aging, enriched for MCI [16]. Participants were required to be at an age of $\geq$60 years, have intact auditory and visual acuity, speak English, and have a study partner. Before enrollment, participants completed a clinical interview, detailed medical history and record review, and neuropsychological protocol and were excluded for a diagnosis other than NC, early MCI [17], or MCI [18], MRI contraindication, history of neurological disease (e.g., stroke, epilepsy), heart failure, major psychiatric illness, head injury, and systemic or terminal illness affecting longitudinal participation. A diagnosis of NC required a score of "0" on the Clinical Dementia Rating (CDR) semistructured participant and proxy interview, and no objective neuropsychological impairment (i.e., all scores $>$ -1.5 standard deviation from normative mean). A diagnosis of early MCI required a score of 0.5 on the CDR with no objective neuropsychological impairment. A diagnosis of MCI required a score of 0 or 0.5 on the CDR and objective neuropsychological impairment as evidenced by performance of ≤ -1.5 standard deviation in one or more domains. The enrollment neuropsychological protocol was designed in accordance with the intended study focus on AD and related dementia risk to include particularly detailed characterization of learning and memory performances, and

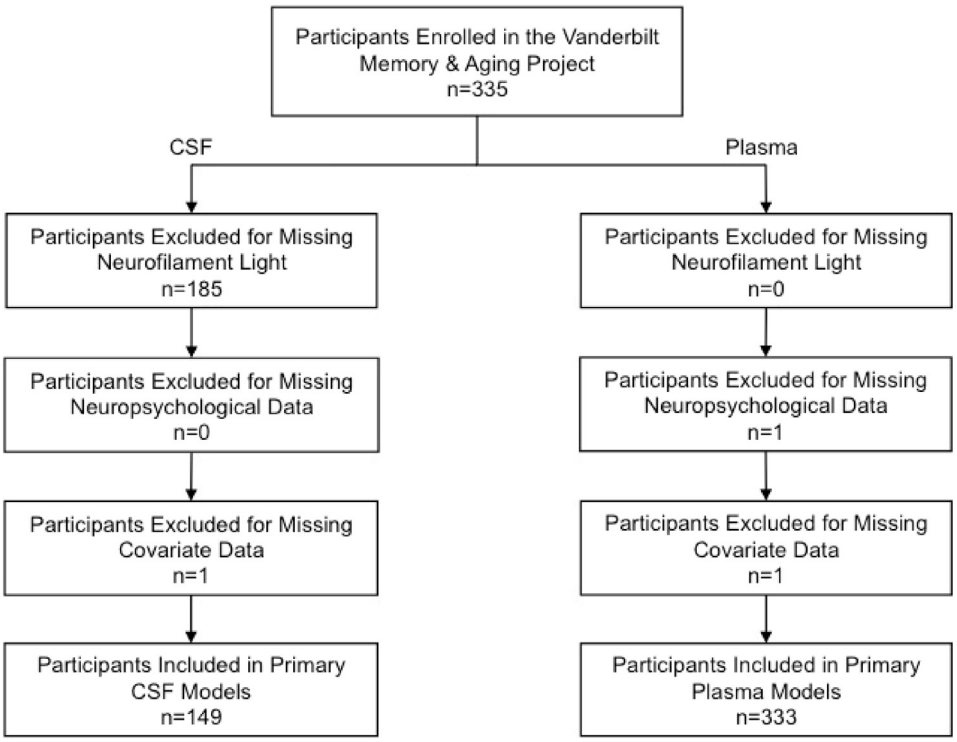

Fig. 1. Participant inclusion and exclusion diagram. Abbreviation: CSF, cerebrospinal fluid.

participants were recruited for participation in a memory-focused clinical research study. Consequently, most participants in the MCI group exhibit amnestic neuropsychological profiles. Once enrolled, participants completed a comprehensive evaluation, including (but not limited to) fasting blood draw, medical history, medication review, physical examination, neuropsychological assessment, and optional lumbar puncture. Participants were excluded from the present study for missing covariate or neuropsychological data (Fig. 1). The Vanderbilt University Medical Center Institutional Review Board approved the protocol. Written informed consent was obtained before data collection.

2.2. Fluid collection and biochemical analyses

All participants completed a morning fasting venous blood draw. Plasma was separated from whole blood by centrifugation at 2000g and 4°C for 15 minutes. An optional lumbar puncture was performed with polypropylene syringes using a Sprotte 25-gauge spinal needle in an intervertebral lumbar space. Both plasma and CSF samples were aliquoted in 0.5 mL polypropylene tubes and stored at −80°C pending analyses. Plasma NFL levels were measured using an in-house Simoa method [13], which is based on the same monoclonal antibodies as the ELISA used to measure CSF NFL (NF-light; UmanDiagnostics). All samples were

Table 1
Participant characteristics

	Total n = 333	NC n = 174	Early MCI n = 27	MCI n = 132	*P* value
Demographic and health characteristics					
Age, years	73 ± 7	72 ± 7	73 ± 6	73 ± 8	.63
Sex, % male	59	59	74	56	.22
Race, % White non-Hispanic	86	87	85	86	.89
Education, years	16 ± 3	16 ± 2	16 ± 3	15 ± 3	**<.001**[*]
APOE-ε4 carriers, %	35	29	22	44	**.01**[†]
FSRP, total score[‡]	12.4 ± 4.2	11.9 ± 4.1	13.4 ± 3.2	13.0 ± 4.3	**.04**
Montreal Cognitive Assessment, total score	25.3 ± 3.3	27.0 ± 2.2	25.4 ± 2.4	23.1 ± 3.4	**<.001**[*†]
Plasma neurofilament light, pg/mL	20.1 ± 10.7	17.5 ± 9.2	17.9 ± 7.8	25.2 ± 16.5	**<.001**[*†]
CSF neurofilament light, pg/mL[§]	1066 ± 580	930 ± 448	1088 ± 465	1250 ± 712	**.002**[*]
Neuropsychological outcomes					
Language					
Boston Naming Test	26.8 ± 3.1	27.9 ± 2.0	26.6 ± 2.4	25.4 ± 3.9	**<.001**[*¶]
Animal Naming	19.0 ± 5.4	21.0 ± 4.9	19.4 ± 3.4	16.2 ± 5.2	**<.001**[*†]
Information processing speed					
WAIS-IV Coding	53 ± 13	57 ± 12	53 ± 11	46 ± 12	**<.001**[*†]
DKEFS Number Sequencing[#]	43 ± 20	36 ± 13	42 ± 13	51 ± 26	**<.001**[*†]
Executive function					
Executive function composite	0.003 ± 0.9	0.43 ± 0.6	0.17 ± 0.4	−0.60 ± 1.0	**<.001**[*†]
DKEFS Tower Test	14.9 ± 4.7	16.1 ± 4.3	16.2 ± 3.5	13.0 ± 4.7	**<.001**[*†]
DKEFS Letter-Number Switching[**]	117 ± 94	86 ± 34	93 ± 22	164 ± 131	**<.001**[*†]
DKEFS Color-Word Inhibition[#]	69 ± 24	60 ± 14	75 ± 15	81 ± 30	**<.001**[*¶]
Letter Fluency (FAS)	38.7 ± 11.7	42.9 ± 11.4	37.9 ± 11.1	33.3 ± 9.9	**<.001**[*]
Visuospatial skills					
Hooper Visual Organization Test	24.4 ± 3.1	25.3 ± 2.4	24.7 ± 2.2	23.2 ± 3.6	**<.001**[*]
Episodic memory					
Episodic memory composite	−0.002 ± 1.0	0.57 ± 0.7	−0.06 ± 0.8	−0.75 ± 0.8	**<.001**[*¶]
CVLT-II Total Learning	40.4 ± 12	46.9 ± 9.4	40.1 ± 9.7	31.9 ± 9.6	**<.001**[*¶]
CVLT-II Long Delay Free Recall	8.0 ± 4.3	10.5 ± 3.3	7.6 ± 3.5	4.9 ± 3.5	**<.001**[*¶]
CVLT-II Recognition	2.4 ± 1.0	3.0 ± 0.7	2.3 ± 0.8	1.7 ± 0.9	**<.001**[*¶]
BFLT Total Learning	113 ± 41	136 ± 30	110 ± 28	82 ± 35	**<.001**[*¶]
BFLT Long Delay Free Recall	26.9 ± 10.6	32.6 ± 7.5	28.0 ± 6.6	19.1 ± 9.9	**<.001**[*†¶]
BFLT Recognition	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	**<.001**[*¶]

NOTE. Significant ($P < .05$) results indicated in bold.

Abbreviations: APOE, apolipoprotein E; BFLT, Biber Figure Learning Test; CSF, cerebrospinal fluid; CVLT-II, California Verbal Learning Test second edition; DKEFS, Delis-Kaplan Executive Function System; FSRP, Framingham Stroke Risk Profile; MCI, mild cognitive impairment; NC, normal cognition; WAIS-IV, Wechsler Adult Intelligence Scale IV.

[*] $P < .05$ for NC versus MCI.

[†] $P < .05$ for early MCI versus MCI.

[‡] FSRP minus age points, total = 6.5 ± 3.1, NC = 6.1 ± 2.9, early MCI = 7.4 ± 2.6, MCI = 6.9 ± 3.3 ($P = .03$).

[§] n = 149.

[¶] $P < .05$ for NC versus early MCI.

[#] Represents time to completion (s).

[**] Represents log time to completion (s).

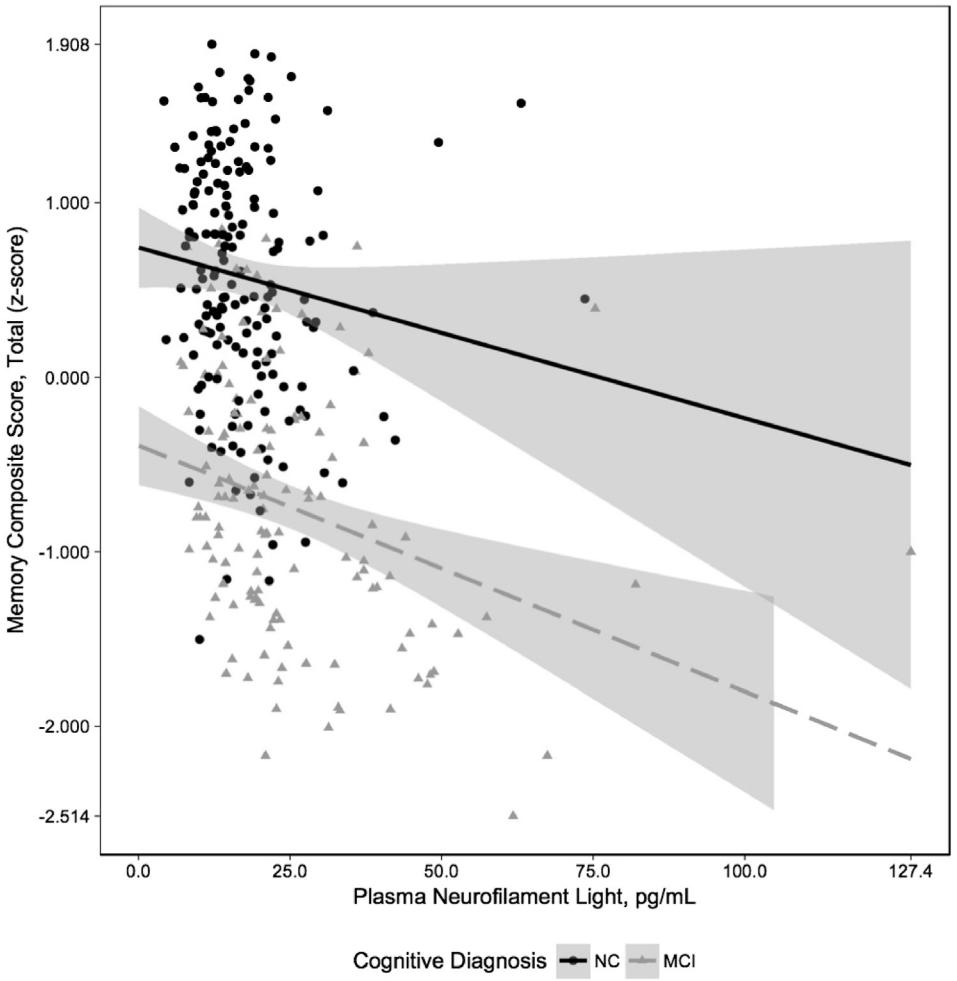

Fig. 2. Plasma NFL and episodic memory scatterplot by diagnostic status. Abbreviations: NFL, neurofilament light; MCI, mild cognitive impairment; NC, normal cognition.

analyzed in batch by board-certified laboratory technicians who were blinded to clinical information. For both biomarkers, intraassay coefficients of variation were <10%.

2.3. Neuropsychological assessment

Participants completed a comprehensive assessment of language, information processing speed, executive function, visuospatial skills, and episodic memory (Table 1). Measures differed from tests used to screen and select participants for study inclusion or determine cognitive diagnosis. To minimize multiple comparisons, z-scores were derived separately for composite episodic memory and executive function performances using a latent variable approach previously described [19]. Briefly, the memory composite was calculated with item-level data from the California Verbal Learning Test, Second Edition (CVLT-II) and the Biber Figure Learning Test (BFLT). As previously described [19], using a bifactor latent variable model, each item was treated as a raw continuous variable and loaded on a general factor, as well as on a test-specific factor (i.e., CVLT-II or BFLT) to remove potentially confounding test effects. Both test models included Trials 1 to 5 Total Learning, List B Learning, Immediate Recall, Delayed Recall, and Recognition raw scores. The executive function composite was calculated with item-level data from the Delis–Kaplan Executive Function System (D-KEFS) Color–Word Inhibition Test, D-KEFS Tower Test, Letter Fluency (FAS) Test, and D-KEFS Letter–Number Switching Test with each set of raw scores treated as a continuous variable.

2.4. Covariate details

As previously described [20], Framingham Stroke Risk Profile (FSRP [21]) scores were calculated by applying points by sex for age, systolic blood pressure accounting for antihypertensive medication usage, diabetes mellitus, cigarette smoking, left ventricular hypertrophy, atrial fibrillation, and cardiovascular disease, including coronary heart disease, angina, or myocardial infarction (heart failure was a parent study exclusion). Apolipoprotein E (*APOE*) genotyping was performed on whole blood. *APOE-ε4* status was defined as positive (*APOE ε2/ε4*, *APOE ε3/ε4*, *APOE ε4/ε4*) or negative (*APOE ε2/ε2*, *APOE ε2/ε3*, *APOE ε3/ε3*).

2.5. Analytical plan

Unadjusted Spearman rank correlations compared plasma and CSF concentrations. Ordinary least squares regression related each biomarker to neuropsychological performances. Excluding early MCI due to small sample size, models were repeated testing a *NFL biomarker* × *cognitive diagnosis* interaction and repeated stratifying by NC and MCI. Models adjusted for age, sex, race/ethnicity, education, *APOE-ε4*, and FSRP (excluding points assigned for age). For a head-to-head comparison of the magnitude of associations between plasma and CSF NFL with neuropsychological performance, regression analyses were repeated including both plasma and CSF NFL predictors in each model to assess whether either NFL variable accounted for unique variance in neuropsychological performance. Head-to-head analyses with dual predictor models were repeated with stratification by diagnostic group (NC, MCI). Significance was set a priori at $P < .05$. Effect sizes were generated for all statistically significant findings by calculating the change in R^2 from the addition of the predictor after all covariates were included in the model (i.e., ΔR^2). Sensitivity analyses were conducted by repeating all models with exclusion of outliers >4 standard deviation from mean values. Analyses were conducted using R version 3.4.3 (www.r-project.org).

3. Results

3.1. Participant characteristics

Neuropsychological assessment was performed within 3 ± 11 days of plasma sample collection and within 38 ± 38 days of CSF sample collection. Lumber puncture was always performed on a separate day from the neuropsychological assessment. Plasma analyses included samples from 333 adults aged 60–92 years. Plasma NFL ranged from 4 to 127 pg/mL. CSF analyses included samples from 149 adults aged 60–90 years. CSF NFL ranged from 268 to 4025 pg/mL. One plasma value and 3 CSF values were identified as statistical outliers but retained in all statistical models after sensitivity analyses revealed similar beta values when excluding these cases from analyses. See Table 1 for detailed participant characteristics. Plasma and CSF NFL correlated in the total sample (n = 149, r = 0.50, $P < .0001$) and within each diagnostic group (NC = 78, r = 0.37, $P = .0007$; MCI = 56, r = 0.49, $P < .0002$).

3.2. Plasma NFL and neuropsychological performance

Increased plasma NFL was related to worse performance in all cognitive domains (P values ≤.008, ΔR^2 values = 0.006–0.07) except information processing speed, including Coding ($P = .25$) and Number Sequencing ($P = .09$). Plasma NFL interacted with cognitive diagnosis on Boston Naming Test performance ($P = .03$, $\Delta R^2 = 0.09$), with the association present in MCI ($P = .03$, $\Delta R^2 = 0.15$) but not NC participants ($P = .16$). Results

Table 2
Plasma neurofilament light and neuropsychological performance

	Main effect				Diagnostic interaction				NC only			MCI only		
	β	95% CI	*P* value	$\Delta R^{2\dagger}$	β	95% CI	*P* value	ΔR^{2*}	β	95% CI	*P* value	β	95% CI	*P* value	ΔR^{2*}
Boston Naming Test	−0.04	−0.07, −0.02	**.002**	0.05	−0.06	−0.11, −0.01	**.03**	0.09	−0.03	−0.06, 0.01	.16	−0.05	−0.09, −0.004	**.03**	0.15
Animal Naming	−0.08	−0.12, −0.04	**.0004**	0.06	−0.05	−0.14, 0.05	.32	–	−0.07	−0.16, 0.02	.13	−0.08	−0.13, −0.03	**.004**	0.13
WAIS-IV Coding	−0.06	−0.16, 0.04	.25	–	0.002	−0.21, 0.21	.99	–	0.03	−0.17, 0.23	.80	−0.05	−0.18, 0.07	.40	–
DKEFS Number Sequencing	0.14	−0.02, 0.30	.09	–	0.10	−0.25, 0.44	.58	–	0.10	−0.12, 0.32	.35	0.16	−0.11, 0.43	.26	–
Executive Function Composite	−0.01	−0.02, −0.01	**<.00001**	0.04	−0.01	−0.02, 0.01	.35	–	−0.01	−0.01, 0.003	.15	−0.02	−0.03, −0.01	**.0006**	0.02
Hooper Visual Organization Test	−0.05	−0.08, −0.02	**.0003**	0.07	−0.03	−0.09, 0.02	.27	–	−0.04	−0.09, 0.002	.06	−0.06	−0.10, −0.02	**.005**	0.06
Episodic Memory Composite	−0.01	−0.02, −0.002	**.008**	0.006	−0.01	−0.02, 0.003	.12	–	0.004	−0.01, 0.02	.55	−0.01	−0.02, −0.002	**.02**	0.02

NOTE. Significant ($P < .05$) results indicated in bold.

Abbreviations: β, unstandardized beta reflects the change in outcome as a function of one-unit increase in the raw value of the predictor; DKEFS, Delis-Kaplan Executive Function System; MCI, mild cognitive impairment; NC, normal cognition; WAIS-IV, Wechsler Adult Intelligence Scale Fourth Edition.

*Change in R^2 accounted for by the predictor after entering all covariates into the model.

†Change in R^2 accounted for by the interaction term after entering all covariates into the model.

stratified by diagnosis revealed plasma NFL was related to worse episodic memory composite ($P = .02$, $\Delta R^2 = 0.02$ Fig. 2), executive function composite ($P = .0006$, $\Delta R^2 = 0.02$), Hooper Visual Organization Test ($P = .005$, $\Delta R^2 = 0.06$), and Animal Naming ($P = .004$, $\Delta R^2 = 0.13$) performances in MCI participants. No associations in the NC group achieved a priori significance ($P \geq .06$). See Table 2 for details. When models were restricted to participants with CSF (n = 149), all plasma main effect associations persisted (P values $\leq .03$, ΔR^2 values = 0.02–0.05) except for episodic memory composite ($P = .22$). No diagnostic interactions with plasma NFL emerged, but in stratified analyses, associations in MCI persisted for the executive functioning composite ($P = .03$, $\Delta R^2 = 0.09$), Hooper Visual Organization Test ($P = .02$, $\Delta R^2 = 0.10$), and Animal Naming ($P = .01$, $\Delta R^2 = 0.02$). Plasma associations in MCI were no longer present for the episodic memory composite ($P = .56$) and Boston Naming Test ($P = .08$) in this smaller sample. As in the larger plasma sample, no associations were present in the NC group when stratifying by diagnosis. See Supplementary Table 1 for details.

3.3. CSF NFL and neuropsychological performance

Increased CSF NFL was related to worse episodic memory composite ($P = .04$, $\Delta R^2 = 0.02$) and Animal Naming performances ($P = .01$, $\Delta R^2 = 0.03$). CSF NFL interacted with cognitive diagnosis on episodic memory composite performance ($P = .04$, $\Delta R^2 = 0.02$ Fig. 3), with the association present in NC ($P = .008$, $\Delta R^2 = 0.07$) but not MCI participants ($P = .58$). Results stratified by diagnosis revealed the association between CSF NFL and Animal Naming was restricted to the MCI group ($P = .03$, $\Delta R^2 = 0.08$). The remaining stratified models were null in both groups ($P \geq .17$). See Table 3 for details.

3.4. Head-to-head comparisons between plasma and CSF NFL associations with neuropsychological performance

When both plasma and CSF NFL were included as dual predictors in single regression models, plasma but not CSF NFL was related to worse executive function composite (plasma $P = .004$, $\Delta R^2 = 0.04$; CSF $P = .80$), Hooper

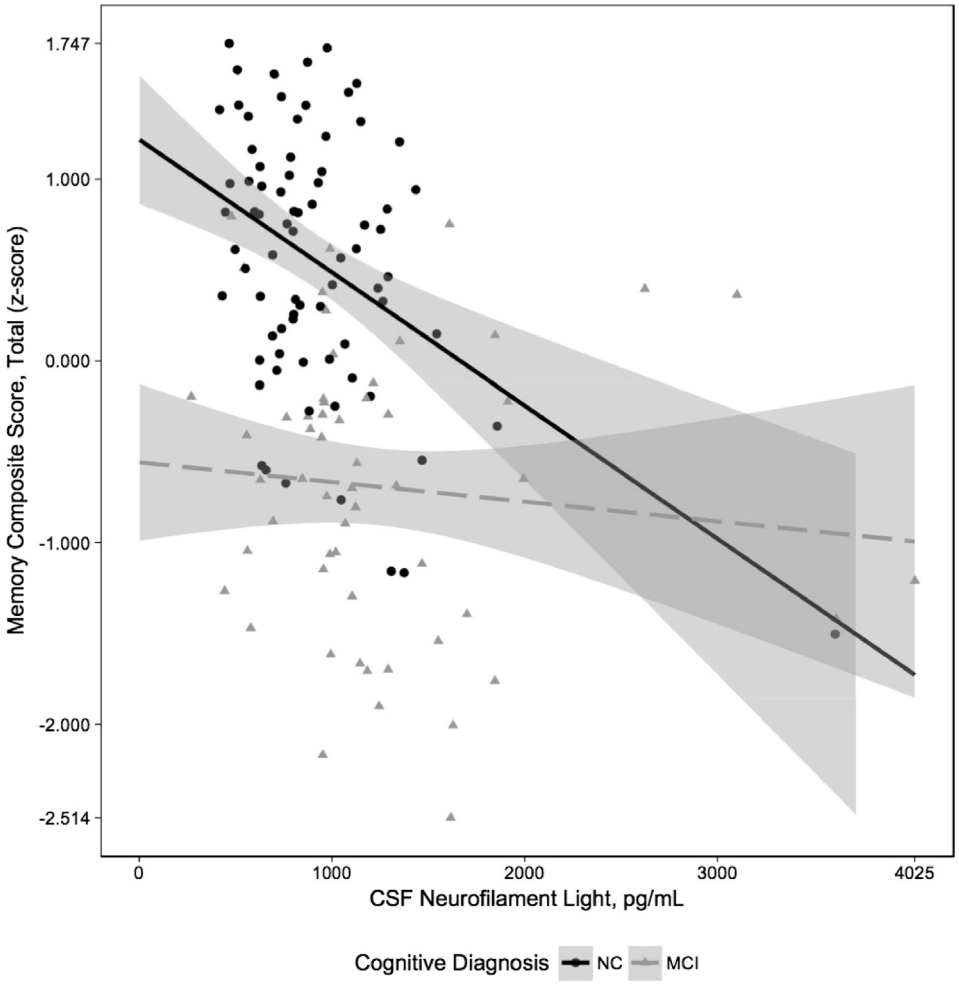

Fig. 3. CSF NFL and episodic memory scatterplot by diagnostic status. Abbreviations: CSF, cerebrospinal fluid; NFL, neurofilament light; MCI, mild cognitive impairment; NC, normal cognition.

Table 3
CSF neurofilament light and neuropsychological performance

	Main effect				Diagnostic interaction				NC only				MCI only			
	β	95% CI	*P* value	ΔR^{2*}	β	95% CI	*P* value	$\Delta R^{2\dagger}$	β	95% CI	*P* value	ΔR^{2*}	β	95% CI	*P* value	ΔR^{2*}
Boston Naming Test	−0.0006	−0.001, 0.0002	.15	–	−0.0004	−0.002, 0.001	.63	–	−0.0007	−0.002, 0.0003	.17	–	-0.0005	−0.002, 0.0008	.46	–
Animal Naming	−0.002	−0.003, −0.0004	**.01**	0.03	−0.0005	−0.003, 0.002	.75	–	−0.001	−0.004, 0.001	.34	–	−0.002	−0.004, −0.0002	**.03**	0.08
WAIS-IV Coding	−0.0005	−0.004, 0.003	.80	–	−0.003	−0.011, 0.004	.41	–	0.003	−0.004, 0.009	.43	–	−0.002	−0.007, 0.002	.32	–
DKEFS Number Sequencing	−0.0005	−0.005, 0.004	.85	–	−0.006	−0.017, 0.005	.27	–	0.004	−0.002, 0.010	.21	–	−0.002	−0.011, 0.007	.64	–
Executive Function Composite	−0.0002	−0.0004, 0.0001	.17	–	0.00004	−0.0004, 0.0005	.87	–	−0.0001	−0.0004, 0.0002	.51	–	−0.0002	−0.0005, 0.0002	.40	–
Hooper Visual Organization Test	−0.0007	−0.002, 0.0002	.13	–	−0.0004	−0.002, 0.002	.66	–	−0.0005	−0.0018, 0.0008	.45	–	−0.0008	−0.002, 0.0007	.29	–
Episodic Memory Composite	−0.0002	−0.0005, −0.00001	**.04**	0.02	0.0005	0.0000, 0.001	**.04**	0.02	−0.0005	−0.0009, −0.0001	**.008**	0.07	−0.00008	−0.0004, 0.0002	.58	–

NOTE. Significant ($P < .05$) results indicated in bold.
Abbreviations: β, unstandardized beta reflects the change in outcome as a function of one-unit increase in the raw value of the predictor; CSF, cerebrospinal fluid; DKEFS, Delis-Kaplan Executive Function System; MCI, mild cognitive impairment; NC, normal cognition; WAIS-IV, Wechsler Adult Intelligence Scale IV.
*Change in R^2 accounted for by the predictor after entering all covariates into the model.
†Change in R^2 accounted for by the interaction term after entering all covariates into the model.

Visual Organization Test (plasma $P = .02$, $\Delta R^2 = 0.03$; CSF $P = .56$), and Coding performances (plasma $P = .04$, $\Delta R^2 = 0.02$; CSF $P = .61$). A similar pattern emerged for Boston Naming Test but did not achieve the a priori significance threshold (plasma $P = .05$; CSF $P = .51$). Neither plasma nor CSF NFL significantly accounted for variance in episodic memory composite ($P \geq .59$), Animal Naming ($P \geq .06$), or Number Sequencing performances ($P \geq .46$) in these dual predictor models. When restricted to the NC group, CSF but not plasma NFL was related to worse episodic memory composite performance (CSF $P = .01$, $\Delta R^2 = 0.07$; plasma $P = .92$). Neither plasma nor CSF NFL was related to any other neuropsychological domains among the NC group in head-to-head comparison models ($P \geq .11$). When restricted to the MCI group, plasma but not CSF NFL was related to worse executive function composite (plasma $P = .04$, $\Delta R^2 = 0.08$; CSF $P = .69$) and Hooper Visual Organization Test (plasma $P = .04$, $\Delta R^2 = 0.07$; CSF $P = .82$) performances. Neither plasma nor CSF NFL was related to language, information processing speed, or episodic memory performances among the MCI group in head-to-head comparison models ($P \geq .10$). See Table 4 for details.

4. Discussion

We evaluated plasma and CSF NFL associations with comprehensive neuropsychological performance in community-dwelling older adults. CSF NFL was related to cognition in our participants without clinical dementia or stroke, aligning with previous reports in more clinically symptomatic older adults with MCI and clinical AD [2,7]. Plasma and CSF NFL levels were moderately correlated in our sample, consistent with effects reported in the Alzheimer's Disease Neuroimaging Initiative [12,15]. In head-to-head comparisons, plasma NFL appeared to account for more variance in cognition, including associations with information processing speed, executive functioning, and visuospatial skills. Plasma NFL associations with executive functioning and visuospatial skills persisted when the sample was restricted to individuals with MCI, whereas no plasma NFL associations were observed within the NC group. By contrast, CSF NFL was uniquely related to episodic memory performance within the NC group but otherwise did not account for any variance in cognition beyond that of plasma NFL. While effects ranged from small to medium, these findings were observed in the context of comprehensive covariate adjustment, adding plausibility to the interpretive value of these biomarker associations despite modest effects. This novel evidence supports the utility of CSF and plasma NFL as biomarkers of early adverse cognitive aging.

Notably, plasma NFL did not relate to cognition within the NC group. Given that plasma and CSF NFL are more strongly correlated in other clinical groups with greater

Table 4
Head-to-head comparisons of plasma and CSF neurofilament light in dual predictor models of neuropsychological performance

	Plasma NFL				CSF NFL			
Total sample	β	95% CI	*P* value	ΔR^{2*}	β	95% CI	*P* value	$\Delta R^{2\dagger}$
Boston Naming Test	−0.05	−0.09, 0.0008	.054	–	−0.0003	−0.001, 0.0005	.51	–
Animal Naming	−0.06	−0.14, 0.02	.16	–	−0.001	−0.003, 0.0001	.06	–
WAIS-IV Coding	−0.23	−0.45, −0.009	**.04**	0.02	0.001	−0.003, 0.005	.61	–
DKEFS Number Sequencing	0.12	−0.19, 0.43	.46	–	−0.001	−0.007, 0.004	.65	–
Executive Function Composite	−0.02	−0.03, −0.006	**.004**	0.04	−0.00003	−0.0003, 0.0002	.80	–
Hooper Visual Organization Test	−0.07	−0.12, −0.009	**.02**	0.03	−0.0003	−0.001, 0.0007	.56	–
Episodic Memory Composite	−0.004	−0.02, 0.01	.59	–	−0.0002	−0.0005, 0.00003	.09	–
NC only								
Boston Naming Test	−0.05	−0.12, 0.02	.15	–	−0.0008	−0.002, 0.0002	.12	–
Animal Naming	−0.003	−0.19, 0.18	.97	–	−0.001	−0.004, 0.001	.35	–
WAIS-IV Coding	0.12	−0.32, 0.56	.59	–	0.003	−0.004, 0.01	.39	–
DKEFS Number Sequencing	0.10	−0.33, 0.53	.65	–	0.004	−0.002, 0.01	.19	–
Executive Function Composite	−0.01	−0.03, 0.007	.18	–	−0.0001	−0.0004, 0.0002	.38	–
Hooper Visual Organization Test	−0.04	−0.13, 0.04	.32	–	−0.0006	−0.002, 0.0007	.36	–
Episodic Memory Composite	0.001	−0.02, 0.03	.92	–	−0.0005	−0.0009, −0.0001	**.01**	0.07
MCI only								
Boston Naming Test	−0.07	−0.15, 0.02	.11	–	0.0002	−0.001, 0.002	.81	–
Animal Naming	−0.09	−0.21, 0.02	.10	–	−0.001	−0.003, 0.001	.33	–
WAIS-IV Coding	−0.24	−0.57, 0.09	.14	–	−0.0001	−0.006, 0.006	.96	–
DKEFS Number Sequencing	0.25	−0.33, 0.83	.40	–	−0.004	−0.01, 0.006	.40	–
Executive Function Composite	−0.03	−0.05, −0.001	**.04**	0.08	0.00009	−0.0003, 0.0005	.69	–
Hooper Visual Organization Test	−0.11	−0.21, −0.005	**.04**	0.07	0.0002	−0.002, 0.002	.82	–
Episodic Memory Composite	−0.004	−0.02, 0.02	.74	–	−0.00005	−0.0004, 0.0003	.79	–

NOTE. Results are for plasma and CSF NFL when both predictors were simultaneously entered into the regression model after all covariates. Significant ($P < .05$) results indicated in bold.

Abbreviations: CSF, cerebrospinal fluid; β, unstandardized beta reflects the change in outcome as a function of one-unit increase in the raw value of the predictor; DKEFS, Delis-Kaplan Executive Function System; MCI, mild cognitive impairment; NC, normal cognition; NFL, neurofilament light; WAIS-IV, Wechsler Adult Intelligence Scale IV.

*Change in R^2 accounted for by plasma NFL after entering all covariates and CSF NFL into the model.

†Change in R^2 accounted for by CSF NFL after entering all covariates and plasma NFL into the model. Significant ($P < .05$) results are indicated in bold.

white matter disease severity [13,14], it is plausible that plasma NFL may be a slightly more robust biomarker in MCI than NC due to increased solute exchange to the blood with worsening blood-brain barrier (BBB) integrity. Findings from dynamic contrast arterial imaging that depicts increased hippocampal BBB permeability in MCI compared to age-matched NC controls offer strong evidence that BBB integrity is compromised in MCI [22]. Moreover, recent findings from Bowman and colleagues [23] showed an association between increased BBB permeability measured by serum/CSF albumin ratio and worse cognitive decline in older adults with MCI. However, to date, no direct comparison of BBB permeability with plasma NFL concentrations or the slope of the correlation of plasma NFL with CSF NFL in the context of neurodegeneration has been reported to examine this hypothesis closer, though a small pilot study suggests blood NFL concentration is unaffected by BBB permeability [24]. Moreover, the exact clearance pathway(s) by which NFL enters the CSF and plasma have not been well characterized in the literature, and it is possible that the CSF and plasma NFL levels might reflect separate, albeit co-occurring, underlying neural injury processes. Such physiological distinctions could account for the diagnostic interactions observed here. Replication studies with larger sample sizes and longitudinal modeling will further elucidate possible distinctions between plasma and CSF NFL in detecting subtle indicators of adverse brain aging.

Fluid biomarkers provide in vivo evidence of neuropathological changes underlying AD and related dementias, improving early diagnostic capabilities, enhancing participant selection for clinical trials, and deepening our understanding of the pathological substrates of adverse cognitive aging [25]. Our study provides novel evidence supporting the utility of plasma NFL, a biomarker of large-caliber neuroaxonal injury, as a promising, relatively accessible blood-based biomarker with relevance to early cognitive changes in advanced age. Our findings highlight the importance of better understanding potential mechanisms of age-related neuroaxonal injury in the context of AD pathology development and progression. Prior work has suggested plasma NFL may be a useful biomarker of cognitive decline in AD and other neurodegenerative conditions, with greater specificity for AD compared to movement disorders [26]. Such improved specificity may be due to neuroaxonal degradation occurring secondary to neuronal death during the onset and

progression of AD-related cortical atrophy. Furthermore, emerging evidence leveraging CSF Aβ42 and hyperphosphorylated tau have linked these core AD pathologies to white matter microstructural damage among aging adults [5] even in familial AD [27,28]. These findings suggest damage to the cerebral white matter (and underlying axons) may be more directly involved in the AD pathological cascade than previously recognized. Future research should continue to explore the role of neuroaxonal injury in contributing to cognitive decline both within and beyond the amyloid cascade hypothesis.

Study strengths include detailed neuropsychological assessment, core biomarker processing laboratory, and comprehensive covariate ascertainment. Emphasizing biomarkers beyond the amyloid cascade hypothesis [29] offers an opportunity to enhance our understanding of concomitant pathological pathways driving adverse cognitive aging. Limitations include the cross-sectional design precluding inferences about causality. Generalizability is restricted given the well-educated, predominantly White older sample. Finally, multiple comparisons were made increasing the possibility of a false-positive finding. Replication in more diverse ethnic/racial groups of older adults and leveraging longitudinal models would further elucidate how CSF and plasma NFL concentrations relate to early cognitive trajectory in AD and related dementias.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.dadm.2019.08.008.

RESEARCH IN CONTEXT

1. Systematic review: For our PubMed search, we used various combinations of terms "neurofilament light" (NFL), "cerebrospinal fluid" (CSF), "plasma," "cognition," "memory," "Alzheimer's," and "dementia" using Boolean search logic. Additional references were identified using works cited in articles located from search terms. Articles related to neurodegeneration and cognitive decline were used to determine prior findings of NFL levels in CSF and plasma across the advanced aging spectrum.
2. Interpretation: Our findings suggest plasma and CSF NFL (reflecting large caliber neuroaxonal injury) relate to cognition among nondemented older adults. Plasma NFL associations were particularly robust in participants with mild cognitive impairment, suggesting its promise as a widely accessible biomarker relevant to cognitive functioning in prodromal stages of Alzheimer's disease.
3. Future directions: Future research should assess how plasma and CSF NFL relate to longitudinal cognitive trajectory, as well as replicate present findings in more demographically diverse participant samples.

References

[1] Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci 2005;233:183–98.
[2] Skillback T, Farahmand B, Bartlett JW, Rosen C, Mattsson N, Nagga K, et al. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology 2014;83:1945–53.
[3] Bacioglu M, Maia LF, Preische O, Schelle J, Apel A, Kaeser SA, et al. Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. Neuron 2016;91:494–6.
[4] Osborn KE, Liu D, Samuels LR, Moore EE, Cambronero FE, Acosta LMY, et al. Cerebrospinal fluid β-amyloid42 and neurofilament light relate to white matter hyperintensities. Neurobiol Aging 2018;68:18–25.
[5] Moore EE, Hohman TJ, Badami FS, Pechman KR, Osborn KE, Acosta LMY, et al. Neurofilament relates to white matter microstructure in older adults. Neurobiol Aging 2018;70:233–41.
[6] Melah KE, Lu SY, Hoscheidt SM, Alexander AL, Adluru N, Destiche DJ, et al. Cerebrospinal fluid markers of Alzheimer's disease pathology and microglial activation are associated with altered white matter microstructure in asymptomatic adults at risk for Alzheimer's disease. J Alzheimer's Dis 2016;50:873–86.
[7] Rolstad S, Berg AI, Eckerstrom C, Johansson B, Wallin A. Differential impact of neurofilament light subunit on cognition and functional outcome in memory clinic patients with and without vascular burden. J Alzheimers Dis 2015;45:873–81.

[8] Idland AV, Sala-Llonch R, Borza T, Watne LO, Wyller TB, Braekhus A, et al. CSF neurofilament light levels predict hippocampal atrophy in cognitively healthy older adults. Neurobiol Aging 2017;49:138–44.
[9] Ritchey M, Libby LA, Ranganath C. Cortico-hippocampal systems involved in memory and cognition: the PMAT framework. Prog Brain Res 2015;219:45–64.
[10] Knopman DS, Jack CR Jr, Lundt ES, Wiste HJ, Weigand SD, Vemuri P, et al. Role of beta-amyloidosis and neurodegeneration in subsequent imaging changes in mild cognitive impairment. JAMA Neurol 2015; 72:1475–83.
[11] Kantarci K, Schwarz CG, Reid RI, Przybelski SA, Lesnick TG, Zuk SM, et al. White matter integrity determined with diffusion tensor imaging in older adults without dementia: influence of amyloid load and neurodegeneration. JAMA Neurol 2014;71:1547–54.
[12] Mattsson N, Andreasson U, Zetterberg H, Blennow K. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol 2017;74:557–66.
[13] Gisslen M, Price RW, Andreasson U, Norgren N, Nilsson S, Hagberg L, et al. Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. EBioMedicine 2016;3:135–40.
[14] Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, Malaspina A, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS One 2013;8:e75091.
[15] Pereira JB, Westman E, Hansson O. Association between cerebrospinal fluid and plasma neurodegeneration biomarkers with brain atrophy in Alzheimer's disease. Neurobiol Aging 2017;58:14–29.
[16] Jefferson AL, Gifford KA, Acosta LM, Bell SP, Donahue MJ, Taylor Davis L, et al. The vanderbilt memory & aging project: study design and baseline cohort overview. J Alzheimer's Dis 2016;52:539–59.
[17] Aisen PS, Petersen RC, Donohue MC, Gamst A, Raman R, Thomas RG, et al. Clinical core of the Alzheimer's disease neuroimaging initiative: progress and plans. Alzheimers Dement 2010;6:239–46.
[18] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:270–9.
[19] Kresge HA, Khan OA, Wegener MA, Liu D, Terry JG, Nair S, et al. Subclinical compromise in cardiac strain relates to lower cognitive performances in older adults. J Am Heart Assoc 2018;7:e007562.
[20] Jefferson AL, Liu D, Gupta DK, Pechman KR, Watchmaker JM, Gordon EA, et al. Lower cardiac index levels relate to lower cerebral blood flow in older adults. Neurology 2017;89:2327–34.
[21] D'Agostino RB, Wolf PA, Belanger AJ, Kannel WB. Stroke risk profile: adjustment for antihypertensive medication. the framingham Study. Stroke 1994;25:40–3.
[22] Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-brain barrier breakdown in the aging human hippocampus. Neuron 2015;85:296–302.
[23] Bowman GL, Dayon L, Kirkland R, Wojcik J, Peyratout G, Severin IC, et al. Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults. Alzheimers Dement 2018;14:1640–50.
[24] Kalm M, Bostrom M, Sandelius A, Eriksson Y, Ek CJ, Blennow K, et al. Serum concentrations of the axonal injury marker neurofilament light protein are not influenced by blood-brain barrier permeability. Brain Res 2017;1668:12–9.
[25] Ahmed RM, Paterson RW, Warren JD, Zetterberg H, O'Brien JT, Fox NC, et al. Biomarkers in dementia: clinical utility and new directions. J Neurol Neurosurg Psychiatry 2014;85:1426–34.
[26] Lin YS, Lee WJ, Wang SJ, Fuh JL. Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. Sci Rep 2018;8:17368.
[27] Araque Caballero MA, Suarez-Calvet M, Duering M, Franzmeier N, Benzinger T, Fagan AM, et al. White matter diffusion alterations precede symptom onset in autosomal dominant Alzheimer's disease. Brain 2018;141:3065–80.
[28] Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. New Engl J Med 2012;367:795–804.
[29] Behl C, Ziegler C. Beyond amyloid–widening the view on Alzheimer's disease. J Neurochem 2017;143:394–5.