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Featured Article

Differential effects of neurodegeneration biomarkers on subclinical cognitive decline

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Abstract

Introduction: Neurodegeneration appears to be the biological mechanism most proximate to cognitive decline in Alzheimer's disease. We test whether t-tau and alternative biomarkers of neurodegeneration—neurogranin and neurofilament light protein (NFL)—add value in predicting subclinical cognitive decline.

Methods: One hundred fifty cognitively unimpaired participants received a lumbar puncture for cerebrospinal fluid and at least two neuropsychological examinations (mean age at first visit = 59.3 ± 6.3 years; 67% female). Linear mixed effects models were used with cognitive composite scores as outcomes. Neurodegeneration interactions terms were the primary predictors of interest: age × NFL or age × neurogranin or age × t-tau. Models were compared using likelihood ratio tests.

Results: Age × NFL accounted for a significant amount of variation in longitudinal change on preclinical Alzheimer's cognitive composite scores, memory composite scores, and learning scores, whereas age × neurogranin and age × t-tau did not.

Discussion: These data suggest that NFL may be more sensitive to subclinical cognitive decline compared to other proposed biomarkers for neurodegeneration.

Keywords: Alzheimer's disease; Biomarkers; Cognition; Neurodegeneration; Amyloid; Cognitive decline

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1. Introduction

Establishing biomarkers that are predictive of cognitive decline before the onset of dementia is expected to facilitate early intervention in AD. Recently, the amyloid, tau, and neurodegeneration [AT(N)] research framework has been proposed as a biologically based method for classifying individuals into varying risk categories [1]. In doing so, the aim is to use biomarker status to predict rate of cognitive decline and onset of dementia symptoms [2]. However, it is not yet clear which combination of biomarkers lends the greatest predictive value.

In the current AT(N) framework, it is proposed that amyloidosis can be measured with cerebrospinal fluid (CSF) biomarkers Aβ42 or Aβ42/Aβ40, neurofibrillary tangles with phosphorylated tau (p-tau), and neurodegeneration with total tau (t-tau) [1]. Yet this framework will continue to undergo refinements as new biomarkers are discovered and tested. Indeed, a recently published framework from the National Institute on Aging–Alzheimer's Association (NIA-AA) suggested that other biomarkers of neurodegeneration—including neurofilament light protein (NFL) and neurogranin (NG)—should be investigated for potential added value in predicting cognitive decline [2].

NFL is a key cytoarchitectural protein present primarily in large-caliber myelinated axons [3]. As such, increased NFL in CSF suggests degeneration or damage of these axons. NG, on the other hand, is expressed within dendritic spines on postsynaptic neurons and plays a key role in plasticity, synapse repair, and long-term potentiation [4]. Increased concentrations of CSF NG signify a loss of synaptic integrity [5–7].

A small number of studies have compared these biomarkers across diseases and stages of dementia, as well as examined their diagnostic accuracy [8–10]. Other research has investigated their relationships with longitudinal amyloid accumulation, structural brain changes, cognition, and brain metabolism in older populations of participants with varying diagnoses (e.g., cognitively unimpaired, mild cognitive impairment [MCI], and AD dementia) [8,11]. Yet less is known about the specific role these biomarkers play in predicting longitudinal, subclinical cognitive decline in younger populations. This is the major goal of the present study. Our primary hypothesis is that NFL and NG will be independently associated with subclinical cognitive decline and that they will provide additional predictive value compared to t-tau.

2. Methods

2.1. Participants

Demographic characteristics and biomarker levels for all participants are available in Table 1. One hundred fifty participants (67% female) were recruited from the Wisconsin Registry for Alzheimer's Prevention (WRAP) [12]. This observational cohort consists of participants who were cognitively unimpaired at baseline and middle-aged, with and without parents with AD. All participants are community dwelling and underwent examination (including lumbar puncture for research purposes) at the University of Wisconsin–Madison. Lumbar punctures were performed between 2009 and 2014, and neuropsychological examinations were performed between 2005 and 2017. Cognitive data were taken from wave 2 of the WRAP study onward because of the expansion of the cognitive battery at that time. The current sample was enriched for AD risk via a parental history of AD (N = 108; 72%) and included some participants positive for at least one allele of the known AD genetic risk factor *apolipoprotein E* ε4 (*APOE* ε4) (N = 56; 37%). Participants with dementia or MCI were excluded from this study, and no participants who had converted to MCI or dementia over the course of their cognitive visits were included.

2.2. Standard protocol approvals, registrations, and patient consents

The University of Wisconsin's institutional review board approved all portions of this study, and each participant provided written informed consent before all procedures.

2.3. Cerebrospinal fluid analyses

Cross-sectional CSF was used in the present study. CSF biomarker collection, assays, and postprocessing analysis

Table 1
Participant characteristics

Sample characteristics	Value
2 Cognitive visits, N (% of sample)	150 (100)
3 Cognitive visits, N (% of sample)	141 (94)
4 Cognitive visits, N (% of sample)	101 (67)
5 Cognitive visits, N (% of sample)	17 (11)
Age at baseline cognitive visit, years	59.3 (6.3)
Age at LP, years	61.0 (6.5)
Age difference between LP and cognitive visits, years	1.7 (2.9)
Female, N (% female)	101 (67)
Parental history of AD, N (% positive)	108 (72)
APOE ε4, N (% positive)	56 (37)
WRAT-3 Reading Subtest Raw Score	51.6 (4.3)
MMSE	29.3 (0.9)
NFL, pg/mL	676 (350)
Neurogranin, pg/mL	388 (176)
$A\beta_{42}/A\beta_{40}$	0.09 (0.02)
P-tau, pg/mL	47 (18)
T-tau, pg/mL	325 (125)
Amyloid positive, N (% of sample)	46 (31)
P-tau positive, N (% of sample)	20 (13)
Amyloid and P-tau positive, N (% of sample)	9 (6)

Values are mean (standard deviation) except where otherwise indicated.

Abbreviations: LP, lumbar puncture; AD, Alzheimer's disease; *APOE* ε4, apolipoprotein E gene ε4; WRAT, Wide Range Achievement Test; MMSE, Mini–Mental State Examination; NFL, neurofilament light protein; $A\beta_{42}/A\beta_{40}$, amyloid beta 42 and amyloid beta 40 peptide ratio; p-tau, tau phosphorylated at threonine 181; t-tau, total tau.

to account for batch-to-batch variation have been described previously [13–15]. We measured $A\beta_{42}$, $A\beta_{40}$, and tau phosphorylated at threonine 181 (p-tau), biomarkers that distinguish patients with dementia due to AD from controls [16] and are indicative of conversion from mild cognitive impairment to dementia [17]. In addition to these AD biomarkers, we examined markers of neurodegeneration: t-tau, NFL, and NG. These biomarkers have been associated with cognitive decline in MCI and are elevated in AD patients compared to controls [8]. To measure global amyloidosis, we conducted analyses using $A\beta_{42}/A\beta_{40}$ (rather than $A\beta_{42}$ alone) given that it is more closely associated with amyloid plaque burden measured with molecular brain imaging [18].

2.4. Cognitive composite scores

Longitudinal tests of cognition were used in the present study. To reduce measurement errors, improve the longitudinal stability of cognitive outcomes, and reduce type 1 errors associated with multiple comparisons, composite scores were computed for learning, memory, and executive function domains, as well as the preclinical Alzheimer's cognitive composite (PACC) [19]. Composite scores were created by computing z-scores from raw scores using the population means and standard deviations for each constituent test across all visits (hence, each individual participant's number of visits is accounted for). Then, the z-scores within each cognitive domain were averaged to produce the final composite. The tests falling into each composite are as follows:

PACC: Rey Auditory Verbal Learning Test (RAVLT) [20] total trials 1–5, Wechsler Memory Scale–Revised Logical Memory delayed recall [21], Wechsler Abbreviated Intelligence Scale–Revised [22], Digit Symbol Coding total items completed in 90 seconds, and the Mini–Mental State Examination [23]. This composite differs slightly from the originally proposed composite [19], which includes the total recall score from the Free and Cued Selective Reminding Test [24] rather than the RAVLT.

Learning: RAVLT [20] total trials 1–5, Wechsler Memory Scale–Revised Logical Memory [21] immediate recall, and the Brief Visuospatial Memory Test (BVMT-R) immediate recall [25].

Memory: RAVLT long-delay free recall [20], Wechsler Memory Scale–Revised Logical Memory delayed recall [21], and the BVMT-R delayed recall.

Executive functioning: Trail Making Test Part B (TMT B) [26] total time to completion, Stroop Neuropsychological Screening Test color-word interference total items completed in 120 seconds [27], and the Wechsler Abbreviated Intelligence Scale–Revised [22] Digit Symbol Coding total items completed in 90 seconds. Because higher raw scores on the TMT B are indicative of poorer performance, the z-score for this test was reversed so that higher composite scores were indicative of better performance.

2.5. Statistical analyses

Pearson correlations were performed between biomarkers for descriptive purposes. For primary analyses, complete cases were used in linear mixed effects models within the R lme4 package [28], where the PACC and composite scores for memory, executive function, learning were used as separate outcomes [15]. In all analyses, a reading score from each participant wave 2 visit (their baseline visit for this study) was included as a covariate to control for overall educational and intellectual attainment: the Wide Range Achievement Test 3rd Edition reading subtest [29]. Fixed effects included sex, *APOE* ε4 positivity, Wide Range Achievement Test reading score, age at each cognitive visit (centered around the mean baseline age of the sample), age difference in years between the single time point LP and each cognitive testing session, amyloid positivity ($A\beta42/A\beta40 \leq 0.09$) [30], phosphorylated tau positivity (p-tau $\geq$ 59.50 pg/mL) [30], age $\times$ amyloid positivity, age $\times$ p-tau positivity. In addition to these covariates, each model included one of the following terms of interest and its interaction with age: NFL or NG or t-tau. These variables were standardized before statistical analysis. All models included random effects of intercept and slope nested within subject. Nested models with and without the interaction term of interest were compared using the Akaike information criterion (AIC) and likelihood ratio tests. Statistical significance was inferred at a familywise alpha of 0.05, and a Bonferroni correction was applied for the three primary models tested within each cognitive composite (final $P = .017$). Variance inflation factors were examined to assess for model multicollinearity.

2.6. Data availability

For purposes of replicating procedures and results, the data used in this study can be made available upon request.

3. Results

Table 2 shows Pearson correlations between biomarkers. For descriptive purposes, readers should note the relatively high correlation between t-tau and p-tau, and the relatively low correlations between NFL and other biomarkers.

Table 2
Pearson correlation matrix between biomarkers used in the present study

Biomarker	$A\beta_{42}/A\beta_{40}$	P-tau	T-tau	Neurogranin	NFL
$A\beta_{42}/A\beta_{40}$	1				
P-tau	−0.14	1			
T-tau	−0.31	0.80	1		
Neurogranin	−0.28	0.64	0.74	1	
NFL	−0.12	0.26	0.32	0.10	1

Abbreviations: NFL, neurofilament light protein; $A\beta_{42}/A\beta_{40}$, amyloid beta 42 and amyloid beta 40 peptide ratio; p-tau, tau phosphorylated at threonine 181; t-Tau, total tau.

Table 3
Statistical summary of the preclinical Alzheimer's cognitive composite (PACC), memory composite, and learning composite models, including beta coefficients and standard errors

	Linear mixed effects models								
	PACC			Memory composite			Learning composite		
Predictor variable	T-tau	NG	NFL	T-tau	NG	NFL	T-tau	NG	NFL
Age (centered)	−0.031*	−0.031*	−0.032*	−0.030†	−0.029†	−0.032*	−0.025*	−0.024*	−0.027*
	(0.006)	(0.007)	(0.006)	(0.009)	(0.009)	(0.009)	(0.007)	(0.007)	(0.007)
Sex	0.158‡	0.153‡	0.143‡	0.356*	0.344*	0.344*	0.188‡	0.179‡	0.186‡
	(0.070)	(0.070)	(0.068)	(0.103)	(0.103)	(0.102)	(0.077)	(0.076)	(0.077)
APOE ε4	−0.030	−0.032	−0.031	−0.151	−0.146	−0.142	−0.114	−0.104	−0.104
	(0.070)	(0.071)	(0.069)	(0.103)	(0.104)	(0.102)	(0.078)	(0.078)	(0.077)
WRAT Score	0.040*	0.039*	0.030*	0.056*	0.054*	0.049*	0.039*	0.037*	0.035*
	(0.008)	(0.008)	(0.008)	(0.012)	(0.012)	(0.012)	(0.009)	(0.009)	(0.009)
Age difference	0.008	0.007	0.003	0.041*	0.041*	0.038*	0.040*	0.040*	0.038*
	(0.007)	(0.007)	(0.007)	(0.009)	(0.009)	(0.009)	(0.007)	(0.007)	(0.007)
Amyloid positivity	−0.101	−0.075	−0.038	−0.169	−0.101	−0.108	−0.139	−0.079	−0.120
	(0.082)	(0.081)	(0.077)	(0.120)	(0.117)	(0.111)	(0.093)	(0.090)	(0.086)
P-tau positivity	−0.049	0.0001	0.065	0.041	0.189	0.157	0.078	0.216	0.121
	(0.110)	(0.110)	(0.096)	(0.162)	(0.161)	(0.139)	(0.125)	(0.123)	(0.107)
T-tau	0.044			0.068			0.028		
	(0.041)			(0.060)			(0.046)		
NG		0.009			−0.034			−0.068	
		(0.040)			(0.058)			(0.044)	
NFL			−0.012			0.017			0.040
			(0.041)			(0.059)			(0.046)
Age × amyloid positivity	−0.008	−0.008	0.001	−0.012	−0.012	−0.002	−0.006	−0.006	0.001
	(0.009)	(0.009)	(0.008)	(0.013)	(0.013)	(0.012)	(0.010)	(0.010)	(0.009)
Age × P-tau positivity	−0.018	−0.018	−0.001	−0.052†	−0.052†	−0.032	−0.029	−0.031	−0.015
	(0.014)	(0.014)	(0.012)	(0.020)	(0.020)	(0.017)	(0.016)	(0.016)	(0.014)
Age × T-tau	0.0001			0.006			0.004		
	(0.005)			(0.007)			(0.005)		
Age × NG		0.0002			0.006			0.004	
		(0.005)			(0.007)			(0.005)	
Age × NFL			−0.021*			−0.016†			−0.011‡
			(0.004)			(0.006)			(0.005)
Constant	−2.080*	−2.029*	−1.557*	−2.944*	−2.845*	−2.605*	−1.989*	−1.935*	−1.806*
	(0.403)	(0.403)	(0.398)	(0.591)	(0.591)	(0.596)	(0.443)	(0.439)	(0.449)
Observations	559	559	559	559	559	559	559	559	559
AIC	548.5	549.6	511.2	866.8	868.1	860.1	661.8	660.4	656.7

Abbreviations: *APOE* ε4, apolipoprotein E gene ε4; WRAT, Wide Range Achievement Test; age difference, years between lumbar puncture and cognitive examinations; p-tau, tau phosphorylated at threonine 181; t-tau, total tau; NG, neurogranin; NFL, neurofilament light protein; AIC, Akaike information criterion.
*$P < .001$.
†$P < .01$.
‡$P < .05$.

Summary statistics for the PACC, memory, and learning composite models are displayed in Table 3 and statistics for the executive function model are displayed in Table 4. Plots for the PACC, memory composite, and learning composite are in Figs. 1–3, respectively (while linear mixed effects analyses were performed across all participants regardless of biomarker status, Figs. 1–3 display results for biomarker negative and biomarker positive groups for illustrative purposes).

PACC: Likelihood ratio tests indicated that age × NFL accounted for a significant amount of variation in longitudinal change on PACC scores (χ [2](1) = 26.9, $\beta = -0.021$, $P < .001$), whereas age × NG (χ [2] (1) = 0.001, $\beta = 0.0002$, $P = .96$) and age × t-tau (χ [2](1) = 0.0004, $\beta = 0.0001$, $P = .99$) did not. As seen in Table 3, the full NFL model (including the age × NFL interaction) had the lowest AIC of all PACC models.

Memory: Likelihood ratio tests indicated that age × NFL also accounted for a significant amount of variation in longitudinal change on the memory composite (χ [2](1) = 7.8, $\beta = -0.016$, $P = .005$), whereas age × NG (χ [2](1) = 0.74, $\beta = 0.006$, $P = .39$) and age × t-tau (χ [2](1) = 0.59, $\beta = 0.006$, $P = .44$) did not. As seen in Table 3, the full NFL model (including the age × NFL interaction) had the lowest AIC of all memory composite models.

Learning: Likelihood ratio tests indicated that age × NFL also accounted for a significant amount of variation in longitudinal change on the learning composite (χ [2](1) = 5.89, $\beta = -0.011$, $P = .015$), whereas age × NG (χ [2](1) = 0.67, $\beta = 0.004$, $P = .42$) and age × t-tau

Table 4
Statistical summary of the executive function composite models, including beta coefficients and standard errors

Linear mixed effects models			
	Executive function composite		
Predictor variable	T-tau	NG	NFL
Age (centered)	−0.068*	−0.068*	−0.066*
	(0.008)	(0.008)	(0.008)
Sex	0.072	0.077	0.066
	(0.096)	(0.096)	(0.097)
APOE ε4	0.148	0.149	0.144
	(0.095)	(0.096)	(0.095)
WRAT Score	0.037*	0.038*	0.035†
	(0.011)	(0.011)	(0.011)
Age difference	0.029*	0.030*	0.028*
	(0.008)	(0.008)	(0.008)
Amyloid positivity	−0.033	−0.057	−0.046
	(0.108)	(0.106)	(0.102)
P-tau positivity	0.012	−0.035	−0.018
	(0.149)	(0.148)	(0.131)
T-tau	−0.039		
	(0.055)		
NG		−0.007	
		(0.054)	
NFL			−0.050
			(0.052)
Age × amyloid positivity	−0.0002	−0.001	−0.002
	(0.009)	(0.009)	(0.009)
Age × P-tau positivity	0.005	0.004	0.002
	(0.014)	(0.014)	(0.012)
Age × T-tau	−0.003		
	(0.005)		
Age × NG		−0.002	
		(0.005)	
Age × NFL			−0.0001
			(0.004)
Constant	−1.898*	−1.941*	−1.797†
	(0.541)	(0.541)	(0.552)
Observations	559	559	559
AIC	519.4	520.0	519.2

Abbreviations: *APOE* ε4, apolipoprotein E gene ε4; WRAT, Wide Range Achievement Test; age difference, years between lumbar puncture and cognitive exams; p-tau, tau phosphorylated at threonine 181; t-tau, total tau; NG, neurogranin; NFL, neurofilament light protein; AIC, Akaike information criteria.
*$P < .001$.
†$P < .01$.

(χ [2](1) = 0.41, β = 0.004, P = .52) did not. As seen in Table 3, the full NFL model (including the age × NFL interaction) had the lowest AIC of all learning composite models.

Executive function: No biomarker interaction terms (age × NFL, age × NG, age × t-tau) were significant for the executive function composite (Table 4). Multicollinearity was not a significant issue in any model (all variance inflation factors < 3).

4. Discussion

The AT(N) research framework aims to create a biologically based definition of Alzheimer's disease and to classify individuals based on etiology and risk of future cognitive decline [1]. However, it is not clear which specific biomarkers will produce the greatest value in predicting cognitive decline before the onset of dementia. Here, we demonstrate that—in a cognitively unimpaired, late middle-aged cohort of individuals at risk for AD—higher levels of NFL are associated with cognitive decline on the PACC as well as learning and memory cognitive composites after accounting for amyloid and p-tau. Further, NFL exhibits stronger associations with cognitive outcomes compared to NG or t-tau.

Although the currently proposed AT(N) framework includes t-tau as a biomarker for neurodegeneration, the utility of this measure in the context of AD remains unclear. It is typically correlated with p-tau, making it difficult to draw conclusions about its independent influence or to build robust statistical models including both these biomarkers [2,31]; indeed, in this sample, the Pearson correlation between p-tau and t-tau is 0.80 (see Table 2 for full CSF biomarker correlation matrix). Still, as mentioned in the Results section, multicollinearity diagnostics were normal for all models herein. The fact that t-tau was not a significant predictor of cognitive decline in the present study underscores the need for more research on additional biomarkers for predicting incipient dementia.

The lack of robust findings for NG was unexpected as synaptic degeneration is thought to impact the progression from healthy cognition to dementia and may be predictive of neuronal loss [6,7,32]. In a cross-sectional study of 132 cognitively unimpaired participants from the WRAP and Wisconsin ADRC cohorts, NG was associated with poorer performance on the RAVLT delayed recall test [33]. Yet, there was no similar relationship found longitudinally for the composite memory score tested here. One possibility is that changes in cognitive composites may be more difficult to detect but are less confounded by measurement errors and are therefore more robust when detected. In longitudinal studies, NG has been observed to predict conversion from MCI to frank AD dementia, raising the possibility that increased NG is a robust predictor of cognitive decline only later in the disease course [34,35]. In partial support of this hypothesis, NG has also been associated with longitudinal cognitive decline, but only in amyloid-positive individuals [8]. Similarly, NG has been shown to be associated with regional brain atrophy only in amyloid-positive participants [36]. It is possible that the relationship between NG and cognitive decline is insufficiently robust to be measurable early in the disease or that elevated NG is an important factor only among individuals who have accumulated measurable AD neuropathology burden.

Yet the literature examining differences in biomarkers across neurodegenerative diseases suggests other interpretations for the lack of t-tau and NG findings in the present study. Although t-tau has been considered a marker of gross neurodegeneration and axonal atrophy, some observations

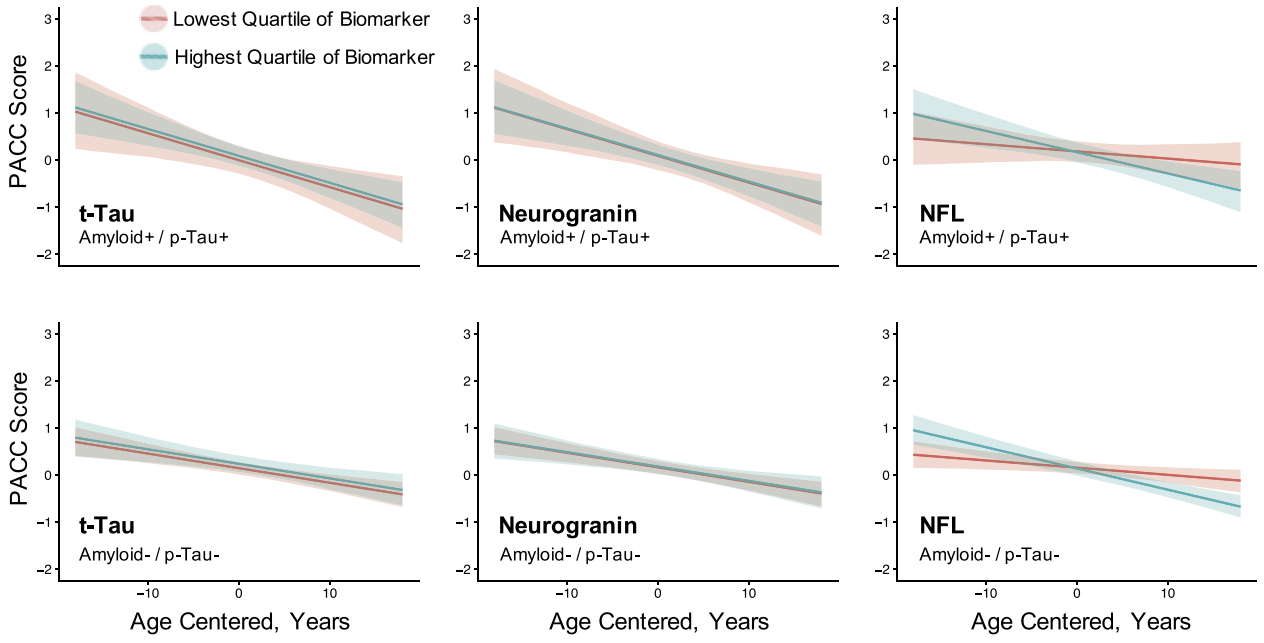

Fig. 1. Linear relationships between age (centered on the sample mean), Alzheimer's biomarker status, and standardized cognitive scores on the preclinical Alzheimer's cognitive composite (PACC), adjusted for covariates. The top row represents individuals positive for both amyloid and p-tau pathology, whereas the bottom row represents individuals negative on these biomarkers. Although linear mixed effects analyses were performed across all participants regardless of biomarker status, results for biomarker negative and biomarker positive groups are displayed here for illustrative purposes. Blue represents the highest quartile of t-tau, neurogranin, and NFL, and red the lowest quartile. Higher NFL, but not t-tau or neurogranin, was associated with longitudinal cognitive decline independent of amyloid and p-tau concentrations. Abbreviation: NFL, neurofilament light protein.

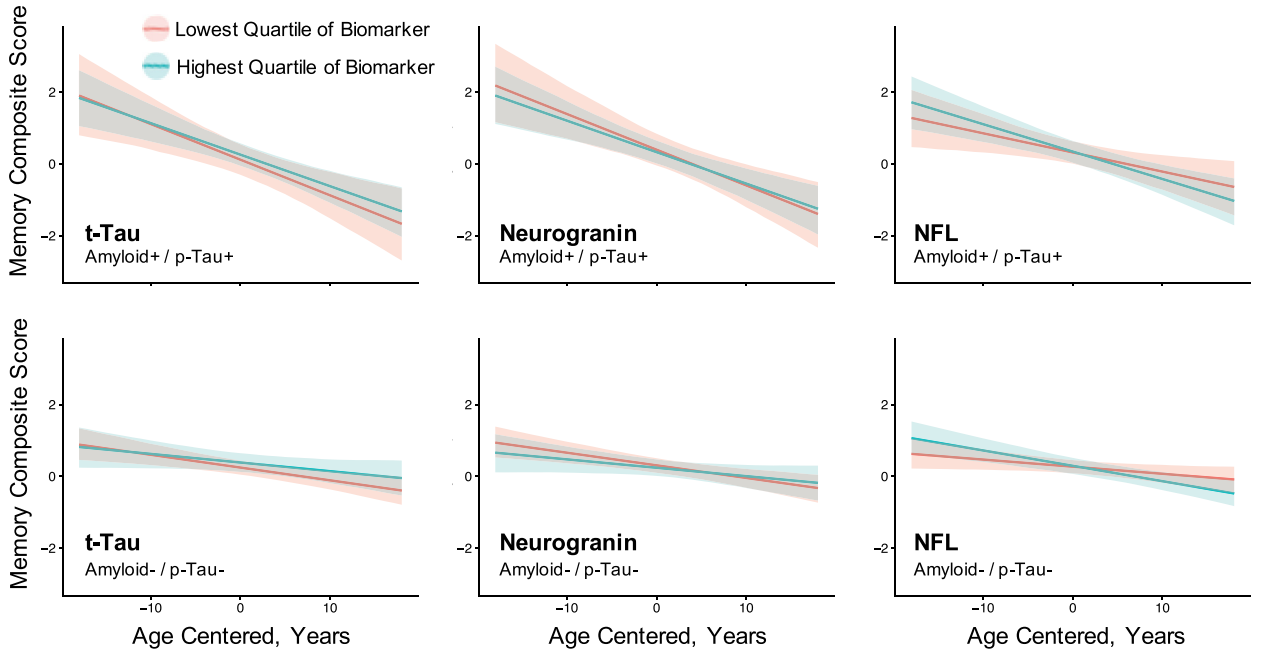

Fig. 2. Linear relationships between age (centered on the sample mean), Alzheimer's biomarker status, and standardized scores on the memory composite, adjusted for covariates. The top row represents individuals positive for both amyloid and p-tau pathology, whereas the bottom row represents individuals negative on these biomarkers. Although linear mixed effects analyses were performed across all participants regardless of biomarker status, results for biomarker-negative and biomarker-positive groups are displayed here for illustrative purposes. Blue represents the highest quartile of t-tau, neurogranin, and NFL, and red the lowest quartile. Higher NFL, but not t-tau or neurogranin, was associated with longitudinal cognitive decline independent of amyloid and p-tau concentrations. Abbreviation: NFL, neurofilament light protein.

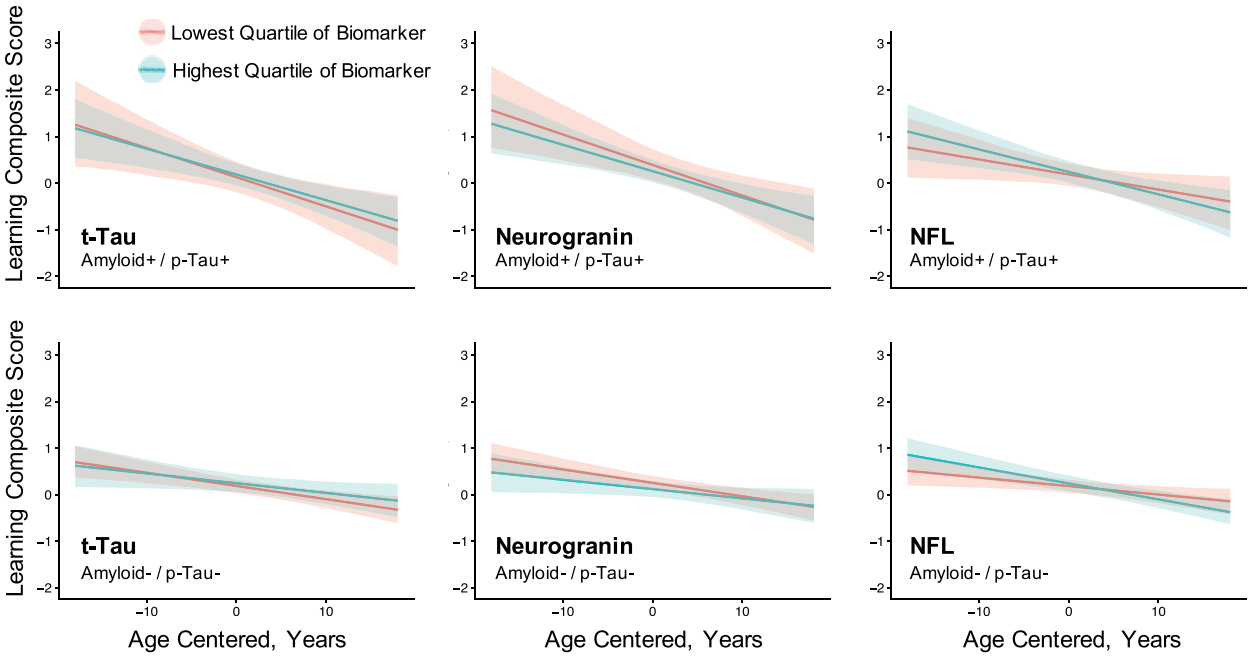

Fig. 3. Linear relationships between age (centered on the sample mean), Alzheimer's biomarker status, and standardized scores on the learning composite, adjusted for covariates. The top row represents individuals positive for both amyloid and p-tau pathology, whereas the bottom row represents individuals negative on these biomarkers. Although linear mixed effects analyses were performed across all participants regardless of biomarker status, results for biomarker-negative and biomarker-positive groups are displayed here for illustrative purposes. Blue represents the highest quartile of t-tau, neurogranin, and NFL, and red the lowest quartile. Higher NFL, but not t-tau or neurogranin, was associated with longitudinal cognitive decline independent of amyloid and p-tau concentrations. Abbreviation: NFL, neurofilament light protein.

do not fit with this interpretation. For example, t-tau elevations appear to be relatively specific to AD; t-tau concentrations are typically lower in patients with other neurodegenerative diseases, such as Parkinson's disease dementia, Lewy body dementia, and progressive supranuclear palsy [37–41]. Of course, this is not always the case: tau is elevated in Creutzfeldt-Jakob disease, adding further complexity to the role of t-tau in neurodegeneration [42]. With respect to NG, it is important to note that it is not entirely clear what this biomarker represents. If NG was a specific marker for synaptic degeneration, one would expect elevated levels in other neurodegenerative dementias; yet similar to CSF tau, CSF NG elevation is strikingly AD specific and may in fact be linked to amyloid-related synaptic damage [43,44].

An alterative interpretation, therefore, is that these biomarkers are specific to AD pathophysiology; that is, rather than reflecting overall neurodegeneration, CSF tau and NG are excreted from neurons in an AD-specific process, whereby tau undergoes hyperphosphorylation and neurons truncate and subsequently secrete t-tau, p-tau, and NG [45]. Neurofibrillary tangle development, compromised axonal transport, and degeneration may then occur in these affected neurons, which would follow the elevated concentrations of t-tau, p-tau, and NG detectable in CSF. This interpretation of CSF tau is supported by both animal and human data: Maia et al. found an Aβ-dependent increase in tau secretion into CSF in APP-transgenic mice in the absence of neurodegeneration [46]. In addition, stable isotope labeling experiments in humans revealed increased tau secretion into CSF in Aβ-positive cases [47]. To the best of our knowledge, similar data for NG do not yet exist.

Interpretations are more straightforward for NFL as a marker of neurodegeneration. NFL is present in large-caliber myelinated axons connecting temporal and frontal lobes [3,48] and is a crucial component of the neuronal cytoskeleton [49,50]. It is robustly elevated in many neurodegenerative diseases [48], appears to be relatively independent of amyloid and tau levels [8,36,51], and correlates with symptomology, progression, and survival [51,52]. From a disease mechanism standpoint, this research suggests an important role for axonal cytoarchitecture in the development of dementia. NFL may be an especially promising biomarker for neurodegeneration because it may be measurable in plasma [53]. Furthermore, because NFL was associated with cognitive decline while controlling for Aβ42/Aβ40 and p-tau in the present study, it may be useful as a predictive biomarker independent of obvious AD neuropathology. Future studies should test whether the results observed here can be replicated in blood-based tests of NFL.

There are several limitations of the present study that deserve note. First, although CSF $A\beta_{42}/A\beta_{40}$ and p-tau are widely used metrics of AD neuropathology, they do not capture regional variation in the deposition of amyloid and tau that may play a crucial role in predicting cognitive

decline [54]. Studies using amyloid and tau positron emission tomography (PET) will be invaluable for determining whether regional protein accumulation does in fact add value in predicting cognitive decline before the onset of dementia. The clinical significance of the cognitive decline observed in this study remains unclear, although longitudinal study of this population will lend insight into whether subclinical cognitive decline on these composite tests is a robust and acute predictor of MCI or dementia. In addition, generalizability to other populations may be difficult: the vast majority of this sample is Caucasian and highly educated. Intensive recruitment of underrepresented populations is currently underway.

It is also worth noting the lack of amyloid-related cognitive decline in this study. Other studies have demonstrated that beta-amyloid deposition is associated with cognitive decline [55–58], including on individual cognitive tests [30], and on cognitive composite scores [15]. Many of the previous studies had larger sample sizes than the present study and examined relationships between different independent variables (CSF vs. PiB-PET) with different dependent variables (individual cognitive tests vs. composite scores). Clearly, more work will be required to understand the independent effect of amyloid on cognitive decline, and whether specific neurodegenerative processes (like axonal degeneration) mediate this relationship.

Although the results of this study provide data that may guide selection of markers within the AT(N) framework, other modalities are also expected to show utility. [^{18}F] fluorodeoxyglucose positron emission tomography and structural magnetic resonance imaging are common methods of indexing neurodegenerative processes [2], and additional sensitive brain imaging metrics are undergoing testing and development—including synaptic vesicle glycoprotein 2A for indexing synaptic density [59]. Ultimately, research comparing the utility of each of these techniques will be crucial for creating a valid, biologically based definition of AD etiology and risk of cognitive decline.

Although continuous variables are useful for describing biological phenomena from a research perspective, cut points may ultimately be more useful for bringing research results into clinical care. Because the AT(N) framework aims to categorize individuals based on biomarker status, an important next step in this research will be to create clinically relevant cut points for neurodegeneration biomarkers, including NFL. To that end, it will be crucial to follow these participants longitudinally to determine whether NFL is also predictive of faster decline to MCI or dementia, rather than the subclinical decline measured here.

5. Conclusion

The data presented here suggest that NFL may lend additional value to the AT(N) framework and that it may be more sensitive in detecting cognitive decline before the onset of dementia than either t-tau or NG. Our findings underscore the idea that axonal degeneration may play an important role in cognitive decline before the onset of dementia due to AD—or perhaps independent of AD, given that NFL was associated with cognitive decline independently of Aβ and p-tau neuropathology. This study also calls for more research on how t-tau, NG, NFL, and other neurodegeneration biomarkers contribute to the pathogenesis of Alzheimer's disease.

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RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed peer-reviewed literature using traditional methods (e.g., PubMed, Google Scholar). There are a number of papers published on neurodegeneration biomarkers across disease stages (cognitively unimpaired, mild cognitive impairment, and dementia) but fewer that examine longitudinal change in a cognitively unimpaired cohort.
2. Interpretation: The data presented here suggest that NFL may lend additional value to the AT(N) framework and that it may be more sensitive in detecting cognitive decline before the onset of dementia than either t-tau or neurogranin. Our findings underscore the idea that axonal degeneration may play an important role in cognitive decline before the onset of dementia due to AD—or perhaps independent of AD, given that NFL was associated with cognitive decline independently of Aβ and p-tau neuropathology.
3. Future directions: Research is needed to understand the biological mechanism linking increased NFL and cognitive decline, as well as research using more easily attained blood-based biomarkers.

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