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Serum Nf-L and GFAP are associated with incident dementia and dementia mortality in older adults: The Cardiovascular Health Study

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COMPETING INTERESTS

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CONSENT STATEMENT

All CHS participants provided voluntary written informed consent.

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Abstract

INTRODUCTION: Circulating neurofilament light chain (Nf-L) and glial fibrillary acidic protein (GFAP) have been independently associated with dementia risk. Their additive association, and their associations with dementia-specific mortality have not been investigated.

METHODS: We associated serum Nf-L, GFAP, total tau and ubiquitin carboxyl-terminal hydrolase-L1, measured in 1,712 dementia-free adults, with 19-year incident dementia and dementia-specific mortality risk, and with 3-year cognitive decline.

RESULTS: In adjusted models, being in the highest vs. lowest tertile of Nf-L or GFAP associated with a hazard ratio (HR) of 1.49 (1.20–1.84) and 1.38 (1.15–1.66) for incident dementia, and 2.87 (1.79–4.61) and 2.76 (1.73–4.40) for dementia-specific mortality. Joint third vs. first tertile exposure further increased risk; HR=2.06 (1.60–2.67) and 9.22 (4.48–18.9). Nf-L was independently associated with accelerated cognitive decline.

DISCUSSION: Circulating Nf-L and GFAP may, independently and jointly, provide useful clinical insight regarding dementia risk and prognosis.

Keywords

3MSE; blood-based biomarkers; cognition; dementia; longitudinal; mortality; risk factor; serum

1. INTRODUCTION

Blood-based biomarkers of neurodegeneration are potentially critical tools in the global fight against Alzheimer's disease and related dementias [1]. As neurodegenerative biomarkers, these analytes are not necessarily specific for a single pathophysiology but, as markers of neuronal injury and death, may provide predictive and staging information across dementia subtypes, analogous to the broad use of high-sensitivity troponin as a biomarker of myocyte damage in cardiac disease [2]. Moreover, if shown to predict future incidence of dementia among individuals at risk, biomarkers of preclinical disease could assist in recruitment to future preventive trials that may enable disease-modifying intervention prior to a critical loss of neurons.

Recent advances in quantification have brought at least two such biomarkers to the fore: neurofilament light chain (Nf-L; a biomarker of axonal injury) and glial fibrillary acidic protein (GFAP; a marker of astrocytic injury). Although both proteins have been associated with dementia risk [3–7], their potential as complementary biomarkers capturing related disease processes, and their association with dementia-specific mortality remain to be explored [8, 9].

To determine the prospective independent and additive associations of Nf-L and GFAP with dementia-related outcomes, we used the highly sensitive neurology four-plex A assay (N4PA) to quantify serum levels of Nf-L, GFAP, and two other brain-derived proteins, total tau (t-tau) and ubiquitin carboxyl-terminal hydrolase L1 (UCHL1), in older adults enrolled in the Cardiovascular Health Study (CHS). We previously found that Nf-L and

GFAP, in particular, were associated with worsening white matter lesions over a 5-year period [10]. Building on those findings, we leverage the 19-years of follow-up in CHS and its representation of individuals in their eighth and ninth decades–when dementia incidence is highest but representation in research often low–to relate serum N4PA biomarkers to incident dementia, dementia-specific mortality, and trajectories of cognitive decline.

2. METHODS

2.1. Study population and design

CHS is a prospective cohort study of older adults that recruited from four field centers in the US [11]. Eligibility required individuals to be aged ≥65 years, planning to remain in their area of residence for at least three years, and to not be institutionalized, wheelchair-dependent or undergoing cancer treatment. CHS was approved by site-specific institutional review boards and at the University of Washington Coordinating Center. All participants provided voluntary written informed consent.

Enrollment and baseline data collection of 5,201 participants, randomly identified and recruited from Medicare-eligibility lists, commenced in 1989–90 and was followed by a second round of enrollment of 687 predominantly African American individuals in 1992–93. Annual follow-up visits took place through 1998–99, whereafter bi-annual telephone calls, cardiovascular event and mortality adjudication, and Medicare claim records were used to collect data on health status and relevant events.

The current study investigated the 2,145 CHS participants who provided fasting blood samples at the 1996–97 CHS clinical examination that were used to quantify N4PA levels. Because this examination included a routine oral glucose tolerance test, eligibility required that participants be free of treated diabetes. Prior to statistical analysis, we further excluded 134 individuals who had clinical dementia at the 1996–97 visit—hereafter referred to as baseline–and 299 who had incomplete data.

2.2. Data collection

2.2.1. Serum biomarkers—Previously unthawed serum samples from 1996–97 were used to measure Nf-L, GFAP, t-tau, and UCHL1 on the Simoa™ Human Neurology 4-Plex A assay (N4PA, Quanterix™) at the CHS Central Laboratory at the University of Vermont. The inter-assay coefficients of variation were 8.2% for GFAP, 9.3% for Nf-L, 10.1% for t-tau, and 21.6% for UCHL1.

2.2.1. Assessment of dementia morbidity and mortality status—The ancillary CHS Cognition Study adjudicated incident cases of dementia that occurred between 1992–93 and 1998–99. Thereafter, participants at the Pittsburgh CHS field center continued to be followed prospectively for incident dementia through 2015 [12, 13]. For the other three sites, incident dementia cases were identified through the following auxiliary sources: appointment of a proxy due to impaired cognitive function, medication use (Donepezil, Galantamine, Memantine, Rivastigmine, or Tacrine), Medicare claims that included relevant ICD-9 codes (290.xx, 294.xx, 331.0, 331.1, 331.2, 331.82, 331.83, 331.9, or 438.0), and

adjudicated cause of death. Dementia-specific mortality refer to dementia being noted as the adjudicated cause of death [14].

2.2.2. Assessment of cognitive scores—The modified mini-mental status examination (3MSE, [15]) and the digit symbol substitution test (DSST) were administered annually from 1989–90 through 1998–99. When participants were unable to attend an examination in-person, published telephone-based measures of global cognitive function were obtained [16].

2.2.3. Covariates—Data used for covariate adjustment were collected at the same clinical examination as the serum samples used for N4PA protein measurements (1996–97). Age, sex, race, education, smoking status, alcohol intake and physical functioning were self-reported. Medication use was obtained using a validated medication inventory [17], and physical measurements were taken on-site personnel. Cardiovascular disease histories were obtained from participant and/or proxy interviews, and adjudicated by central committees [18]. Estimated glomerular filtration rate (eGFR) was calculated using creatinine and cystatin [19]. Genotyping of the apolipoprotein E (*APOE*) gene was performed only for participants who consented to genetic data collection, using polymerase chain reaction [20].

2.3. Statistical analysis

Primary analysis comprised of Cox proportional hazards regression models used to assess the independent associations between the N4PA proteins–both on a continuous scale and categorized by tertiles–and the risk of incident dementia and dementia-specific mortality. The linear trend across tertiles was tested using the median of each tertile as a continuous variable. We adjusted for baseline age, sex, race, education, and study site in the first model; and baseline body mass index, smoking status, alcohol consumption status, systolic blood pressure, total cholesterol, fasting plasma glucose, eGFR, C-reactive protein, activities of daily living limitation, *APOE* ε4 carrier status, antihypertensive medication use, lipid lowering medication use, and history of coronary heart disease, myocardial infarction, stroke and heart failure, additionally in Model 2. Our third model additionally included a mutual adjustment of the N4PA biomarkers to evaluate their independent contributions.

In sensitivity analysis, we excluded events occurring within the first two years to account for possible undiagnosed cases. Post-hoc, we explored whether the observed associations were equally strong in participants on either side of the median baseline age (77 years) to determine the utility of these biomarkers at early- vs. late-old age. Similarly, to determine whether biomarkers were differentially useful immediately vs. long before dementia diagnosis or death, we repeated analyses in data stratified by median time-to-dementia diagnosis, and median time-to-death. In secondary analysis, we evaluated the potential interaction between NF-L and GFAP in their association with the risk of incident dementia and dementia-specific mortality, using the interaction term between Nf-L and GFAP levels, and comparing associations estimates across the nine joint-tertile groups (i.e., Nf-L_{T1}–$GFAP_{T1}$, Nf-L_{T1}–$GFAP_{T2}$, Nf-L_{T1}–$GFAP_{T3}$ etc.).

To account for the potential misclassification in dementia diagnosis at clinic sites where auxiliary data sources were used, we incorporated probabilistic bias analysis (PBA) in

Cox regression models. This method corrects for the expected misclassification and adds appropriate uncertainty in the estimates of the parameters of interest and has been successfully applied to CHS data previously [21]. In this study, PBA was implemented using a model-based approach, where the probability of correct classification was estimated by logistic regression with age, sex, race, educational attainment, and the N4PA protein levels as the predictors. A bootstrapped method with 10,000 iterations was used to obtain the averaged parameters of interest and standard errors.

Finally, to determine the association between the N4PA proteins and three-year (scores obtained between 1996–97 and 1998–99) 3MSE and DSST trajectories, we used the time/protein interaction term coefficient from random-intercept linear mixed effect models. Most participants contributed to all three time points: 1,461 for 3MSE and 1,412 for DSST. Additionally, 162 and 199 participants contributed two time points, and 89 and 101 provided only baseline data to the 3MSE and DSST analysis, respectively. In addition to the covariates adjusted for previously, we included a sex/time interaction term to address the longitudinal confounding by sex on the trajectories of cognitive decline.

Regarding descriptive statistics, we report data as median (25^{th} percentile—75^{th} percentile) and correlation estimates as Spearman's rank coefficients. Fisher's Exact and Kruskal-Wallis rank sum tests were used to compare biomarkers across N4PA tertiles. N4PA levels were natural log transformed and converted to z-scores prior to linear modelling. All tests were two-sided and considered statistically significant at $p< 0.05$. Analyses were performed using R software version 4.1.3 [22].

3. RESULTS

The baseline study population was 69 to 96 years old and included 1,042 women (61%) and 218 (13%) Black participants. The N4PA biomarkers were modestly correlated, with the strongest correlation observed between Nf-L and GFAP (ρ=0.46). Participants with lower Nf-L were more likely to be Black, and less likely to have a history of cardiovascular disease or be on antihypertensive medication ($p< 6.1 \times 10^{-04}$). On the other hand, those with lower GFAP had lower average CRP and fasting glucose levels ($p< 3.1 \times 10^{-05)}$). For both biomarkers, lower levels were more likely to be observed in males, and with younger age, lower t-tau and UCHL1, and higher BMI and eGFR ($p< 6.3 \times 10^{-04}$). The distribution of these and other demographics and dementia risk factors across Nf-L and GFAP tertiles are reported in Table 1 (p-values and extended summary, including across t-tau and UCHL1 tertiles in eTable 1).

Participants were followed for a median of 11.6 (6.8–16.7) years. During this time, 975 incident dementia cases were ascertained using adjudicated or auxiliary data (median time-to-diagnosis, 8.6 [5.0–13.0] years) and 183 dementia-specific deaths occurred (median time-to-dementia-death=11.8 [8.5–14.5] years).

3.1. Circulating N4PA proteins and clinical dementia outcomes

Elevated baseline Nf-L and GFAP levels associated with a graded increase in incident dementia and dementia-specific mortality risk (eFigure 1, $P_{trend}< .001$ for all Nf-L and

GFAP analyses). In fully adjusted models (Model 3), participants in the highest vs. lowest tertile of Nf-L or GFAP were at a respective 49% (hazard ratio [HR], 1.49; 95% CI, 1.20–1.84) and 38% (HR, 1.38; CI, 1.15–1.66; p< .001) higher risk of incident dementia, and more than a 2.5-fold increased risk of dementia-specific mortality (HR, 2.87; 95% CI, 1.79–4.61; p< .001 for Nf-L, and HR, 2.76; 95% CI, 1.73–4.40; p< .001 for GFAP). Association estimates corresponding to risk per standard deviation higher protein level are reported in (Table 2).

For t-tau, a non-linear pattern of association was observed for both outcomes, consisting of a larger difference in risk between the first and second, compared to the third, tertile (eFigure 1, P_{trend}> .01). Upon full adjustment, t-tau only associated with dementia-specific mortality when the second and first, but not the extreme tertiles were compared. UCHL1 was not robustly associated with either outcome. Excluding dementia cases within the first two years of follow-up did not substantively alter our findings. Summary statistics of all tested models are presented in eTable 2.

In post-hoc analyses by stratified groups, we observed stronger Nf-L and GFAP associations with incident dementia in the older (≥77 years) vs. younger group. This pattern was not observed for dementia-specific mortality. Association estimates did not differ between early and late-developing case groups (Figure 1, eTable 3).

We next evaluated the independent and joint associations of Nf-L and GFAP with incident dementia and dementia-specific mortality using tertiles of each (Figure 2). Compared to the joint reference group with the lowest values of each, the category with the highest tertiles of both biomarkers experienced a two-fold higher risk of dementia morbidity [HR=2.06 (1.60–2.67)] and a nine-fold higher risk of dementia mortality [HR=9.22 (4.48–18.9)]. In general, having higher levels of either protein was associated with an increase in the risk of these clinical outcomes, regardless of the concurrent protein level, (i.e., higher Nf-L tertiles were positively associated with risk within individual tertiles of GFAP, and vice versa. In models using continuous protein levels, we observed a statistically significant interaction between Nf-L and GFAP in their association with dementia-specific mortality (p= .01), but not with incident dementia (p= .35).

3.2. Circulating N4PA proteins and cognitive decline

Higher baseline Nf-L and GFAP levels associated with a sharper 3-year decline in both measures of cognitive function (Table 3). GFAP did not contribute to these outcomes independent of Nf-L. Surprisingly, UCHL1 levels were associated with improved 3MSE scores when Nf-L, GFAP and t-tau were adjusted for (Model 3). Post-hoc, we observed a more significant Nf-L-associated decrease in cognitive scores among older (≥77 years) compared to younger groups (eTable 3).

4. DISCUSSION

In this prospective investigation of 1,712 cognitively healthy older adults, circulating Nf-L and GFAP were each associated with higher risk of dementia incidence and mortality independent of baseline demographics, body composition, lifestyle, vascular risk profile,

and cardiovascular disease history. These associations were graded and additive, with the strongest associations observed when Nf-L and GFAP levels were simultaneously elevated. Nf-L was also independently associated with a faster 3-year decline in cognitive function. We observed no robust associations between serum t-tau or UCHL1 and any of these outcomes.

Our results affirm the global associations between elevated serum Nf-L and GFAP and dementia risk, and add to the existing literature in at least three specific domains. First, we were able to address the role of age in these associations. While older age generally associates with higher Nf-L and GFAP, association strength differs by prevalent disease and age group [3, 23]. Reported associations between Nf-L or GFAP, and incident dementia or cognitive decline are all age-adjusted; namely these biomarkers associate with higher risk independent of their association with age. In addition to a positive age-independent association, our results also demonstrate that Nf-L and GFAP are most useful among adults beyond their eight decade, whereas they appear to be less useful among older adults below this age. While the lack of a statistically significant interaction by age precludes our ability to make strong conclusions about age differences, our results suggest that these biomarkers provide prognostic information among the oldest old.

Secondly, we address the role of the durability of biomarker measurements to cognitive outcomes. Although previous prospective investigations of Nf-L, GFAP and t-tau in relation to dementia-specific outcomes have spanned up to 17 years [6], only one study has, to our knowledge, specifically addressed the role of event proximity [24]. In the Chicago Health and Aging Project (CHAP) cohort, higher baseline Nf-L and GFAP associated with higher 16-year odds of incident Alzheimer's disease and mild cognitive impairment [24]; however, when stratified to time-to-diagnosis intervals, these associations only retained significance across the first eight years (0, 0–4, and 4–8-year groups). This attenuation in statistical significance might reflect limited statistical power in the group representing diagnoses after eight years (≤48 cases per outcome) compared to the other groups (≥110 cases per outcome). When comparing equally powered groups in CHS, we observed no difference between the associations of single Nf-L and GFAP measurements with incident dementia diagnoses occurring within or after ~9 years, or dementia-specific death occurring within or after ~12 years.

Our results also suggest that these markers remain strongly associated with even small changes in cognition. In line with previous studies [25–29], we observed that higher levels of circulating Nf-L and GFAP associate with faster cognitive decline among participants without clinical cognitive impairment. Nf-L and GFAP are, therefore, associated with subtle changes, often long before clinical pathology is present. Taken together, our findings support the value of Nf-L or GFAP measurements at a single time point in older adults regarding both short-term cognitive change and long-term dementia risk and prognosis. We also report an association between higher baseline UCLH-1 with a smaller reduction in 3MSE over three years, after adjusting for the other N4PA markers, for the first time. We did not observe complementary evidence to validate this finding in our study, but note that UCHL1 could be worth exploring in further research as a candidate for the measurement of cognitive reserve.

Finally, we address the independent vs. combined clinical utility of Nf-L and GFAP. Circulating Nf-L and GFAP correlated only moderately in CHS (ρ=0.46) and other cohorts [7, 24], reflecting their independent role in neurodegeneration. In the longest previous study to date, Beyer *et al.* found that baseline plasma GFAP, but not Nf-L, was associated with higher odds of AD within 17 years (N=308, including 68 cases, mean age of 67 years) [6]. Verberk *et al.* [5] reported that Nf-L, but not GFAP, was no longer associated with risk of clinical progression over 15 years when both were entered into the same model (N=300, including 27 cases, mean age of 61 years). Ebenau *et al.* [7] simultaneously included Nf-L, GFAP and hippocampal volume in their models (N=256, mean age of 61 years); Nf-L lost statistical significance in its association with MSSE score trajectories, and with the risk of clinical progression (N cases ≈ 41).

Important considerations when comparing our findings with these studies include the differences in the cohort mean ages (78 years in CHS vs. 67 years in the cohort investigated by Beyer *et al.*, and 61 years in both the Verberk *et al.* and Ebenau *et al.* investigations), statistical power, and confounder adjustment (comprehensive adjustment in CHS compared to largely demographic adjustment elsewhere). In CHS, the mutually adjusted associations of Nf-L and GFAP with incident dementia morbidity and mortality were equally strong overall and they appeared to be at least independent if not synergistic when evaluating joint categories. While both proteins also associate with three-year cognition score trajectories, associations with GFAP are likely reflective of the corresponding Nf-L association, and not of an independent GFAP contribution.

4.1. Strengths and limitations

Our study is notable for both specific strengths and limitations. The cohort was large and well-characterized with extensive phenotyping and long follow-up. We leveraged the strength of well-powered PBA-augmented incident dementia data, with the specificity of adjudicated dementia-specific mortality data–both of which spanned almost two decades of follow-up–with the short-term continuous evaluation of pre-clinical cognitive decline. At the same time, we were unable to assess the trajectories of these markers over time and how that relates to their associations with dementia outcomes and overall utility. The limited number of dementia-specific mortality cases also resulted in wide confidence intervals in stratified analyses.

As an observational study, the results may also be biased by measurement error in covariates or incomplete adjustment for confounding. Dementia outcomes may have been incompletely ascertained, as they were indirectly determined for many participants, rather than diagnosed. The measures of cognitive function we investigated do not comprehensively capture the complexity of dementia-related cognitive decline, and are sensitive to bias introduced by overrepresenting participants with better cognitive status who are more likely to return for repeated measures. Additionally, the use of serum, as opposed to plasma samples, particularly for t-tau that is known to reflect lower levels in the former, might have affected our ability to observe true positive associations. Finally, although CHS has previously reported circulating beta amyloid to be of limited utility in predicting dementia [30], newer

biomarkers, such as isoforms of phosphorylated tau, continue to emerge and will require comparison with biomarkers like Nf-L and GFAP.

In conclusion, in this study of over 1,700 older adults, Nf-L and GFAP were associated with incident dementia morbidity and mortality in a consistent, graded, and independent manner. They retained durable associations many years into follow-up yet were also able to detect subtle declines in three-year cognitive trajectory. As new preventative therapies for dementing diseases are developed, these biomarkers appear well-positioned to identify high-risk individuals who may receive maximal benefit from these interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

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ABBREVIATIONS

3MSE	modified mini-mental status examination
ADL	activities of daily living
BP	blood pressure
CHS	Cardiovascular Health Study
CVD	cardiovascular disease
DSST	digit symbol substitution test
eGFR	estimated glomerular filtration rate
GFAP	glial fibrillary acidic protein
MMSE	mini-mental status examination
N4PA	neurology four-plex assay
Nf-L	neurofilament light chain

PBA	probabilistic bias analysis
UCHL1	ubiquitin carboxyl-terminal hydrolase L1

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RESEARCH IN CONTEXT

Systematic review:

Google scholar was used to review the literature on Nf-L and GFAP in the context of dementia-related outcomes. We noted a lack of prospective community-based cohort studies and representation of participants beyond eight decades of life. We also found no investigations on associations with dementia-specific mortality risk.

Interpretation:

Nf-L and GFAP measurements associate with both short-term cognitive change and long-term risk of incident dementia and dementia-specific mortality, independent of demographics, body composition, lifestyle, and vascular risk profile. These biomarkers are at least as useful among the oldest old as among older adults below this age.

Future directions:

Equally powered longitudinal studies should compare the potential clinical utility of single Nf-L and GFAP measures in older adults to (1) repeated Nf-L and GFAP measurements and (2) other established (e.g., beta-amyloid) and emerging (e.g., p-tau isoforms) circulating biomarkers that are more specific to Alzheimer's disease.

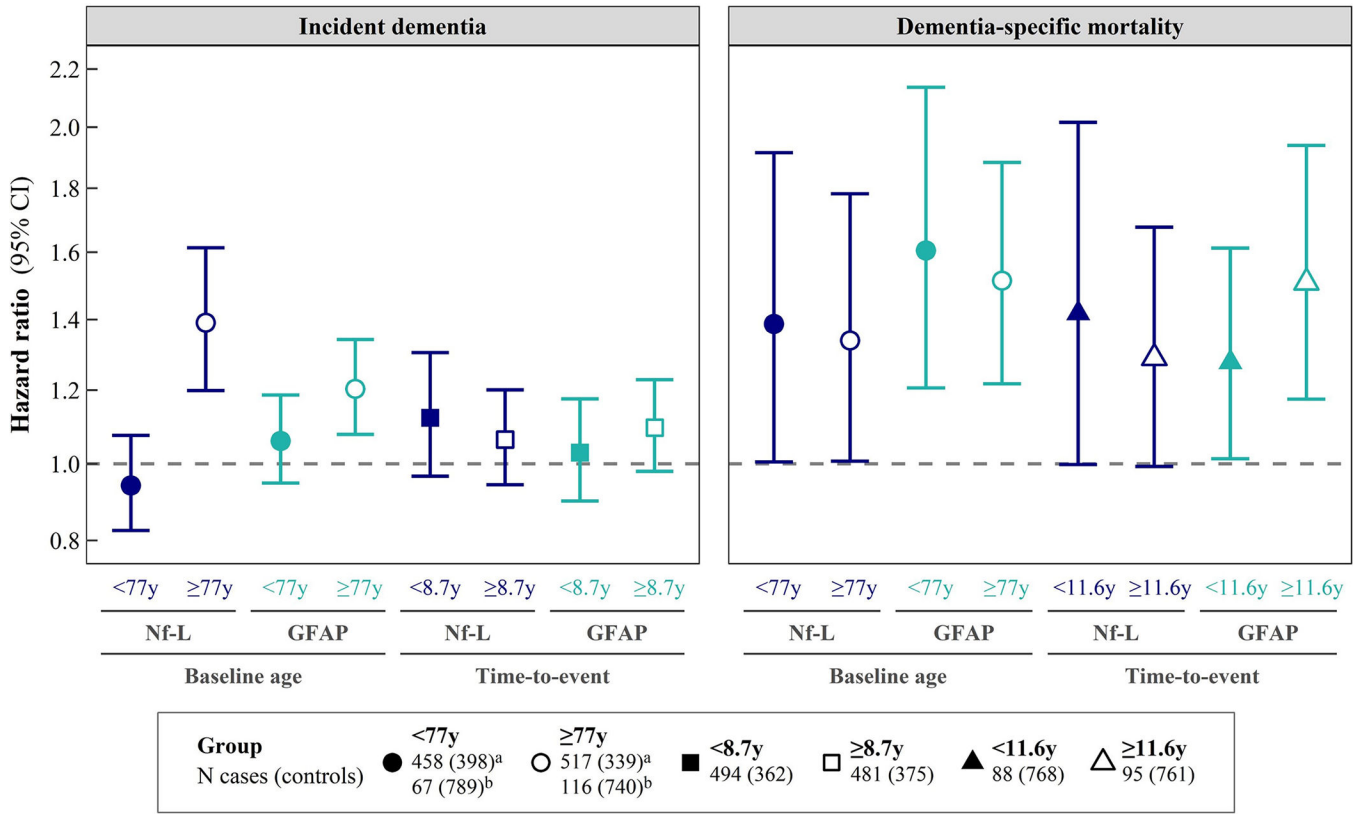

Figure 1.

Comparison of the Nf-L and GFAP associated risk of incident dementia (left) and dementia-specific mortality (right) between median age- and time-to-event stratified groups.

Notes: [a] Incident dementia; [b] Dementia-specific mortality. Blue color indicates Nf-L. Green color indicates GFAP. Compared groups were baseline age <77 vs. ≥77 years; time-to-event (incident dementia) <8.7 vs. ≥8.7 years, and time-to-event (dementia-specific mortality) <11.6 vs. ≥11.6 years. Hazard ratios represent risk per standard deviation log(pg/mL) protein. Model adjusted for age, sex, black race, clinic, education, body mass index, waist circumference, smoking status, alcohol consumption status, systolic blood pressure, total cholesterol, fasting plasma glucose, estimated glomerular filtration rate, C- reactive protein, activities of daily living limitation, *APOE* ε4 carrier status, antihypertensive medication use, lipid lowering medication use, prevalent coronary heart disease, myocardial infarction, stroke, and heart failure, total tau, UCHL1, and Nf-L or GFAP.

Abbreviations: CI, confidence interval; GFAP, glial fibrillary acidic protein; Nf-L, neurofilament light chain.

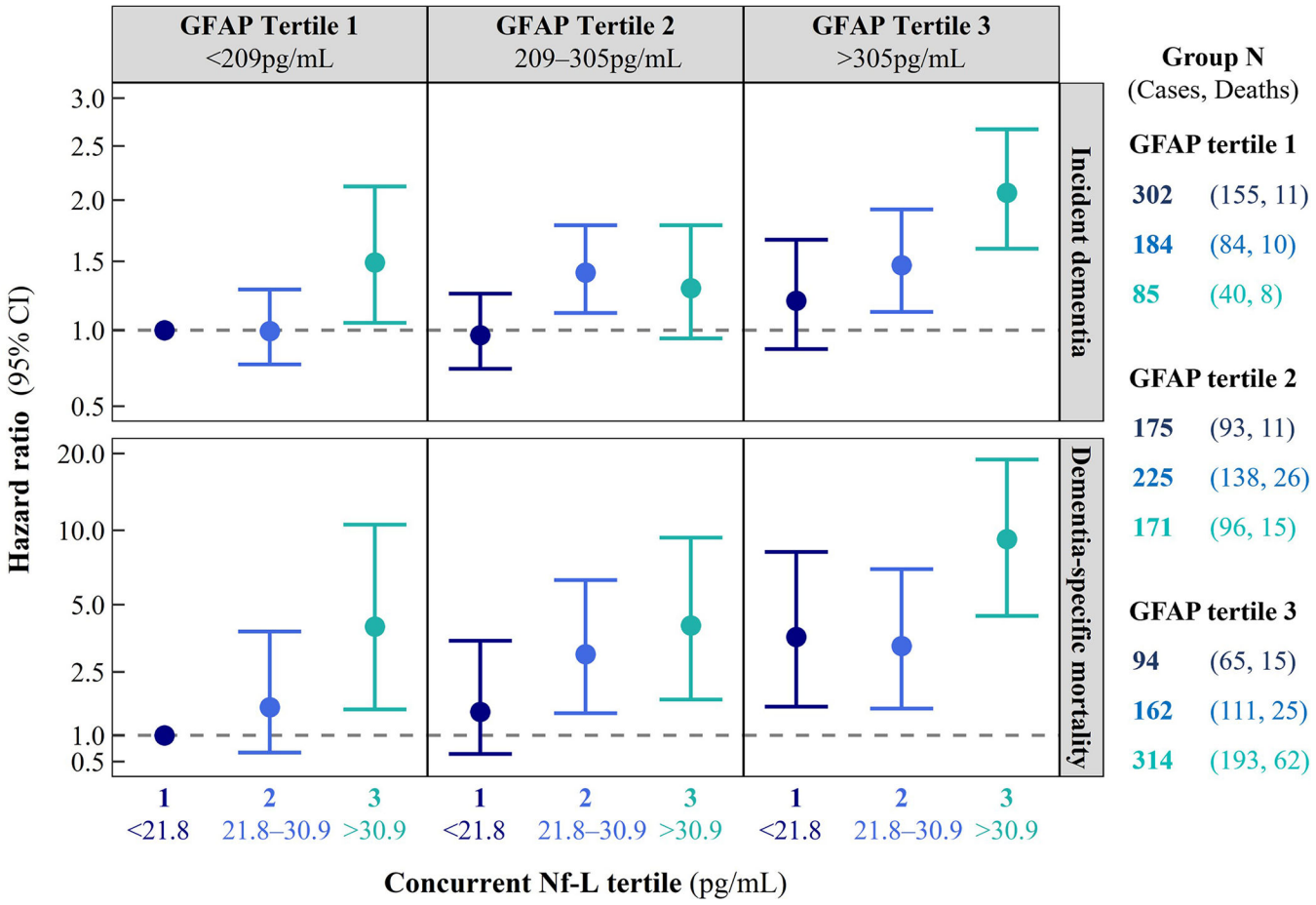

Figure 2.

Hazard ratios for incident dementia (top) and dementia-specific mortality (bottom) across joint Nf-L and GFAP tertiles.

Notes: Hazard ratios represent risk compared to joint reference group (Concurrently Nf-L tertile 1 and GFAP tertile 1). Model adjusted for age, sex, black race, clinic, education, body mass index, waist circumference, smoking status, alcohol consumption status, systolic blood pressure, total cholesterol, fasting plasma glucose, estimated glomerular filtration rate, C-reactive protein, activities of daily living limitation, *APOE* ε4 carrier status, antihypertensive medication use, lipid lowering medication use, prevalent coronary heart disease, myocardial infarction, stroke, and heart failure, total tau and UCHL1.

Abbreviations: CI, confidence interval; GFAP, glial fibrillary acidic protein; Nf-L, neurofilament light chain; UCHL1, ubiquitin carboxyl-terminal hydrolase L1.

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Table 1

Baseline characteristics of the 1,712-person study sample presented across Nf-L and GFAP tertiles

Characteristics[a]	Nf-L tertiles (pg/mL)			GFAP tertiles (pg/mL)		
	T1 (<21.8)	T2 (21.8–30.9)	T3 (>30.9)	T1 (<209)	T2 (209–305)	T3 (>305)
No. with data	**571**	**571**	**570**	**571**	**571**	**570**
Age, years	75 (74–78)	77 (75–80)	79 (76–83)	76 (74–78)	77 (75–80)	79 (76–83)
Female sex, N (%)	383 (67)	337 (59)	322 (57)	289 (51)	347 (61)	406 (71)
Black race, N (%)	125 (22)	55 (10)	38 (6.7)	86 (15)	76 (13)	56 (9.8)
High school graduate, N (%)	450 (79)	462 (81)	476 (84)	451 (79)	472 (83)	465 (82)
Current smoker, N (%)	47 (8.2)	36 (6.3)	41 (7.2)	59 (10)	38 (6.7)	27 (4.7)
Alcohol consumer, N (%)	270 (47)	256 (45)	257 (45)	285 (50)	270 (47)	228 (40)
Body mass index, kg/m^2	28 (25–30)	26 (24–29)	25.3 (23–28)	27 (25–30)	26 (24–29)	25.3 (23–28)
History of CVD, N (%)[b]	111 (19)	136 (24)	188 (33)	140 (25)	135 (24)	160 (28)
Systolic BP, mmHg	133 (122–144)	133 (122–148)	136 (123–151)	133 (121–144)	134 (123–147)	135 (123–151)
Total cholesterol, mg/dL	200 (175–225)	198 (173–226)	199 (174–227)	193 (169–222)	199 (175–224)	203 (178–231)
Fasting glucose, mg/dL	96 (91–104)	96 (89–103)	95 (89–103)	98 (92–105)	96 (90–103)	94 (89–100)
eGFR, mL/min/1.73m^2	77 (68–85)	70 (61–80)	60 (47–71)	74 (63–83)	70 (59–80)	65 (53–77)
C-reactive protein, mg/L	2.4 (1.1–4.8)	2.2 (1.0–4.7)	2.2 (0.9–4.4)	2.46 (1.18–59)	2.42 (15–53)	1.85 (0.9–4.2)
***APOE* ε4 allele carrier**, N (%)	122 (21)	141 (25)	129 (23)	112 (20)	134 (24)	146 (26)
N4PA biomarkers						
Nf-L, pg/mL	18 (16–20)	26 (24–28)	40 (34–52)	21 (17–27)	25 (20–33)	33 (25–44)
GFAP, pg/mL	205 (160–262)	248 (196–321)	327 (239–442)	170 (144–190)	248 (228–271)	401 (347–471)
Total tau, pg/mL	0.3 (0.2–0.4)	0.3 (0.2–0.4)	0.3 (0.2–0.5)	0.3 (0.2–0.4)	0.3 (0.2–0.4)	0.3 (0.2–0.5)
UCHL1, pg/mL	25 (20–35)	30 (23–39)	33 (26–44)	26 (20–35)	29 (22–39)	33 (26–42)
Cognitive function						
3MSE, score	96 (92–98)	96 (92–99)	95 (91–98)	96 (92–98)	96 (92–98)	95 (91–98)
DSST, score	42 (35–50)	41 (32–49)	38 (29–46)	41 (34–50)	40 (32–49)	39 (30–48)
ADL limitation, N (%)[c]	82 (14)	74 (13)	100 (18)	89 (16)	74 (13)	93 (16)

Notes:

[a] Data presented as median (Q1–Q3).

[b] Includes coronary heart disease, myocardial infarction, stroke, and heart failure

[c] Reported limitation of at least one activities of daily living.

Abbreviations: 3MSE: modified mini-mental status examination; ADL: activities of daily living; BP: blood pressure; CVD: cardiovascular disease; DSST: digit symbol substitution test; eGFR: estimated glomerular filtration rate; GFAP: glial fibrillary acidic protein; Nf-L: neurofilament light chain; UCHL1: ubiquitin carboxyl-terminal hydrolase L1.

Table 2

Association of serum N4PA protein levels with incident dementia and dementia-specific mortality risk

Model	Nf-L		GFAP		Total tau		UCHL1	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Incident dementia								
1	1.21 (1.12–1.30)	<0.001	1.18 (1.10–1.26)	<0.001	1.03 (0.97–1.10)	0.33	1.03 (0.97–1.09)	0.41
2	1.17 (1.08–1.28)	<0.001	1.16 (1.09–1.24)	<0.001	0.99 (0.93–1.06)	0.83	1.01 (0.95–1.08)	0.70
3	1.14 (1.03–1.26)	0.01	1.12 (1.04–1.21)	0.003	0.96 (0.89–1.05)	0.39	0.93 (0.85–1.01)	0.08
Dementia-specific mortality								
1	1.57 (1.37–1.81)	<0.001	1.68 (1.47–1.91)	<0.001	1.18 (1.03–1.36)	0.02	1.11 (0.97–1.27)	0.13
2	1.58 (1.35–1.86)	<0.001	1.67 (1.44–1.93)	<0.001	1.14 (0.98–1.33)	0.09	1.11 (0.97–1.27)	0.14
3	1.37 (1.11–1.69)	0.004	1.55 (1.31–1.83)	<0.001	1.03 (0.85–1.25)	0.73	0.87 (0.72–1.05)	0.14

Notes: Hazard ratios represent risk per standard deviation log(pg/mL) protein. Model 1 adjusted for age, sex, black race, clinic, and education. Model 2 additionally adjusted for body mass index, waist circumference, smoking status, alcohol consumption status, systolic blood pressure, total cholesterol, fasting plasma glucose, estimated glomerular filtration rate, C- reactive protein, activities of daily living limitation, *APOE* ε4 carrier status, anti-hypertensive medication use, lipid lowering medication use, prevalent coronary heart disease, myocardial infarction, stroke, and heart failure. Model 3 additionally included mutual N4PA protein adjustment. Probabilistic bias analysis was applied to account for misclassification in auxiliary dementia data sources.

Abbreviations: CI, confidence interval; HR, hazard ratio; GFAP, glial fibrillary acidic protein; Nf-L, neurofilament light chain; UCHL1, ubiquitin carboxyl-terminal hydrolase L1.

Table 3

Serum N4PA biomarker associations with three-year cognitive function score trajectories

Model	Nf-L		GFAP		Total tau		UCHL1	
	β (95%CI)	p	β (95%CI)	p	β (95%CI)	p	β (95%CI)	p
3MSE								
1	–0.43 (–0.56; –0.30)	<0.001	–0.25 (–0.37; –0.12)	<0.001	–0.05 (–0.17; 0.08)	0.49	0.07 (–0.06; 0.20)	0.29
2	–0.43 (–0.56; –0.30)	<0.001	–0.24 (–0.37; –0.12)	<0.001	–0.05 (–0.17; 0.08)	0.48	0.07 (–0.06; 0.20)	0.28
3	–0.47 (–0.62; –0.31)	<0.001	–0.07 (–0.21; 0.08)	0.36	–0.01 (–0.17; 0.15)	0.90	0.24 (0.08; 0.39)	0.003
DSST								
1	–0.33 (–0.51; –0.16)	<0.001	–0.21 (–0.38; –0.03)	0.02	–0.01 (–0.18; 0.17)	0.96	–0.04 (–0.21; 0.14)	0.69
2	–0.33 (–0.51; –0.16)	<0.001	–0.21 (–0.38; –0.04)	0.02	–0.01 (–0.18; 0.16)	0.91	–0.04 (–0.21; 0.14)	0.68
3	–0.34 (–0.55; –0.13)	0.002	–0.07 (–0.27; 0.13)	0.49	0.09 (–0.12; 0.30)	0.38	0.03 (–0.18; 0.24)	0.76

Notes: β represents the protein/time interaction estimate (score change per year per standard deviation log(pg/mL) protein). Model 1 adjusted for age, sex, black race, clinic, and education. Model 2 additionally adjusted for body mass index, waist circumference, smoking status, alcohol consumption status, systolic blood pressure, total cholesterol, fasting plasma glucose, estimated glomerular filtration rate, C- reactive protein, activities of daily living limitation, *APOE* ε4 carrier status, antihypertensive medication use, lipid lowering medication use, prevalent coronary heart disease, myocardial infarction, stroke, and heart failure. Model 3 additionally included mutual N4PA protein adjustment.

Abbreviations: 3MSE: modified mini-mental status examination; CI, confidence interval; DSST: digit symbol substitution test; GFAP, glial fibrillary acidic protein; Nf-L, neurofilament light chain; UCHL1, ubiquitin carboxyl-terminal hydrolase L1.