

FEATURED ARTICLE

Association of plasma biomarkers, p-tau181, glial fibrillary acidic protein, and neurofilament light, with intermediate and long-term clinical Alzheimer's disease risk: Results from a prospective cohort followed over 17 years

Hannah Stocker^{1,2} | Léon Beyer^{3,4} | Laura Perna⁵ | Dan Rujescu⁶ |
Bernd Holleczek⁷ | Konrad Beyreuther¹ | Julia Stockmann^{3,4} | Ben Schöttker^{1,2} |
Klaus Gerwert^{3,4} | Hermann Brenner^{1,2}

¹ Network Aging Research, Heidelberg University, Heidelberg, Germany

² Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany

³ Center for Protein Diagnostics (ProDi), Ruhr-University Bochum, Bochum, Germany

⁴ Faculty of Biology and Biotechnology, Department of Biophysics, Ruhr-University Bochum, Bochum, Germany

⁵ Department of Translational Research in Psychiatry – Max Planck Institute of Psychiatry, Munich, Germany

⁶ Department of Psychiatry, University of Vienna, Vienna, Austria

⁷ Saarland Cancer Registry, Saarbrücken, Germany

Correspondence

Hannah Stocker, PhD, MPH, Bergheimer Straße 20, D-69115 Heidelberg, Germany.
E-mail: h.stocker@dkfz-heidelberg.de

Hannah Stocker and Léon Beyer share first authorship.

Funding information

the Baden-Württemberg Ministry of Science, Research and Arts; the German Federal Ministry of Education and Research; the German Federal Ministry of Family, Senior Citizens, Women and Youth; the Saarland Ministry of Social Affairs, Health, Women and Family; the Network Aging Research at Heidelberg University

Abstract

Introduction: Blood biomarkers for Alzheimer's disease (AD) are the future of AD risk assessment. The aim of this study was to determine the association between plasma-measured phosphorylated tau (p-tau181), glial fibrillary acidic protein (GFAP), and neurofilament light (NfL) levels and risk of clinical AD incidence with consideration to the impact of cardiovascular health.

Methods: Within a community-based cohort, biomarker levels were measured at baseline using single molecule array technology in 768 participants (aged 50–75) followed over 17 years. Associations among biomarkers and AD, vascular dementia, and mixed dementia incidence were assessed.

Results: GFAP was associated with clinical AD incidence even more than a decade before diagnosis (9–17 years), while p-tau181 and NfL were associated with more intermediate AD risk (within 9 years). Significant interaction was detected between cardiovascular health and p-tau181/NfL.

Discussion: GFAP may be an early AD biomarker increasing before p-tau181 and NfL and the effect modifying role of cardiovascular health should be considered in biomarker risk stratification.

KEYWORDS

Alzheimer's disease, blood biomarkers, cardiovascular risk, risk stratification, vascular dementia

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial License](https://creativecommons.org/licenses/by-nc/4.0/), which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Alzheimer's & Dementia* published by Wiley Periodicals LLC on behalf of Alzheimer's Association.



1 | BACKGROUND

Alzheimer's disease (AD) is an irreversible neurodegenerative disease characterized by amyloid plaques and tau tangles in the brain, with pathological changes present decade(s) before clinical symptoms.¹ Blood biomarkers for AD are the future of AD prescreening and risk assessment in older adults.²⁻⁵ Recent evidence indicates that phosphorylated tau (p-tau181) in blood can predict amyloid beta (A β) and tau pathologies⁶ and neurofilament light (NfL) chain reflects neurodegeneration.⁷⁻⁹ Single molecule array (Simoa)-measured p-tau181 in plasma has exhibited very promising results with strong ability to discern AD pathology and identify AD across the continuum.^{3,6,10,11} NfL and glial fibrillary acidic protein (GFAP) in blood have also shown a strong ability to determine AD/dementia and cognitive decline.^{10,12-17} With the US Food and Drug Administration (FDA) approval of the first disease-modifying therapy, risk assessment through blood biomarkers could become crucial to identifying individuals who may benefit most from treatment.

Previous studies of p-tau181 measured in blood have had limited follow-up,^{3,18} but it has been theorized that p-tau181 levels may rise more than a decade before diagnosis.¹⁹ Additionally, GFAP has also been suggested to be an early marker of AD but long-term association evidence is scarce. Epidemiological studies with extensive follow-up investigating the ability of these biomarkers to detect AD/dementia risk more than a decade before diagnosis are lacking. Furthermore, the effect-modifying role of important risk factors such as apolipoprotein E (APOE) status, the greatest genetic risk factor for AD,²⁰ and cardiovascular health, which plays a paramount role in dementia and its progression,²¹ should be assessed to better inform risk stratification based upon these biomarkers.

Therefore, the aim of this study was to determine the association between plasma measured p-tau181, GFAP, and NfL levels at baseline and risk of clinical AD incidence in a community-based cohort study prospectively followed over 17 years. Secondarily, disease specificity was assessed through comparison to vascular dementia (VD) and mixed dementia (MD) incidence, and the impact of cardiovascular health at baseline and APOE genotype on the association between plasma biomarkers and dementia risk was explored.

2 | METHODS

2.1 | Study participants and data collection

The analyses are based on a nested case-control (NCC) study within the ESTHER study, a population-based prospective cohort study of community-dwelling older adults in Germany. Briefly, ESTHER consists of 9940 participants (50–75 years old at baseline) recruited by general practitioners (GPs) in a statewide study in Saarland, Germany from 2000 to 2002.^{22,23} Participants completed standardized health questionnaires, provided blood samples, and GPs provided medical information. Comprehensive monitoring of major disease incidence and mortality was conducted through participant and GP follow-up 2, 5, 8, 11,

HIGHLIGHTS

- Glial fibrillary acidic protein (GFAP) was associated with clinical Alzheimer's disease (AD) incidence 9–17 years before diagnosis.
- Phosphorylated tau (p-tau181)/neurofilament light (NfL) were only associated with AD incidence within 9 years of diagnosis.
- GFAP had the highest AD diagnosis prediction accuracy of all biomarkers.
- Cardiovascular health significantly modified the biomarker–AD risk association.

RESEARCH IN CONTEXT

1. Systematic Review: Extensive research regarding phosphorylated tau (p-tau181), glial fibrillary acidic protein (GFAP), and neurofilament light (NfL) measured in blood has exemplified the ability of these biomarkers to identify Alzheimer's disease (AD) and/or dementia across the continuum, mostly in clinical studies with limited follow-up.
2. Interpretation: In our study, GFAP was associated with clinical AD incidence even more than a decade before diagnosis (9–17 years), while p-tau181 and NfL were associated with more intermediate AD risk (within 9 years). Significant interaction was detected between cardiovascular health and p-tau181/NfL. Our findings suggest that GFAP may be a very early AD biomarker increasing before p-tau181 and NfL and that the effect-modifying role of cardiovascular health should be considered biomarker risk stratification.
3. Future Directions: Future research is necessary to confirm our findings and to determine the timepoint at which these biomarkers become clinically useful tools as well as confirm the interaction with cardiovascular health.

14, and 17 years after recruitment for all participants. Furthermore, data were linked to the Saarland cancer registry and death certificates were obtained from local health authorities. The ESTHER study was approved by the Ethics Committee of the Medical Faculty at Heidelberg University and the Physicians' Board of Saarland.

AD, VD, and MD diagnoses were collected from participants' GPs during the 14- and 17-year follow-ups as previously reported.^{23,24} Briefly, GPs of all ESTHER participants were contacted at the 14-year and 17-year follow-ups and asked to provide dementia diagnosis information as well as all available medical records of other specialized providers (Figure S1 in supporting information). The current guidelines in Germany for AD diagnosis follow the National Institute on Aging



and the Alzheimer's Association²⁵ or the International Working group (IWG)-2 criteria, for VD diagnosis the National Institute of Neurological Disorders and Stroke (NINDS)-Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) criteria,²⁸ and for MD diagnosis the IWG-criteria for mixed dementia.^{26,27}

Due to resource restrictions, biomarker measurements were not completed for the entire ESTHER cohort. Therefore, a NCC approach was used and biomarker levels were measured only at baseline. The sample included controls (participants confirmed by own GP to be without dementia diagnosis throughout 17 years of follow-up) chosen at random ($n = 507$) from the ESTHER study and all participants with an exclusive diagnosis of AD between baseline and the 17-year follow-up ($n = 145$; Figure S1). To investigate disease specificity, several VD and MD cases occurring between baseline and 17 years of follow-up included in a previous study²⁹ were additionally included ($n = 116$). Briefly, these were VD and MD cases that were identified in the first round of dementia diagnoses collection and not all VD, MD, or unspecified dementia cases from the ESTHER study were measured due to limited funds. Blood used for the biomarker measurements was drawn at baseline when all included participants were without dementia diagnosis (confirmed by own GP).

2.2 | Simoa measurements

Simoa technology was used to measure p-tau181, GFAP, and NFL in a single batch in plasma drawn at baseline. Lithium-heparin samples were stored upon arrival at -80°C . Prior to analysis on the Simoa HD-X Analyzer by Quanterix, samples were thawed at room temperature and mixed thoroughly. After a centrifugation step at $10000 \times g$ for 5 minutes, samples were applied to a conical 96-well plate (Quanterix) and measurements were carried out immediately. For the calculation of levels, lot-specific calibrators included in the kits were measured as well as one low and one high concentrated lot-specific control. In this study the commercially available Simoa Neurology 4-Plex E Advantage Kit and Simoa pTau-181 Advantage V2 Kit (Quanterix) were used according to manufacturer's instructions and with on-board automated 4x sample dilution. Ethylenediaminetetraacetic acid plasma samples are recommended for the Simoa measurement of $A\beta_{40}$ and $A\beta_{42}$ and the levels measured in our sample using heparin samples were extremely low or not detected. Therefore, $A\beta_{40}$ and $A\beta_{42}$ measurements were excluded.

2.3 | APOE genotype

APOE genotype was determined based on allelic combinations of the single nucleotide polymorphisms (SNPs) rs7412 and rs429358 using predesigned TaqMan SNP genotyping assays (Applied Biosystems). Genotypes were analyzed in an endpoint allelic discrimination read using the Bio-RAD CFX Connect System (Bio-Rad Laboratories). In the case of missing APOE data ($n = 64$), available quality controlled, imputed genetic data was used ($n = 37$; imputation conducted using

the Michigan Imputation Server, where SHAPEIT2 was used to phase the data and Minimac 4 was used to impute to the HRC Version r1.1.24 reference panel²³).

2.4 | Ten-year risk of fatal cardiovascular disease assessment

The 10-year risk of fatal cardiovascular disease (CVD) was determined from baseline data through the European Society of Cardiology SCORE (Systematic Coronary Risk Evaluation), which is a validated risk score based upon age, sex, smoking status, systolic blood pressure, and the total cholesterol to high-density lipoprotein ratio.³⁰ The SCORE risk estimates were calculated only in participants without diabetes. Individuals with $> 5\%$ SCORE risk estimates or diabetes were considered to have moderate to high (mod-high) 10-year risk of fatal CVD, while participants without diabetes and $\leq 5\%$ SCORE risk estimates were considered to have low to moderate (low-mod) 10-year risk of fatal CVD. Multiple imputation ($n = 20$) for data missing at random was carried out following the Markov chain Monte Carlo method, and the imputed dataset was used to calculate the SCORE.³¹

2.5 | Statistical methods

Descriptive statistics were used to provide information regarding participant characteristics in the entire sample as well as in age-stratified groups (aged 50–64 and 65–75 years at baseline), while Chi-square, *t*-tests, and Mann-Whitney *U* tests were used to compare both AD cases, VD cases, and MD cases to controls (individuals without dementia diagnosis) as appropriate. Cox proportional hazards regression analyses adjusted for age at baseline and sex were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the outcomes: incident AD, MD, and VD diagnoses occurring between baseline and 17 years. AD diagnoses were split into two time periods in the main analyses, within the first 9 years and between 9 and 17 years after baseline. In a sensitivity analysis, an additional time period (AD within 6 years) was used.

Independent variables included: APOE $\epsilon 4$ status ($\geq 1 \epsilon 4$ allele vs. no $\epsilon 4$ allele), age at baseline, sex, 10-year risk of fatal CVD, p-tau181 levels, GFAP levels, and NFL levels. The biomarker levels were right-skewed and therefore were natural log-transformed. The *z*-score of the log-transformed biomarker levels were tested individually in the Cox models. Additionally, the biomarkers were used as categorical variables, in which participants in the highest (Q5) and second-highest (Q4) quintile were compared to the lowest three quintiles (Q1–Q3) as the reference group. End of observation included date of dementia diagnosis, date of death, and date of 14-year or 17-year follow-up (date of response from the GP regarding dementia diagnosis status). The 14-year follow-up was used if 17-year follow-up information was not available.

Multiple imputation for data missing at random (GFAP [$n = 4$], NFL [$n = 3$]) was carried out following the Markov chain Monte Carlo



method.³¹ In analyses including *APOE*, individuals with missing *APOE* status were excluded ($n = 27$).

Area under the receiver operating characteristic (ROC) curves (AUCs) were calculated for AD diagnosis (0–9 years, 0–6 years, 9–17 years, 0–17 years) based upon natural log-transformed p-tau181, GFAP, and NfL baseline levels. ROC contrast analysis using the DeLong test was conducted to compare for significant differences between curves.³² The dose–response relationships between biomarker levels and AD was assessed using restricted cubic spline (RCS) functions with four knots at the 5th, 35th, 65th, and 95th percentiles of biomarker plasma levels. Additionally, stratified and interaction analyses (Cox regression and RCS) by 10-year fatal CVD risk status (low–moderate vs. moderate–high) and *APOE* $\epsilon 4$ status was completed for all outcomes. Finally, Pearson correlation and linear regression were used to explore the relationship between biomarker levels and age.

All analyses were conducted using SAS software, version 9.4 (SAS Institute). Statistical tests were two sided and conducted at an α -level of 0.05.

3 | RESULTS

Of a total of 768 participants, 145, 66, and 50 participants were diagnosed with AD, VD, and MD, respectively, between baseline and 17 years of follow-up, and 507 remained without dementia diagnosis throughout follow-up. The mean length of follow-up was 9.9 and 15.2 years in dementia cases and controls, respectively. The Pearson correlation coefficients for age and p-tau181, GFAP, and NfL were 0.26, 0.50, and 0.57, respectively (Figure S2 in supporting information).

3.1 | AD

The characteristics of study participants according to AD status are shown in Table 1. The mean age at baseline 67 and 61 years in AD cases and controls, respectively. There were more females (61%), a higher proportion of *APOE* $\epsilon 4$ positivity (51%), and higher proportion of moderate to high 10-year risk of fatal CVD (55%) among AD cases compared to controls.

The highest average baseline levels of p-tau181 and NfL were found in participants diagnosed within the first 5 years of follow-up, with somewhat lower levels in participants diagnosed later (Figure 1A and 1C). Baseline GFAP levels were, however, significantly elevated compared to controls regardless of the length of time to diagnosis, even in those diagnosed 13–17 years after baseline (Figure 1B). In the age stratified groups (50–64 and 65–75 years at baseline), GFAP levels in participants diagnosed with AD during follow-up were significantly higher than controls regardless of age group or diagnosis time period (Table S1 in supporting information). P-tau181 and NfL levels were largely only significantly higher in participants diagnosed in the first 9 years, but not between 9 and 17 years of follow-up.

P-tau181 (per standard deviation [SD] increase of log-transformed levels) was associated with increased risk of incident AD diagnosis

between baseline and 9 years and baseline and 17 years (Table 2: HR, 95% CI: AD 0–9years, 1.39, 1.05–1.85; AD 0–17 years, 1.19, 1.002–1.41), but not between 9 and 17 years (HR, 95% CI: 1.08, 0.87–1.34). Conversely, baseline GFAP (per SD increase of log-transformed levels) was associated to an increased risk of incident clinical AD diagnosis occurring between 9 and 17 years after baseline (HR, 95% CI: AD 0–9years, 1.40, 1.06–1.84; AD 9–17 years, 1.75, 1.36–2.26; AD 0–17 years, 1.58, 1.30–1.91).

The disease prediction accuracy as measured by the AUC value of p-tau181, GFAP, NfL, and all predictors combined after 17 years of follow-up was 0.610, 0.729, 0.676, and 0.735, respectively (Figure 2). The disease prediction accuracy based upon p-tau181 and GFAP at baseline was much higher for AD diagnoses occurring within the first 6 (Figure S3 in supporting information) and 9 years than between 9 and 17 years of follow-up, while the accuracy of GFAP remained more consistent, even in predicting diagnoses between 9 and 17 years of follow-up. The resulting AUCs based upon varying combinations of biomarkers is presented in Table S2 in supporting information. When adding p-tau181 and NfL to models already including GFAP, only modest increases in disease prediction accuracy were evident. The dose–response relationship between biomarker levels and AD is shown in Figure 3. Increasing levels above the median of all biomarkers were significantly associated with increasing risk of AD.

There was significant interaction between p-tau181 ($P = .04$) and NfL ($P < .01$) levels and 10-year risk of fatal CVD (Figure 4 and Table S3 in supporting information). The dose–response relationship between p-tau181, GFAP, and NfL levels and AD diagnosis (0–17 years) according to fatal CVD risk is shown in Figure 4. No significant interaction was seen between biomarker levels and *APOE* status (Table S4 in supporting information).

3.2 | Vascular and mixed dementia

The characteristics of study participants according to VD and MD status are shown in Table S5 in supporting information. VD and MD cases had higher baseline levels of plasma biomarkers, p-tau181, GFAP, and NfL than controls. However, higher plasma levels of p-tau181 were not significantly associated to higher VD or MD risk after adjustment of covariates, asserting AD specificity of p-tau181 (Table S6 in supporting information). There was a 36% and 94% increase in risk of clinical VD incidence per SD increase of log-transformed GFAP and NfL levels, respectively (HR, 95% CI: GFAP, 1.36, 1.01–1.84; NfL, 1.94, 1.48–2.55). GFAP and NfL levels were also significantly associated with higher risk of MD incidence during follow-up (HR, 95% CI: GFAP per SD increase, 1.74, 1.23–2.46; NfL per SD increase, 1.54, 1.09–2.16).

There was no interaction detected between the plasma biomarkers and 10-year risk of fatal CVD or *APOE* status (Tables S3 and S4).

Participant characteristics and the results of the Cox regression analyses for the biomarkers excluding outliers, which yielded very minimal differences, can be found in Tables S7 and S8 in supporting information.



TABLE 1 Participant characteristics: AD cases by diagnosis time period and controls

	AD (0–9 years)	AD (9–17 years)	AD (0–17 years)	Controls	P value ¹	P value ²	P value ³
n	51	94	145	507			
Baseline biomarker plasma levels pg/ml, mean ± SD; median (min-max)							
p-tau181	2.4 ± 1.5; 2.0 (0.8–7.5)	1.9 ± 1.2; 1.6 (0.2–7.7)	2.1 ± 1.3; 1.7 (0.2–7.7)	1.7 ± 1.2; 1.5 (0.1–17.9) n = 504	<.0001	.04	<.0001
GFAP	151.4 ± 122.1; 125.0 (6.0–875.0)	123.6 ± 56.2; 116.5 (31.7–335.0)	133.3 ± 86.0; 118.0 (6.0–875.0)	87.0 ± 46.7; 78.2 (13.3–529.0) n = 504	<.0001	<.0001	<.0001
NfL	23.0 ± 10.2; 21.0 (0.7–60.6)	18.9 ± 9.5; 16.6 (7.8–80.0)	20.3 ± 9.9; 17.8 (0.7–80.0)	15.8 ± 8.4; 14.1 (3.9–79.3)	<.0001	<.0001	<.0001
Additional predictors							
APOE ε4 –	29 (60.4)	38 (43.2)	67 (49.3)	364 (73.5)			
APOE ε4 +	19 (39.6)	50 (56.8)	69 (50.7)	131 (26.5)	.05	<.0001	<.0001
Male	24 (47.1)	32 (34.0)	56 (38.6)	229 (45.2)			
Female	27 (52.9)	62 (66.0)	89 (61.4)	278 (54.8)	.80	<.05	.16
Age at baseline	68.8 ± 4.4	65.6 ± 5.2	66.7 ± 5.2	61.2 ± 6.5	<.0001	<.0001	<.0001
Education,							
< 9 years	39 (76.5)	77 (81.9)	116 (80.0)	394 (77.7)			
≥ 10 years	12 (23.5)	17 (18.1)	29 (20.0)	113 (22.3)	.84	.36	.56
CVD risk,^a							
low-mod	15 (29.4)	50 (53.2)	65 (44.8)	294 (58.0)			
mod-high	36 (70.6)	44 (46.8)	80 (55.2)	213 (42.0)	<.0001	.39	<.01

Notes: Non-imputed data presented as frequency (%) for categorical values and mean ± SD for continuous variables. Imputed data are presented only for CVD risk. APOE ε4 +: ≥ 1 ε4 allele, APOE ε4 -: no ε4 allele

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; CVD, cardiovascular disease; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; n, number of participants; p-tau181, phosphorylated tau; SD, standard deviation.

^aCVD risk based upon European Society of Cardiology SCORE, low-moderate (mod): 0%–5% 10-year risk of fatal cardiovascular disease, mod-high: > 5% 10-year risk of fatal cardiovascular disease + participants with diabetes.

¹P value for comparison between incident AD cases (0–9 years) and controls.

²P value for comparison between incident AD cases (9–17 years) and controls.

³P value for comparison between incident AD cases (0–17 years) and controls.

4 | DISCUSSION

GFAP levels were associated with greater risk of clinical AD incidence even more than a decade (9–17 years) before diagnosis, while higher p-tau181 and NfL levels were only associated with risk of clinical AD incidence within 9 years of diagnosis in a community-based cohort study prospectively followed over 17 years. Additionally, higher p-tau181 and NfL levels were only associated with a higher risk of clinical AD incidence in participants with low to moderate risk of 10-year fatal CVD with significant interaction evident between the biomarkers and risk of 10-year fatal CVD. Finally, APOE status did not modify the association between the biomarkers and risk of dementia.

GFAP, a marker of astrogliosis, has previously shown the ability to predict dementia with several studies indicating an AD-specific relationship.^{17,33,34} Elevated GFAP levels are indicative of abnormal activation of astrocytes that often surround amyloid plaques. In one other previous longitudinal study, higher GFAP levels were associated

with AD 4 to 8 years prior to diagnosis.³⁵ In our study, GFAP levels at baseline were robustly associated with risk of AD incidence even many years before diagnosis and the disease prediction accuracy of GFAP was highest among the included biomarkers with only modest improvements apparent when adding p-tau181 or NfL to a model already including GFAP. These results suggest that GFAP may be a very early marker for AD and could be a critical early risk identifier.

p-tau181 has been suggested to have the highest level of maturity for clinical utility due to its high diagnostic accuracy, ability to differentiate AD from other neurodegenerative disorders, and ability to track progression of AD-specific neurodegeneration.^{3,4,18,36,37} Longitudinal studies investigating p-tau181 have included limited follow-up times.^{3,10,18} In a study of dominantly inherited AD, it was shown that some patients saw an initial increase in p-tau181 levels as early as two decades before symptoms at around the time of amyloid aggregation, suggesting that levels of p-tau181 specifically rise in response to amyloid.¹⁹ Currently, our study is the only to investigate the

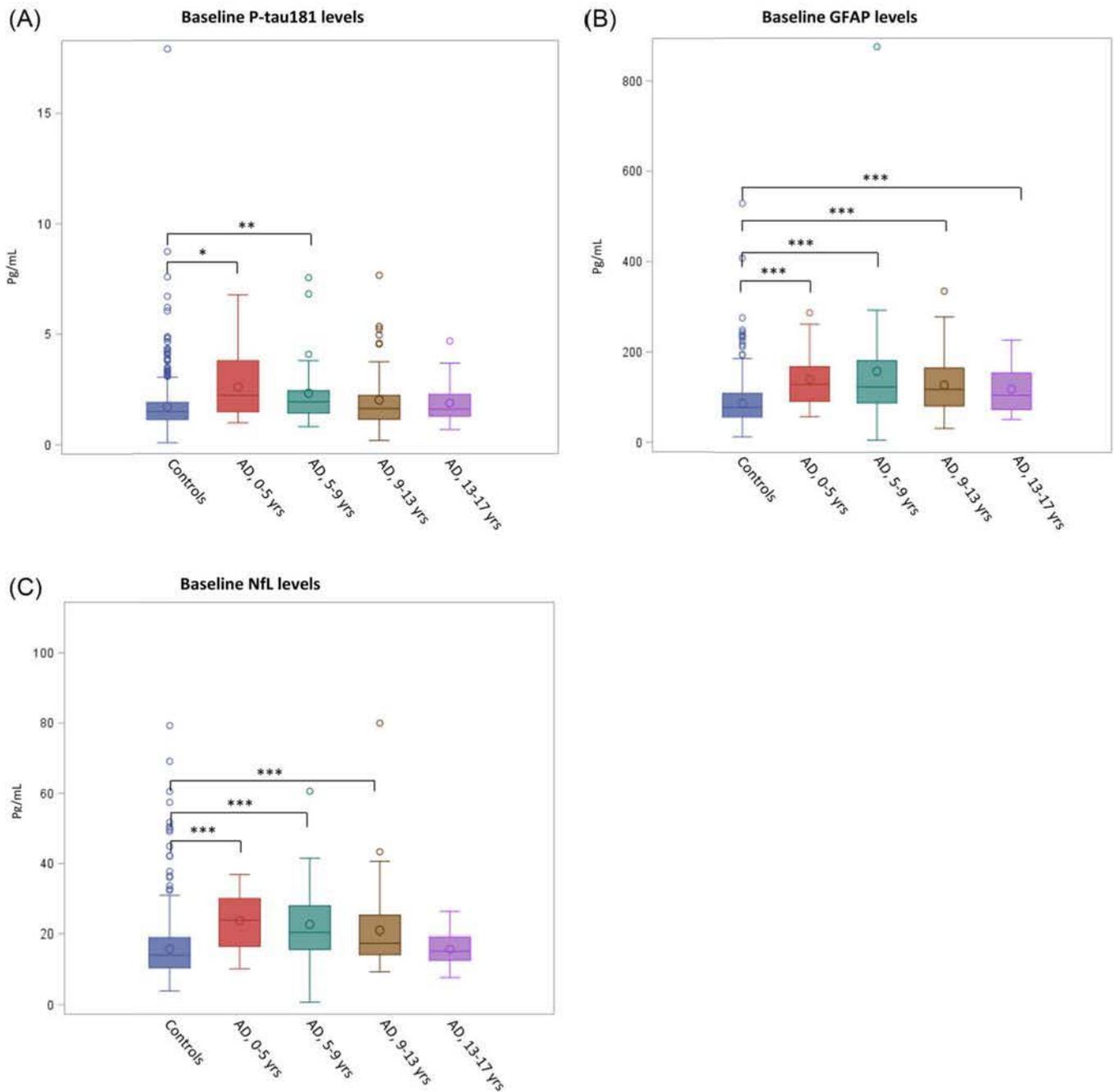


FIGURE 1 Baseline plasma biomarker levels by incident AD diagnosis time period (years after baseline) and in controls. A, Baseline p-tau181 levels, B, Baseline GFAP levels. C, Baseline NfL levels. AD, Incident AD diagnosis within 17 years by diagnosis time period. All, $n = 768$; Controls (participants without dementia diagnosis throughout 17 years), $n = 507$; AD 0–5 yrs, $n = 19$; AD 5–9 years, $n = 32$; AD 9–13 years, $n = 56$; AD 13–17 years, $n = 38$. Mann-Whitney U tests were used to test for statistically significant differences between cases and controls as indicated by: * $P < .01$; ** $P < .001$; *** $P < .0001$. AD, Alzheimer's disease; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau181, phosphorylated tau

association between p-tau181 and sporadic AD diagnosis with a follow-up longer than 9 years. The discrepancy between the theoretical idea that p-tau181 levels rise in sporadic AD decades before symptoms and our results, which lacked significance between 9 and 17 years, warrant further longitudinal cohort studies with longer follow-up.

NfL in plasma is a marker of neurodegeneration that has shown strong associations to and discriminatory ability of AD and

dementia.^{10,12,13,15,16} We have shown in our analyses an association between higher baseline plasma levels of NfL and higher risk of clinical AD only within the first 9 years of follow-up in line with previous studies and similar to the pattern seen with p-tau181 levels.

A significant interaction between plasma levels and 10-year risk of fatal CVD was evident, with participants at lower CVD risk having higher AD risk per SD increase in p-tau181 and NfL levels.

TABLE 2 Cox regression results: HRs for risk of incident clinical AD diagnosis (0–9 years, 9–17 years, 0–17 years)

	N	AD (0–9 years) HR (95% CI)	P-value	n	AD (9–17 years) HR (95% CI)	P-value	n	AD (0–17 years) HR (95% CI)	P-value
N	51			94			145		
p-tau181 (cont.)	51	1.39 (1.05–1.85)	.02	94	1.08 (0.87–1.34)	.50	145	1.19 (1.002–1.41)	<.05
Q1–Q3 (< 1.74 pg/ml)	18	Ref.		54	Ref.		72	Ref.	
Q4 (1.74–2.24 pg/ml)	14	1.47 (0.71–3.06)	.30	18	1.01 (0.58–1.73)	.98	32	1.20 (0.78–1.84)	.41
Q5 (> 2.25 pg/ml)	19	1.94 (0.99–3.82)	.05	22	1.15 (0.69–1.92)	.59	41	1.42 (0.95–2.11)	.09
GFAP (cont.)	51	1.40 (1.06–1.84)	.02	94	1.75 (1.36–2.26)	<.0001	145	1.58 (1.30–1.91)	<.0001
Q1–Q3 (< 102.00 pg/ml)	16	Ref.		41	Ref.		57	Ref.	
Q4 (102.00–136.99 pg/ml)	14	1.35 (0.64–2.85)	.43	20	0.86 (0.49–1.53)	.61	34	1.01 (0.64–1.59)	.96
Q5 (> 136.99 pg/ml)	21	2.91 (1.43–5.93)	<.01	33	2.84 (1.72–4.67)	<.0001	54	2.65 (1.76–3.98)	<.0001
NfL (cont.)	51	1.19 (0.86–1.63)	.29	94	1.26 (0.95–1.68)	.11	145	1.22 (0.98–1.51)	.07
Q1–Q3 (< 17.60 pg/ml)	16	Ref.		52	Ref.		68	Ref.	
Q4 (17.60–23.14 pg/ml)	12	1.32 (0.61–2.84)	.48	22	0.94 (0.56–1.57)	.80	34	1.03 (0.68–1.58)	.88
Q5 (> 23.14 pg/ml)	23	2.47 (1.23–4.97)	.01	20	1.40 (0.80–2.46)	.24	43	1.73 (1.13–2.65)	.01
APOE ε4 –	29	Ref.		38	Ref.		67	Ref.	<.0001
APOE ε4 +	19	1.58 (0.89–2.83)	.12	50	3.09 (2.02–4.73)	<.0001	69	2.32 (1.66–3.25)	
Male	24	Ref.		32	Ref.		56	Ref.	.31
Female	27	1.11 (0.64–1.92)	.72	62	1.38 (0.90–2.11)	.14	89	1.18 (0.85–1.66)	
Age at baseline per year increase	51	1.23 (1.17–1.31)	<.0001	94	1.14 (1.10–1.18)	<.0001	145	1.16 (1.13–1.20)	<.0001
CVD risk, low–mod	15	Ref.		50	Ref.		65	Ref.	.87
mod–high	36	1.29 (0.56–2.99)	.54	44	0.84 (0.46–1.51)	.55	80	0.96 (0.60–1.54)	

Notes: All analyses adjusted for age and sex. All continuous biomarker predictors are per standard deviation increase in natural log-transformed biomarker level measured at baseline. CVD risk based upon European Society of Cardiology SCORE, low–mod (mod): 0%–5% 10-year risk of fatal cardiovascular disease, mod–high: > 5% 10-year risk of fatal cardiovascular disease + participants with diabetes. APOE ε4 +: ≥ 1 ε4 allele, APOE ε4 -: no ε4 allele. Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; CVD, cardiovascular disease; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; n, number of participants; p-tau181, phosphorylated tau; SD, standard deviation.

Cardiovascular health plays an influential role at all stages of AD development and can affect the rate in which the disease progresses.²¹ Often AD co-occurs with vascular pathology or individuals with mostly vascular pathology additionally exhibit amyloid and tau accumulation.³⁸ It is more likely that those individuals with low risk of fatal CVD develop a more pure form of AD, which could explain the greater association between p-tau181 levels and AD risk. There is a known discrepancy between clinical and neuropathological data and the possibility of misdiagnosis must be considered.³⁹ Another possible explanation could be that those individuals at higher risk for CVD may die before developing AD symptoms. Cardiovascular health should be taken into account when considering the use of plasma biomarkers as AD risk assessment tools due to its paramount role in AD and its progression.

4.1 | Clinical implications

This study provides unique and innovative information critical for future research and translation of plasma biomarkers as risk stratification tools in a disease in which pathological changes precede diagno-

sis by decade(s). With the monumental decision by the FDA to approve adunamucab and likely in the future additional disease-modifying therapies, it is critical that a feasible and cost-effective method for screening older adults in the community is established. Previous studies have suggested that p-tau181 is an early marker of AD pathological changes. Based upon our results, further studies need to confirm when p-tau181 levels rise to better inform clinical risk assessment. GFAP levels however exhibited potential for longer-term risk assessment and should be considered in clinical risk assessment. In a multi-step symptom prevention strategy, GFAP could be used as a first step indicating the need for more frequent monitoring, while p-tau181 could indicate more imminent risk of future clinical AD. A multi-step approach could be critical for detecting disease pathology when modifying treatments provide most benefit.

4.2 | Strengths and weaknesses

The greatest strengths of this study include the exceptional length of follow-up (17 years) and baseline biomarker measurements in dementia diagnosis-free participants. Additionally, this is the first study to



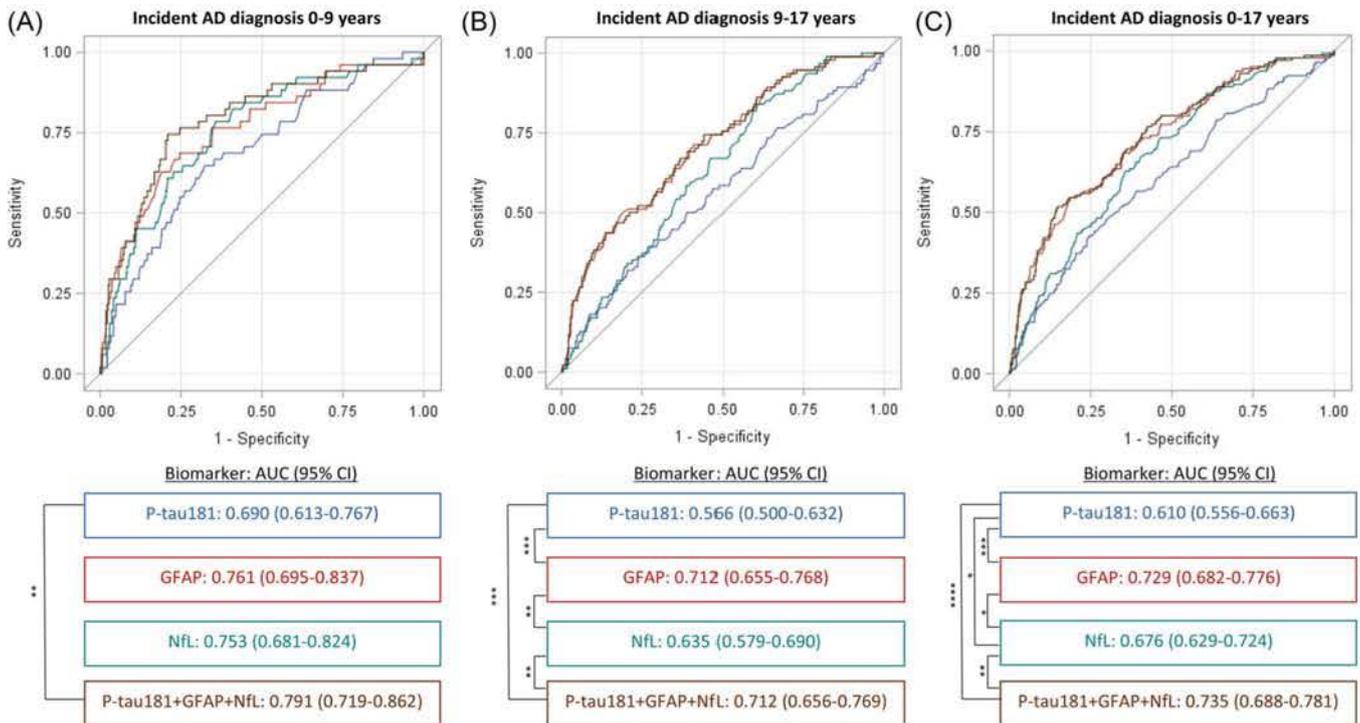


FIGURE 2 ROC curves and contrast for incident AD diagnosis within 17 years based upon baseline biomarker levels. Biomarker levels are natural log-transformed levels from baseline. AUC including 95% CIs are reported below ROC curves. ROC contrast analysis using the DeLong test was conducted to compare for significant differences between curves as indicated by: * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$. AD, Alzheimer's disease; AUC, area under the ROC curve; CI, confidence intervals; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau181, phosphorylated tau; ROC, receiver operating characteristic

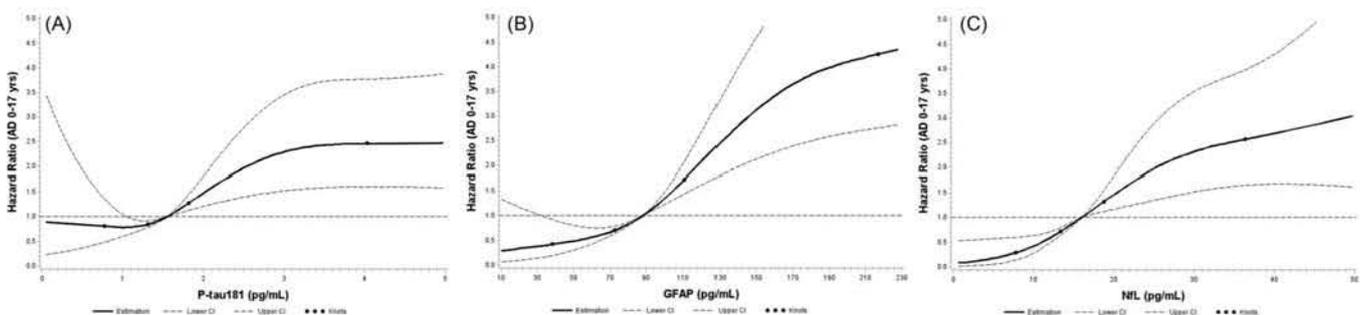


FIGURE 3 Dose-response relationship between plasma biomarker levels at baseline and incident AD diagnosis within 17 years ([A] p-tau181, [B] GFAP, [C] NfL). Restricted cubic spline function of biomarker levels with four knots at the 5th, 35th, 65th, and 95th percentiles of biomarker levels and the median as the reference. AD, Alzheimer's disease; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau181, phosphorylated tau

measure p-tau181 with such length of follow-up, exhibit the long-term association between GFAP and clinical AD risk, as well as explore the effect modification of cardiovascular health in biomarker risk stratification.

This study also has several weaknesses. First, the possibility of dementia misdiagnosis/underdiagnosis or delayed diagnosis as the dementia diagnoses in the ESTHER study were clinical diagnoses reported by numerous practitioners using heterogeneous diagnostic procedures, which may be inferior to diagnostic standards that can

be achieved in highly specialized academic settings. This is, however, the nature of community-based cohort studies, which portray common practice in such a setting. One advantage of a population-based study set in the community (ESTHER) is the inclusion of a more representative sample that may not be possible in a specialized academic setting. Additionally, dementia neuropathologies are complex where AD pathology seldom occurs in isolation,³⁸ further complicating diagnoses. Additionally, the lack of $A\beta$ concentration measurements limits comparability and amyloid classification; however, $A\beta$

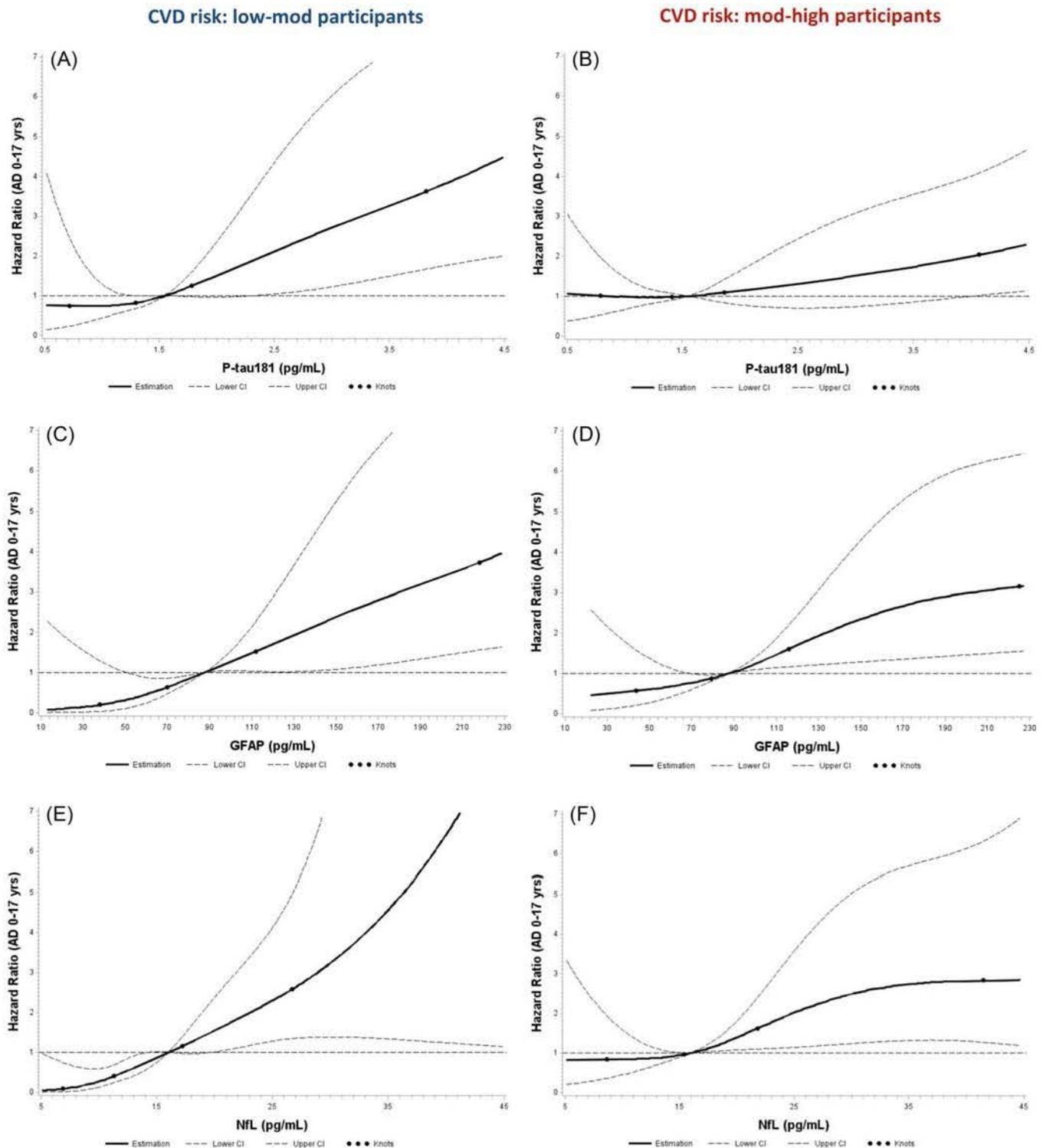


FIGURE 4 Dose–response relationship between p-tau181, GFAP, and NFL baseline levels and incident AD diagnosis by 10-year risk of fatal CVD. Restricted cubic spline function of p-tau181 (A and B), GFAP (C and D), and NFL (E and F) levels with four knots at the 5th, 35th, 65th, and 95th percentiles of biomarkers levels and the median as the reference. CVD risk low-moderate (mod): $\leq 5\%$ 10-year risk of fatal cardiovascular disease, CVD risk mod-high: $> 5\%$ 10-year risk of fatal cardiovascular disease + participants with diabetes. There was significant interaction between p-tau181 ($P = .04$) and NFL ($P < .01$) levels and 10-year risk of fatal CVD. AD, Alzheimer's disease; CVD, cardiovascular disease; GFAP, glial fibrillary acidic protein; NFL, neurofilament light; p-tau181, phosphorylated tau

concentration in blood is notoriously difficult to measure and it has been shown that p-tau181 accurately predicts A β pathology.⁶ We have previously measured the secondary structure of A β in ESTHER using an immuno-infrared-sensor, which is not dependent on the concentration of A β , and saw an extremely high association to AD diagnosis within 14 years (odds ratio, 23).²⁹ This type of structural marker might offer a solution to this important issue.

4.3 | Conclusion

In this prospective longitudinal cohort study followed over 17 years, we have shown that higher GFAP levels were associated with greater risk of clinical AD incidence even more than a decade (9–17 years) before diagnosis, while higher p-tau181 and NFL levels were only associated with risk of clinical AD incidence within 9 years of diagnosis. Significant interaction between biomarker plasma levels and 10-year risk of fatal CVD asserts the need to consider cardiovascular health when assessing clinical AD risk. Further longitudinal studies are needed to confirm the long-term risk associations to better inform clinical applicability pivotal to the initiation of disease-modifying therapies at the most beneficial stage in AD progression.

ACKNOWLEDGMENTS

The ESTHER study was supported by grants from the Baden-Württemberg Ministry of Science, Research and Arts; the German Federal Ministry of Education and Research; the German Federal Ministry of Family, Senior Citizens, Women and Youth; the Saarland Ministry of Social Affairs, Health, Women and Family; and the Network Aging Research at Heidelberg University.

CONFLICTS OF INTEREST

The authors Hannah Stocker, Léon Beyer, Laura Perna, Julia Stockmann, Bernd Holleczeck, Ben Schöttker, Klaus Gerwert, and Hermann Brenner have no competing interests to declare. Dan Rujescu has received consulting fees from and served on a Data Safety Monitoring Board/Advisory Board for Janssen, Germany. Konrad Beyreuther has received payments for lectures from Novartis, Germany.

REFERENCES

1. Jack CR, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14:535-562.
2. Nabers A, Perna L, Lange J, et al. Amyloid blood biomarker detects Alzheimer's disease. *EMBO Mol Med*. 2018;10:e8763.
3. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol*. 2020;19:422-433.
4. Ashton NJ, Leuzy A, Karikari TK, et al. Alzheimer's disease biomarker roadmap 2020: fluid biomarkers: strategic roadmap for the translation of tau biomarkers in the clinic: an international task force. *Alzheimers Dement*. 2020;16:e039557.
5. Stockmann J, Verberk IMW, Timmesfeld N, et al. Amyloid- β misfolding as a plasma biomarker indicates risk for future clinical Alzheimer's disease in individuals with subjective cognitive decline. *Alzheimers Res Ther*. 2020;12:169.
6. Barthélemy NR, Horie K, Sato C, Bateman RJ. Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. *J Exp Med*. 2020;217(11):e20200861.
7. Li D, Mielke MM. An update on blood-based markers of Alzheimer's disease using the SiMoA platform. *Neurol Ther*. 2019;8:73-82.
8. Fyfe I. Neurofilament light chain – new potential for prediction and prognosis. *Nat Rev Neurol*. 2019;15:557.
9. Leuzy A, Cullen NC, Mattsson-Carlgrén N, Hansson O. Current advances in plasma and cerebrospinal fluid biomarkers in Alzheimer's disease. *Curr Opin Neurol*. 2021;34:266-274.
10. Cullen NC, Leuzy A, Palmqvist S, et al. Individualized prognosis of cognitive decline and dementia in mild cognitive impairment based on plasma biomarker combinations. *Nature Aging*. 2021;1:114-123. <https://doi.org/10.1038/s43587-020-00003-5>
11. Hansson O, Cullen N, Zetterberg H, Initiative AsDN, Blennow K, Mattsson-Carlgrén N. Plasma phosphorylated tau181 and neurodegeneration in Alzheimer's disease. *Ann Clin Transl Neur*. 2021;8:259-265.
12. Ashton NJ, Leuzy A, Lim YM, et al. Increased plasma neurofilament light chain concentration correlates with severity of post-mortem neurofibrillary tangle pathology and neurodegeneration. *Acta neuropathol comm*. 2019;7. <https://doi.org/10.1186/s40478-018-0649-3>
13. de Wolf F, Ghanbari M, Licher S, et al. Plasma tau, neurofilament light chain and amyloid- β levels and risk of dementia; a population-based cohort study. *Brain*. 2020;143:1220-1232.
14. Thijsen EH, Verberk IM, Stoops E, Boxer AL, Teunissen CE. Amyloid, pTau, NFL, and GFAP as biomarkers for Alzheimer's disease: clinical study results of AD blood biomarkers. *Alzheimers Dement*. 2020;16:e038179.
15. Sugarman MA, Zetterberg H, Blennow K, et al. A longitudinal examination of plasma neurofilament light and total tau for the clinical detection and monitoring of Alzheimer's disease. *Neurobiol Aging*. 2020;94:60-70.
16. Simrén J, Leuzy A, Karikari TK, et al. Ultrasensitive blood biomarkers to predict cognitive decline and diagnose Alzheimer's disease in the absence of AT (N) classification as the reference standard: biomarkers (non-neuroimaging)/Longitudinal change over time. *Alzheimers Dement*. 2020;16:e041808.
17. Oeckl P, Halbgebauer S, Anderl-Straub S, et al. Glial fibrillary acidic protein in serum is increased in Alzheimer's disease and correlates with cognitive impairment. *J Alzheimers Dis*. 2019;67:481-488.
18. Moscoso A, Grothe MJ, Ashton NJ, et al. Longitudinal associations of blood phosphorylated tau181 and neurofilament light chain with neurodegeneration in Alzheimer disease. *JAMA Neurology*. 2021;78:396-406.
19. Barthélemy NR, Li Y, Joseph-Mathurin N, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat Med*. 2020;26:398-407.
20. Van Cauwenbergh C, Van Broeckhoven C, Sleegers K. The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genet Med*. 2016;18:421-430.
21. Santos CY, Snyder PJ, Wu W-C, Zhang M, Echeverria A, Alber J. Pathophysiological relationship between Alzheimer's disease, cerebrovascular disease, and cardiovascular risk: a review and synthesis. *Alzheimers Dement: DADM*. 2017;7:69-87.
22. Löw M, Stegmaier C, Ziegler H, Rothenbacher D, Brenner H. Epidemiological investigations of the chances of preventing, recognizing early and optimally treating chronic diseases in an elderly population (ESTHER study). *Dtsch Med Wochenschr*. 2004;129(49), 2643-2647.
23. Stocker H, Perna L, Weigl K, et al. Prediction of clinical diagnosis of Alzheimer's disease, vascular, mixed, and all-cause dementia by a polygenic risk score and APOE status in a community-based cohort



- prospectively followed over 17 years. *Mol Psychiatry*. 2020;26:5812–5822. <https://doi.org/10.1038/s41380-020-0764-y>
24. Perna L, Wahl H, Weberpals J, et al. Incident depression and mortality among people with different types of dementia: results from a longitudinal cohort study. *Soc Psychiatry Psychiatr Epidemiol*. 2019;54:793–801.
 25. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia 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;7:263–269.
 26. Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol*. 2014;13:614–629.
 27. Deutsche Gesellschaft für Neurologie. *S3-Leitlinie "Demenzen"*. Springer-Verlag GmbH; 2016.
 28. Román GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies: report of the NINDS-AIREN International Workshop. *Neurology*. 1993;43:250.
 29. Stocker H, Nabers A, Perna L, et al. Prediction of Alzheimer's disease diagnosis within 14 years through A β misfolding in blood plasma compared to APOE4 status, and other risk factors. *Alzheimers Dement*. 2019;16:283–291.
 30. Conroy RM, Pyörälä K, Fitzgerald Ae, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J*. 2003;24:987–1003.
 31. Schafer JL. *Analysis of Incomplete Multivariate Data*. Chapman and Hall/CRC; 1997. <https://doi.org/10.1201/9780367803025>
 32. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988; 44(3):837–845.
 33. Verberk IM, Laarhuis MB, van den Bosch KA, et al. Serum markers glial fibrillary acidic protein and neurofilament light for prognosis and monitoring in cognitively normal older people: a prospective memory clinic-based cohort study. *Lancet Healthy Longevity*. 2021;2:e87–e95.
 34. Chatterjee P, Pedrini S, Stoops E, et al. Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease. *Transl Psych*. 2021;11(27). <https://doi.org/10.1038/s41398-020-01137-1>
 35. Rajan KB, Aggarwal NT, McAninch EA, et al. Remote blood biomarkers of longitudinal cognitive outcomes in a population study. *Ann Neurol*. 2020;88:1065–1076.
 36. Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med*. 2020;26:379–386.
 37. Thijssen EH, La Joie R, Wolf A, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med*. 2020;26:387–397.
 38. Boyle PA, Yu L, Wilson RS, Leurgans SE, Schneider JA, Bennett DA. Person-specific contribution of neuropathologies to cognitive loss in old age. *Ann Neurol*. 2018;83:74–83.
 39. Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005–2010. *J Neuropathol Exp Neurol*. 2012;71:266–273.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Stocker H, Beyer L, Perna L, et al. Association of plasma biomarkers, p-tau181, glial fibrillary acidic protein, and neurofilament light, with intermediate and long-term clinical Alzheimer's disease risk: Results from a prospective cohort followed over 17 years. *Alzheimer's Dement*. 2023;19:25–35. <https://doi.org/10.1002/alz.12614>

