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RESEARCH ARTICLE

Serum biomarkers as prognostic markers for Alzheimer's disease in a clinical setting

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Abstract

INTRODUCTION: Blood-based glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and phosphorylated tau (pTau) have shown promising prognostic potential in Alzheimer's disease (AD), but their applicability in clinical settings where comorbidities are prevalent remains uncertain.

METHODS: Simoa assays quantified GFAP, NfL, and pTau181 in retrospectively retrieved prediagnostic serum samples from 102 AD patients and 21 non-AD controls.

RESULTS: Higher serum GFAP levels predicted earlier clinical presentation and faster subsequent Mini-Mental State Examination decline in AD patients. Serum NfL levels were increased in patients with arterial hypertension (AHT), kidney dysfunction, and a history of stroke and only demonstrated predictive value for time to clinical AD presentation after adjustment for these comorbidities. Serum pTau181 instability during long-term storage at −20°C prevented its prognostic evaluation in retrospectively retrieved serum samples.

DISCUSSION: Serum GFAP is a robust prognostic marker for AD progression, whereas NfL is impacted by various comorbidities, which complicates the interpretation of its prognostic value.

KEYWORDS
Alzheimer's disease, blood biomarker, comorbidity, diagnosis, glial fibrillary acidic protein, longitudinal, memory clinic, neurofilament light, phosphorylated tau, preclinical, prognosis

Rik Vandenberghe, Koen Poesen, and Charlotte E. Teunissen shared senior authorship

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Highlights

- Serum GFAP levels predict time to clinical AD presentation.
- Serum NfL levels are increased by hypertension, kidney disease, and stroke history.
- Prognostic value of serum NfL in AD is only evident after comorbidity correction.
- Serum levels of GFAP, but not NfL, increase over time within prediagnostic AD stages.

1 | BACKGROUND

Alzheimer's disease (AD) is characterized by a long preclinical phase marked by the aggregation of amyloid beta (Aβ) without apparent cognitive symptoms.[1,2] If disease-modifying therapies could be started within this preclinical phase, irreversible brain damage and the ensuing cognitive decline may be prevented. Blood-based biomarkers have consistently shown high performance in detecting preclinical Aβ pathology.[3–7] However, the duration of the preclinical AD phase is highly variable.[8,9] Information about the risk of cognitive onset within a clinically relevant time frame would increase the power of clinical trials and guide treatment strategies in clinical practice.[10] Recent studies in cognitively unimpaired (CU) older adults have demonstrated good prognostic value of blood-based biomarkers, particularly glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and phosphorylated tau (pTau).[7,11–16] These studies have predominantly been conducted in population-based cohorts with a low prevalence of comorbidities. Therefore, it remains uncertain how the prognostic capabilities of blood-based biomarkers are influenced by comorbidities that are prevalent in memory clinic populations. In this study, we retrospectively retrieved the serum samples taken from patients that were eventually diagnosed with clinical AD in the memory clinic but for whom blood sampling occurred on average 4 years prior to clinical presentation. Due to the retrospective nature of the study and the storage of the retrospectively retrieved clinical serum samples at −20°C rather than −80°C, which is typically used in research settings, we first investigated the stability of serum GFAP, NfL, and pTau181 levels at −20°C. For biomarkers with stable serum levels over the long term at −20°C, we aimed to assess whether their prediagnostic levels could predict the time to clinical presentation and subsequent cognitive decline. We also investigated how serum biomarkers changed within the prediagnostic time frame. Lastly, we evaluated the extent to which these biomarkers were influenced by comorbidities – like arterial hypertension (AHT), kidney dysfunction, diabetes, hyperlipidemia, and a history of stroke – and how this impacted their prognostic performance in AD.

2 | METHODS

2.1 | Study population

This retrospective cohort study involved analyzing data from the Confirmed Prediagnostic AD (COPRA) cohort consisting of patients diagnosed with AD at the memory clinic of University Hospitals Leuven between September 13, 2006, and January 15, 2021, according to published criteria.[17] A retrospective search of memory clinic records identified 1666 patients with an AD diagnosis. Finally, 102 AD patients were included based on the availability of a prediagnostic serum sample taken during a routine clinical visit for reasons other than cognitive complaints. The term "prediagnostic" was used to refer to the time period preceding the first memory clinic visit in which cognitive deficits could be objectified by means of neuropsychological or clinical testing. The AD diagnosis was confirmed through cerebrospinal fluid (CSF) or amyloid-positron emission tomography (PET) biomarker evidence in 50 (49%) patients. For 20 patients without CSF or amyloid-PET biomarker evidence (38%), fluorodeoxyglucose (FDG)-PET (n = 8) or structural MRI (n = 10) support, or both (n = 2), was available. The remaining 32 AD patients were included based on a consistent, clinically probable AD diagnosis in at least three consecutive memory clinic visits across an average time interval of 3 years (range 1–8 years) and supported by neuropsychological assessments. See supplementary methods and Figure S1 for further diagnostic and inclusion criteria. Clinical, neuropsychological, and comorbidity information was derived from hospital records.

A clinical disease control cohort of 21 patients with cognitive impairment due to non-AD causes called the Dementia Control cohort of Frontotemporal and Lewy Body Dementia (DoCFaD) was retrospectively retrieved in the same way as described for the COPRA cohort (supplementary methods). A third cohort of 150 Aβ− CU older adults (Aβ load < 23.5 Centiloids throughout follow-up) was selected from the community-recruited prospective Flemish Prevent AD Cohort KU Leuven (F-PACK).[11]

Ethics approval for all study procedures was obtained from the Ethics Committee of University Hospitals Leuven.

2.2 | Blood collection and biomarker measurements

For AD and non-AD patients, serum samples were retrospectively retrieved from the biobank at University Hospitals Leuven where they were stored at −20°C for a median of 12 years (range 5–16 years). For CU older adults, serum samples were prospectively collected and stored at −80°C for a median of 9 years (range 1–12 years). GFAP and NfL concentrations were quantified using the commercial N4PE Simoa kit within one batch according to the manufacturer's instructions

(Catalogue No. 103670, Quanterix, Billerica, Massachusetts, USA). $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ were not included in this study since the majority of measurements did not reach the lower limits of quantification (supplementary methods). Serum pTau181 was quantified using the pTau181 Advantage V2 Simoa kit (Catalogue No. 103714, Quanterix). Serum creatinine was measured in the earliest prediagnostic sample of each COPRA patient using either isotope dilution mass spectrometry (IDMS)-traceable methods ($n = 101$) or calculated back to the IDMS-equivalent serum creatinine concentration ($n = 1$).[18] The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI 2021 equation and used as a proxy of kidney (dys)function.[19]

2.3 | Statistical analysis

All analyses were performed in R version 4.2.2. Normality was assessed using D'Agostino–Pearson test, and outliers were detected using Rosner's test. Biomarker concentrations were transformed using either a natural log (GFAP and pTau) or reciprocal square root transformation (NfL) to approximate normality and eliminate outliers (Figure S2). To facilitate the comparison of effect sizes, transformed biomarker values were standardized through conversion to *z*-scores (based on the mean and standard deviations of transformed baseline biomarker values in the COPRA cohort). Demographic, clinical, and biomarker differences between cohorts were evaluated using two-paired *t*-tests, Mann–Whitney *U*-tests, or χ^2 tests, as appropriate. Biomarker stability in serum samples stored at −20°C was tested using linear multivariate regression models with storage time as predictor and biomarker levels as outcome. These models were adjusted for age at blood sampling, sex, and Mini-Mental State Examination (MMSE) score at clinical presentation as well as time from sampling to clinical presentation in order to limit bias for progression status.

Cox proportional hazards models examined the predictive abilities of serum biomarkers for the time to clinical presentation (survival package version 3.5.5). Linear mixed-effects (LME) models including random effects for subject (intercept) and years since blood sampling (slope) evaluated the predictive value of serum biomarkers for MMSE decline after clinical presentation (lme4 package version 1.1.33). Predictive values of biomarkers for MMSE decline were compared between AD patients and CU older adults with similar follow-up times through the inclusion of a three-way interaction term (diagnosis*time*biomarker). Since measurement of AD progression using MMSE scores is only reliable for follow-up periods longer than 3 years, these analyses were restricted to individuals with at least 3 years of MMSE follow-up.[20,21]

To assess longitudinal biomarker changes, LME models with time as predictor and biomarker levels as outcome were constructed using all available serial serum samples as well as including only serial samples taken at least 2 years prior to clinical presentation. Longitudinal changes were compared between AD patients and CU older adults through the inclusion of a two-way interaction term (cohort*time).

Lastly, the influence of prevalent comorbidities on serum biomarker levels in prediagnostic AD phases was assessed using Pearson correlation tests for continuous variables and *t*-tests for binary variables. The comorbidities demonstrating a significant influence on biomarker levels were then included as covariates in secondary Cox proportional hazards and LME models of the respective biomarkers.

Effect sizes of Cox proportional hazards models and linear models were reported adjusted for age at blood sampling, sex, and MMSE at clinical presentation (unless otherwise specified) as well as unadjusted. *P* values were corrected for multiple comparisons using Bonferroni correction. Statistical significance was set at a two-tailed $p < 0.05$.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors conducted a literature review using PubMed. While several blood biomarkers have demonstrated promising prognostic value in AD, further investigation into the impact of comorbidities is necessary to assess their clinical applicability.
2. **Interpretation**: Our findings suggest that prediagnostic blood-based GFAP provides clear prognostic information about time to clinical presentation and MMSE decline thereafter. Blood-based NfL, on the other hand, is influenced by several comorbidities, which hampers the interpretation of its prognostic value in clinical settings.
3. **Future directions**: Future studies should validate our results in a prospective manner, particularly in the context of prevalent comorbidities.

3 | RESULTS

3.1 | Cohort characteristics

AD patients and non-AD disease controls were matched with respect to age and sex. The prevalence of all tested comorbidities in AD patients was similar to that in disease controls (Table 1) and higher than in CU older adults (Table S1). Serum GFAP and NfL levels in AD patients did not change with increasing storage time at −20°C (Figure S3, Table S2). In contrast, pTau181 levels decreased with increasing storage time, and pTau181 was therefore not included in further analyses. AD patients demonstrated higher prediagnostic GFAP levels than non-AD disease controls, but no difference in prediagnostic NfL levels was found. Prediagnostic serum samples from AD patients demonstrated higher GFAP as well as NfL levels than baseline serum samples from CU older adults (Figure S4).

3.2 | Association with time to clinical AD presentation

High prediagnostic GFAP levels were associated with a shorter time to clinical presentation in AD patients (hazard ratio [HR] = 1.4, 95%

TABLE 1 Demographics of clinical study cohorts.

	AD (COPRA)	Disease controls (DoCFaD)
N	102	21
Age, mean (SD), years	69 (9)	65 (9)
Female, No. (%)	61 (60)	12 (57)
eGFR, median (IQR), mL/min/1.73 m^2	84 (30)	NA
Comorbidities, *N* (%)		
AHT	65 (64)	14 (67)
Diabetes mellitus	23 (23)	7 (33)
Hyperlipidemia	54 (53)	15 (71)
History of stroke	9 (9)	2 (10)
Pre-diagnostic time frame, mean (SD), years[a]	4 (3)	3 (3)
MMSE, median (IQR), /30[b]	24 (6)	26 (4)
Clinical follow-up time, median (IQR), years[c]	5 (2)	NA
Serum levels, median (IQR)		
Creatinine, mg/dL[d]	0.85 (0.27)	NA
GFAP, pg/mL	189 (122)	137 (66)*
NfL, pg/mL	22.9 (19.0)	17.0 (28.4)

Abbreviations: AD, Alzheimer's disease; AHT, arterial hypertension; COPRA, confirmed Prediagnostic AD; DoCFaD, Dementia Control of Frontotemporal and Lewy Body Dementia; GFAP, glial fibrillary acidic protein; IQR, interquartile range; MMSE, Mini-Mental State Examination; NA, not available; NfL, neurofilament light chain; SD, standard deviation.

[a]The prediagnostic time frame equals the time interval between first blood sampling and clinical presentation.

[b]The reported MMSE score is the MMSE score at clinical presentation.

[c]Clinical follow-up time is the time between the MMSE test at clinical presentation and the last memory clinic visit with MMSE testing.

[d]1 mg/dL (conventional unit) = 88.4 µmol/L (Système International [SI] unit).

**P* value of comparison with AD cohort <0.001.

confidence interval [CI]: 1.1–1.7, $p = 0.01$, Figure 1A). Prediagnostic serum NfL levels were not associated with time to clinical presentation (HR = 1.2, 95% CI: 0.9–1.6, $p = 0.30$, Figure 1B). Similar effect estimates were obtained when models were unadjusted for covariates (Table S3).

3.3 | Prediction of cognitive decline after clinical presentation

For 67 AD patients, cognitive performance was longitudinally assessed for at least 3 years following clinical presentation (median time interval of 5 years, range 3–10 years, Table S4). High GFAP levels during the prediagnostic stage were predictive of faster MMSE decline ($\beta_{GFAP*time} = -0.557$, 95% CI: −0.888 to −0.226, $p = 0.002$, Figure 1C). The predictive effect of prediagnostic GFAP levels for MMSE decline in AD patients was higher than that of baseline GFAP levels in CU older adults of whom the majority remained cognitively stable during follow-up (5% converted to a clinical dementia rating score of 0.5 during follow-up, $\beta_{GFAP*time*Group} = -0.452$, 95% CI: −0.694 to −0.190, $p = 0.001$). Prediagnostic NfL levels did not predict MMSE decline in either cohort (Figure 1D, Table S5). Effect estimates were comparable when unadjusted for covariates (Table S5).

3.4 | Pre-diagnostic serum biomarker changes in AD

For 43 AD patients, longitudinal blood samples taken across an average follow-up time of 6 years (range 2–13 years, Table S6) spanning pre- and post-diagnostic phases were available. Serum GFAP ($\beta_{time} = 0.114$, 95% CI: 0.074–0.153, $p < 0.001$) as well as serum NfL levels ($\beta_{time} = 0.117$, 95% CI: 0.074–0.157, $p < 0.001$) increased over time within the AD cohort across the total follow-up period. Effect sizes were comparable between GFAP and NfL.

For 13 AD patients, serial blood samples were available within the prediagnostic phase (Table S6). These serial prediagnostic samples were taken across an average follow-up time of 4 years (range 2–6 years). Only GFAP ($\beta_{time} = 0.180$, 95% CI: 0.109–0.248, $p < 0.001$, Figure 2A) and not NfL ($\beta_{time} = 0.092$, 95% CI: −0.002–0.194, $p = 0.34$, Figure 2B) demonstrated significant longitudinal increases in the prediagnostic phase. These prediagnostic serum GFAP increases occurred more rapidly than the GFAP increases observed in CU older adults ($\beta_{time*Group} = 0.158$, 95% CI: 0.089–0.229, $p < 0.001$, Figure S5). Effect estimates were comparable when unadjusted for covariates (Table S7).

3.5 | Influence of comorbidities

Within the AD cohort, prediagnostic serum GFAP levels were not associated with the eGFR (Figure 3A) or with any of the examined comorbidities (Figure 3B). Prediagnostic serum NfL levels were higher in subjects with a low eGFR ($\rho = -0.65$, $p < 0.001$, Figure 3C) and were also elevated in patients with AHT ($p = 0.001$) or a history of stroke ($p = 0.008$, Figure 3D). After adjustment for these comorbidities, serum NfL demonstrated predictive value for the time to clinical AD presentation (HR = 1.7, 95% CI: 1.2–2.3, $p = 0.003$). In contrast, a comorbidity-adjusted model for the prediction of MMSE decline following clinical AD presentation showed no predictive value of prediagnostic NfL levels ($p = 0.49$, Figure S6, Table S8).

4 | DISCUSSION

This retrospective cohort study showed that prediagnostic AD patients – whose serum biomarker levels were measured on average 4 years (range 0–11 years) prior to clinical presentation – exhibit elevated GFAP levels compared to patients in the prediagnostic phase of non-AD cognitive disorders. Higher prediagnostic GFAP levels predicted a shorter time to clinical AD presentation as well as faster cognitive decline thereafter. GFAP increased during the prediagnostic AD phase. Prediagnostic NfL levels were comparable between AD

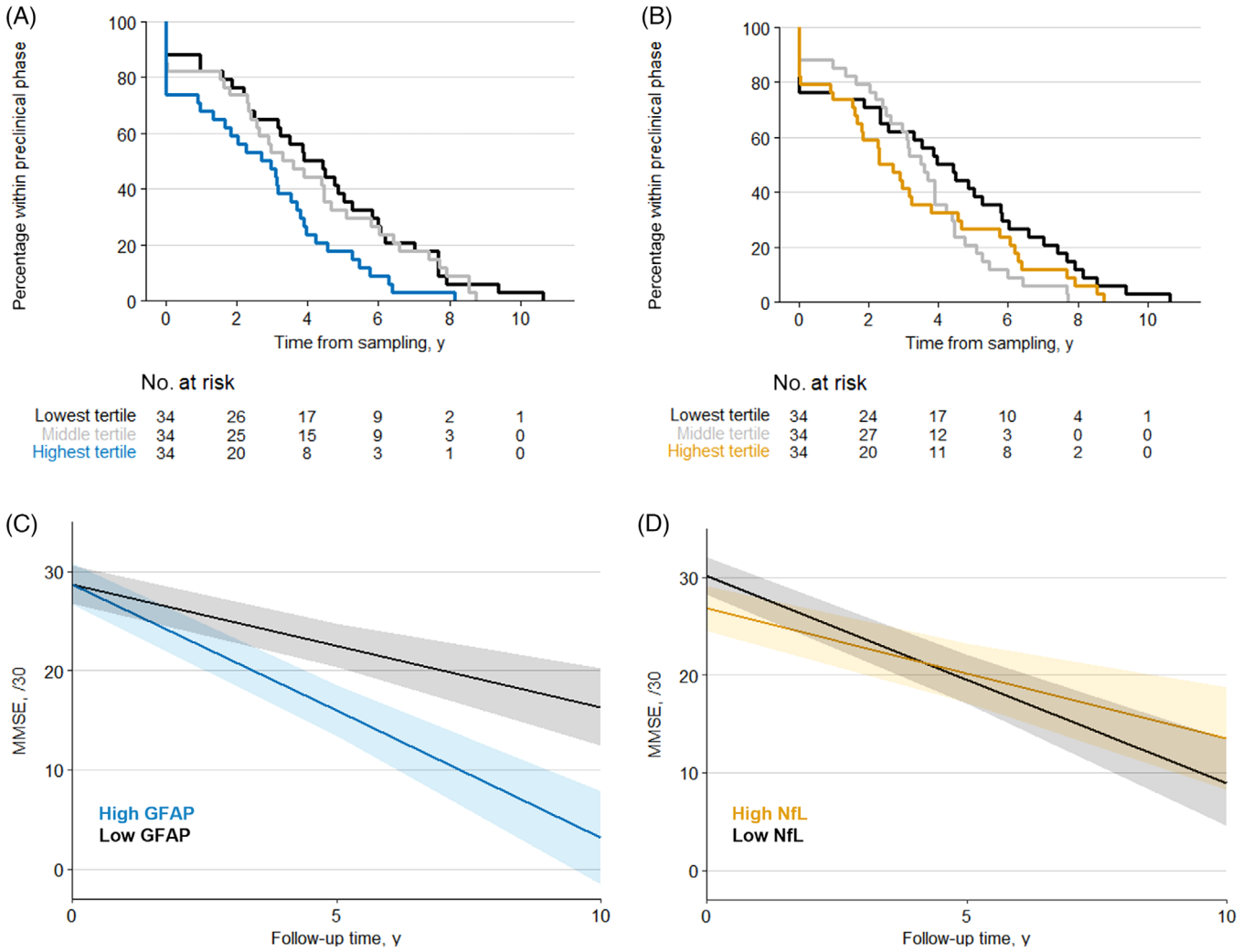

FIGURE 1 Association of prediagnostic serum biomarker levels with AD progression. (A and B) Kaplan–Meier curves demonstrate the association between prediagnostic serum biomarker levels and time to clinical AD presentation through stratification in tertiles. (A) GFAP levels were evaluated at the lowest tertile (< 18 pg/mL, black), the middle tertile (18–30 pg/mL, gray), and the highest tertile (> 30 pg/mL, blue). (B) NfL levels were evaluated at the lowest tertile (< 165 pg/mL, black), the middle tertile (165–230 pg/mL, gray), and the highest tertile (> 230 pg/mL, yellow). (C and D) Interaction plots demonstrate MMSE change over time for high (GFAP > 189 pg/mL in blue [C], NfL > 23 pg/mL in yellow [D]) and low (black) prediagnostic serum biomarker levels, respectively, adjusted for age at blood sampling, sex, and MMSE at clinical presentation. AD, Alzheimer's disease; GFAP, glial fibrillary acidic protein; HR, hazard ratio; MMSE, Mini-Mental State Examination; NfL, neurofilament light chain.

and non-AD patients. In AD, prediagnostic NfL levels also demonstrated predictive value for time to clinical presentation – albeit only when adjusted for comorbidities like AHT, kidney dysfunction, and a history of stroke – but not for cognitive decline after clinical presentation. Serum pTau181 levels decreased with increasing storage time at −20°C, which prevented evaluation of the prognostic value of pTau181 in this cohort.

Our findings support the use of serum GFAP as a prognostic marker for AD in clinical settings, both in terms of time to clinical presentation and cognitive decline following clinical presentation. Similarly, a previous large-scale population-based study demonstrated that plasma GFAP could predict clinical AD incidence more than a decade prior to diagnosis.[22] Two other studies where participants were followed over time periods of respectively 3 and 16 years, equally demonstrated the potential of blood-based GFAP as a risk biomarker for clinical AD onset.[7,23] Blood-based GFAP has also been shown to predict MMSE decline in preclinical AD cohorts within follow-up times of 3–5 years after blood sampling.[7,24,25] We now showed that prediagnostic GFAP levels are also predictive for cognitive decline following clinical presentation, indicating that GFAP is an early marker for disease progression across the AD continuum from early stages.

Prior studies predominantly included population-based cohorts well-controlled for comorbidities. Consequently, information about the translatability of the prognostic value of blood-based biomarkers to clinical settings – where comorbidities are prevalent – is lacking. This is important as the impact of comorbidities on serum biomarker levels could potentially confound their prognostic utility for AD. The current study was performed in a population that presented to the

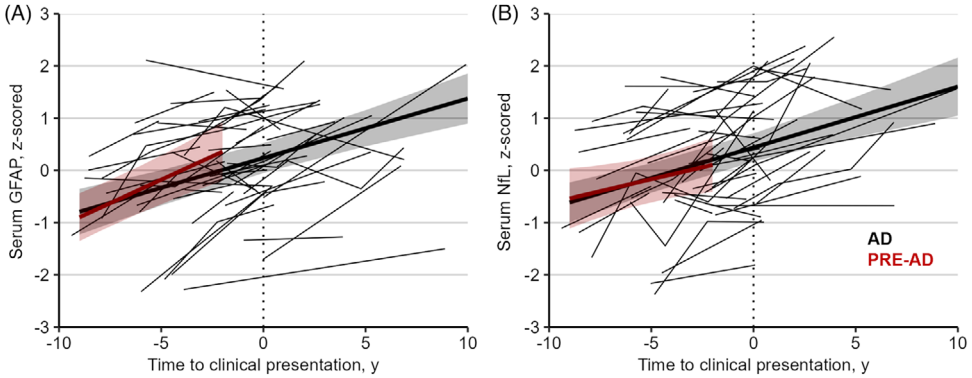

FIGURE 2 Prediagnostic serum biomarker changes in AD. Spaghetti plots show individual trajectories of serum GFAP (A) and NfL (B) levels in AD patients. The vertical dotted lines indicate the time of clinical presentation. Linear fits with 95% CIs of relationship between time and serum biomarker levels were derived from LME models and are superimposed. The black line indicates the serum biomarker change across the entire follow-up period ($n = 43$). The red line indicates the serum biomarker change within the prediagnostic time frame (at least 2 years prior to onset, $n = 13$). AD, Alzheimer's disease; CI, confidence interval; LME, linear mixed-effects; PRE-AD, prediagnostic AD; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain.

memory clinic with clinical AD, but for whom prediagnostic serum samples, taken up to a decade before clinical presentation, were available. In this AD cohort, the prevalence of comorbidities such as AHT, diabetes, hyperlipidemia, and a history of stroke was higher than that in the community-recruited CU cohort. This allowed assessment of the influence of comorbidities on prediagnostic biomarker levels as well as their prognostic value in AD. For GFAP, no associations with any of the aforementioned comorbidities were found. NfL, on the other hand, was elevated in patients with AHT, a history of stroke, or a low eGFR, like previously reported in population-based studies.[26–30] Although an earlier population-based study stated that comorbidities, such as kidney dysfunction (measured by serum creatinine levels) had only a minimal effect on the ability of serum NfL to predict AD conversion within a 4-year period, we only found prognostic value for NfL after adjustment for comorbidities at the time of sampling.[31] This contrast might be explained by the lower prevalence of comorbidities in the previous cohort.

With respect to the prediction of cognitive decline following clinical presentation, we found no significant effect for prediagnostic serum NfL levels in AD patients with or without comorbidity adjustment, similar to what was observed in CU older adults without Aβ pathology. This corroborates prior findings of limited to no predictive value of NfL for MMSE decline in preclinical phases.[7,24,25] In contrast, in populations spanning the AD continuum, thus including clinical stages, blood-based NfL was able to predict cognitive decline across average time periods of 2–6 years.[15,32,33] These findings suggest that the dynamic phase of NfL release into the bloodstream coincides with symptom onset. Although blood-based NfL has been demonstrated to rise 15 years before expected symptom onset in autosomal dominant AD, they progressively increase thereafter, reaching their peak rates of change within the clinical disease phase.[34] Conversely, other blood-based biomarkers, including GFAP, reach their peak rates of change in earlier preclinical stages.[35,36] Likewise, we observed GFAP increases within the prediagnostic AD phase, which occurred more rapidly than longitudinal GFAP increases in CU older adults without evidence of Aβ pathology. In contrast, NfL increases were only observed across the total follow-up time, with no significant differences in longitudinal NfL changes during the prediagnostic phase compared to those observed in Aβ-negative CU older adults. This suggests that NfL increases predominantly occur around or after symptom onset, consistent with prior reports in both AD and FTD.[34–38]

In addition to GFAP, blood-based pTau previously showed good prognostic value for AD through accurate prediction of clinical AD onset as well as cognitive decline.[16,24,25,39,40] We were not able to evaluate the prognostic value of serum pTau181 due to its instability when stored at −20°C. The biomarker utility of pTau181 has so far predominantly been evaluated in plasma.[3,5,41,42] In the current study, all biomarkers were quantified in serum since our clinical laboratory systematically performs long-term storage of serum rather than plasma samples in case serological tests are ordered. However, serum pTau181 concentrations correlate strongly with their plasma counterparts and have demonstrated comparable performances to detect AD in both clinical and preclinical stages.[11,42–44] Similarly, serum and plasma measurements of both GFAP and NfL have demonstrated strong correlations and are both commonly used in biomarker studies.[7,12,45,46]

A correct understanding of the predictive value of blood-based biomarkers for AD onset and further cognitive decline is pivotal in clinical settings. It facilitates the early identification of patients at risk for developing clinical symptoms while also providing the means to evaluate the patient-specific risks and benefits associated with the initiation of disease-modifying treatments. Altogether our results suggest that GFAP might be valuable in this clinical risk assessment as it provides reliable prognostic information that allows a straightforward interpretation with minimal impact of comorbidities. Alternatively, the high impact of comorbidities on blood-based NfL levels complicates its implementation as a prognostic marker in the clinic. To assess the real-world performance of GFAP for clinical implementation, future studies

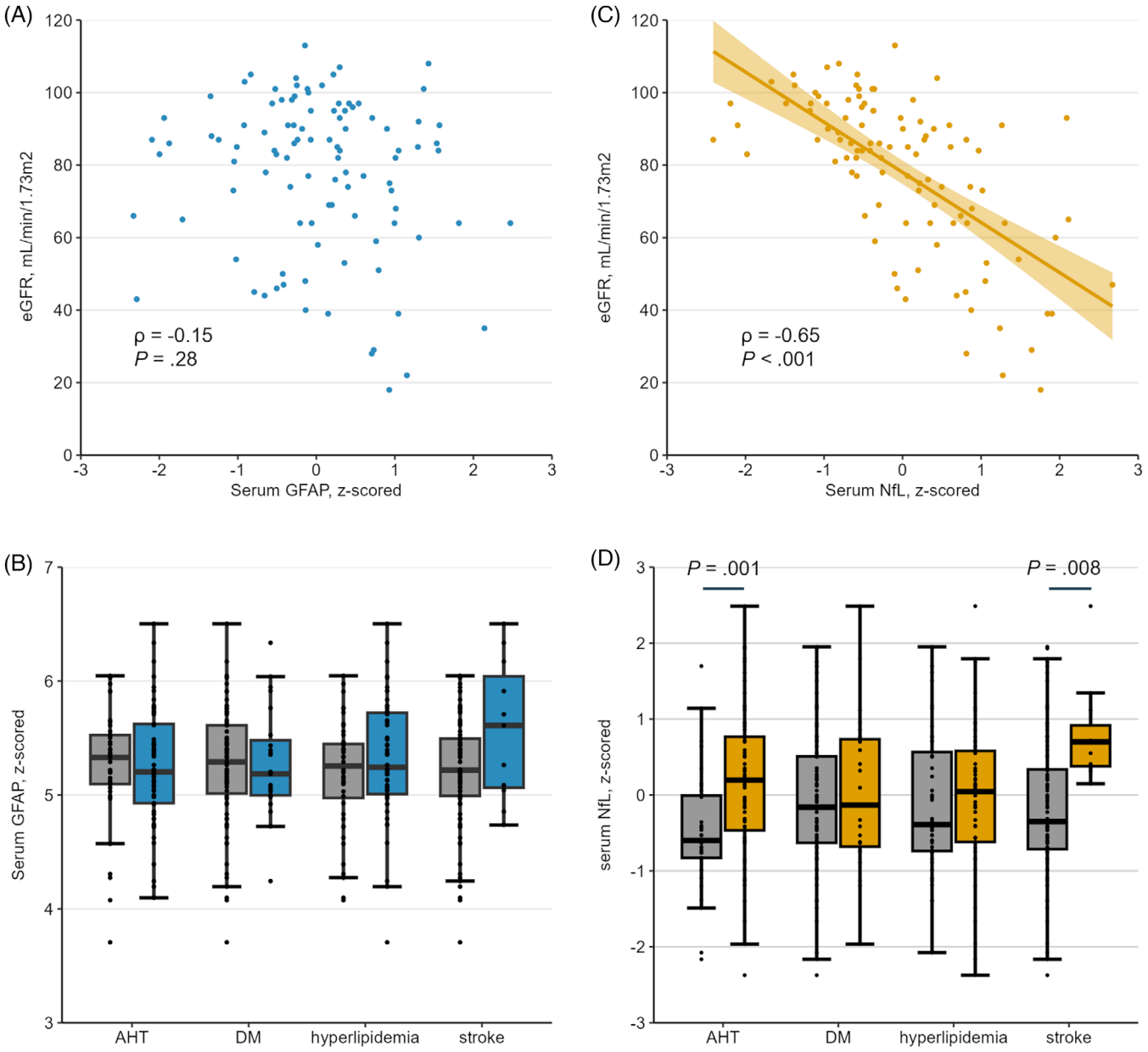

FIGURE 3 Association of prediagnostic serum biomarker levels with comorbidities in a memory clinic setting. (A and C) Scatter plots show associations between eGFR and respectively serum GFAP (A, blue) and NfL (C, yellow) levels after transformation and standardization. (B and D) Box-and-whisker plots show differences in prediagnostic serum GFAP (B, blue) and NfL (D, yellow) levels based on presence of certain comorbidities. The middle line of the box represents the median, while the upper and lower lines denote the interquartile range. The whiskers indicate 1.5 × interquartile range. *P* values were computed with *t*-tests using transformed and standardized serum biomarker levels and were Bonferroni-corrected for multiple comparisons. AHT, arterial hypertension; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain.

should validate the prognostic performance of GFAP – particularly in a context of prevalent comorbidities – in a prospective manner.

A limitation of the current study is that only half of AD diagnoses were based on biomarker evidence. To limit the inclusion of patients without underlying AD pathology, we only included patients in which a clinical AD diagnosis was supported by neuropsychological evidence and remained consistent during at least three consequent memory clinic consultations. Moreover, several cases without biomarker evidence did have structural MRI or FDG-PET support ($n = 20$). Another limitation is that the proportion of AD patients for which prediagnostic serum samples were available was small (9%) compared to all AD patients presenting to the memory clinic and that these prediagnostic serum samples were taken during clinical visits (ie, for non-neurological complaints). This could have introduced a bias toward a higher prevalence of comorbidities than would normally be observed in a memory clinic population as well as toward larger differences in comorbidity prevalence between the AD cohort and the academic cohort of CU older adults. Moreover, due to the retrospective nature of our

study, we could not determine the exact time point of clinical AD onset. Instead, the term "prediagnostic" was used to refer to the time period prior to presentation to the memory clinic with objective cognitive impairment. Consequently, the AD severity at which patients presented to the clinic also differed among patients. In response, we included time to clinical presentation as a continuous variable and adjusted all models for MMSE score at clinical presentation. Moreover, the serum samples of the COPRA cohort were convenience samples from the hospital, which likely introduced variability in the number of freeze-thaw cycles and centrifugation or storage delays. Prior studies showed that freeze-thaw cycles influenced blood-based levels of Aβ, total tau, and pTau181,[47,48] whereas GFAP and NfL levels were unaffected for up to at least four freeze-thaw cycles.[45,47,48] Similarly, delayed storage has been shown to decrease blood-based Aβ, total tau, and, to a lesser extent, pTau181 levels, whereas no such effects were observed for GFAP or NfL.[47,48] Of note, these pre-analytical effects on blood-based Aβ levels might explain why the majority of serum Aβ measurements in the convenience samples of the COPRA cohort did not reach the limit of detection. Lastly, the sample processing (eg, centrifugation, aliquoting) and storage conditions (-20°C versus -80°C) of the serum samples differed between the AD patients and CU older adults. Direct comparisons were therefore not the main focus of the current study but were included as supplementary information given the clinical relevance of different predictive values and longitudinal biomarker changes between prediagnostic AD patients (in the decade leading up to clinical presentation) and CU older adults.

In conclusion, elevated prediagnostic serum GFAP levels are a predictor of a shorter time to clinical presentation in AD as well as faster cognitive decline following clinical presentation. In contrast, serum NfL is influenced by various comorbidities that complicate the interpretation of its predictive capability for AD progression. These findings highlight the potential utility of serum GFAP as a prognostic biomarker in the early identification of AD progression and, consequently, early intervention in clinical settings. Conversely, caution is required when assessing prediagnostic serum NfL since contextual factors (eg, comorbidities) need to be carefully evaluated in order to correctly interpret its prognostic utility.

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CONFLICT OF INTEREST STATEMENT

The authors S.D.M., E.R.B., and J.M.S. have no competing interests to disclose. R.V.'s institution has had a clinical trial agreement for phase 1 and 2 studies with GE Healthcare, which provided [18F]flutemetamol for this study, as well as with Alector, AviadoBio, Biogen, Denali, Eli Lilly, Johnson & Johnson, Roche/Genentech, Wave, and UCB. R.V.'s institution has consultancy agreements for participation in DSMB (R.V. as a consultant) with AC Immune and Novartis. K.P. has a consultancy agreement with Eisai. K.P.'s institution has biomarker trial agreements with ADx NeuroSciences, Euroimmun, and Fujirebio. K.P. is a member of the scientific advisory board of Stichting Alzheimer Onderzoek/Fondation Recherche Alzheimer, Belgium. C.E.T. performed contract research for Acumen, ADx Neurosciences, AC-Immune, Alamar, Aribio, Axon Neurosciences, Beckman-Coulter, BioConnect, Bioorchestra, Brainstorm Therapeutics, Celgene, Cognition Therapeutics, EIP Pharma, Eisai, Eli Lilly, Fujirebio, Grifols, Instant Nano Biosensors, Merck, Novo Nordisk, Olink, PeopleBio, Quanterix, Roche, Toyama, and Vivoryon. She is editor-in-chief of Alzheimer's Research and Therapy and serves on the editorial boards of *Medidact Neurologie*/Springer and *Neurology: Neuroimmunology & Neuroinflammation*. She has had consultancy/speaker contracts for Eli Lilly, Merck, Novo Nordisk, Olink, and Roche. K.A. currently holds the position of associate director at Eli Lilly and Company, but her contributions to this study occurred prior to assuming this role. Author disclosures are present in Supporting Information.

CONSENT STATEMENT

All participants provided written informed consent in accordance with the declaration of Helsinki.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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