Longitudinal associations of serum biomarkers with early cognitive, amyloid and grey matter changes

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Abstract

Blood-based biomarkers have been extensively evaluated for their diagnostic potential in Alzheimer's disease. However, their relative prognostic and monitoring capabilities for cognitive decline, amyloid-β (Aβ) accumulation and grey matter loss in cognitively unimpaired elderly require further investigation over extended time periods.

This prospective cohort study in cognitively unimpaired elderly (n = 185, mean age [range] = 69 [53-84] years, 48% female) examined the prognostic and monitoring capabilities of glial fibrillary acidic protein (GFAP), neurofilament light (NfL), $A\beta_{1-42}/A\beta_{1-40}$ and phosphorylated tau (pTau)181 through their quantification in serum. All participants underwent baseline Aβ-PET, MRI and blood sampling as well as two-yearly cognitive testing. A subset additionally underwent Aβ-PET (n = 109), MRI (n = 106) and blood sampling (n = 110) during follow-up (median time interval [range] = 6.1 [1.3-11.0] years). Matching plasma measurements were available for $A\beta_{1-42}/A\beta_{1-40}$ and pTau181 (both n = 140).

Linear mixed-effects models showed that high serum GFAP and NfL predicted future cognitive decline in memory ($\beta_{GFAP*time}$= -0.021, P_{FDR} = .007 and $\beta_{NfL*time}$ = -0.031, P_{FDR} = .002) and language ($\beta_{GFAP*time}$ = -0.021, P_{FDR} = .002 and $\beta_{NfL*time}$ = -0.018, P_{FDR} = .03) domains. Low serum $A\beta_{1-42}/A\beta_{1-40}$ equally but independently predicted memory decline ($\beta_{A\beta1-42/A\beta1-40*time}$ = -0.024, P_{FDR} = .02). Whole-brain voxelwise analyses revealed that low $A\beta_{1-42}/A\beta_{1-40}$ predicted Aβ accumulation within the precuneus and frontal regions, high GFAP and NfL predicted grey matter loss within hippocampal regions and low $A\beta_{1-42}/A\beta_{1-40}$ predicted grey matter loss in lateral temporal regions. Serum GFAP, NfL and pTau181 increased over time, while $A\beta_{1-42}/A\beta_{1-}$

$_{40}$ decreased only in Aβ-PET-negative elderly. NfL increases associated with declining memory ($\beta_{NfLchange*time}$ = -0.030, P_{FDR} = .006) and language ($\beta_{NfLchange*time}$ = -0.021, P_{FDR} = .02) function and serum $A\beta_{1-42}/A\beta_{1-40}$ decreases associated with declining language function ($\beta_{A\beta1-42/A\beta1-40*time}$ = -0.020, P_{FDR} = .04). GFAP increases associated with Aβ accumulation within the precuneus and NfL increases associated with grey matter loss. Baseline and longitudinal serum pTau181 only associated with Aβ accumulation in restricted occipital regions. In head-to-head comparisons, serum outperformed plasma $A\beta_{1-42}/A\beta_{1-40}$ (ΔAUC = 0.10, $P_{DeLong,FDR}$ = .04), while both plasma and serum pTau181 demonstrated poor performance to detect asymptomatic Aβ-PET positivity (AUC = 0.55 and 0.63, respectively). However, when measured with a more phospho-specific assay, plasma pTau181 detected Aβ-positivity with high performance (AUC = 0.82, $P_{DeLong,FDR}$ < .007).

In conclusion, serum GFAP, NfL and $A\beta_{1-42}/A\beta_{1-40}$ are valuable prognostic and/or monitoring tools in asymptomatic stages providing complementary information in a time- and pathology-dependent manner.

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Abbreviations: Aβ = amyloid-β; AAL = Automatic Anatomical Labeling; AUC = area under the curve; AMYPAD = Amyloid Imaging to Prevent Alzheimer's disease; AVF = Animal Verbal Fluency; AVLT DR = Auditory Verbal Learning test: delayed recall; AVLT TL = Auditory Verbal Learning test: total learning; BNT = Boston Naming Test; BSRT DR = Buschke Selective Reminding Test: delayed recall; BSRT TR = Buschke Selective Reminding Test: total retention; CAT = Computational Anatomy Toolbox; CDR = Clinical Dementia Rating; CU = cognitively unimpaired; FDR = false discovery rate; F-PACK = Flemish Prevent Alzheimer's Disease Cohort KU Leuven; GFAP = glial fibrillary acidic protein; LVF = Letter Verbal Fluency; MMSE = Mini-Mental State Examination; MNI = Montreal Neurological Institute; NfL = neurofilament light; PACC = Preclinical Alzheimer Cognitive Composite; PALPA = Psycholinguistic Assessment of Language Processing in Aphasia; PiB = Pittsburgh Compound B; pTau = phosphorylated tau; RFT = random field theory; RPM = Raven's Progressive Matrices; SUVR = standardised uptake value ratio; TIV = total intracranial volume; TMT = Trial Making Test

Introduction

Alzheimer's disease has a long asymptomatic phase characterised by the formation of Alzheimer's disease-specific hallmarks – amyloid-β (Aβ) plaques and phosphorylated tau (pTau) tangles – as well as downstream consequences like astrogliosis and neurodegeneration.[1,2] If initiated within this asymptomatic phase, disease-modifying treatments may be able to avert or

slow down downstream consequences and potentially prevent symptomatic Alzheimer's disease onset. Therefore, clinical trials in Alzheimer's disease have started to target cognitively unimpaired (CU) elderly demonstrating Alzheimer's disease pathology. This recruitment has historically been based on genetic risk together with PET- and CSF-based evidence of Alzheimer's disease pathology.[3] However, more recently, blood-based measures have provided a more scalable alternative. The most promising blood-based biomarkers to date are the $A\beta_{1-42}/A\beta_{1-40}$ ratio – particularly when quantified in plasma using mass-spectrometry methods – and pTau species – quantified in plasma using either immunoassays or mass-spectrometry – as they reflect Alzheimer's disease hallmarks from preclinical stages.[4–11] Glial fibrillary acidic protein (GFAP) – presumably reflecting astrogliosis – and neurofilament light (NfL) – reflecting neurodegeneration – have also shown promise as blood-based biomarkers for Alzheimer's disease from respectively preclinical and prodromal stages.[12–21] The use of blood-based biomarkers to pre-screen potential clinical trial participants may drive down costs as well as invasiveness and increase the scalability of recruitment procedures. Of note, rates of cognitive decline and neurodegeneration in preclinical Alzheimer's disease are heterogeneous[22–24] and accompany Aβ accumulation prior to significant Aβ pathology[25,26] with Aβ accumulation slowing down at later Aβ stages, regardless of clinical stage.[27,28] The ability to select asymptomatic individuals who are likely to exhibit Aβ accumulation, grey matter loss and/or cognitive decline in the years following inclusion would thus increase the power of clinical trials to detect therapeutic effects on these key outcome measures.

Although evidence of the predictive value of blood-based biomarkers for cognitive decline,[14,15,35–39,16,21,29–34] Aβ accumulation[21,33,40] and grey matter loss[14,15,29,33,38,39,41] is emerging, most studies have limited follow-up periods or lack domain-specific assessments, thus potentially missing the opportunity to observe substantial pathological changes in asymptomatic stages. Moreover, head-to-head comparisons of prognostic and monitoring performances of blood biomarkers for simultaneous pathological events (i.e. cognitive decline, Aβ accumulation as well as grey matter loss in the same cohort) are lacking. Additional information about the ability of blood biomarkers to predict cognitive decline over extended time periods and insight into how these prognostic capabilities relate to Aβ accumulation or grey matter loss would better contextualise the advantage of blood-based biomarker implementation in clinical trial recruitment as well as their potential clinical utility once drugs become available. Moreover, the

Alzheimer's Association appropriate use recommendations for blood biomarkers in Alzheimer's disease state that more observational studies on longitudinal blood-based biomarker changes and their relationship with clinically relevant outcomes, as well as more established biomarkers – like Aβ-PET and MRI – are needed to assess their monitoring potential.[42]

Both the $A\beta_{1-42}/A\beta_{1-40}$ ratio and pTau181 levels are higher in plasma than in serum and plasma measurements are generally used for their quantification in blood-based biomarker studies. Recent evidence suggests that plasma is a more suitable matrix for $A\beta_{1-42}/A\beta_{1-40}$ quantification, whereas serum pTau181 has demonstrated strong correlations with plasma pTau181 as well as similar performance to detect clinical Alzheimer's disease.[9,43,44] However, there is a lack of head-to-head comparisons of the clinical performance of plasma and serum measurements of $A\beta_{1-42}/A\beta_{1-40}$ and pTau181 in asymptomatic stages of the disease. In contrast, for both GFAP and NfL, serum and plasma measurements demonstrated near-perfect correlations and both matrices are commonly used in biomarker studies for clinical as well as asymptomatic Alzheimer's disease.[12,13,15–17,45–48] In addition to the matrix in which the analyte is measured, it is crucial to recognise the significance of the assay used for its quantification. This importance has been substantiated in previous research showing that the ADx252 antibody-based plasma pTau181 assay consistently outperforms assays based on the AT270 antibody – which we previously showed to cross-react with pTau175 – with respect to detecting Alzheimer's disease.[5,7,11,49] However, these comparative studies have exclusively focussed on their performance within clinical disease stages.

In this prospective cohort study, we investigated whether baseline measures of serum GFAP, NfL, $A\beta_{1-42}/A\beta_{1-40}$ and pTau181 in CU elderly can predict changes in cognitive performance across memory, language and executive functioning domains as well as changes in Aβ load or grey matter volume over a median time period of 6.1 (range 1.3–11.0) years. As a secondary objective, we assessed whether longitudinal changes in these serum biomarkers accompanied cognitive decline, Aβ accumulation and grey matter loss. Finally, we aimed to contextualise our serum-based findings by comparing the performances of serum and plasma measurements of $A\beta_{1-42}/A\beta_{1-40}$ as well as pTau181 with respect to classifying CU individuals by Aβ-PET status.

Materials and methods

Study population

The Flemish Prevent Alzheimer's Disease Cohort KU Leuven (F-PACK) is a community-recruited observational cohort of CU elderly being followed over 10 years by the Laboratory for Cognitive Neurology at KU Leuven. Inclusion criteria were age between 50 and 80 years and normal cognition as defined by a Mini-Mental State Examination (MMSE) score ≥ 27, a Clinical Dementia Rating (CDR) = 0 and neuropsychological test scores within published norms. Further details on eligibility criteria were described previously.[50,51] Recruitment took place between March 2009 and May 2020 and followed a two-factorial genetic stratification scheme with an equal number of sex- and education-matched participants per 5-year age bin, as well as an equal number of *APOE-ε4* and *BDNF val66met* carriers versus non-carriers in each bin. All participants gave written informed consent in accordance with the declaration of Helsinki.

F-PACK participants were eligible for inclusion in the current study if they underwent neuropsychological assessment, Aβ-PET as well as blood sampling at baseline in addition to at least one follow-up visit with neuropsychological testing. This yielded a total study population of 185 participants. Neuropsychological assessment was performed every two years for up to five times (median follow-up time [range] = 6.1 [1.3-11.0] years). A subset underwent serial blood sampling (n = 110) and imaging (n = 109) at two (n = 72 for blood, n = 71 for imaging), three (n = 36), or four (n = 2) time points (median follow-up time [range] = 6.3 [2.6–10.8] years). All participants provided written informed consent according to the Declaration of Helsinki and study protocols were approved by the Ethics Committee of University Hospitals Leuven (S66140, S51125, S57168, S61444).

Serum collection and analysis

Blood was collected in Vacutainer serum separator tubes (cat#367985, Becton Dickinson, Franklin Lakes, NJ, USA) at University Hospitals Leuven. Samples were inverted five times and centrifuged at 1,200*g* for 10 min at 4°C. The supernatant was aliquoted in polypropylene cryovials (cat#363401, Thermo Fisher Scientific, Waltham, MA, USA) and snap frozen in liquid nitrogen followed by storage at -20°C for 24h prior to long-term storage at -80°C.

Biomarker measurements were performed at Amsterdam UMC. Serum GFAP, NfL, $A\beta_{1-42}$ and $A\beta_{1-40}$ were quantified using the Simoa Neurology 4-Plex E kit (cat#103670, Quanterix, Billerica, MA, USA) according to manufacturer's instructions. Serum pTau181 was quantified using the Simoa pTau-181 Advantage V2 kit (cat#104111, Quanterix, Billerica, MA, USA). Prior to biomarker measurements, serum samples were thawed, vortexed and centrifuged at 10,000*g* for 10 min. Longitudinal samples from the same subject were measured within the same plate. All GFAP, NfL, pTau181 and $A\beta_{1-40}$ measurements exceeded the detection limits, while two follow-up $A\beta_{1-42}$ measurements (0.6%) did not. In addition, two follow-up samples demonstrated extreme high outliers for $A\beta_{1-42}/A\beta_{1-40}$ despite low $A\beta_{1-42}$ concentrations due to unusually low $A\beta_{1-40}$ measurements (< 2.4 pg/mL) and were omitted (Supplementary Fig. 1). Omitted $A\beta_{1-42}/A\beta_{1-40}$ ratios and those with $A\beta_{1-42}$ values below the detection limits were imputed to 0.01 (minimal value/2). Intra- and inter-assay coefficients of variation ranged between 1.5-6.7% and 4.4-16.1%, respectively (Supplementary Table 1). For a subset of participants, matching plasma $A\beta_{1-42}/A\beta_{1-40}$ (n = 140) and pTau181 (n = 141) measurements were available. Plasma $A\beta_{1-42}/A\beta_{1-40}$ was measured using the Amyblood assay.[4,6] Plasma pTau181 was measured with the AT270-based Quanterix Simoa pTau-181 Advantage V2 kit – to match the serum-based measurements – as well as with a phospho-specific ADx252-based Simoa assay (ADx NeuroSciences, Ghent, Belgium), which has only been analytically validated for use in plasma.[7] One subject with matching pTau181 measurements was omitted from inter-matrix comparisons due to a Quanterix plasma pTau181 measurement below the detection limit.

Neuropsychological assessment

Cognitive performance was assessed through extensive neuropsychological assessment covering episodic memory, language and executive functioning domains. Episodic memory function was examined through the Rey's Auditory Verbal Learning Test (AVLT) by means of total learning (AVLT TL, /75), delayed recall (AVLT DR, %) and recognition (/15) parameters as well as through the 12-item Buschke Selective Reminding Test (BSRT) by means of the average total retention (BSRT TR, /12) and delayed recall (BSRT DR, /12) parameters. Language processing was assessed by the Boston Naming Test (BNT, /60), Animal Verbal Fluency (AVF, #words) and the Psycholinguistic Assessment of Language Processing in Aphasia (PALPA, /30) item 49. Executive functioning was measured through the Standard Raven's Progressive Matrices (RPM,

/60), Trial Making Test B over A (TMT B/A) and Letter Verbal Fluency (LVF, #words). For each domain, composite scores were calculated by converting individual test scores to standardised z-scores (based on averages & standard deviations of baseline results within the Aβ-PET-negative subset) and subsequent averaging per domain.

Amyloid-β PET

Aβ-PET scans were acquired on a 16-slice Biograph PET/CT scanner (Siemens, Erlangen, Germany) at University Hospitals Leuven using [^{18}F]flutemetamol (n = 256) or [^{11}C]Pittsburgh Compound B ([^{11}C]PiB (n = 78) tracers with net injection doses of respectively 155 ± 16 and 271 ± 39 MBq. PET data obtained in the 90-120 min and 30-60 min post-injection window for [^{18}F]flutemetamol and [^{11}C]PiB, respectively, were reconstructed into 6 x 5 minute frames using the ordered subset expectation maximisation iterative algorithm (4 iterations x 16 subsets for all baseline PET & 102 follow-up PET scans, 4 iterations x 21 subsets for 47 follow-up scans obtained within the Amyloid Imaging to Prevent Alzheimer's Disease (AMYPAD) project).[51–53] Standardised uptake value ratio (SUVR) images (voxel size 2 x 2 x 2 mm^3) were constructed using a participant-specific cerebellar grey matter reference region based on 90-110 min (for [^{18}F]flutemetamol) or 40-60 min (for [^{11}C]PiB) post-injection PET measurements. Subjects with longitudinal Aβ-PET data (n = 109) underwent either two (n = 50) or three (n = 17) [^{18}F]flutemetamol scans or one [^{11}C]PiB scan combined with one (n = 21), two (n = 179) or three (n = 2) [^{18}F]flutemetamol scans. Composite SUVRs were calculated in a cortical volume of interest consisting of five bilateral cortical regions derived from the Automated Anatomical Labeling (AAL) atlas: frontal (AAL areas 3–10, 13–16, and 23–28), parietal (AAL 57–70), anterior cingulate (AAL 31–32), posterior cingulate (AAL 35–36) and lateral temporal (AAL 81–82 and 85–90), and were converted to Centiloids.[6,50,52] Aβ-positivity was defined as a tracer uptake ≥ 23.5 Centiloids, a neuropathologically validated threshold identifying Thal phase 3-to-5.[7,52–54] For voxelwise analyses, Centiloid maps were constructed as previously described.[7]

Structural MRI

High-resolution structural MRI was performed using a 3 Tesla scanner (Philips, Best, Netherlands) in all but one participant (occupational welder).[52] One or two follow-up T1-weighted MRI scans were available for 106 subjects with a median follow-up period of 6.3

(range 2.6–10.8) years. All baseline scans and 121 T1-weighted follow-up scans were obtained using a turbo field echo sequence.[50] The remaining 23 follow-up T1-weighted scans were obtained using a 3D magnetisation-prepared rapid gradient-echo sequence within AMYPAD.[53] A subset of 180 F-PACK subjects additionally underwent a 2D T2-weighted fluid attenuated inversion recovery image at baseline (TR/TE/TI = 11000/125/2800 ms, voxel size = 0.45 x 0.45 x 5 mm^3, 24 slices). The lesion segmentation toolbox was applied on T2-weighted images and corresponding T1-weighted images to generate white matter lesion probability maps (kappa = 0.05) from which white matter lesion volumes were extracted.

Voxel-based morphometry

T1-weighted images were segmented and non-linearly resampled to Montreal Neurological Institute (MNI) space using the default Computational Anatomy Toolbox (CAT12, http://dbm.neuro.uni-jena.de/cat/) pipeline for longitudinal data and total intracranial volume (TIV) was extracted. Resulting modulated grey matter maps were smoothed with an 8 mm Gaussian filter.

Statistical analyses

Statistical analyses were performed using R version 4.1.2. Normality and homoscedasticity were tested with D'Agostino-Pearson test and Levene's test, respectively. Serum biomarker concentrations were ln transformed (GFAP, NfL and pTau181) or squared ($A\beta_{1-42}/A\beta_{1-40}$) prior to parametric analyses or if required to meet model assumptions (i.e. normality and homoscedasticity of residuals). For comparisons of demographic variables between Aβ-PET based subgroups, unpaired two-sided *t*-tests, Mann-Whitney *U* tests or X^2 tests were used, as appropriate. Between-group differences in Aβ-PET load and grey matter volume were assessed through voxelwise comparisons of respectively Centiloid maps and grey matter maps using two-sample *t*-tests in SPM12. Spearman correlations were calculated between serum biomarkers as well as between biomarkers and white matter lesion volumes. Annual rates of change in cognition, Aβ-PET Centiloids and serum biomarkers were assessed using linear mixed-effects models (R package *lme4*) including random effects for subject (intercept) and years since baseline (slope). All models were corrected for age and sex. *APOE-ε4* genotype and education were included as covariates for prediction of cognitive decline if they demonstrated a significant effect in multivariate models (Supplementary Table 2). Rates of change in Aβ-PET load were

adjusted for *APOE-ε4* genotype since asymptomatic *APOE-ε4* carriers have higher Aβ accumulation rates than non-carriers while rates of grey matter loss were adjusted for TIV.[53] The differences between Aβ-subgroups were examined through inclusion of an interaction term between baseline Aβ-status and years since baseline. Linear mixed-effects models were also constructed in Aβ-subgroups separately. The unadjusted subject-specific slopes of linear mixed-effects models for longitudinal serum biomarker changes were used as a measure of annual serum biomarker rate of change in secondary linear mixed-effects models described below.

As a primary outcome analysis, the performance of serum biomarkers to predict cognitive decline, Aβ accumulation and grey matter loss was examined using linear mixed-effects models with the interaction term between serum biomarker levels and time since baseline blood sampling – further referred to as time – as predictor. In cognition-based analyses, $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratios were inverted and biomarkers were entered as z-scores to facilitate comparisons. Domain-averaged cognitive scores as well as individual neuropsychological test scores were used as outcome variables. The CART imputation method (R package *mice*) was used to create 20 imperatively imputed datasets in order to account for individual missing values. Confidence intervals were calculated using bootstrapping (n = 1,000). Reported P values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) method and a $P_{FDR} < .05$ was considered significant. For each cognitive outcome variable, a parsimonious model of the optimal predictors was identified through automatic backward selection (R package *lmerTest*). The contribution of white matter lesion volumes to these parsimonious models was also examined. Linear mixed-effects models for Aβ accumulation and grey matter loss were constructed at the voxel-level using the VoxelStats package implemented in Matlab R2018b.[55] Significance thresholds were set at a cluster-level whole-brain Random Field Theory (RFT) adjusted threshold of $P_{RFT} < .05$ with voxel-level set at $P_{uncorrected} < .001$.[55]

As a secondary analysis, the concurrence of serum biomarker changes with cognitive changes as well as their monitoring potential for Aβ accumulation and grey matter loss was assessed using linear mixed-effects models with the interaction term between time and annual serum biomarker rate of change as the predictor. All equations used in linear mixed-effects models are listed in **supplementary methods**.

Lastly, the ability of serum and plasma biomarkers to classify CU elderly based on baseline Aβ-PET status was evaluated for both $A\beta_{1-42}/A\beta_{1-40}$ and pTau181 using unpaired *t*-tests and receiver operating characteristic analysis. Areas under the curve (AUCs) were compared using DeLong tests.

Results

Cohort characteristics and participant follow-up

Baseline cohort characteristics of all 185 included F-PACK participants are summarised in Table 1, stratified by baseline Aβ-PET status. At baseline, serum GFAP levels were higher in Aβ+ (CL > 23.5) subjects while $A\beta_{1-42}/A\beta_{1-40}$ ratios were lower compared to the Aβ- group. No differences in serum NfL nor pTau181 were found. Aβ+ subjects had a higher Aβ load throughout the cerebral cortex (Supplementary Fig. 2). No differences in grey matter volume were observed. Serum GFAP and NfL demonstrated the strongest inter-biomarker correlations ($\rho = 0.47$, $P_{FDR} < .001$, Supplementary Fig. 3). Serum NfL as well as $A\beta_{1-42}/A\beta_{1-40}$ demonstrated significant correlations – albeit weak - with pTau181 ($\rho = 0.22$, $P_{FDR} = .008$ and $\rho = -0.20$, $P_{FDR} = .01$, respectively). Serum NfL additionally associated with white matter lesion volume ($P_{FDR} = .03$, Supplementary Figure 4).

Subjects were longitudinally followed for a median period of 6.1 (range 1.3–11.0) years with respectively 100%, 74%, 61%, 24% and 13% completing the 2-, 4-, 6-, 8- and 10-year follow-up visit. Thirty-six participants dropped out following loss of motivation ($n = 19$), health ($n = 7$), mobility problems ($n = 5$) or death ($n = 5$) and three subjects refused an MRI scan at follow-up (Fig. 1). In follow-up neuropsychological assessments, there were missing values for MMSE (0.2%), AVLT DR (0.3%), AVLT Recognition (0.6%), BSRT TR (0.6%), BSRT DR (2%), BNT (7.3%), PALPA item 49 (7.6%), TMT A (0.2%) and TMT B (0.9%) tests. Throughout follow-up, ten subjects (5%) progressed from a CDR of 0 to 0.5, of which three were Aβ+. CDR converters had higher serum NfL levels ($P = .02$) than those with a stable CDR, but no differences were observed for any other serum biomarker.

Longitudinal trajectories of cognition, amyloid load and grey matter volume

Age-, sex- and education-independent decreases in memory ($P < .001$) and language ($P < .001$) function were observed across the cohort as well as within Aβ subgroups (Fig. 2A-B). For both domains, Aβ+ elderly declined faster than Aβ- elderly ($P < .007$). Executive functioning remained stable (Fig. 2C). Out of 109 subjects with serial Aβ-PET, 12 (11%) were Aβ+ at baseline. Longitudinal PET-based Aβ accumulation was observed in both Aβ- and Aβ+ subgroups, but was steeper in the latter ($P < .001$, Fig. 2D). All Aβ+ subjects remained Aβ+ at follow-up, with the exception of one participant whose follow-up Centiloid score fell just below the positivity threshold. Fifteen subjects (9%) that were Aβ- at baseline had crossed the threshold of Aβ-positivity at follow-up. Age-, sex- and *APOE*-independent Aβ accumulation predominantly occurred within the precuneus and frontal cortical regions (Supplementary Fig. 5A). Age-, sex- and TIV-independent grey matter loss overlapped with Aβ accumulation but was more widespread throughout the cerebral cortex (Supplementary Fig. 5B).

Prognostic capabilities of serum biomarkers

Predictive value of serum biomarkers for cognitive decline

High baseline GFAP or NfL levels and low baseline $A\beta_{1-42}/A\beta_{1-40}$ predicted a more rapid longitudinal decline in memory performance with highly comparable effect sizes (Table 2, Fig. 3A) and this independently of age, sex and education. In addition, high baseline GFAP and NfL were equally predictive of a steeper longitudinal decline in language performance. Despite not reaching significance, the predictive performance of serum $A\beta_{1-42}/A\beta_{1-40}$ for language decline was not inferior to those of serum GFAP and NfL (Table 2). Serum pTau181, on the other hand, was not predictive for a longitudinal decline in memory nor language performance and was thereby outperformed by serum GFAP, NfL as well as $A\beta_{1-42}/A\beta_{1-40}$. The predictive value of serum biomarkers for memory decline was mainly driven by the AVLT TL and the BSRT TR scores, while the predictive value for language decline was predominantly driven by AVF and/or BNT scores for GFAP and NfL, respectively (Supplementary Fig. 6). No serum biomarker was predictive for longitudinal changes in the composite nor individual executive functioning test

scores (Table 2). Unadjusted effect estimates of the predictive values for cognitive decline were similar to those adjusted for age, sex and education and are shown in Supplementary Table 3.

Backward selection of predictor variables showed that the effects of serum GFAP and NfL were interdependent: NfL ($P < .001$) cancelled out the predictive value of GFAP ($P = .21$) for memory decline, while the opposite was true for language decline ($P < .001$ for GFAP, $P = .19$ for NfL). In addition to NfL, serum $A\beta_{1-42}/A\beta_{1-40}$ ($P = .008$) was also retained in the parsimonious model for predicting memory decline. White matter lesion volume significantly predicted memory decline as well ($P_{FDR} = .04$), but was not retained in the parsimonious model.

Predictive value of serum biomarkers for amyloid-β accumulation

Voxelwise linear mixed-effects models for Aβ accumulation corrected for age, sex and *APOE-ε4* showed that low baseline $A\beta_{1-42}/A\beta_{1-40}$ predicted faster Aβ accumulation within the precuneus and frontal lobes (Fig. 3B). For GFAP and NfL, no significant predictive values for Aβ accumulation were found. For serum pTau181, a small significant cluster was observed within the superior occipital lobe (Supplementary Fig. 7).

Predictive value of serum biomarkers for grey matter loss

Voxelwise linear mixed-effects models for grey matter loss corrected for age, sex and TIV revealed that both baseline GFAP and NfL predicted grey matter loss within the hippocampus. GFAP additionally predicted grey matter loss within the precuneus. Serum $A\beta_{1-42}/A\beta_{1-40}$ predicted grey matter loss within the lateral temporal lobe (Fig. 3C).

Trajectories of serum biomarker changes

Serum GFAP ($\beta_s = 0.022$, $P_{FDR} < .001$), NfL ($\beta_s = 0.040$, $P_{FDR} < .001$) and pTau181 ($\beta_s = 0.060$, $P_{FDR} < .001$) levels increased over time, while $A\beta_{1-42}/A\beta_{1-40}$ decreased ($\beta_s = -8*10^{-5}$, $P_{FDR} < .001$). Serum GFAP, NfL and pTau181 increases were found in both Aβ-subgroups. Serum $A\beta_{1-42}/A\beta_{1-40}$, on the other hand, decreased only in Aβ- elderly, while the ratio remained unchanged in the Aβ+ group (Supplementary Fig. 8). Longitudinal changes in serum biomarkers persisted after correction for age and sex (Supplementary Fig. 9).

Associations of serum biomarker changes with cognitive decline

Increasing serum NfL levels were associated with decreasing memory function (β_s = -0.030, P_{FDR} = .006) independent of age, sex and education (Supplementary Table 4). This association was stronger than those observed for GFAP, $A\beta_{1-42}/A\beta_{1-40}$ and pTau181, which did not reach significance. In addition, NfL increases (β_s = -0.021, P_{FDR} = .02) as well as $A\beta_{1-42}/A\beta_{1-40}$ decreases (β_s = -0.020, P_{FDR} = .04) associated with decreasing language performance and this with comparable effect sizes. However, only NfL changes and not $A\beta_{1-42}/A\beta_{1-40}$ changes were retained in the parsimonious model of language decline. GFAP and pTau181 changes did not associate with any cognitive changes. Unadjusted effect estimates were similar to those adjusted for age, sex and education and are shown in Supplementary Table 5.

Associations of serum biomarker changes with amyloid-β accumulation

Voxelwise linear mixed-effects models for longitudinal Aβ accumulation corrected for age, sex and *APOE-ε4* genotype revealed sparse but significant clusters within the precuneus as well as within frontal and medial temporal areas that demonstrated Aβ accumulation with rising GFAP levels (Fig. 4B). Longitudinal pTau181 changes demonstrated associations with longitudinal Aβ accumulation in a small cluster within the superior occipital lobe (Supplementary Fig. 7B). No significant clusters were found for serum NfL nor $A\beta_{1-42}/A\beta_{1-40}$.

Associations of serum biomarker changes with grey matter loss

Voxelwise linear mixed-effects models for grey matter loss corrected for age, sex and TIV revealed that serum NfL increases were related to grey matter loss within anterior and posterior regions of the cingulate gyrus and within the paracentral lobule as well as within the inferior parietal lobe (Fig. 4C). Contrarily, no significant clusters were found for GFAP, $A\beta_{1-42}/A\beta_{1-40}$ nor pTau181.

Head-to-head comparisons of serum and plasma biomarker performance to detect brain amyloid-β status

Since plasma is most commonly used for $A\beta_{1-42}/A\beta_{1-40}$ and pTau181 quantification and might be considered a more suitable matrix than serum, we compared the performances of plasma and serum measurements of both biomarkers to detect Aβ-PET status in order to contextualise our

serum-based findings. Plasma as well as serum $A\beta_{1-42}/A\beta_{1-40}$ were higher in Aβ+ than Aβ- CU elderly, yet the performance of $A\beta_{1-42}/A\beta_{1-40}$ was higher when measured in serum compared to plasma (ΔAUC = 0.10, $P_{DeLong,FDR}$ = .04, Supplementary Fig. 10A-C). Neither plasma nor serum pTau181 levels differed between Aβ+ and Aβ- CU elderly and in both matrices pTau181 demonstrated poor performances to detect Aβ-PET status when measured with the Quanterix AT270-based Simoa assay (AUC = 0.55 and 0.63, respectively, $P_{DeLong,FDR}$ = .10). In contrast, ADx252-based plasma pTau181 measurements were elevated in Aβ+ individuals and could accurately distinguish the two groups (AUC = 0.82, 95% CI 0.72 – 0.92) thereby outperforming serum and plasma AT270-based Simoa measurements ($P_{DeLong,FDR}$ < .007, Supplementary Figure 10D-G). Serum $A\beta_{1-42}/A\beta_{1-40}$ (r = 0.65, P_{FDR} < .001) as well as pTau181 (r = 0.68, P_{FDR} < .001) correlated with their plasma counterparts. However, for pTau181, inter-matrix correlations were much stronger for measurements conducted with the same assay (Supplementary Fig. 11).

Discussion

This prospective longitudinal study examined the prognostic and monitoring capabilities of serum biomarkers in asymptomatic stages across a median time period of 6.1 (range 1.3-11.0) years. Serum GFAP, NfL and $A\beta_{1-42}/A\beta_{1-40}$ were equally predictive for memory and language decline. While the predictive values of serum GFAP and NfL were highly interdependent, serum $A\beta_{1-42}/A\beta_{1-40}$ predicted memory decline independently. Low serum $A\beta_{1-42}/A\beta_{1-40}$ additionally predicted Aβ accumulation in the precuneus and frontal regions and grey matter loss within temporal regions, all typically affected in early Alzheimer's disease. Moreover, GFAP and NfL both predicted grey matter loss within the hippocampus, yet GFAP was also predictive of grey matter loss within the precuneus. We also found that GFAP increases were linked to Aβ accumulation within the precuneus while NfL increases were associated with decreasing memory and language performance as well as general grey matter loss. Serum pTau181 could not accurately predict nor monitor cognitive decline, Aβ accumulation or grey matter loss.

According to previous studies of blood-based GFAP as a predictor of cognitive decline in CU elderly, increased GFAP levels predict a decline in global cognition and, more specifically, memory, similar to our findings.[16,56] In contrast, high GFAP has previously also been associated

with subsequent decline in executive functioning, but not language performance, likely due to a difference in the cognitive tests contributing to the respective composite scores or a shorter follow-up time (3 versus 6 years).[16] Another study with equally short follow-up of 3.6 years did, however, report predictive capabilities of plasma NfL as well as $A\beta_{1-42}/A\beta_{1-40}$ for memory decline, in line with our results in serum over extended time periods.[34] In addition to GFAP, we also found serum NfL to be predictive for language decline. The domain-specific results presented here build further on recent studies with comparable follow-up periods that reported predictive capabilities of both GFAP and NfL for global cognitive decline.[21,57] With regard to grey matter loss, predictive abilities have previously been shown for both GFAP and NfL, yet mostly restricted to more advanced stages with either overt Aβ burden or dementia diagnosis[32,33,38] or within autosomal dominant mutation carriers.[58] This contrasts with our findings in CU elderly likely due to shorter follow-up times in previous studies (median < 3 years). Similar to the current report, presymptomatic familial Alzheimer's disease mutation carriers with high blood-based GFAP levels experienced greater hippocampal volume loss and cognitive decline within a six-year period compared to those with low GFAP levels at baseline.[58]

The predictive values of serum GFAP and NfL for grey matter loss and cognitive decline were not independent. However – as reported previously[13,16] – GFAP and NfL only moderately correlated (Spearman's $\rho = 0.47$), reflecting their different cellular origins and consequently different neuropathologic substrate. Moreover, we found that GFAP was relatively more related to early Alzheimer's disease-specific processes as it was elevated upon the presence of asymptomatic Aβ pathology. We also observed that steeper GFAP increases were associated with faster Aβ accumulation in Aβ-vulnerable regions (i.e. frontal regions), thus further supporting it's Aβ-dependence. Moreover, GFAP was not only associated with grey matter loss within the hippocampus – a phenomenon shared by other dementia types – but also within the precuneus, which is relatively more characteristic of Alzheimer's disease.[59] These findings align with pathological studies showing that reactive astrocytes colocalise with Aβ deposits and exacerbate Aβ accumulation.[60–62] In contrast, NfL was not related to Aβ load or its accumulation over time and was elevated in CDR converters, of which the majority was Aβ-negative. Instead, we demonstrated an association of NfL with white matter lesions. Moreover, steeper serum NfL increases were associated with a faster language and memory decline as well as a more rapid loss of grey matter in regions not typically affected in early Alzheimer's disease (i.e. paracentral

lobule), thus further illustrating its non-specificity for Alzheimer's disease. Similarly, faster NfL increases have been linked to a higher risk of all-cause dementia in CU elderly and reflected a faster decline in the Preclinical Alzheimer Cognitive Composite (PACC) score across the Alzheimer's disease continuum.[16,32] Another study in non-demented subjects showed no associations of NfL changes with declining MMSE scores, modified PACC scores nor cortical thickness.[63] Possible explanations are the limited maximal follow-up of 6 years – compared to 11 years in the current study – and/or lower sensitivity of the employed cognitive tests. Of note, GFAP, like NfL, is not specific for Alzheimer's disease, as previous reports have shown GFAP and NfL increases in non-Alzheimer's disease dementias as well as good predictive performances of both biomarkers for conversion to all-cause dementia.[16,17,19,64] Alternative pathways in which these biomarkers might be linked to cognitive decline are vascular, α-synuclein or TDP-43 pathology or other mechanisms yet to be investigated.

Serum $A\beta_{1-42}/A\beta_{1-40}$ predicted memory decline independently of GFAP and NfL, as reported previously.[34] A recent study with a comparable follow-up period to the current study did, however, not find predictive abilities of plasma $A\beta_{1-42}/A\beta_{1-40}$ for cognitive decline in CU elderly.[21] However, herein cognition was assessed globally using either MMSE or modified PACC scores. Our domain-specific analyses of cognitive decline did reveal predictive value of $A\beta_{1-42}/A\beta_{1-40}$ for memory decline, illustrating that cognitive changes might be too subtle to detect through assessment of global cognition in these early asymptomatic stages. In addition, we found serum $A\beta_{1-42}/A\beta_{1-40}$ to be predictive of longitudinal Aβ accumulation within regions typically affected in early Alzheimer's disease (i.e. precuneus, frontal regions). Similarly, a study in nondemented elderly for predicting Aβ accumulation found the largest effect size for plasma $A\beta_{1-42}/A\beta_{1-40}$ – as measured by ELISAs – followed by Mesoscale-based measurements of plasma pTau217 with no predictive value for pTau181, nor NfL, measured with respectively Mesoscale and Simoa immunoassays.[33] Serum $A\beta_{1-42}/A\beta_{1-40}$ also demonstrated predictive value for grey matter loss within temporal brain regions (i.e. medial, inferior and superior), in line with a previous report in CU elderly.[38] This is clinically relevant considering the role of these regions in memory function and their early involvement in Alzheimer's disease pathophysiology.[65] In contrast to GFAP, NfL and pTau181 – which increased in both Aβ+ and Aβ- CU elderly – serum $A\beta_{1-42}/A\beta_{1-40}$ decreased over time in Aβ- but not in Aβ+ CU elderly. Moreover, unlike GFAP changes, $A\beta_{1-42}/A\beta_{1-40}$ changes did not associate with cortical Aβ accumulation, thus extending

previous findings within the TRAILBLAZER trial to the asymptomatic Alzheimer's disease phase.[66] Together with the lack of association between longitudinal serum $A\beta_{1-42}/A\beta_{1-40}$ changes and memory decline or grey matter loss observed here, this suggests that serum $A\beta_{1-42}/A\beta_{1-40}$ decreases early in the disease course but may then stabilise. Similarly, cortical Aβ accumulation has been shown to occur only when plasma $A\beta_{1-42}/A\beta_{1-40}$ is already low and plasma $A\beta_{1-42}/A\beta_{1-40}$ decreases predominantly prior to reaching the threshold of 23.5 Centiloids, after which it reaches a plateau.[33,67] Moreover, a recent longitudinal study in a comparable cohort followed across the same time frame (median 6 years) only found plasma $A\beta_{1-42}/A\beta_{1-40}$ decreases in Aβ-individuals.[68] Previous studies argued against quantification of $A\beta_{1-42}/A\beta_{1-40}$ in serum due to its poor stability after multiple freeze-thaw cycles and the lower relative concentration of $A\beta_{1-42}$ in serum compared to plasma.[43,69] Whereas plasma might be preferred in most clinical and research settings, all serum $A\beta_{1-42}/A\beta_{1-40}$ measurements in the current study were performed immediately after first thawing in a cohort of CU elderly in which $A\beta_{1-42}$ levels are overall still high. Moreover, a direct comparison of serum $A\beta_{1-42}/A\beta_{1-40}$ performance relative to plasma $A\beta_{1-42}/A\beta_{1-40}$ did not show inferior performance to detect asymptomatic Aβ-PET burden. Altogether, this suggests that the serum $A\beta_{1-42}/A\beta_{1-40}$ measurements reported here are equivalent to plasma-based measurements.

The performance of pTau181 as a blood-based biomarker for Alzheimer's disease has also primarily been investigated in plasma. The current study shows that serum pTau181 – quantified by an AT270-based assay – has limited prognostic and monitoring capability. However, AT270-based serum pTau181 measurements have previously shown strong correlations with their plasma counterpart as well as similar performances to detect Alzheimer's disease in clinical stages.[9,11,44] We now extended these findings to the asymptomatic phase, but neither plasma nor serum pTau181 differed between Aβ+ and Aβ- individuals. Consequently, in both matrices, pTau181 demonstrated poor performances to detect Aβ pathology, thereby contrasting earlier plasma-based findings.[9,21,29] Notably, CU participants underwent extensive screening covering multiple cognitive domains prior to inclusion in the current study likely placing them in earlier disease stages than CU cohorts based on global cognitive measures. When quantified using the more phospho-specific ADx252 antibody, plasma pTau181 detected asymptomatic Aβ-positivity with higher performance compared to AT270-based measurements consistent with prior findings in clinical Alzheimer's disease.[5,11,49] This suggests that the

performance of blood-based pTau181 strongly depends on the employed immunoassay rather than the matrix in which it was measured. Further research between these findings and ADx252-based serum pTau181 measurements is needed once the assay has undergone rigorous analytical validation for use in serum, and would be valuable considering the widespread use of serum in healthcare settings. Moreover, given the consistently superior diagnostic and prognostic performance of plasma pTau217 compared to AT270-based pTau181, studies directly comparing the prognostic and monitoring performances of ADx252-based pTau181 to that of other blood-based biomarkers, especially pTau217, are needed.

The strengths of this study are the well-defined community-recruited F-PACK cohort, which is independent of cohorts previously used in prognostic and monitoring-based assessments and which underwent extensive neuropsychological assessments covering multiple cognitive domains as well as imaging and blood-based follow-up across extended time periods. Moreover, the F-PACK cohort was enriched for genetic Alzheimer's disease risk factors (i.e. *APOE-ε4* and *BDNF val66met* carriership) which promotes the translatability of the presented results to presymptomatic Alzheimer's disease patients, which are also enriched for these risk factors compared to the general population. The simultaneous evaluation of different biomarkers within this cohort allowed assessment of their relative prognostic and monitoring capabilities for different clinical and biomarker-based outcomes. Moreover, the incidence of comorbidities like hypertension (39%) and hypercholesterolemia (38%) reflect those of the general Flemish population.[70] In contrast, our study cohort had a low prevalence of diabetes (2% versus 5-18% of elderly Flemish population) and was predominantly white preventing the examination of ethnic disparities. Another limitation is the absence of head-to-head comparisons with other pTau species. In addition, PET, MRI and blood sampling were not performed as consistently as cognitive testing during follow-up, since they were not included in the original study design. We used statistical methods accounting for interindividual variation in time intervals, but the estimated effects for Aβ accumulation and grey matter loss might be less accurate than those for cognitive decline. In addition, Aβ isoforms were quantified using immunoassays despite the better performance of mass-spectrometry methods,[10] which might underestimate their performance. Lastly, despite enrichment for Alzheimer's disease risk through genetic stratification, the number of Aβ+ elderly was relatively low, which prevented subgroup analyses of biomarker performance.

In conclusion, our findings suggest that serum biomarkers have differential prognostic and monitoring value in asymptomatic Alzheimer's disease. Serum GFAP and NfL are valuable prognostic markers for cognitive and grey matter changes in asymptomatic elderly and serum $A\beta_{1-42}/A\beta_{1-40}$ provides complementary Alzheimer's disease dependent prognostic information, particularly in the earliest stages. NfL can also serve as a monitoring tool for asymptomatic grey matter loss and cognitive decline in an Aβ-independent manner. Instead, serum GFAP appears to be linked to an Aβ-dependent trajectory. The presented evidence concerning the long-term prognostic and monitoring capabilities of blood-based biomarkers may eventually aid in providing patients with early prognostic information and clinical intervention as well as the ability to monitor their response to treatment.

Data availability

Anonymised data are available from the corresponding author on reasonable request for the sole purpose of recreating study procedures or results presented in the current study. Such data transfer will be regulated through a material transfer agreement.

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Competing interests

RV's institution has had a clinical trial agreement for phase 1 and 2 studies with GE Healthcare, which provided [^{18}F]flutemetamol for this study. RV's institution has clinical trial agreements (RV as PI) with Biogen, Eli Lilly, J&J, Prevail, Roche/Genentech, Wave and UCB. RV's

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Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Participant follow-up. Overview of participant progression through the 10-year follow-up (median [IQR]: 6 [4] years) at the time of the study. For visualisation purposes, the follow-up period was divided into two-year intervals and per interval the number of participants completing follow-up was listed for each type of examination. Of note, time intervals were used as continuous variables in all analyses.

Figure 2 Longitudinal trajectories of pathological processes in cognitively unimpaired elderly. Spaghetti plots show the individual trajectories in (**A**) memory function, (**B**) language function, (**C**) executive functioning and (**D**) Aβ load. Linear fits with 95% confidence intervals of the relationship between time and the respective outcome variables as well as β_s estimates of time since baseline assessment are shown on top of the plot. β_s estimates were calculated using linear mixed-effects models with random slopes for time and subject-specific random intercepts. Reported β_s estimates were corrected for age and sex as well as, respectively, education and *APOE-ε4* genotype in cognition- and Aβ-based analyses. (**A-C**) More negative β_s estimates indicate steeper slopes of memory, language and executive functioning decline, (**D**) while more positive β_s estimates indicate steeper slopes for Aβ accumulation. The horizontal dashed line indicates the threshold of Aβ-positivity. Significance levels were corrected for multiple

comparisons using the Benjamini-Hochberg FDR method: $^{*}P_{FDR} < .05$, $^{\dagger}P_{FDR} < .01$, $^{\ddagger}P_{FDR} < .001$.

Figure 3 Predictive capabilities of serum biomarkers. (**A**) Coefficient plot visualising the standardised regression coefficient (β_s) estimates of the association between baseline serum biomarker levels and longitudinal cognitive changes in memory, language and executive functioning domains. Reported β_s estimates were adjusted for age, sex and education and are indicated by boxes. Whiskers represent the 95% confidence interval and colours correspond to different serum biomarkers. $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratios were inverted to facilitate biomarker comparison. (**B,C**) Parametric *T* maps show the observed regional relationships of baseline serum GFAP, NfL, and/or $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ with (**B**) Aβ accumulation and (**C**) grey matter loss over time, adjusted for age and sex as well as, respectively, *APOE-ε4* genotype and total intracranial volume. Thresholded maps (voxel-level $P_{\text{uncorrected}} < .001$, cluster level $P_{\text{RFT}} < .05$) were superimposed on the MNI152 template using the Nilearn package in Python (v3.9.13). Crosshairs were positioned within significance peaks (with the exception of the *T* map of GFAP for grey matter loss since it would have hidden the cluster within the hippocampus) and indicate the cut positions of the coronal, sagittal and transverse planes.

Figure 4 Monitoring capabilities of serum biomarkers. (**A**) Coefficient plot visualising the β_s estimates of the association between longitudinal serum biomarker changes and cognitive changes in memory, language and executive functioning domains, respectively. Reported β_s estimates were adjusted for age, sex and education and are indicated by boxes. $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratios were inverted to facilitate biomarker comparison. Whiskers represent the 95% confidence interval and colours correspond to different serum biomarkers. (**B,C**) Parametric *T* maps show the regional relationships between annual increases in serum GFAP and NfL as well as decreases in serum $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratios with (**B**) Aβ accumulation and (**C**) grey matter loss over time, adjusted for age and sex as well as, respectively, *APOE-ε4* genotype and total intracranial volume. Thresholded maps (voxel-level $P_{\text{uncorrected}} < .001$, cluster level $P_{RFT} < .05$) were superimposed on the MNI152 template using the Nilearn package in Python (v3.9.13).

Crosshairs were positioned within significance peaks and indicate the cut positions of the coronal, sagittal and transverse planes.

Table 1 Baseline cohort characteristics

Characteristic	All CU participants	Aβ+	Aβ−	*P*
Number, N	185	23	162	NA
Age, y	69 ± 6	71 ± 5	68 ± 6	**0.02**
Sex, female	89 (48)	8 (35)	81 (50)	0.25
APOE-ε4 carriers, n (%)	83 (45)	17 (74)	66 (41)	**0.006**
Education, y	15 [5]	15 [4]	15 [5]	0.95
White matter lesions (mm^3)	7691 [8273]	8204 [6229]	7602 [9344]	0.54
Memory performance (z-score)	−0.04 ± 0.74	−0.34 ± 0.75	0.00 ± 0.73	**0.04**
Language performance (z-score)	−0.02 ± 0.74	−0.18 ± 0.75	0.00 ± 0.74	0.27
Executive functioning (z-score)	−0.01 ± 0.57	−0.08 ± 0.55	0.00 ± 0.55	0.54
Aβ-PET load (CL)	5.7 [12.6]	44.7 [39.6]	3.9 [9.8]	**<0.001**
CDR (/3)	0 [0]	0 [0]	0 [0]	1.00
MMSE (/30)	29 [1]	29 [1]	29 [1]	0.99
Serum GFAP (pg/mL)	121 [70]	150 [67]	117 [67]	**0.01**
Serum NfL (pg/mL)	16.4 [9.5]	17.2 [11.7]	16.1 [8.6]	0.12
Serum $A\beta_{1-42}/A\beta_{1-40}$	0.062 [0.013]	0.050 [0.010]	0.063 [0.012]	**<0.001**
Serum pTau181 (pg/mL)	1.06 [0.68]	1.38 [0.84]	0.98 [0.65]	0.07

Continuous data are expressed as mean ± SD when normally distributed and median [IQR] when not. Categorical data are expressed as number (%). Comparisons between cohort subgroups were made using either unpaired t-tests (normal data), Mann–Whitney U tests (non-normal data) or $\chi 2$ tests (categorical data). *P* values corresponding to subgroup comparisons are shown on the right and were indicated in bold if significant.

Table 2 Predictive values of serum biomarkers for cognitive decline

Model	β_s	95% CI	P_{uncor}	P_{FDR}
Memory				
GFAP	−0.021	−0.036 to −0.008	**0.004**	**0.007**
NfL	−0.031	−0.048 to −0.015	**<0.001**	**0.002**
$A\beta_{1-42}/A\beta_{1-40}$	−0.024	−0.042 to −0.004	**0.01**	**0.02**
pTau181	−0.004	−0.021 to 0.012	0.63	0.63
Language				
GFAP	−0.021	−0.033 to −0.009	**<0.001**	**0.002**
NfL	−0.018	−0.032 to −0.004	**0.01**	**0.03**
$A\beta_{1-42}/A\beta_{1-40}$	−0.011	−0.025 to 0.005	0.16	0.22
pTau181	0.008	−0.006 to 0.022	0.23	0.23
Executive functioning				
GFAP	0.005	−0.006 to 0.015	0.38	0.57
NfL	0.004	−0.010 to 0.017	0.57	0.57
$A\beta_{1-42}/A\beta_{1-40}$	−0.005	−0.018 to 0.009	0.44	0.57
pTau181	0.005	−0.007 to 0.017	0.42	0.57

Standardised regression coefficient (β_s) estimates of the interaction term between serum biomarker levels and time from blood sampling derived from linear mixed-effects models with corresponding 95% CIs and *P* values both corrected (P_{FDR}) and uncorrected (P_{uncor}) for multiple comparisons are shown. 95% CIs were calculated using bootstrapping (n = 1,000). *P* values were indicated in bold when significant. The $A\beta_{1-42}/A\beta_{1-40}$ ratio was inverted in analyses to facilitate comparisons between biomarkers. All models were corrected for age, sex and education.

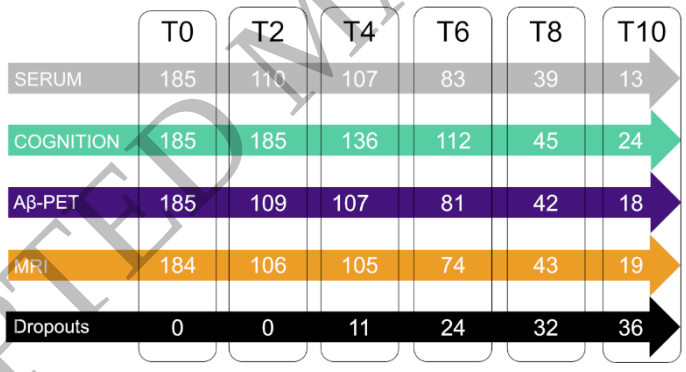

Figure 1
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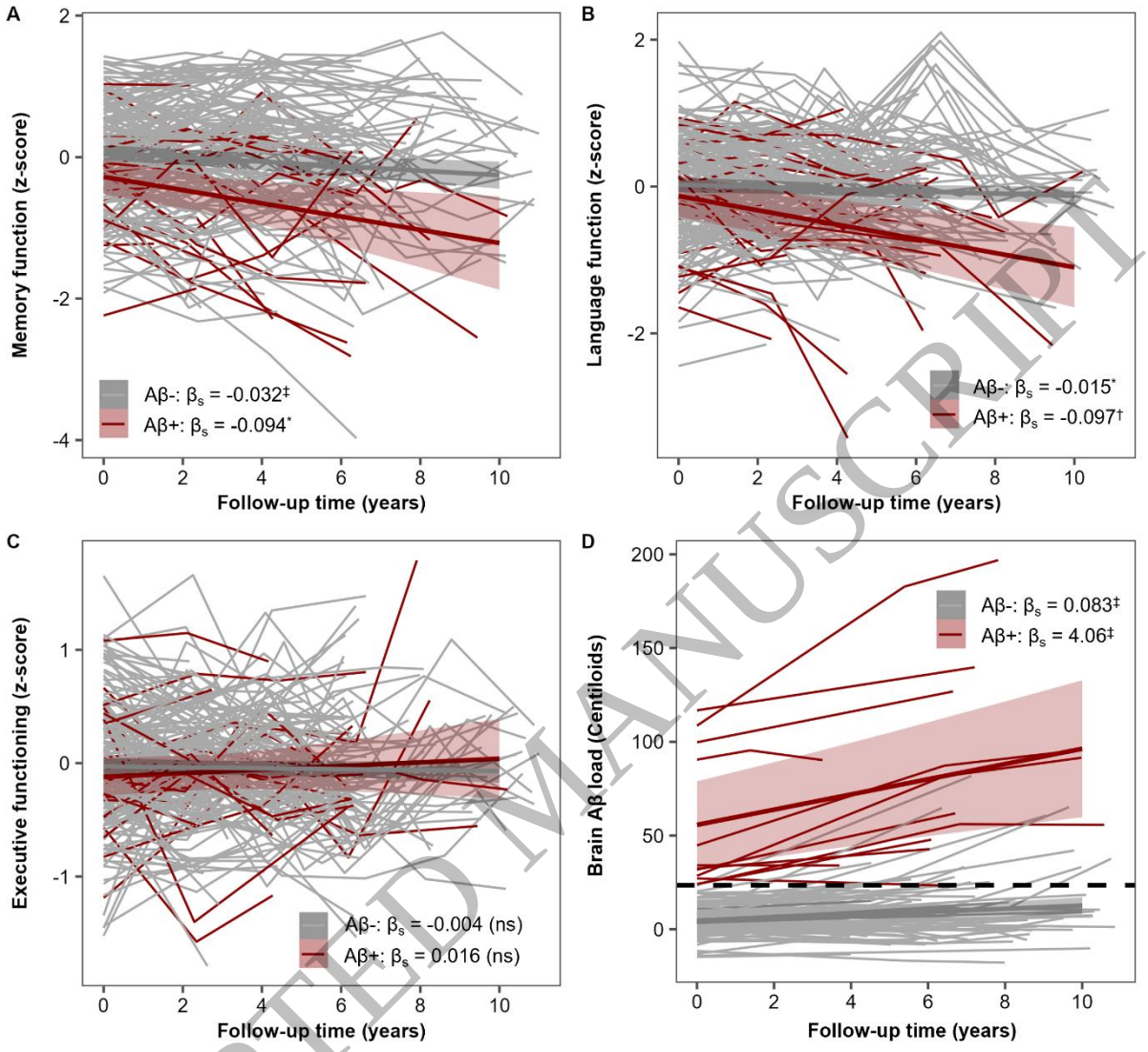

Figure 2
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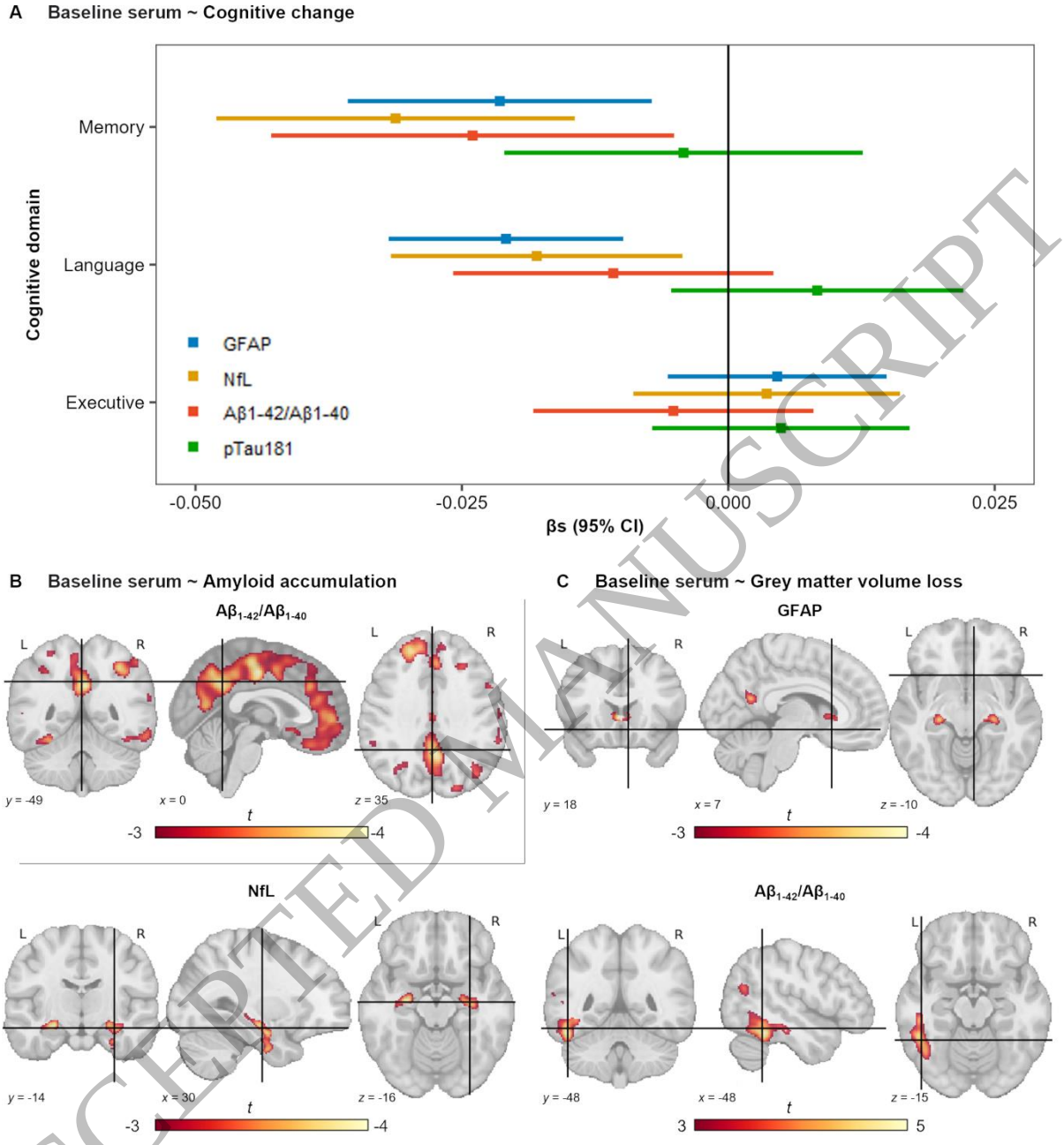

Figure 3
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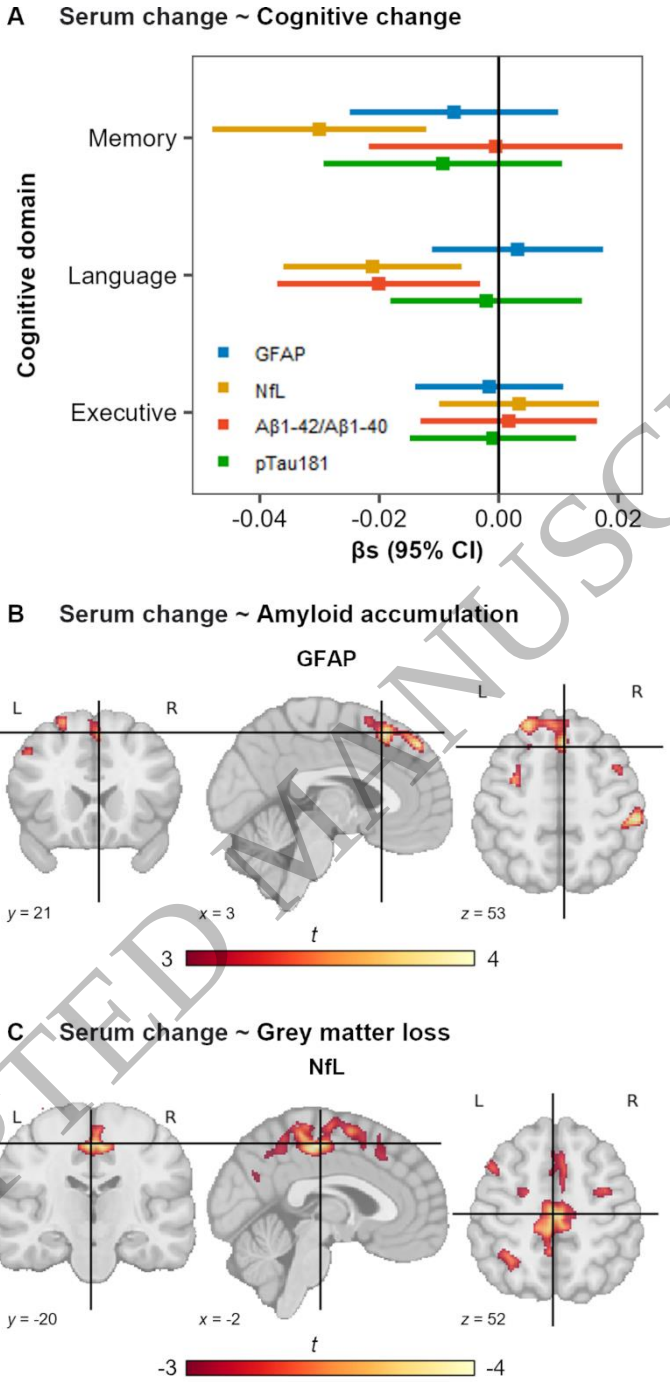

Figure 4
90x182 mm (x DPI)