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Abstract

Background and Objectives

Patients' comorbidities can affect Alzheimer disease (AD) blood biomarker concentrations. Because a limited number of factors have been explored to date, our aim was to assess the proportion of the variance in fluid biomarker levels explained by the clinical features of AD and by a large number of non–AD-related factors.

Methods

MEMENTO enrolled 2,323 individuals with cognitive complaints or mild cognitive impairment in 26 French memory clinics. Baseline evaluation included clinical and neuropsychological assessments, brain MRI, amyloid-PET, CSF (optional), and blood sampling. Blood biomarker levels were determined using the Simoa-HDX analyzer. We performed linear regression analysis of the clinical features of AD (cognition, AD genetic risk score, and brain atrophy) to model biomarker concentrations. Next, we added covariates among routine biological tests, inflammatory markers, demographic and behavioral determinants, treatments, comorbidities, and preanalytical sample handling in final models using both stepwise selection processes and least absolute shrinkage and selection operator (LASSO).

Results

In total, 2,257 participants were included in the analysis (median age 71.7, 61.8% women, 55.2% with high educational levels). For blood biomarkers, the proportion of variance explained by clinical features of AD was 13.7% for neurofilaments (NfL), 11.4% for p181-tau, 3.0% for Aβ-42/40, and 1.4% for total-tau. In final models accounting for non–AD-related factors, the variance was mainly explained by age, routine biological tests, inflammatory markers, and preanalytical sample handling. In CSF, the proportion of variance explained by clinical features of AD was 24.8% for NfL, 22.3% for Aβ-42/40, 19.8% for total-tau, and 17.2% for p181-tau. In contrast to blood biomarkers, the largest proportion of variance was explained by cognition after adjustment for covariates. The covariates that explained the largest proportion of variance were also the most frequently selected with LASSO. The performance of blood biomarkers for predicting A+ and T+ status (PET or CSF) remained unchanged after controlling for drivers of variance.

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MEMENTO Study Group coinvestigators are listed in Appendix 2 as supplemental content.

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Glossary

Aβ = β-amyloid; **AD** = Alzheimer disease; **ALT** = alanine aminotransferase; **AUC** = area under the curve; **BMI** = body mass index; **CDR** = Clinical Dementia Rating; **CDR SoB** = CDR-Sum of Boxes; **FCSRT** = Free and Cued Selective Reminding Test; **HDL** = high-density lipoprotein; **IL** = interleukin; **LAG-CRB** = Genomic Analysis Laboratory-Biological Resource Centre; **LASSO** = least absolute shrinkage and selection operator; **MCI** = mild cognitive impairment; **NfL** = neurofilament light chain; **p181-tau** = 181-phosphorylated tau; **TMT-B** = Trail Making Test part B.

Discussion

This comprehensive analysis demonstrated that the variance in AD blood biomarker concentrations was mainly explained by age, with minor contributions from cognition, brain atrophy, and genetics, conversely to CSF measures. These results challenge the use of blood biomarkers as isolated stand-alone biomarkers for AD.

Introduction

Over the past decade, biological markers of Alzheimer disease (AD) have increasingly been used to guide AD diagnosis. The National Institute on Aging and the Alzheimer's Association introduced the AT(N) classification research framework, which defines AD on the basis of biomarkers without considering the clinical presentation.[1] Recently, the US Food and Drug Administration granted accelerated approval for the use of aducanumab and lecanemab (then converted to a traditional approval few months later for lecanemab) based on their ability to significantly reduce amyloid plaques because this surrogate endpoint "reflects the effect of the drug on an important aspect of the disease."[2]

Traditionally, the pathologic hallmarks of AD, such as β-amyloid (Aβ) peptides and phosphorylated tau levels, and markers of neurodegeneration, including neurofilament light chain (NfL) and total-tau, were primarily measured in the CSF, typically in specialized clinical centers. However, recent technical advancements using ultra-sensitive assays have enabled the measurement of these biomarkers in blood. Blood-based biomarker measurements offer advantages such as easier and more rapid collection, lower costs, and better patient acceptance compared with the corresponding CSF measurements. Multiple studies have consistently shown correlations of these markers with amyloid status,[3-5] their ability to distinguish AD from other neurodegenerative diseases,[6] as well as their potential in predicting cognitive decline[7-9] or progression from mild cognitive impairment (MCI) to dementia.[10-14]

The use of blood biomarkers for clinical purposes in the evaluation of patients with cognitive impairment and dementia (population screening, diagnosis, and disease monitoring in clinical trials) has been discussed extensively[13-15] and raises concerns regarding their widespread availability. Ideal biomarkers should exhibit abnormal levels exclusively in affected individuals and be unaffected by unrelated factors. However, recent studies have shown that the concentrations of these blood biomarkers are affected by various conditions, including chronic kidney disease, myocardial infarction, body mass index (BMI), diabetes, dyslipidemia, arterial hypertension, ethnicity, and preanalytical sample handling.[16-20] However, these studies focused on few variables, and there may be other unidentified covariates. Furthermore, even when there is a statistical association, it remains unclear how much of the variability of the measure is explained by these covariates, particularly when compared with conventional clinical features of AD, such as cognition, imaging, and genetic risk score.

To address these questions, we used data from the MEMENTO cohort, a large multicentric French memory clinic cohort for which comprehensive data collection was implemented. Our primary objective was to estimate the variance explained in blood and CSF biomarker concentrations by clinical features of AD and other covariates. In addition, we evaluated the effect of these covariates on the performance of blood biomarkers for predicting amyloid (A+) or tau (T+) pathologic status, as assessed by CSF or PET measurements.

Methods

Study Population

The MEMENTO cohort recruited a total of 2,323 non-demented participants from 26 French memory clinics between 2011 and 2014. Participants were referred to the clinics by their general practitioners (49%) or other physicians (17%) or sought consultations independently (34%). Participants were consecutively recruited if they presented a cognitive complaint with a Clinical Dementia Rating (CDR) score at 0 (normal) or 0.5 (a proxy of mild cognitive impairment). Level of cognitive impairment was evaluated using a comprehensive battery of neuropsychological tests, and the intensity of cognitive complaints was assessed using visual analog scales. Details of the cohort have been published previously.[21] Main exclusion criteria were a history of head trauma with persistent neurologic deficits, stroke in the past 3 months or with persistent neurologic deficits, brain tumor, epilepsy, schizophrenia, known alteration in familial AD genes, and illiteracy. Written informed consent was

obtained from all participants, and the study protocol received approval from the ethics committee "CPP Sud-Ouest et Outre-Mer III." The study was registered at ClinicalTrials.gov (NCT01926249).

Data Collection

At the time of enrolment, participants underwent comprehensive clinical and neuropsychological assessment. The CDR scale was administered and a neuropsychological test battery to evaluate memory, executive functions, language, and attention domains.[21] To ensure standardized test scoring, investigators and neuropsychologists received training. Medications and comorbidities were obtained from medical records or reported by the participants. In addition, participants were requested to provide a blood sample at their local biochemistry department for routine biological measurements, including glucose, lipids, creatinine, blood counts, hemoglobin, liver enzymes, and electrolytes.

Neuropsychological Evaluation

Verbal episodic memory was assessed using the Free and Cued Selective Reminding Test (FCSRT), following the procedure proposed by Grober and Buschke[22] and adapted to the French population.[23] Episodic memory performance was determined by summing the 3 total (free and cued) recalls. Executive functions were assessed using the Trail Making Test part B (TMT-B).[24] In this article, we considered the time required for a good move (i.e., the total time divided by the total number of correct moves until test completion). The CDR scale is widely used to assess cognition and function for staging dementia.[25] It includes 6 domains (memory, orientation, judgment, community affairs, home hobbies, and personal care) scored from 0 (no impairment) to 3 (severe impairment) by a trained physician based on interviews with both participants and their informants. For the analysis, we considered the sum of 6 domains (CDR-SoB, 0–18).

MRI and Amyloid PET Imaging

At the time of inclusion in the cohort, participants underwent brain MRI, and 1-mm isotropic 3-dimensional T1-weighted images and 2D T2-weighted fluid-attenuated inversion recovery images were acquired. The Centre pour l'Acquisition et le Traitement des Images in Paris, France,[26] conducted preacquisition standardization and centralized quality checks on image acquisition. They applied postprocessing pipelines for standardized measurement of intracranial volumes using SPM12, hippocampal volumes using SACHA,[27] and cortical thicknesses using FreeSurfer 5.3.

To evaluate the amyloid load by amyloid PET, participants had the opportunity to participate in 2 ancillary studies: Insight-PreAD[28] at baseline and AMYGING (NCT02164643) during follow-up (2 years on average after inclusion in MEMENTO). Depending on the clinical site, the radiotracer administered was 18F-florbetapir (Amyvid) or 18F-flutemetamol (Vizamyl). Each tracer has a specific threshold for amyloid positivity (florbetapir >0.88, flutemetamol >1.063).[29]

CSF Biomarkers

Lumbar puncture was an optional procedure. For participants who accepted, their CSF was collected into polypropylene tubes following standardized protocols. The CSF samples were transferred to the local CSF bank within 4 hours after collection and centrifuged at 1,000*g* and 4°C for 10 minutes. After centrifugation, the CSF samples were aliquoted into polypropylene tubes and stored at −80°C. Aβ 42 peptide (Aβ-42), Aβ-40, total-tau, and 181-phosphorylated tau (p181-tau) levels were determined in a central location using INNOTEST kits (Fujirebio, Ghent, Belgium). NfL levels were assessed using the Single Molecular Array Ultra-sensitive Immunoassay (Simoa) with commercial kits on a Quanterix HD-X analyzer (Quanterix, Billerica, MA). The assay had a sensitivity cutoff of 17.4 pg/mL. Only CSF collected within 90 days after blood sampling was included in the analysis.

Blood Biomarkers

At the time of inclusion, blood samples for biobanking were collected and stored at −80°C in a central biobank, the Genomic Analysis Laboratory-Biological Resource Centre (LAG-CRB) at the Pasteur Institute, Lille (BB-0033-00071). The baseline samples were used for whole-genome sequencing and analysis of inflammatory markers and AD blood biomarkers.

Genomic DNA from peripheral blood samples was extracted at LAG-CRB using Gentra Puregene blood kits (Qiagen, Hilden, Germany). The genotypes of 35 single-nucleotide polymorphisms associated with AD dementia risk, including *APOE* genotype, were determined. A genetic risk score was computed as the sum of the risk alleles for AD weighted by their estimated effect sizes.[30] The genetic risk score was computed without the *APOE* genotype to exclude its potentially large effect due to its high frequency and large effect size.

The levels of serum inflammatory markers (referred to as "inflammatory markers" in the subsequent text), including interleukin (IL)-6, IL-10, IL-12p70, IL-18, tumor necrosis factor-α, RANTES (CCL5), and IP-10 (CXCL10), were measured using the commercial Bio-Plex Pro Human kit (Bio-Rad, Hercules, CA).

Baseline AD blood biomarkers were measured using commercial Simoa immunoassay kits on a Quanterix HD-X analyzer. Plasma Aβ42, Aβ40, and total-tau were measured using the Neurology 3-Plex A Advantage kit, serum p181-tau using the p181-tau Advantage V2 kit, and serum NfL using the NF-light Advantage kit. The lower limits of detection for the kits were (in pg/mL) 0.69 for NfL, 0.33 for p181-tau, 0.25 for total-tau, 2.7 for Aβ40, and 0.56 for Aβ42.[31] The measurements were conducted at the Bordeaux University Hospital research platform (PARS). The internal coefficient of variation was 8.0% for Aβ-40, 9.7% for Aβ-42, 10.9% for total-tau, 11.1% for p181-tau, and 12.7% for NfL.

Table 1 Baseline Sample Characteristics: Whole Cohort (n = 2,257) and CSF Subsample (n = 305): The MEMENTO Cohort

	Overall (n = 2,257)	CSF subsample (n = 305)
Age, median (Q1–Q3)	71.7 (65.6–77.2)	69.0 (63.6–74.4)
Female, n (%)	1,394 (61.8)	152 (49.8)
High educational level[a], n (%)	1,244 (55.2)	172 (56.4)
ApoE ε4 carriers, n (%)	648 (29.9)	119 (40.8)
CDR, n (%)		
0.0	921 (41.0)	105 (34.4)
0.5	1,325 (59.0)	200 (65.6)
MMSE, median (Q1–Q3)	28 (27–29)	28 (27–29)
Amyloid status[b] (n = 900)		
Negative	666 (74.0)	217 (71.1)
Positive	234 (26.0)	88 (28.9)
Blood sample time, n (%)		
Before 10 AM	1,091 (63.8)	173 (71.8)
After 10 AM	619 (36.2)	68 (28.2)
Blood biomarker, pg/mL, median (Q1–Q3)		
NfL	18.2 (13.4–25.0)	17 (12.5–23.5)
Aβ42	10.9 (8.9–13.1)	10.5 (8.6–12.5)
Aβ40	194 (165.0–226.0)	190 (158.0–218.0)
Aβ42/Aβ40 (×100)	5.6 (4.8–6.5)	5.6 (4.8–6.6)
Total-tau	1.9 (1.4–2.6)	1.8 (1.4–2.5)
p181-tau	0.9 (0.6–1.4)	0.9 (0.6–1.4)
CSF biomarker, pg/mL, median (Q1–Q3)		
NfL	—	1,230 (900–1,880)
Aβ42	—	1,104 (727–1,435)
Aβ40	—	13,789 (10,957–16,550)
Aβ42/Aβ40	—	8.6 (5.4–11.0)
Total-tau	—	292 (212–434)
p181-tau	—	55 (44–72)

Abbreviations: Aβ = β-amyloid; CDR = Clinical Dementia Rating scale; MMSE = Mini-Mental State Examination; NfL = neurofilament light chain.
[a] High educational level: baccalaureate and above.
[b] Amyloid positivity was defined using PET or CSF (see Methods).

The evaluation of CSF markers, genetic single-nucleotide polymorphisms, and blood markers was performed by investigators who were blinded to the clinical data.

Statistical Analysis

Variables are presented as median and interquartile, or frequency and percentage as appropriate. Correlations between CSF and blood biomarker levels were evaluated using the nonparametric Spearman rank correlation test. To evaluate the proportion of biomarker variance explained, we used R^2. R^2 values range from 0 to 1, with a value of 1 signifying a perfect model that explains all of the variance in the data. For clearer interpretation, we present R^2 values as a percentage (0%–100%), representing the proportion of variance explained by each variable in the overall model. The linear regression assumptions of normality and homoscedasticity of the residuals were assessed graphically, and collinearity was evaluated using the variance inflation factor.

Identification of Characteristics Associated With Biomarker Concentrations

In the first step, linear regression models were used to estimate the associations between the biomarkers of interest and clinical features of AD as explanatory variables. These features of AD, which have previously been shown to be associated with incident AD,[10] were selected based on their expected ability to explain a substantial proportion of the biomarkers' variance.

The clinical features of AD included cognitive performance (CDR-SoB, FCSRT, and TMT-B, as described above), morphological MRI features (normalized hippocampal volume [hippocampal volume/intracranial volume] and mean cortical thickness in the entorhinal, inferior temporal, middle temporal, and inferior parietal cortices; fusiform gyrus; and precuneus according to the Desikan atlas, corresponding to the Dickerson signature of AD[32]), and AD genetic risk factors (*APOE* ε4 status and a genetic risk score excluding *APOE*). These variables were included in a multivariable linear regression model without selection. The AD biomarkers (i.e., Aβ-42, Aβ-40, tau, p181-tau, and NfL) and Aβ-42/Aβ-40 and p181-tau/tau ratio data were log transformed to achieve a normal distribution and then standardized. Subgroup analyses were performed by stratifying according to the baseline global CDR (0 vs 0.5), amyloid status on amyloid-PET scan or abnormal CSF Aβ-42 level (whichever was available first), and educational level (the French baccalaureate corresponded to a high educational level).

Next, we explored non–AD-related factors associated with blood and CSF biomarker concentrations. A wide range of candidate variables were tested, including age, sex, routine biological measurements, blood inflammatory markers, behavioral and cardiovascular risk factors (systolic and diastolic blood pressure, heart rate, BMI, alcohol and tobacco consumption, and physical activity), treatments (if ≥50 individuals were exposed), comorbidities (if present in ≥50 individuals), and preanalytical sample handling (delay in hours from collection to freezing, duration of storage at −80°C, time of day for collection, and fasting status). eTable 1 presents a comprehensive list of the candidate variables.

Using individual biomarkers as outcomes, we performed univariate linear regression analyses with each candidate covariate included separately (i.e., 1 model per biomarker and covariate). To account for type I error inflation in the context of multiple testing, a false discovery rate correction was applied, and a statistical significance level was set at 0.05. Then, we computed multivariate linear regression including significantly associated clinical features of AD and non–AD-related factors, and a stepwise selection (based on p-values <0.05) was performed to determine the final models. In parallel, a least absolute shrinkage and selection operator (LASSO) approach was implemented to select among all clinical features of AD and non–AD-related factor variables associated with biomarker concentrations. Owing to the instability of LASSO in variable selection, 100 bootstrap samples were generated from the original data set. We selected variables independently for each bootstrap sample, determining the optimal tuning parameter (λ) through a fivefold cross-validation process. The results of this selection process are the selection frequency (%) for each variable (i.e., the number of times the variable was finally selected). The final model for each biomarker included covariates statistically significant after stepwise selection and the corresponding LASSO selection. Overall and partial R^2 values were derived from the final models to determine the proportion of variance that the models explained.

Correction of Blood Biomarker Concentrations to Predict Pathologic Status

To test whether the prediction accuracy of disease status could be improved, a correction was applied to the blood biomarker concentrations. This correction accounted for the effects of medication intake, comorbidities, laboratory results, and inflammatory markers. The coefficients derived from the final multiple linear regression models served as the basis for these adjustments. For instance, individuals exposed to certain treatments exhibited an X-point increase in p181-tau levels compared with untreated individuals. To determine the corrected level for individuals under this treatment, we predicted the level that would be observed in the absence of this treatment by subtracting X from the actual level. The value corrected on the basis of the laboratory results was computed using the sample mean. The aim of this adjustment was to evaluate the extent to which accounting for covariates improved the correlations of blood biomarkers with CSF markers and had better discriminatory performance in predicting amyloid positivity on PET or pathologic levels of CSF Aβ-42 (<750 pg/mL) or p181-tau (>60 pg/mL). The change in discriminative performance was assessed by calculating the difference in the area under the curve (AUC) between the corrected and uncorrected blood biomarkers. Confidence intervals for the differences in AUC values were obtained through bootstrap resampling.

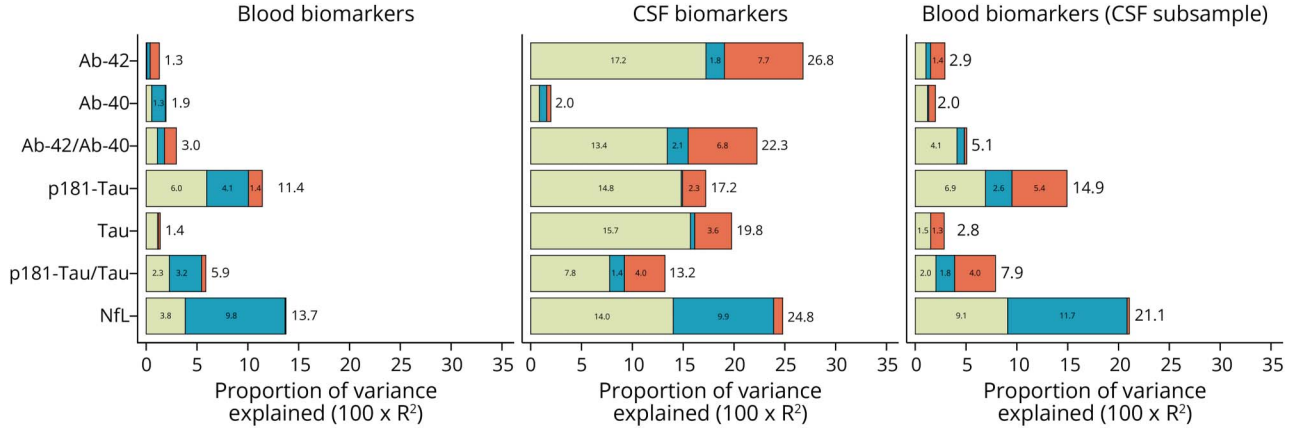
Statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC).

Results

Population Characteristics

Blood biomarkers of 2,257 participants were assessed at the time of inclusion in MEMENTO (Table 1). The median age of the participants was 72 years, with 61.8% being women and 55.2% having a high educational level. Table 1 presents the baseline characteristics of the participants, 305 (13.5%) of whom underwent lumbar puncture within 90 days (median 7 days) after inclusion.

Figure 1 Proportions of Variance Explained by Clinical Features of AD for Blood and CSF Biomarker Concentrations: The MEMENTO Cohort



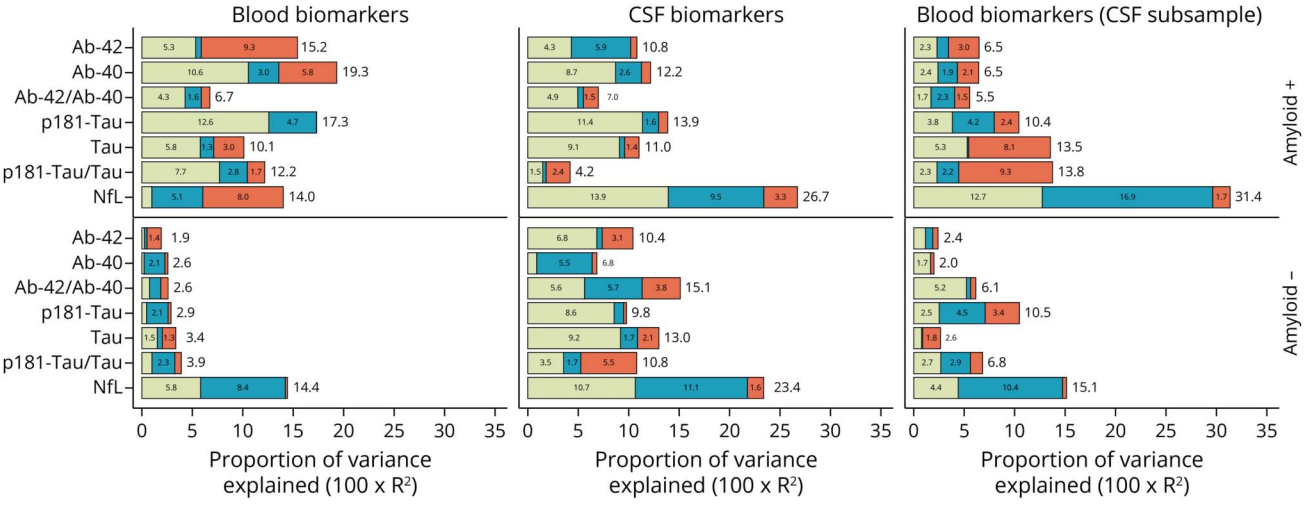
Green: neuropsychological tests, blue: imaging, and red: genetic. Blood biomarkers: n = 2,257; CSF subsample: n = 305. Aβ = β-amyloid; p181-tau = 181-phosphorylated tau.

Variance Explained by Clinical Features of AD

The proportion of variance of blood biomarkers explained by clinical features of AD (i.e., cognitive performance, AD genetic risk score, normalized hippocampal volume, and cortical signature on brain MRI) is presented in Figure 1. The amount of variance explained by the clinical features was as follows (in decreasing order): 13.7% for NfL, 11.4% for p181-tau, 5.9% for the p181-tau/tau ratio, 3.0% for the Aβ-42/Aβ-40 ratio, 1.9% for Aβ-40, 1.4% for total-tau, and 1.3% for Aβ-42. Similar results were obtained for blood biomarkers in the CSF subsample, although a slightly higher proportion of the variance was explained. Regarding CSF biomarkers, the clinical features of AD explained a larger proportion of the variance, particularly for Aβ-42 (26.8%), NfL (24.8%), Aβ-42/Aβ-40 ratio (22.3%), total-tau (19.8%), and p181-tau (17.2%).

To account for the potential of amyloid positivity, baseline cognitive status, or educational level to modulate levels of fluid biomarkers and their association with clinical features of AD, we conducted stratified analyses (Figure 2 and eFigure 1). The proportion of variance in blood biomarkers explained by the clinical features of AD was substantially higher in the amyloid-positive subgroup compared with the amyloid-negative subgroup. The proportion of variance explained was roughly comparable according to CDR or educational level. Regarding CSF biomarkers, the effect of CDR,

Figure 2 Proportions of the Variance Explained by Clinical Features of AD for Blood and CSF Biomarker Concentrations According to Amyloid Status: The MEMENTO Cohort



Green: neuropsychological tests, blue: imaging, and red: genetic. Blood biomarkers: Amyloid+ n = 234, Amyloid− n = 666; CSF subsample: Amyloid+ n = 88, Amyloid− n = 217. Aβ = β-amyloid; p181-tau = 181-phosphorylated tau.

Table 2 Variance Explained by the Factors Associated With the Measure of Blood and CSF Biomarker Concentration: The MEMENTO Cohort

		Proportion of variance explained (100 × R^2) in the final models									
	N	Overall	Clinical features of AD	Age	Sex	Routine biological measurements	Blood inflammatory markers	Behavorial risk factors	Treatments	Comorbidities	Preanalytical sample handling
Blood biomarkers											
Aβ-42	2,215	8.3	1.3			4.0	1.6		0.6	0.4	0.5
Aβ-40	2,213	11.5	0.3	6.1		2.5	1.0		0.8	0.2	0.6
Aβ-42/ Aβ-40	2,210	8.7	1.5	4.2			0.3		0.2		2.4
p181-tau	2,057	20.5	6.7	9.2		2.5	0.7	0.6	0.8		
Total-tau	2,224	12.3	0.6		0.7	2.7	2.0		0.8	1.1	4.5
p181-tau/tau	2,028	14.3	2.2	7.2		1.9	0.6	0.6	0.3	0.6	0.9
NfL	2,255	46.0	0.3	35.9		5.0	0.4	3.5	0.2	0.2	0.5
CSF biomarkers											
Aβ-42	303	29.7	21.6	8.1							NA
Aβ-40	297	2.2	1.2	1.1							NA
Aβ-42/ Aβ-40	297	28.7	18.1	10.5							NA
p181-tau	303	21.5	13.8	3.1		4.6					NA
Total-tau	302	29.1	15.2	6.1		5.6				2.3	NA
p181-tau/tau	302	21.4	10.7	7.4						3.4	NA
NfL	286	26.0	11.9	14.1							NA

Abbreviations: Aβ = β-amyloid; AD = Alzheimer disease; NfL = neurofilament light chain; NA = not applicable.
Only variables significantly associated ($p < 0.05$) with biomarker concentrations contribute to R^2 calculation. A comprehensive list of the variables is provided in Table 3 and eTable 2.

amyloid positivity, and educational level on the proportions of variance explained by clinical AD correlates was minor.

Variance Explained by Age and Other Associated Covariates

Beyond clinical features of AD, a comprehensive analysis was conducted to identify other factors associated with biomarker levels, including demographics, routine biological measurements, inflammatory markers, behavioral and cardiovascular risk factors, alcohol consumption, physical activity, treatments, comorbidities, and preanalytical sample handling. Table 2 summarizes the overall variance explained by the final models and the contribution of each category of covariates. Data on the assumptions of homoscedasticity and normality of residuals are presented in the supplementary materials. We found no evidence of heteroscedasticity of residuals (eFigure 2), and their distributions were close to normal (eFigure 3). We found no substantial collinearity across covariates (all variance inflation factors <5; eTable 2).

In the final models, a larger proportion of the variance in blood biomarkers was explained by age (Aβ-40: 6.1%, Aβ-42/Aβ-40: 4.2%, p181-tau: 9.2%, p181-tau/tau: 7.2%, and NfL: 35.9%) or non–AD-related factors (Aβ-42: 7.1%, Aβ-40: 5.1%, Aβ-42/Aβ-40: 2.9%, p181-tau: 4.6%, p181-tau/tau: 4.9%, and NfL: 9.8%). Conversely, clinical features of AD accounted for a smaller fraction, except for p181-tau (6.7%, including 3.1% for genetic score, 3.1% for cognitive function, and 0.5% for AD signature on brain MRI; Table 3). Overall, sex, inflammatory markers, behavioral and cardiovascular risk factors, treatments, and comorbidities made a limited contribution to the explained variance. The standard biological measurements explained a larger fraction of variance compared with the clinical features of AD for Aβ-42 (4.0% for creatinine [2.85%], alanine aminotransferase [ALT; 0.61%], and triglycerides [0.50%]), Aβ-40 (2.5% for creatinine [1.68%], alkaline phosphatase [0.45%], platelets [0.28%], and ALT [0.14%]), total-tau (2.7% for ALT [0.66%], high-density lipoprotein [HDL] cholesterol

Table 3 AD and Non–AD-Related Factors Explaining Blood Biomarker Variance: The MEMENTO Cohort

	β (95% CI)	Proportion of variance explained (%)	LASSO selection (%)
Aβ-42/Aβ-40			
Clinical features of AD			
APOE ε4	−0.229 (−0.319 to −0.139)	1.25	38
ROI cortical thickness	−0.511 (−0.851 to −0.170)	0.29	0
Non–AD-associated covariates			
Age	−0.026 (−0.031 to −0.021)	4.20	96
Fasting status	−0.153 (−0.295 to −0.010)	1.68	67
Duration of storage at −80°C	−0.101 (−0.152 to −0.049)	0.74	14
IP-10	−0.000 (−0.000 to −0.000)	0.33	18
α-Adrenoreceptor antagonists	−0.139 (−0.272 to −0.006)	0.18	1
P181-tau			
Clinical features of AD			
APOE ε4	0.274 (0.188 to 0.360)	2.65	48
FCSRT	−0.016 (−0.024 to −0.009)	2.38	81
TMT-B	0.022 (0.009 to 0.035)	0.65	38
ROI cortical thickness	−0.463 (−0.812 to −0.114)	0.54	68
Genetic risk score	0.191 (0.081 to 0.301)	0.49	3
Non–AD-associated covariates			
Age	0.020 (0.015 to 0.026)	9.23	100
Creatinine	0.006 (0.004 to 0.009)	1.09	18
Vitamin K antagonists	0.307 (0.155 to 0.460)	0.80	13
IL-12p70	0.008 (0.004 to 0.011)	0.66	5
Total cholesterol	−0.042 (−0.077 to −0.006)	0.59	0
BMI	−0.017 (−0.026 to −0.007)	0.50	3
Hemoglobin	−0.055 (−0.091 to −0.018)	0.44	3
Alkaline phosphatase	−0.002 (−0.003 to −0.001)	0.39	1
Tobacco consumption	0.077 (0.012 to 0.141)	0.12	1
NfL			
Clinical features of AD			
CDR SoB	0.072 (0.023 to 0.121)	0.27	6
Non–AD-associated covariates			
Age	0.053 (0.048 to 0.057)	35.94	100
Creatinine	0.013 (0.011 to 0.015)	3.93	94
BMI	−0.038 (−0.046 to −0.030)	2.90	94
Hemoglobin	−0.077 (−0.106 to −0.048)	1.02	65
Time of day for collection	0.204 (0.100 to 0.307)	0.43	0
TNFα	0.001 (0.001 to 0.002)	0.42	9
Diastolic blood pressure	−0.007 (−0.011 to −0.003)	0.29	25

Continued

Table 3 AD and Non–AD-Related Factors Explaining Blood Biomarker Variance: The MEMENTO Cohort *(continued)*

	β (95% CI)	Proportion of variance explained (%)	LASSO selection (%)
Tobacco consumption	0.076 (0.026 to 0.126)	0.26	1
Breast cancer	0.196 (0.059 to 0.334)	0.19	0
Vitamin K antagonists	0.150 (0.026 to 0.273)	0.14	0
Vitamin D and analogs	0.077 (0.007 to 0.148)	0.09	0
HDL cholesterol	0.068 (0.005 to 0.132)	0.05	5
Hypertension	0.091 (0.015 to 0.168)	0.03	0
Fasting status	0.191 (0.095 to 0.288)	0.03	0

Abbreviations: AD = Alzheimer disease; BMI = body mass index; CDR SoB = Clinical Dementia Rating-Sum of Boxes; FCSRT = Free and Cued Selective Reminding Test; HDL = high-density lipoprotein; IL = interleukin; IP = inducible protein; LASSO = least absolute shrinkage and selection operator; ROI = region of interest; TMT = Trail Making Test; TNF = tumor necrosis factor.
Details of AD and non–AD-related factors for Aβ-42, Aβ-40, total-tau, and p181-Tau/Tau are available in eTable 2.

[0.55%], chloride [0.52%], hemoglobin [0.49%], and total bilirubin [0.48%]), and NfL (5% for creatinine [3.93%], hemoglobin [1.02%], and HDL cholesterol [0.05%]). Table 3 and eTable 2 present detailed descriptions of the associated factors. Overall, the covariates that explained the largest proportion of the variance were the most frequently selected with LASSO (e.g., 100% for age and p181-tau, 96% for age Aβ-42/Aβ-40, and 94% for creatinine and NfL), whereas those with a small R^2 value were infrequently selected (eTable 2).

The clinical features of AD explained more of the variance in the levels of all CSF biomarkers except NfL. We found significant associations between activated partial thromboplastin time and p181-tau (4.6% of variance explained) and total-tau (5.6%), as well as between migraine and total-tau (2.3%) and

Table 4 Discriminative Performances of Uncorrected and Corrected Blood Biomarker Concentrations to Predict Pathologic Amyloid (A+) or Tau (T+) Status: The MEMENTO Cohort

			Area under the curve (95% CI)		
Outcome to predict	**Blood biomarker as predictor**	**n**	**Uncorrected blood biomarker**	**Corrected blood biomarker**	**Difference (corrected − uncorrected)**
Amyloid positivity (PET)	Aβ42	819	0.625 (0.622 to 0.628)	0.638 (0.635 to 0.641)	0.013 (0.012; 0.014)
	Aβ42/Aβ40	820	0.671 (0.668 to 0.674)	0.651 (0.648 to 0.654)	−0.020 (−0.021; −0.019)
	p181-tau	765	0.739 (0.736 to 0.742)	0.726 (0.723 to 0.729)	−0.014 (−0.014; −0.013)
	NfL	839	0.632 (0.628 to 0.635)	0.599 (0.596 to 0.603)	−0.032 (−0.034; −0.031)
Amyloid positivity (CSF)[a]	Aβ42	289	0.656 (0.651 to 0.661)	0.682 (0.678 to 0.687)	0.026 (0.024; 0.028)
	Aβ42/Aβ40	292	0.702 (0.697 to 0.707)	0.691 (0.686 to 0.696)	−0.011 (−0.012; −0.009)
	p181-tau	268	0.736 (0.731 to 0.741)	0.711 (0.706 to 0.716)	−0.025 (−0.026; −0.024)
	NfL	303	0.666 (0.661 to 0.671)	0.611 (0.605 to 0.616)	−0.056 (−0.059; −0.053)
P181-tau positivity (CSF)[b]	Aβ42	289	0.596 (0.591 to 0.600)	0.599 (0.594 to 0.603)	0.003 (0.001; 0.004)
	Aβ42/Aβ40	285	0.635 (0.630 to 0.640)	0.624 (0.619 to 0.629)	−0.011 (−0.012; −0.010)
	p181-tau	278	0.648 (0.644 to 0.652)	0.658 (0.653 to 0.662)	0.010 (0.008; 0.011)
	NfL	303	0.643 (0.639 to 0.648)	0.623 (0.619 to 0.627)	−0.020 (−0.023; −0.017)

Abbreviations: Aβ = β-amyloid; NfL = neurofilament light chain; p181-tau = 181-phosphorylated tau.
[a] Defined as CSF Aβ42 concentrations <750 pg/mL.
[b] Defined as CSF p181-tau concentrations >60 pg/mL.

p181-tau/tau ratio (3.4%). No other associations were observed among inflammatory markers, behavioral risk factors, and treatments.

Corrected Blood Biomarker Concentrations: Correlations With CSF Measure and Prediction of Pathologic Amyloid (A+) and Tau (T+) Status

Based on final models presented in Tables 2 and 3 and eTable 2, we incorporated the effects of standard biological measurements, inflammatory marker concentrations, treatments, and comorbidities to estimate corrected blood biomarker concentrations. Correlations between uncorrected and corrected blood and CSF levels are presented in eFigure 4. No major differences in correlations were found between the corrected and uncorrected levels, with the largest increases being observed for Aβ-42 (from $r = 0.27$ to $r = 0.33$ after correction) and blood p181-tau (from $r = 0.30$ to $r = 0.33$).

Regarding the discriminative performances of uncorrected and corrected blood biomarker levels for the prediction of amyloid and tau pathologic status, a slight improvement was observed in the performance of blood Aβ42 for predicting positive CSF Aβ-42 status (delta AUC: +0.026, 95% CI 0.024–0.028) and amyloid PET status (delta AUC: +0.013, 95% CI 0.012–0.014). However, the corrected values for other biomarkers showed small differences (<0.01) or lower AUC values (Table 4).

Discussion

In this study, we investigated the factors associated with fluid biomarker concentrations in a large clinical cohort of individuals with subjective cognitive complaints or MCI. Several clinical features of AD, including episodic memory, executive function, normalized hippocampal volume, the cortical signature of AD, *APOE* ε4 status, and AD genetic risk score, accounted for a small proportion of the variance in blood biomarker levels. Moreover, both age and other covariates, such as routine biological tests, inflammatory markers, behavioral risk factors, treatments, comorbidities, and preanalytical sample handling, explained a greater proportion of variance in blood biomarker levels than the clinical features of AD, except for p181-tau (where age explained 9.2% of the variance, clinical features of AD 6.7%, and other covariates 4.6%). Conversely, clinical features of AD accounted for a large fraction of the variance in CSF biomarker levels, surpassing the effects of age by a factor of 10, except for NfL and Aβ-40.

Although numerous variables were considered, a large proportion of the variance in blood biomarker levels remains unexplained (54%–92%). Several hypotheses can account for this observation. First, these peptides are also produced by peripheral organs, such as kidneys, skeletal muscles, and breasts,[33] and the production source cannot be differentiated. However, there is potential for brain-derived tau to offer promising insight in the future.[34] Second, these markers exhibit intrinsic variability, as indicated by the manufacturer, with internal coefficients of variation ranging from 8.0% for Aβ-40 to 12.7% for NfL. Repeated measurements of these markers over time would be valuable to better disentangle the effect of the disease progression to the intraindividual heterogeneity.

Our analysis confirmed the significant associations between creatinine levels and p181-tau, Aβ-42, Aβ-40, NfL, and the p181-tau/tau ratio. However, no significant associations were observed with total-tau or the Aβ-42/Aβ-40 ratio.[16-18,35] In addition, we found associations among BMI, p181-tau, and NfL concentrations.[18] Furthermore, we identified numerous novel associations with medical treatments, inflammatory markers, routine biological measurements, and preanalytical sample handling (Table 3 and eTable 2). The LASSO approach confirmed that the variables explaining the most variance had higher proportion of selection, while covariates with minimal contributions were poorly selected. The specific role of these characteristics is somewhat unclear, and our findings require confirmation in an independent population because we cannot exclude the possibility of false-positive results.

We report contrasting results regarding CSF biomarkers. Age explained a smaller proportion of the variance in CSF biomarker concentrations compared with blood biomarkers, whereas clinical features of AD explained a larger proportion. These differences between CSF and blood biomarkers can be attributed to the blood-brain barrier, which may protect the CSF from the effects of medications, peripheral inflammation, kidney disease, and other factors. As a result, CSF biomarkers are more likely to accurately reflect AD pathology and are less susceptible to the effects of confounding factors compared with their corresponding blood biomarkers. These findings are in line with those of a recent study that demonstrated a substantial effect of blood-brain barrier integrity on the diagnostic performance of AD blood biomarkers for brain pathology.[36]

We also investigated the effect of non–AD-related factors on predictions of amyloid (A+) and tau (T+) status based on AD blood biomarkers. After adjusting for routine laboratory measurements, treatments, and comorbidities, the correlations between blood and CSF biomarkers, as well as the performance of blood biomarkers in predicting A+ or T+ status (based on PET or CSF analysis), remained unchanged. Despite statistically significant results, routine laboratory tests, treatments, and comorbidities had a small effect on biomarker levels, as reported previously.[18] Furthermore, considering these confounders when interpreting biomarker levels in clinical practice could lead to significant complexities and only marginal improvement in predictions of pathologic levels of Aβ-42 or p181-tau.

Our findings raise concerns regarding the clinical usefulness of AD blood biomarkers. Given their limited ability to reflect the clinical features of AD, they may not provide reliable

information for patient management in the absence of confirmatory examinations, such as CSF analysis or PET.[15] Moreover, their added value in predicting dementia compared with conventional clinical information is questionable.[10] Therefore, 1 potential use for these biomarkers could be as a screening tool to identify patients who would benefit from further investigations and those for whom additional action is unnecessary.[15] In this context, our results emphasize the significance of considering age as a key factor when establishing norms or clinical cutoffs.

This study had several strengths. This is a comprehensive analysis of the factors associated with AD blood biomarker levels that explores simultaneously neuropsychological tests, imaging and genetic data, biological and inflammatory markers, comorbidities, treatments, behavioral and cardiovascular risk factors, and preanalytical sample handling. Second, we examined multiple blood biomarkers and used a similar methodological approach for analyzing CSF markers. Finally, the proportion of variance offers easily interpretable findings and depicts the extent to which biomarker levels are explained by various factors beyond statistical significance.

We acknowledge some limitations. First, the CSF subsample was relatively small (n = 305). Although R^2 of AD blood biomarker were higher in this subsample, the overall results remained consistent with the entire cohort. Second, calculating the proportion of variance explained (R^2) through linear models carries inherent limitations. To address these limitations, the biomarker concentrations were log transformed and standardized to enable comparison across analyses. Moreover, we did not use R^2 for variable selection and abstained from drawing conclusions regarding the goodness-of-fit or predictive performance of the models. Second, compared with the MCI individuals of The French Alzheimer Databank, a French registry of all persons consulting French Memory Clinics,[37] our sample has a comparable proportion of female (62% vs 59%), is younger (72 years vs 76 years), and has a higher "high educational level attainment" (55% vs 15%) and a higher Mini-Mental State Examination score (28 vs 25). These differences might have influenced our results, although our conclusions remained consistent after controlling for baseline cognition and educational level. Furthermore, although we studied a clinical population, some of our findings are aligned with those published on the population-based Mayo Clinic Study of Aging,[16,38] regarding APOE, BMI, and renal function. Still, our findings should be extrapolated cautiously to the general population. Finally, p217-tau, p231-tau, and glial fibrillary acidic protein were not included in our analysis because they are not currently available in the MEMENTO database. Compared with the other assays for blood biomarker quantification, the Quanterix commercial kits used in this study have demonstrated only moderate ability to detect abnormal amyloid load and also have moderate correlations with CSF biomarkers.[39]

In conclusion, the concentrations of AD blood biomarkers were predominantly explained by age and various non–AD-related factors, in contrast to CSF markers. Only blood p181-tau exhibited a modest level of variance explained by clinical features of AD in the final models that considered all associated variables including cognition, genetics, and brain MRI. We identified several non–AD-related factors associated with blood biomarker levels. However, even after accounting for these covariates, the correlations between CSF and blood biomarkers, as well as their predictions of A+ and T+ status, did not significantly improve. Our findings emphasize the need for further methodological strategies to identify the factors that contribute to the variability of AD blood biomarker concentrations. Such efforts are essential to help physicians interpret results more effectively in the future.

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Disclosure

V. Bouteloup, I. Pellegrin, B. Dubois, and G. Chene have no conflict of interest to declare. During the past 3 years, V. Planche was a local unpaid investigator or sub-investigator for clinical trials granted by NovoNordisk, Biogen, TauRx Pharmaceuticals, Janssen, Green Valley Pharmaceuticals, and Alector. He received consultant fees for animal studies from Motac Neuroscience Ltd., outside the submitted work. C. Dufouil has no conflict of interest to declare. Go to Neurology.org/N for full disclosures.

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Continued

Appendix 1 *(continued)*

Name	Location	Contribution
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Bruno Dubois, MD, PhD	Alzheimer Research Center IM2A, Salpêtrière Hospital, AP-HP, Sorbonne University, Paris, France	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Genevieve Chene, MD, PhD	Univ. Bordeaux, Inserm; CIC 1401 EC, CHU Bordeaux, France	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Vincent Planche, MD, PhD	Univ. Bordeaux, Inserm, Bordeaux Population Health, UMR1219, Bordeaux; CIC 1401 EC, Pôle Santé Publique, CHU de Bordeaux, France	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Carole Dufouil, PhD	Univ. Bordeaux, Inserm; CIC 1401 EC, CHU Bordeaux, France	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data

Appendix 2 Coinvestigators

MEMENTO Study Group coinvestigators are listed in Appendix 2 as supplemental content.

References

1. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of 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(3):280-292. doi:10.1016/j.jalz.2011.03.003
2. Planche V, Villain N. US Food and Drug Administration approval of aducanumab: is amyloid load a valid surrogate end point for Alzheimer disease clinical trials? *JAMA Neurol*. 2021;78(11):1307-1308. doi:10.1001/jamaneurol.2021.3126
3. Tosun D, Veitch D, Aisen P, et al. Detection of β-amyloid positivity in Alzheimer's Disease Neuroimaging Initiative participants with demographics, cognition, MRI and plasma biomarkers. *Brain Commun*. 2021;3(2):fcab008. doi:10.1093/braincomms/fcab008
4. Ni M, Zhu ZH, Gao F, et al; CANDI Consortium. Plasma core Alzheimer's disease biomarkers predict amyloid deposition burden by positron emission tomography in Chinese individuals with cognitive decline. *ACS Chem Neurosci*. 2023;14(1):170-179. doi:10.1021/acschemneuro.2c00636
5. Moscoso A, Grothe MJ, Ashton NJ, et al; Alzheimer's Disease Neuroimaging Initiative. Time course of phosphorylated-tau181 in blood across the Alzheimer's disease spectrum. *Brain*. 2021;144(1):325-339. doi:10.1093/brain/awaa399
6. Park JE, Gunasekaran TI, Cho YH, et al. Diagnostic blood biomarkers in Alzheimer's disease. *Biomedicines*. 2022;10(1):169. doi:10.3390/biomedicines10010169
7. Mattsson-Carlgren N, Salvadó G, Ashton NJ, et al. Prediction of longitudinal cognitive decline in preclinical Alzheimer disease using plasma biomarkers. *JAMA Neurol*. 2023;80(4):360-369. doi:10.1001/jamaneurol.2022.5272
8. Marks JD, Syrjanen JA, Graff-Radford J, et al; Alzheimer's Disease Neuroimaging Initiative. Comparison of plasma neurofilament light and total tau as neurodegeneration markers: associations with cognitive and neuroimaging outcomes. *Alzheimers Res Ther*. 2021;13(1):199. doi:10.1186/s13195-021-00944-y
9. Palmqvist S, Tideman P, Cullen N, et al; Alzheimer's Disease Neuroimaging Initiative. Prediction of future Alzheimer's disease dementia using plasma phospho-tau combined with other accessible measures. *Nat Med*. 2021;27(6):1034-1042. doi:10.1038/s41591-021-01348-z
10. Planche V, Bouteloup V, Pellegrin I, et al; MEMENTO Study Group. Validity and performance of blood biomarkers for Alzheimer disease to Predict dementia risk in a large clinic-based cohort. *Neurology*. 2023;100(5):e473-e484. doi:10.1212/WNL.0000000000201479
11. Pichet Binette A, Palmqvist S, Bali D, et al. Combining plasma phospho-tau and accessible measures to evaluate progression to Alzheimer's dementia in mild cognitive impairment patients. *Alzheimers Res Ther*. 2022;14(1):46. doi:10.1186/s13195-022-00990-0
12. Hanon O, Vidal JS, Lehmann S, et al; BALTAZAR study group. Plasma amyloid beta predicts conversion to dementia in subjects with mild cognitive impairment: the BALTAZAR study. *Alzheimers Dement*. 2022;18(12):2537-2550. doi:10.1002/alz.12613
13. Teunissen CE, Verberk IMW, Thijssen EH, et al. Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. *Lancet Neurol*. 2022;21(1):66-77. doi:10.1016/S1474-4422(21)00361-6
14. Karikari TK, Ashton NJ, Brinkmalm G, et al. Blood phospho-tau in Alzheimer disease: analysis, interpretation, and clinical utility. *Nat Rev Neurol*. 2022;18(7):400-418. doi:10.1038/s41582-022-00665-2
15. Hansson O, Edelmayer RM, Boxer AL, et al. The Alzheimer's Association appropriate use recommendations for blood biomarkers in Alzheimer's disease. *Alzheimers Dement*. 2022;18(12):2669-2686. doi:10.1002/alz.12756
16. Syrjanen JA, Campbell MR, Algeciras-Schimnich A, et al. Associations of amyloid and neurodegeneration plasma biomarkers with comorbidities. *Alzheimers Dement*. 2022;18(6):1128-1140. doi:10.1002/alz.12466
17. O'Bryant SE, Petersen M, Hall J, Johnson LA; HABS-HD Study Team. Medical comorbidities and ethnicity impact plasma Alzheimer's disease biomarkers: important considerations for clinical trials and practice. *Alzheimers Dement*. 2023;19(1):36-43. doi:10.1002/alz.12647
18. Pichet Binette A, Janelidze S, Cullen N, et al. Confounding factors of Alzheimer's disease plasma biomarkers and their impact on clinical performance. *Alzheimers Dement*. 2023;19(4):1403-1414. doi:10.1002/alz.12787
19. Verberk IMW, Misdorp EO, Koelewijn J, et al. Characterization of pre-analytical sample handling effects on a panel of Alzheimer's disease–related blood-based biomarkers: results from the Standardization of Alzheimer's Blood Biomarkers (SABB) working group. *Alzheimers Dement*. 2022;18(8):1484-1497. doi:10.1002/alz.12510
20. Mielke MM, Dage JL, Frank RD, et al. Performance of plasma phosphorylated tau 181 and 217 in the community. *Nat Med*. 2022;28(7):1398-1405. doi:10.1038/s41591-022-01822-2. Erratum in: Nat Med. 2023;29(11):2954.
21. Dufouil C, Dubois B, Vellas B, et al; MEMENTO cohort Study Group. Cognitive and imaging markers in non-demented subjects attending a memory clinic: study design and baseline findings of the MEMENTO cohort. *Alzheimers Res Ther*. 2017;9(1):67. doi:10.1186/s13195-017-0288-0
22. Grober E, Buschke H, Crystal H, Bang S, Dresner R. Screening for dementia by memory testing. *Neurology*. 1988;38(6):900-903. doi:10.1212/wnl.38.6.900
23. Van der Linden M, Coyette F, Poitrenaud J, et al. L'épreuve de rappel libre/rappel indice à 16 items (RL/RI-16). In: Van der Linden M, Adam S, Agniel A, et al, eds. *L'évaluation des troubles de la mémoire: présentation de quatre tests de mémoire épisodique (avec leur étalonnage)*. Solal Editeur; 2004:25-47.
24. Reischies FM, Neu P. Comorbidity of mild cognitive disorder and depression: a neuropsychological analysis. *Eur Arch Psychiatry Clin Neurosci*. 2000;250(4):186-193. doi:10.1007/s004060070023
25. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology*. 1993;43(11):2412-2414. doi:10.1212/wnl.43.11.2412-a
26. Operto G, Chupin M, Batrancourt B, et al; CATI Consortium. CATI: a large distributed infrastructure for the neuroimaging of cohorts. *Neuroinformatics*. 2016;14(3):253-264. doi:10.1007/s12021-016-9295-8
27. Chupin M, Mukuna-Bantumbakulu AR, Hasboun D, et al. Anatomically constrained region deformation for the automated segmentation of the hippocampus and the amygdala: method and validation on controls and patients with Alzheimer's disease. *Neuroimage*. 2007;34(3):996-1019. doi:10.1016/j.neuroimage.2006.10.035
28. Dubois B, Epelbaum S, Nyasse F, et al; INSIGHT-preAD study group. Cognitive and neuroimaging features and brain β-amyloidosis in individuals at risk of Alzheimer's disease (INSIGHT-preAD): a longitudinal observational study. *Lancet Neurol*. 2018;17(4):335-346. doi:10.1016/S1474-4422(18)30029-2
29. Habert MO, Marie S, Bertin H, et al; CATI Nuclear Medicine network. Optimization of brain PET imaging for a multicentre trial: the French CATI experience. *EJNMMI Phys*. 2016;3(1):6. doi:10.1186/s40658-016-0141-8
30. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al; European Alzheimer's Disease Initiative (EADI); Genetic and Environmental Risk in Alzheimer's Disease (GERAD); Alzheimer's Disease Genetic Consortium (ADGC); Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE). Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet*. 2013;45(12):1452-1458. doi:10.1038/ng.2802
31. Quanterix. Accessed November 14, 2023. quanterix.com/simoa-assay-kits/.
32. Dickerson BC, Bakkour A, Salat DH, et al. The cortical signature of Alzheimer's disease: regionally specific cortical thinning relates to symptom severity in very mild to mild AD dementia and is detectable in asymptomatic amyloid-positive individuals. *Cereb Cortex*. 2009;19(3):497-510. doi:10.1093/cercor/bhn113
33. The Human Protein Atlas. Accessed May 11, 2023. proteinatlas.org.

34. Gonzalez-Ortiz F, Turton M, Kac PR, et al. Brain-derived tau: a novel blood-based biomarker for Alzheimer's disease-type neurodegeneration. *Brain*. 2023;146(3):1152-1165. doi:10.1093/brain/awac407
35. Lehmann S, Schraen-Maschke S, Vidal J, et al. Plasma Aβ42/Aβ40 ratio is independent of renal function. *Alzheimers Dement*. 2023;19(6):2737-2739. doi:10.1002/alz.12949
36. Bellaver B, Puig-Pijoan A, Ferrari-Souza JP, et al. Blood-brain barrier integrity impacts the use of plasma amyloid-β as a proxy of brain amyloid-β pathology. *Alzheimers Dement*. 2023;19(9):3815-3825. doi:10.1002/alz.13014
37. Tifratene K, Robert P, Metelkina A, Pradier C, Dartigues JF. Progression of mild cognitive impairment to dementia due to AD in clinical settings. *Neurology*. 2015;85(4):331-338. doi:10.1212/WNL.0000000000001788
38. Ramanan VK, Graff-Radford J, Syrjanen J, et al. Association of plasma biomarkers of Alzheimer disease with cognition and medical comorbidities in a biracial cohort. *Neurology*. 2023;101(14):e1402-e1411. doi:10.1212/WNL.0000000000207675
39. Janelidze S, Teunissen CE, Zetterberg H, et al. Head-to-head comparison of 8 plasma amyloid-β 42/40 assays in Alzheimer disease. *JAMA Neurol*. 2021;78(11):1375-1382. doi:10.1001/jamaneurol.2021.3180