Plasma Amyloid as Prescreener for the Earliest Alzheimer Pathological Changes

Inge M. W. Verberk, MSc,[1,2,3] Rosalinde E. Slot, MD,[1,2] Sander C. J. Verfaillie, PhD,[1,2,4] Hans Heijst, BSc,[1,3] Niels D. Prins, MD, PhD,[1,2] Bart N. M. van Berckel, MD, PhD,[1,4] Philip Scheltens, MD, PhD,[1,2] Charlotte E. Teunissen, PhD,[1,3]* and Wiesje M. van der Flier, PhD[1,2,5]*

Objective: We investigated the association of plasma amyloid beta (Abeta)40, Abeta42, and total tau (tTau) with the presence of Alzheimer pathological changes in cognitively normal individuals with subjective cognitive decline (SCD).
Methods: We included 248 subjects with SCD (61 ± 9 years, 42% female, Mini-Mental State Examination = 28 ± 2) from the SCIENCe project and Amsterdam Dementia Cohort. Subjects were dichotomized as amyloid abnormal by cerebrospinal fluid (CSF) and positron emission tomography (PET). Baseline plasma Abeta40, Abeta42, and tTau were measured using Simoa technology. Associations between plasma levels and amyloid status were assessed using logistic regression analyses and receiver operating characteristic analyses. Association of plasma levels with risk of clinical progression to mild cognitive impairment (MCI) or dementia was assessed using Cox proportional hazard models.
Results: Fifty-seven (23%) subjects were CSF-amyloid abnormal. Plasma Abeta42/Abeta40 ratio and plasma Abeta42 alone, but not tTau, identified abnormal CSF-amyloid status (plasma ratio: area under the curve [AUC] = 77%, 95% confidence interval [CI] = 69–84%; plasma Abeta42: AUC = 66%, 95% CI: 58–74%). Combining plasma ratio with age and apolipoprotein E resulted in AUC = 83% (95% CI = 77–89%). The Youden cutoff of the plasma ratio gave a sensitivity of 76% and specificity of 75%, and applying this as a prescreener would reduce the number of lumbar punctures by 51%. Using PET as outcome, a comparable reduction in number of PET scans would be achieved when applying the plasma ratio as prescreener. In addition, low plasma ratio was associated with clinical progression to MCI or dementia (hazard ratio = 2.0, 95% CI = 1.4–2.3).
Interpretation: Plasma Abeta42/Abeta40 ratio has potential as a prescreener to identify Alzheimer pathological changes in cognitively normal individuals with SCD.

ANN NEUROL 2018;84:656–666

Alzheimer disease (AD) pathophysiology is hallmarked by extracellular amyloid beta (Abeta) aggregation and intracellular tau deposition, which start 10 to 20 years prior to onset of clinical symptoms.[1–3] Amyloid pathology without cognitive impairment has been defined as the earliest Alzheimer pathological changes.[3–5] Individuals with these earliest Alzheimer changes (ie, abnormal amyloid status) are at increased risk of future cognitive decline[6–8] and clinical progression to dementia.[7,9–11] For this reason, they are an important target group in the context of clinical trials that evaluate antiamyloid therapies.

Low concentrations of Abeta in cerebrospinal fluid (CSF) as well as Abeta visualized on positron emission tomography (PET) scans have been extensively studied and have proven their accuracy in identifying amyloid pathology in the brain.[3,9,12] The available diagnostic tools are, however, invasive (CSF) or expensive (PET), hampering widespread application for diagnosis (eg, in a primary

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.25334

Received Jun 28, 2018, and in revised form Sep 4, 2018. Accepted for publication Sep 4, 2018.

Address correspondence to Drs. Verberk, Alzheimer Center Amsterdam, Department of Neurology and Neurochemistry Laboratory, Department of Clinical Chemistry, Vrije Universiteit Amsterdam, Amsterdam UMC, Boelelaan 1117, 1081 HV Amsterdam, the Netherlands. E-mail: i.verberk@vumc.nl

*C.E.T. and W.M.V.d.F. share senior authorship.

From the [1]Amsterdam Neuroscience; [2]Alzheimer Center Amsterdam, Department of Neurology, VU University Medical Center Amsterdam; [3]Neurochemistry Laboratory, Department of Clinical Chemistry, VU University Medical Center Amsterdam; [4]Department of Radiology and Nuclear Medicine, VU University Medical Center Amsterdam; and [5]Department of Epidemiology and Biostatistics, VU University Medical Center Amsterdam, Amsterdam, the Netherlands.

care setting) and large scale identification of individuals with abnormal amyloid status in the context of recruitment for trials.[13] There is an urgent need for low-invasive and affordable techniques to prescreen for cerebral amyloid pathology, subsequently forwarding fewer individuals toward further invasive and/or expensive testing.

A blood marker would qualify as an easy prescreening tool. Using first generation techniques like enzyme-linked immunosorbent assays (ELISA), studies on blood Abeta led to insufficient accuracy to allow implementation in prescreening.[12] With the recent emergence of novel highly sensitive technologies, the field is now quickly evolving, proving it is possible to use plasma markers to measure brain amyloid pathology.[14–17]

Although highly promising, recent studies used non-automated, labor-intensive techniques, precluding widespread implementation in large numbers of individuals.[15,16] Others chose an automated technique but evaluated the spectrum from full-blown AD dementia to healthy controls,[14] which although essential for validation of the analytical techniques does not translate to the urgent need of easy prescreening, which lies in the group of individuals in the very earliest stages of AD. Aiming to close this gap, we used fully automated, highly sensitive Simoa (single molecule array) technology[18] to measure plasma concentrations of Abeta40, Abeta42, and total tau (tTau) in a large cohort of cognitively unimpaired subjects with subjective cognitive decline (SCD). We aimed to investigate the potential value of plasma Abeta40, Abeta42 and tTau as a prescreening tool for abnormal cerebral amyloid status in cognitively normal individuals. To further evaluate clinical relevance of our plasma markers, we investigated their association with clinical progression to mild cognitive impairment (MCI) or dementia.

Patients and Methods

Subjects

We included 248 subjects labeled as SCD from the ongoing Amsterdam Dementia Cohort and SCIENCe project.[19–21] All subjects visited the memory clinic of the VU University Medical Center Amsterdam (VUmc) between November 2000 and August 2016 for extensive dementia screening that consisted of neurological, physical, and neuropsychological evaluation, biomarker analyses in CSF obtained by lumbar puncture, electroencephalography, and brain magnetic resonance imaging.[19,20] Subjects were labeled as SCD upon multidisciplinary consensus when no abnormalities on clinical or cognitive testing were observed and criteria for MCI, dementia, and other medical conditions potentially causing cognitive decline were not met (ie, no psychiatric diagnosis).[4,22] Inclusion criteria for this study were met when baseline CSF biomarker data and ethylenediaminetetraacetic acid (EDTA) plasma sample collected within 0.5 years from baseline visit were available, and at least 1 follow-up visit was performed. Written consent to use medical data and biomaterials for research purposes was in place, in accordance with the ethical consent by the VU University Amsterdam and with the Helsinki Declaration of 1975.

Clinical Progression

Subjects were followed on an annual basis (mean follow-up = 3 ± 2 years), where neurological, physical, and neuropsychological examination was repeated. Based on these results, the diagnosis was re-evaluated by clinical consensus. Clinical progression was defined as a change in diagnosis to MCI (Petersen criteria until 2012[23] and National Institute on Aging and Alzheimer's Association [NIA-AA] criteria for MCI from 2012 onward,[24] to Alzheimer dementia (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association until 2011[25] and NIA-AA criteria for AD from 2011 onward,[26] or to other types of dementia.[27–30] Time to clinical progression was calculated as the date difference between baseline blood sampling and the date on which clinical progression was first diagnosed. When SCD subjects progressed to MCI first and later to dementia, the date on which MCI was first diagnosed was used to estimate time to clinical progression.

Amyloid Status

CSF concentrations of Abeta42, tTau, and tau phosphorylated at threonine 181 (pTau181) were measured using Innotest ELISAs (Fuijirebio, Ghent, Belgium) by trained technicians who were blinded for clinical diagnosis.[31] CSF Abeta42 levels were adjusted for the drift in CSF biomarker analyses that occurred over the years and subsequently dichotomized as CSF amyloid abnormal (≤813pg/ml) and amyloid normal (>813pg/ml).[32]

For a subset (n = 69, 28%), amyloid PET was available. Subjects were scanned with [^{18}F]florbetaben (n = 33), [^{18}F]florbetapir (n = 20), [^{18}F]flutemetamol (n = 6), or [^{11}C]Pittsburgh compound B (PiB; n = 10) radiotracer. Tracers were infused trough a venous cannula. [^{18}F]Florbetapir and [^{11}C]PIB scans were acquired through 90-minute dynamic scanning (respectively PET/CT Ingenuity TF or Gemini TF [Philips Medical Systems, Best, the Netherlands] and ECAT EXACT HR + scanner [Siemens/CTI, Knoxville, TN]) simultaneously starting with tracer injection using a Medrad (Warrendale, PA) infusion system (approximately

370MBq [^{18}F]florbetapir, 351MBq [^{11}C]PiB). [^{18}F]Florbetaben and [^{18}F]flutemetamol scans were acquired through 20-minute static PET scanning (respectively PET/MR and Gemini TF-64 PET/CT scanner, Philips Medical Systems) starting 90 minutes after tracer injection (approximately 250MBq [^{18}F]florbetaben, 180MBq [^{18}F] flutemetamol). PET scans were visually read and dichotomously scored as either amyloid abnormal or amyloid normal by an experienced nuclear medicine physician (B.N. M.v.B.).

Plasma Analyses

EDTA plasma was obtained through venipuncture. After centrifugation at 1,800 × *g*, EDTA plasma was aliquoted in 0.5ml polypropylene tubes and stored at −80 °C in the VUmc Biobank. Samples were shortly thawed at room temperature and centrifuged at 14,000 × *g* prior to analyses, to prevent any sample debris from interfering in measurement. Plasma levels of Abeta40, Abeta42, and tTau were measured simultaneously using the commercially available Simoa Human Neurology 3-Plex A assay kit (Quanterix, Lexington, MA) on board of the automated Simoa HD-1 analyzer (Quanterix).[18] The manufacturer's instructions were followed, including 1:4 automated on-board automated sample dilution. All samples were analyzed in duplicate, randomly divided over 2 runs that were performed on 2 consecutive days. Research staff was well trained for the analytical procedure.

The triplex assay was in-house analytically validated prior to use according to standardized international protocols.[33] Abeta40 and Abeta42 gave good average interassay variation (Abeta40: 7.4% coefficient of variation [CV], Abeta42: 8.7% CV). Interassay variation was higher for tTau (22.2% CV), caused by poor repeatability of a validation sample with a low tau concentration (only 1.25pg/ml on average). All patient samples showed values above our in-house quantified lower limit of quantification (LLOQ; Abeta40: 0.16pg/ml, Abeta42: 0.34pg/ml, tTau: 0.42pg/ml), except for n = 10 tau measurements. Average intra-assay variation of duplicate measurements was well below the accepted cutoff of 20% CV (Abeta40: 3.1% CV, Abeta42: 3.9% CV, tTau: 5.8% CV). tTau measurements below LLOQ were assigned the measured concentration, as in our opinion this is more accurate than either assigning 0 (underestimation) or assigning the LLOQ value (overestimation). Two tTau values had an intra-assay percentage CV > 20. Upon repetition of measurement, the measured tTau concentration was very alike, and therefore it was decided to use the initial result. Excluding these 12 tTau measurements did not alter statistical outcomes.

Apolipoprotein E Genotyping

Genomic DNA was isolated from EDTA blood. Using a polymerase chain reaction technique, DNA was amplified and subsequently analyzed using the QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, the Netherlands) to establish size, and Sanger sequenced on the ABI130XL to determine apolipoprotein E (APOE) genotype. One or 2 APOE ε4 alleles classified subjects as APOE ε4 carriers, whereas no ε4 allele classified subjects as noncarriers. APOE ε4 carriership was available for 235 (95%) of our subjects.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows, version 22 (IBM, Armonk, NY). A probability level of $p < 0.05$ was considered statistically significant. Plasma Abeta42 and Abeta40 were used as single variables as well as in the ratio Abeta42/Abeta40 multiplied by 1,000. When biomarker data were skewed, natural log transformation was performed prior to correlation and regression analyses (applied for the following variables: plasma tTau, plasma ratio Abeta42/Abeta40, CSF tau, CSF pTau181). Prior to logistic regression analyses and Cox proportional hazards analyses, plasma Abeta40, Abeta42, and natural log-transformed Abeta42/Abeta40*1,000 and tau data were inverted and transformed to *Z* scores so that lower levels imply higher risk and effect sizes are comparable between markers.

Baseline demographics and clinical characteristics were compared using *t* tests, Mann–Whitney *U* tests, and chi-squared tests as appropriate. CSF and plasma biomarker levels were additionally compared using age- and sex-corrected univariate analyses of variance. Associations of plasma biomarker levels and CSF biomarker levels were assessed using Pearson correlation analyses and visualized in scatterplots constructed using R version 3.4.2. The association of plasma biomarkers with CSF-based and PET-based abnormal amyloid status was assessed using logistic regression analysis followed by receiver operating characteristic (ROC) curve analyses. Predicted values of binary logistic regression models were used to combine variables in ROC analysis. To evaluate the potential of the plasma Abeta42/Abeta40 ratio to identify CSF and PET abnormal amyloid status, the coordinates of the corresponding ROC curve were used to establish the Youden cutoff (ie, maximal sum of sensitivity and specificity). For visualization purposes, we applied the sensitivity and specificity levels of the Youden cutoff to calculate how many individuals we would need to screen in total with the blood test to obtain 100 CSF or PET amyloid abnormal subjects. To evaluate the potential of the multivariate model plasma Abeta42/Abeta40 ratio combined with age and APOE ε4 carriership to identify CSF amyloid

abnormal subjects, heat maps were constructed by filling out the logistic regression formula. Finally, we assessed the association of plasma markers with risk of clinical progression to MCI or dementia using Cox proportional hazard models, both unadjusted and adjusted for age and sex. This analysis was repeated excluding subjects who progressed to non-AD dementia. For visualization, Kaplan–Meier survival curves were plotted for clinical progression to MCI or AD dementia with separate lines for low, middle, and high baseline plasma levels of Abeta42 alone and of Abeta42/Abeta40 ratio (data divided into tertiles).

Results

Demographic and Clinical Characteristics

At baseline, the 248 subjects with SCD were on average 61 ± 9 years old, 42% were female, and Mini-Mental State Examination (MMSE) was 28 ± 2. Based on CSF, 57 (23%) subjects had abnormal amyloid status. After an average follow-up of 3 ± 2 years, 35 (14%) subjects showed clinical progression (Table 1). Of the progressors, 23 progressed to MCI, 4 to AD dementia, and 8 to non-AD dementia (4 to frontotemporal dementia, 1 to vascular dementia, 3 to other types of dementia).

Comparing CSF-based amyloid abnormal to amyloid normal subjects, subjects with abnormal CSF amyloid status were on average older, were more frequently female, had lower MMSE scores, and were more frequently APOE ε4 carriers. CSF-based amyloid abnormal subjects progressed more often to MCI or dementia ($p < 0.05$). Also, CSF tTau and CSF pTau181 levels were higher in subjects with abnormal CSF amyloid status compared to subjects with normal amyloid status (see Table 1).

TABLE 1. Demographics, Clinical Characteristics, and Biomarkers of the Total Study Population and Stratified for CSF-Based Amyloid Status

Characteristic	Total Group, n = 248	CSF-Based Amyloid Status	
		Amyloid Normal, n = 191 (77%)	Amyloid Abnormal, n = 57 (23%)
Age, yr	61 (9)	59 (9)	67 (8)[a]
Female gender	103 (42%)	71 (37%)	32 (56%)[b]
MMSE	28.3 (1.5)	28.4 (1.5)	28.0 (1.6)[b]
APOE ε4 carrier	89 (38%)	55 (31%)	34 (62%)[a]
Follow-up duration, yr	2.8 (2.13)	2.8 (2.11)	2.6 (2.21)
Clinical progression	35 (14%)	14 (7.3%)	21 (37%)[a]
Time to progression, yr	2.5 (2.1)	2.9 (2.6)	2.2 (1.62)
CSF Abeta42, pg/ml	1,024 (256)	1,128 (187)	676 (101)[a]
CSF tTau, pg/ml	325 (237)	267 (133)	518 (373)[a]
CSF pTau181, pg/ml	50.2 (25)	44.3 (18)	70.1 (35)[a]
Plasma Abeta40, pg/ml	208 (38)	206 (36.6)	213 (40.4)
Plasma Abeta42, pg/ml	9.90 (1.82)	10.11 (1.84)	9.20 (1.59)[b]
Plasma Abeta42/Abeta40 ratio	48.1 (7.00)	49.5 (6.81)	43.5 (5.51)[a]
Plasma tTau, pg/ml	3.15 (1.02)	3.18 (1.07)	3.06 (0.84)

Baseline demographic features of the total study population, and stratified for amyloid status (amyloid abnormal through CSF Abeta42 ≤ 813pg/ml, amyloid normal through CSF Abeta42 > 813pg/ml). Continuous data are presented as mean (standard deviation) and dichotomous data as n (%). Plasma Abeta42/Abeta40 ratio was multiplied by 1,000. APOE ε4 carriership data were available for 235 subjects, annotated as n/235 (% of 235). Differences between two groups were calculated using *t* tests, Mann–Whitney *U* tests, or chi-squared tests as appropriate.

[a]$p < 0.001$, [b]$p < 0.05$.

Abeta = amyloid beta; APOE = apolipoprotein E; CSF = cerebrospinal fluid; MMSE = Mini-Mental State Examination; pTau181 = tau phosphorylated at threonine 181; tTau = total tau.

Adjusted for age and sex, plasma Abeta42 alone and plasma Abeta42/Abeta40 ratio were lower in subjects with abnormal CSF amyloid status compared to subjects with normal CSF amyloid status (both $p < 0.01$; Table 1). Plasma Abeta40 and plasma tTau did not differ between groups.

Correlations of Plasma and CSF Markers

All plasma measures Abeta40, Abeta42, and tTau were positively correlated with each other (all $r > 0.25$, $p < 0.001$; Table 2 and Fig 1). Plasma Abeta42 and plasma Abeta42/Abeta40 ratio were positively associated with CSF Abeta42 levels (Abeta42: $r = 0.18$, Abeta42/Abeta40 ratio: $r = 0.38$; both $p < 0.001$) and negatively associated with CSF tTau and CSF pTau181 (all: $r < -0.23$, $p < 0.001$). On visual inspection, plasma Abeta42/Abeta40 ratio had the strongest correlations with all CSF biomarkers. There were no associations between plasma Abeta40 or plasma tTau and any of the CSF biomarkers.

Plasma Markers as Predictors of CSF Amyloid Status

Using logistic regression analysis, we found a positive association of plasma Abeta42/Abeta40 ratio (odds ratio [OR] = 3.15, 95% confidence interval [CI] = 2.10–4.74) and of plasma Abeta42 (OR = 1.74, 95% CI = 1.24–2.44) with CSF-based abnormal amyloid status. After adjustment for age and APOE ε4 carriership, the associations remained significant (Abeta42/Abeta40 ratio: OR = 2.35, 95% CI = 1.53–3.61; Abeta42: OR = 1.94, 95% CI = 1.31–2.86). There was no association between plasma Abeta40 alone or plasma tTau and CSF amyloid status.

ROC analyses (Fig 2) revealed an area under the curve (AUC) of 77% (95% CI = 69–84%) for the plasma Abeta42/Abeta40 ratio and of 66% for plasma Abeta42 alone (95% CI = 58–74%). The Youden cutoff of plasma Abeta42/Abeta40 ratio was 45 and yielded a sensitivity of 76% and specificity of 75%. As an example, based on our cohort, we would need to perform 434 lumbar punctures to obtain 100 subjects with abnormal CSF amyloid status. When applying the Youden cutoff of the plasma Abeta42/Abeta40 ratio, the number of lumbar punctures would be reduced by 51% (Fig 3).

When combining plasma Abeta42/Abeta40 ratio with age and APOE ε4 carriership in a multivariate model, discrimination became good, with an AUC of 83% (95% CI = 77–89%).

Subsequently, we used the linear predictor formula of this model to construct heat maps that visualize the probabilities (%) of having abnormal CSF amyloid status based on age and plasma Abeta42/Abeta40 ratio after stratification for APOE ε4 carriership (Fig 4). For example, an APOE ε4 carrier 70 years old with a plasma ratio

TABLE 2. Pearson Correlations of Plasma and CSF Biomarkers

	Plasma				CSF		
	Abeta40	**Abeta42**	**Abeta42/Abeta40**	**tTau**	**Abeta42**	**tTau**	**pTau181**
Plasma							
Abeta40	1.00	0.71[a]	−0.39[a]	0.30[a]	−0.10	0.03	−0.02
Abeta42		1.00	0.34[a]	0.25[a]	0.18[a]	−0.23[a]	−0.24[a]
Abeta42/Abeta40			1.00	−0.09	0.38[a]	−0.35[a]	−0.30[a]
tTau				1.00	−0.01	0.07	0.07
CSF							
Abeta42					1.00	−0.19[a]	−0.14[b]
tTau						1.00	0.93[a]
pTau181							1.00

Correlations of plasma and CSF markers of the total study population. Data are presented as Pearson correlation coefficient (r). Plasma Abeta42/Abeta40 ratio was multiplied by 1,000, and subsequently plasma ratio, plasma tTau, CSF tTau, and CSF pTau181 levels were natural log transformed prior to analysis.

[a]$p < 0.01$, [b]$p < 0.05$.

Abeta = amyloid beta; CSF = cerebrospinal fluid; tTau = total tau; pTau181 = tau phosphorylated at threonine 181.

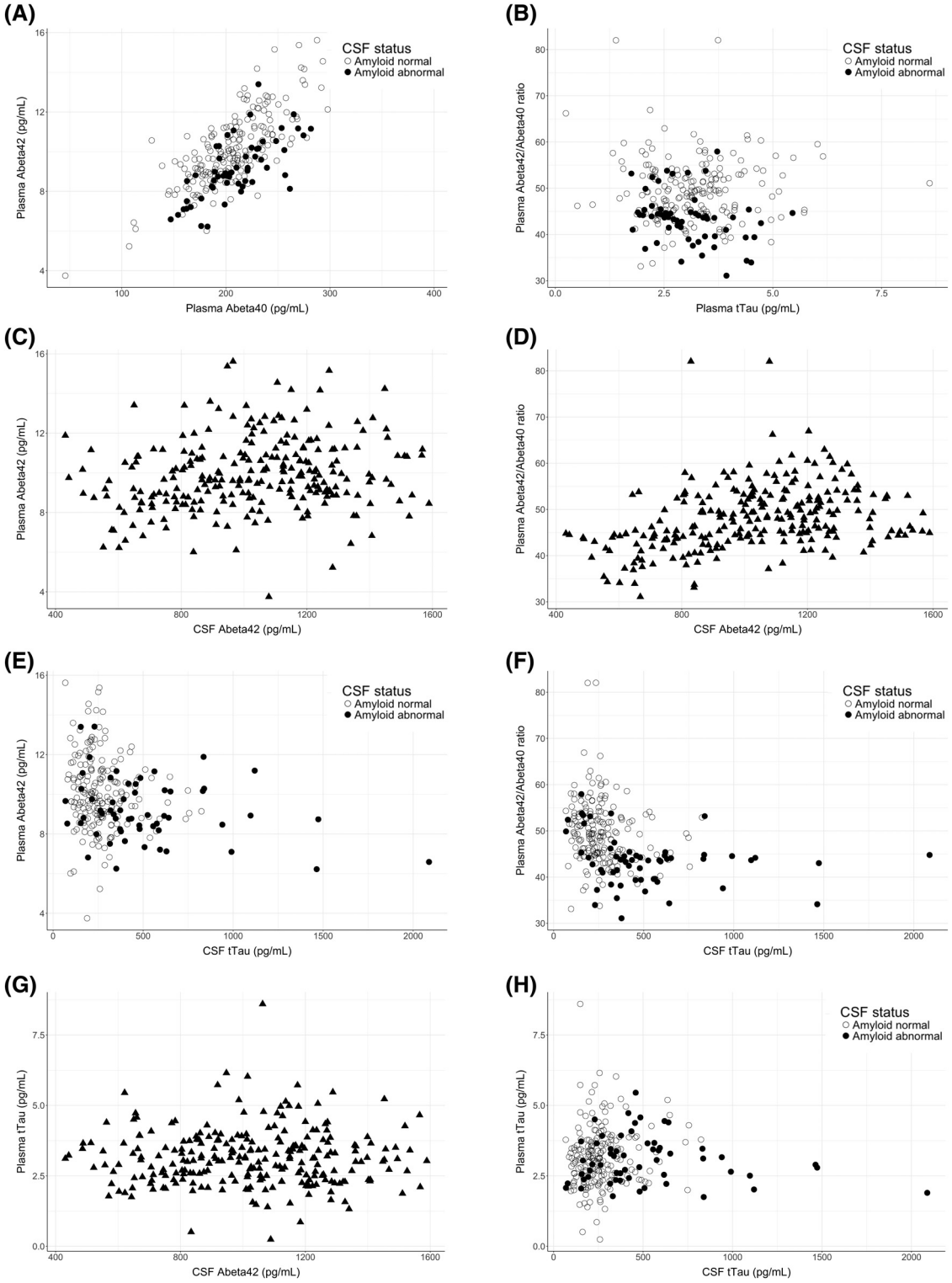

FIGURE 1: Scatterplots of plasma and cerebrospinal fluid (CSF) markers. Scatterplots present the correlation of the plasma marker concentrations (A, B) and the correlation of plasma marker concentrations with CSF marker concentrations (C– H). Triangles = total study population; open circles = subjects with normal CSF amyloid status (ie, CSF amyloid beta [Abeta]42 concentration > 813pg/ml); closed circles = subjects with abnormal CSF amyloid status (ie, CSF Abeta42 concentration ≤ 813pg/ml). tTau = total tau.

of 35 would have a probability of 81% to be CSF amyloid abnormal (ie, 123 lumbar punctures needed to obtain 100 CSF-based amyloid abnormal subjects). By contrast, with this same plasma ratio of 35, the probability of a 70-year-old non–APOE ε4 carrier to be CSF amyloid abnormal is 57% (ie, 175 lumbar punctures

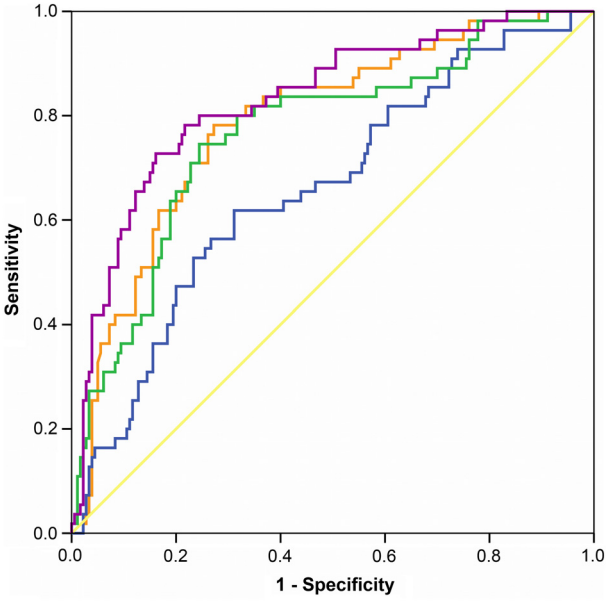

FIGURE 2: Receiver operating characteristic (ROC) curves discriminating cerebrospinal fluid (CSF)-amyloid abnormal from amyloid normal subjects in the nondemented subjects with subjective cognitive decline based on plasma amyloid beta (Abeta)42, plasma ratio Abeta42/Abeta40, and multivariate models including age and apolipoprotein E (APOE) ε4 status. Pink = plasma Abeta42/Abeta40 ratio, APOE ε4 carriership and age; orange = APOE ε4 carriership and age; green = plasma Abeta42/Abeta40 ratio; blue = plasma Abeta42; yellow = 50% reference line.

needed to obtain 100 CSF-based amyloid abnormal subjects), and would be 72% with a plasma ratio of 30 (ie, 138 lumbar punctures needed to obtain 100 CSF-based amyloid abnormal subjects). This illustrates how such a tool could help in prescreening for abnormal amyloid status.

Plasma Markers as Predictors of PET Amyloid Status

For a subset of 69 subjects, amyloid PET was available. Of these, 23 (33%) were amyloid abnormal based on PET imaging. Subjects with abnormal amyloid PET scans had lower plasma Abeta42 compared to subjects with normal amyloid PET scans (uncorrected $p = 0.018$) and tended to have lower plasma Abeta42/Abeta40 ratio ($p = 0.057$). Plasma Abeta40 and plasma tTau did not differ between groups.

Assessing the predictive accuracy of plasma amyloid to discriminate subjects with an abnormal amyloid PET scan from subjects with a normal amyloid PET scan, we found an AUC of 66% (95% CI = 53–79%) for plasma Abeta42 alone and 68% (95% CI = 55–82%) for the plasma Abeta42/Abeta40 ratio. In the multivariate model including age, APOE ε4 status, and plasma Abeta42/Abeta40 ratio, the AUC was 79% (95% CI = 67–91%). The Youden cutoff of plasma Abeta42/Abeta40 ratio was 44 and yielded a sensitivity of 70% and specificity of 78%. As an example, in our cohort 303 PET scans should be performed to obtain 100 subjects with an abnormal amyloid PET scan. Applying the Youden cutoff of the plasma Abeta42/Abeta40 ratio first, the number of PET scans would be reduced by 54% (ie, 431 blood tests result in forwarding 163 individuals to PET scanning of whom 100 will show PET amyloid abnormality).

Plasma Markers as Predictors of Clinical Progression

Finally, we assessed the predictive value of plasma markers for clinical progression. Baseline plasma Abeta42/Abeta40 ratio was lower in SCD subjects with clinical progression to MCI or dementia compared to those who remained stable during the time of study ($p = 0.002$). This decrease lost significance after adjusting for age and sex ($p = 0.09$). Plasma Abeta42 and Abeta40 alone, and plasma tTau did not differ between groups.

Cox proportional hazards analyses showed an association between lower plasma Abeta42/Abeta40 ratio and increased risk of clinical progression to MCI or dementia (hazard ratio [HR] = 2.03, 95% CI = 1.43–2.88), which remained significant after correcting for age and sex (HR = 1.67, 95% CI = 1.15–2.44). Plasma Abeta42, Abeta40, and tTau were not associated with risk of clinical progression to MCI or dementia. Excluding subjects that progressed to non-AD dementia revealed an association between lower baseline plasma Abeta42 alone and Abeta42/Abeta40 ratio and increased risk of clinical progression to MCI or AD (Abeta42: HR = 1.74, 95% CI = 1.19–2.56; Abeta42/Abeta40 ratio: HR = 2.31, 95% CI = 1.55–3.43; Fig 5). Associations remained significant after correcting for age and sex (Abeta42: HR = 1.68, 95% CI = 1.09–2.60; Abeta42/Abeta40 ratio: HR = 1.85, 95% CI = 1.21–2.83). Plasma Abeta40 and tTau were not associated with risk of clinical progression to MCI or AD.

Discussion

In the present study, we found that plasma Abeta42/Abeta40 ratio has potential as a prescreener to identify the earliest Alzheimer pathological changes of the AD continuum in cognitively normal individuals with SCD. Combining the plasma Abeta42/Abeta40 ratio with age and APOE ε4 yielded an accuracy of >80%. This suggests a future where prescreening based on a blood test would allow a reduced need of invasive or expensive methods measuring amyloid such as lumbar puncture or PET scanning. In addition, lower plasma Abeta42/Abeta40 ratio

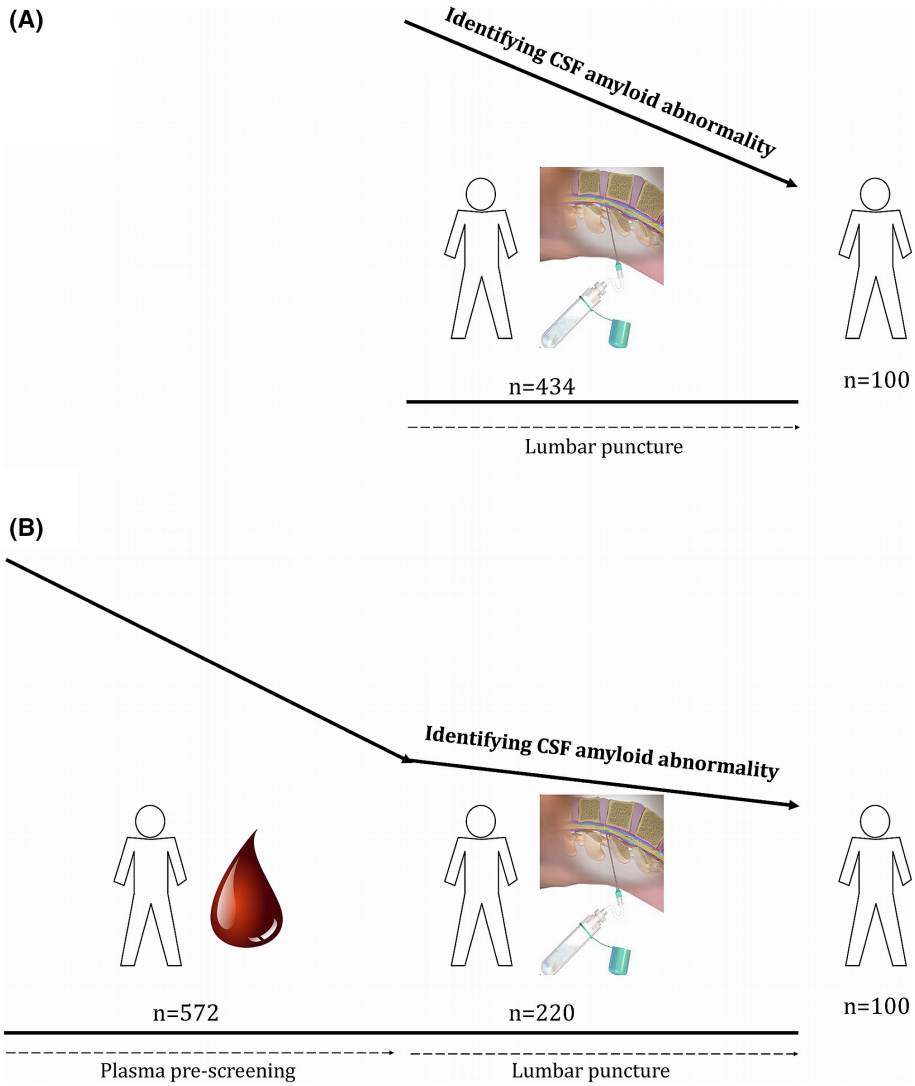

FIGURE 3: For visualization of prescreening potential in a 2-step diagnostics process, prevalence of cerebrospinal fluid (CSF) amyloid abnormality in our cohort (A) and the Youden cutoff of the plasma Abeta42/Abeta40 ratio in our cohort extracted from the receiver operating characteristic (ROC) coordinates table (B; cutoff = 45, sensitivity = 76%, specificity = 75%) were applied. Numbers were extrapolated so that a hypothetical total of 100 CSF-amyloid abnormal subjects would be identified. Plasma Abeta42/Abeta40 ratio was multiplied by 1,000 prior to ROC analysis.

was associated with a 2-fold increased risk of clinical progression to MCI or dementia.

Plasma Abeta42/Abeta40 ratio was lower in CSF amyloid abnormal individuals compared to amyloid normal individuals, and using this ratio we could identify CSF-based amyloid abnormality in our population with an accuracy of 77%. By extrapolating our results, we showed that when applying the optimal plasma Abeta42/Abeta40 ratio cutoff, we could reduce the number of individuals who would need to undergo lumbar puncture by more than half, when first prescreening with this blood test. Although in our cohort Abeta42/Abeta40 ratio was more strongly associated with CSF amyloid status than with PET amyloid status, the prescreening effectivity was comparable. We here chose a cutoff maximizing the sum of sensitivity and specificity, which fits with the goal of prescreening for clinical trial selection. In this context, the impact of missing an amyloid abnormal individual is not very high. The major aim here is to keep costs and invasiveness of screening as low as possible. An alternative goal could be to improve diagnosis of dementia, by applying prescreening in a general practitioner setting. In such a context, cutoffs should be selected favoring sensitivity, as one would not want to miss any diagnosis. We found that the accuracy increased when we additionally included age and APOEε4 carriership. This shows that a blood marker may have great value in combination with a set of simple additional variables. Adding a cognitive screening tool like MMSE or Montreal Cognitive Assessment, or a larger panel

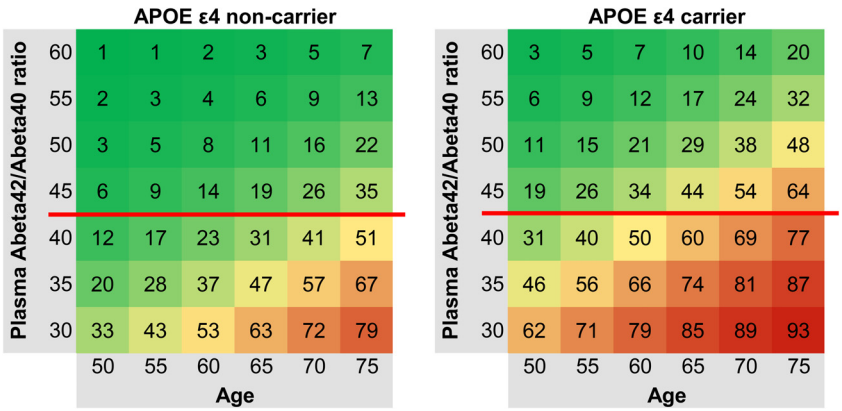

FIGURE 4: Heat maps showing predicted probability of being cerebrospinal fluid (CSF)-amyloid abnormal based on plasma amyloid beta (Abeta)42/Abeta40 ratio and age when stratified for apolipoprotein E (APOE) ε4 carriership. Probabilities are presented as percentages. Red lines indicate the Youden cutoff of plasma Abeta42/Abeta40 ratio. Plasma Abeta42/Abeta40 ratio was multiplied by 1,000 prior to analysis. Heat maps were constructed using a logistic regression predictor formula with constant = −0.879 and betas (B)s B(age) = 0.082, B(plasma Abeta42/Abeta40 ratio) = −0.131, and B(APOE ε4 carriership) = 1.202. Age and plasma ratios were entered as continuous variables, and APOE ε4 carriership as a dichotomous variable with 0 = noncarrier and 1 = carrier.

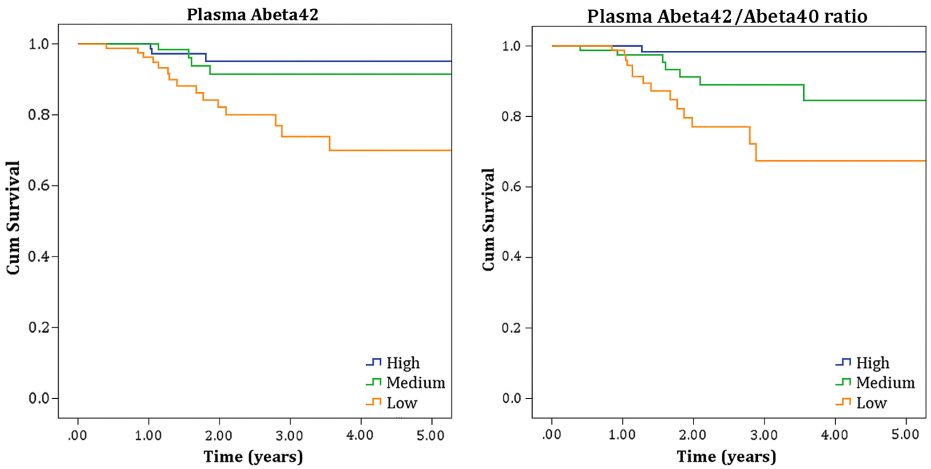

FIGURE 5: Kaplan–Meier survival analysis graphically presenting cognitive decline to or Alzheimer disease (AD) dementia upon follow-up with low (orange), medium (green), or high (blue) baseline plasma Abeta42 (left) or plasma Abeta42/Abeta40 ratio (right). cum = cumulative.

of blood markers might be a promising path to increase both sensitivity and specificity of a prescreening tool.

Our findings expand on recent findings from other groups that focused on plasma Abeta42 and Abeta40 as putative blood biomarkers for Alzheimer pathology.[14–16,34] With sophisticated but laborious immunoprecipitation and mass spectrometry techniques, two groups showed somewhat higher accuracy of plasma amyloid in predicting amyloid status compared to the accuracy reported in the current study.[15,16] The complicated nature of their measurement methods, however, precludes immediate translation to a clinical setting. Two other studies used automated techniques,[14,34] of which one study used the same analytical platform for plasma analysis as we did.[14] Both studies showed comparable findings as the current study. All former studies compared patients across the spectrum from severe disease to healthy controls, which maximizes the contrast between groups. We deliberately chose a cognitively normal sample with SCD, which renders achieving high accuracy more challenging. In our view, cognitively normal individuals who present at memory clinics comprise the target group where a plasma marker should show added benefit. Such benefit in daily practice could only be feasible with an easy to use method, hence our decision to use a straightforward automated analytical technique that would allow large scale measurement of plasma markers on a routine daily basis. Despite having included only cognitively normal subjects in our study, we found a reasonable accuracy for identifying Alzheimer pathophysiology. This is a great leap forward compared to the former generation of plasma amyloid analysis methods.[12] Our results show that a blood marker for Abeta becomes feasible, both in a trial

setting where increasingly individuals with the earliest AD pathological changes are recruited, and also in a clinical (eg, primary care) setting, to facilitate the diagnostic process.

Plasma tTau was not altered in the CSF amyloid abnormal group compared to the amyloid normal group. Moreover, plasma tTau levels were correlated with neither CSF tTau nor CSF pTau181 levels. Former studies have shown diagnostic value of plasma tTau, but only at the stage of full-blown dementia.[35–39] Thus far, no studies have focused on nondemented individuals only. As we sought differences in this nondemented group, effect size was probably too small to be captured using the current method. By contrast, CSF tTau and pTau levels in our sample were already altered in CSF amyloid abnormal subjects compared to amyloid normal subjects, suggesting that the technical sensitivity of the plasma tTau assay used is still insufficient. This reasoning is also supported by the results of our in-house assay validation, in which it was shown that the tTau plasma analysis was performing least well compared to the analysis of the other 2 markers Abeta42 and Abeta40. Alternatively, it might be that plasma tTau levels reflect AD pathology to a lesser extent[38] than tTau levels in CSF do. It might be more effective to measure specific tau isoforms in plasma, such as plasma pTau181.[39] Combining tTau with neurodegeneration biomarkers (eg, neurofilament light)[40] might be another promising alternative to increase diagnostic utility.

Some SCD subjects may harbor very early AD pathological changes,[22] and when comparing an SCD population to a normal aging population they have been found to be more likely to show clinically progression.[41] We found that lower plasma Abeta42/Abeta40 ratio is associated with an increased risk of developing MCI or dementia. It was also found that low CSF Abeta42 concentrations increase the risk of cognitive decline[6] and clinical disease progression.[42] Although the HR for clinical progression of the plasma Abeta42/Abeta40 ratio is lower compared to CSF, the finding of the present study shows clinical validity of the plasma measure.

Among the potential limitations of our study is that we had PET data available for only a small number of individuals, obtained with 4 different tracers, precluding firm conclusions with respect to PET as outcome measure. Second, external validation in an independent cohort should be performed to confirm our findings. Third, we tested our measure in a cohort of SCD individuals and therefore cannot easily translate our findings to the normal aging population. However, we believe that this makes the findings of the current study truly translational to clinical research practice. It has been shown that the presence of subjective memory complaints in itself already represents a higher risk of having high amyloid burden in the brain,[43] making this group particularly interesting for clinical trial participant screening and thus likely to benefit from the prescreening findings we present here. Other strengths of our study are that our study cohort is well defined and follow-up including repeated plasma sampling is still ongoing, providing the opportunity to confirm our longitudinal findings in future.

In conclusion, our results strongly suggest that the plasma Abeta42/Abeta40 ratio, measured with an easy to implement, fully automated platform, could serve as a prescreener, particularly when combined with age and APOE ε4 carriership. These results suggest a future where a blood biomarker is applied as a prescreener to preselect patients for further selection procedure for clinical trials, or for referral to a memory clinic.

Acknowledgment

I.M.W.V., R.E.S., and S.C.J.V. are supported by research grants from Gieskes-Strijbis Fonds and Alzheimer Nederland (NL-17004). PET scan costs were funded by grants from Piramal Imaging, GE Healthcare, Avid Radiopharmaceuticals, and the Center for Translational Molecular Medicine (02N-101; PiB scans). W.M.v.d.F. holds the Pasman chair. The VUmc Biobank is supported by VUmc. The Alzheimer Center Amsterdam is supported by Alzheimer Nederland and Stichting VUmc Fonds, and the clinical database structure was developed with funding from Stichting Dioraphte. The SCIENCe prospective cohort study is supported by Gieske-Strijbis Fonds. Research of the Alzheimer Center Amsterdam is part of the neurodegeneration research program of Amsterdam Neuroscience.

Author Contributions

I.M.W.V., P.S., C.E.T., and W.M.v.d.F. contributed to study concept and design. All authors contributed to data acquisition and analysis. I.M.W.V., C.E.T., and W.M.v.d.F. contributed to drafting the text and figures. All authors critically evaluated and approved the manuscript.

Potential Conflicts of Interest

Nothing to report.

References

1. Jack CR Jr, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 2013;12:207–216.

2. Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012;367:795–804.

3. Dubois B, Hampel H, Feldman HH, et al. Preclinical Alzheimer's disease: definition, #natural |history, and diagnostic criteria. Alzheimers Dement 2016;12:292–323.

4. 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;7:280–292.
5. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018;14:535–562.
6. van Harten AC, Smits LL, Teunissen CE, et al. Preclinical AD predicts decline in memory and executive functions in subjective complaints. Neurology 2013;81:1409–1416.
7. Villemagne VL, Pike KE, Chetelat G, et al. Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. Ann Neurol 2011;69:181–192.
8. Roberts RO, Aakre JA, Kremers WK, et al. Prevalence and outcomes of amyloid positivity among persons without dementia in a longitudinal, population-based setting. JAMA Neurol 2018;75:970–979.
9. Toledo JB, Zetterberg H, van Harten AC, et al. Alzheimer's disease cerebrospinal fluid biomarker in cognitively normal subjects. Brain 2015;138(pt 9):2701–2715.
10. Vos SJ, Xiong C, Visser PJ, et al. Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. Lancet Neurol 2013;12: 957–965.
11. Donohue MC, Sperling RA, Petersen R, et al. Association between elevated brain amyloid and subsequent cognitive decline among cognitively normal persons. JAMA 2017;317:2305–2316.
12. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 2016;15:673–684.
13. Fargo KN, Carrillo MC, Weiner MW, et al. The crisis in recruitment for clinical trials in Alzheimer's and dementia: an action plan for solutions. Alzheimers Dement 2016;12:1113–1115.
14. Janelidze S, Stomrud E, Palmqvist S, et al. Plasma beta-amyloid in Alzheimer's disease and vascular disease. Sci Rep 2016;6:26801.
15. Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid-beta biomarkers for Alzheimer's disease. Nature 2018;554:249–254.
16. Ovod V, Ramsey KN, Mawuenyega KG, et al. Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. Alzheimers Dement 2017;13:841–849.
17. Hansson O, Zetterberg H, Vanmechelen E, et al. Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. Neurobiol Aging 2010;31:357–367.
18. Rissin DM, Kan CW, Campbell TG, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol 2010;28:595–599.
19. van der Flier WM, Pijnenburg YA, Prins N, et al. Optimizing patient care and research: the Amsterdam Dementia Cohort. J Alzheimers Dis 2014;41:313–327.
20. van der Flier WM, Scheltens P. Amsterdam Dementia Cohort: performing research to optimize care. J Alzheimers Dis 2018;62: 1091–1111.
21. Slot RER, Verfaillie SCJ, Overbeek JM, et al. Subjective Cognitive Impairment Cohort (SCIENCe): study design and first results. Alzheimers Res Ther 2018;10:76.
22. Jessen F, Amariglio RE, van Boxtel M, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement 2014;10:844–852.
23. Petersen RC, Smith GE, Waring SC, et al. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999;56:303–308.
24. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:270–279.
25. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–944.
26. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–269.
27. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993;43:250–260.
28. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998;51:1546–1554.
29. Gorno-Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. Neurology 2011;76: 1006–1014.
30. Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 2011;134(pt 9):2456–2477.
31. Mulder C, Verwey NA, van der Flier WM, et al. Amyloid-beta(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. Clin Chem 2010;56:248–253.
32. Tijms BM, Willemse EAJ, Zwan MD, et al. Unbiased approach to counteract upward drift in cerebrospinal fluid amyloid-β 1-42 analysis results. Clin Chem 2018;64:576–585.
33. Andreasson U, Perret-Liaudet A, van Waalwijk van Doorn LJ, et al. A practical guide to immunoassay method validation. Front Neurol 2015;6:179.
34. Nabers A, Perna L, Lange J, et al. Amyloid blood biomarker detects Alzheimer's disease. EMBO Mol Med 2018;10. pii: e8763.
35. Zetterberg H, Wilson D, Andreasson U, et al. Plasma tau levels in Alzheimer's disease. Alzheimers Res Ther 2013;5:9.
36. Mattsson N, Zetterberg H, Janelidze S, et al. Plasma tau in Alzheimer disease. Neurology 2016;87:1827–1835.
37. Deters KD, Risacher SL, Kim S, et al. Plasma tau association with brain atrophy in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis 2017;58:1245–1254.
38. Mielke MM, Hagen CE, Wennberg AMV, et al. Association of plasma total tau level with cognitive decline and risk of mild cognitive impairment or dementia in the Mayo Clinic study on aging. JAMA Neurol 2017;74:1073–1080.
39. Mielke MM, Hagen CE, Xu J, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. Alzheimers Dement 2018;14:989–997.
40. Chatterjee P, Goozee K, Sohrabi HR, et al. Association of plasma neurofilament light chain with neocortical amyloid-beta load and cognitive performance in cognitively normal elderly participants. J Alzheimers Dis 2018;63:479–487.
41. Snitz BE, Wang T, Cloonan YK, et al. Risk of progression from subjective cognitive decline to mild cognitive impairment: the role of study setting. Alzheimers Dement 2018;14:734–742.
42. van Harten AC, Visser PJ, Pijnenburg YA, et al. Cerebrospinal fluid Abeta42 is the best predictor of clinical progression in patients with subjective complaints. Alzheimers Dement 2013;9:481–487.
43. Zwan MD, Villemagne VL, Doré V, et al. Subjective memory complaints in APOEε4 carriers are associated with high amyloid-beta burden. J Alzheimers Dis 2016;49:1115–1122.