Plasma Amyloid as Prescreener for the Earliest Alzheimer Pathological Changes

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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.

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 decline6–8 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 care setting) or screening for AD. A prescreener with high diagnostic accuracy that can reduce the number of invasive diagnostic procedures is desirable. Plasma amyloid analysis is noninvasive and relatively inexpensive compared with commonly used diagnostic tools. Furthermore, plasma levels of amyloid and tau can be measured in an automated manner using Simoa technology and can be analyzed within 1 working day.

Aims: 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.

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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 and National Institute on Aging and Alzheimer’s Association [NIA-AA] criteria for MCI from 2012 onward, to Alzheimer dementia (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association until 2011 and NIA-AA criteria for AD from 2011 onward, or to other types of dementia. 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 (Fujirebio, Ghent, Belgium) by trained technicians who were blinded for clinical diagnosis. CF Abeta42 levels were adjusted for the drift in CSF biomarker analyses that occurred over the years and subsequently dichotomized as CSF amyloid abnormal (≤813 pg/ml) and amyloid normal (>813 pg/ml). For a subset (n = 69, 28%), amyloid PET was available. Subjects were scanned with [18F]florbetaben (n = 33), [18F]florbetapir (n = 20), [18F]flutemetamol (n = 6), or [11C]Pittsburgh compound B (PiB; n = 10) radiotracer. Tracers were infused through a venous cannula. [18F]Florbetapir and [11C]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 [18F]florbetapir, 351MBq [11C]PiB). [18F]Florbetaben and [18F]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 [18F]Florbetaben, 180MBq [18F] 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). 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. 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 carri ship 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 carri ship 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)*          |
| Female gender   | 103 (42%)         | 71 (37%)        | 32 (56%)         |
| MMSE            | 28.3 (1.5)        | 28.4 (1.5)      | 28.0 (1.6)       |
| APOE ε4 carrier | 89 (38%)          | 55 (31%)        | 34 (62%)         |
| 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%)         |
| 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)*       |
| CSF tTau, pg/ml  | 325 (237)        | 267 (133)       | 518 (373)*       |
| CSF pTau181, pg/ml | 50.2 (25)      | 44.3 (18)       | 70.1 (35)*       |
| 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)*     |
| Plasma Abeta42/Abeta40 ratio | 48.1 (7.00) | 49.5 (6.81)     | 43.5 (5.51)*     |
| 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.

* $p < 0.001$, $^b p < 0.05$.

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 e4 carrier, 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 e4 carrier 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 e4 carrier (Fig 4). For example, an APOE e4 carrier 70 years old with a plasma ratio

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**Table 2. Pearson Correlations of Plasma and CSF Biomarkers**

| Plasma | Abeta40 | Abeta42 | Abeta42/Abeta40 | tTau | Abeta42 | tTau | pTau181 |
|--------|---------|---------|-----------------|------|---------|------|---------|
| Plasma |         |         |                 |      |         |      |         |
| Abeta40| 1.00    | 0.71*   | -0.39*          | 0.30*| -0.10   | 0.03 | -0.02   |
| Abeta42| 1.00    | 0.34*   | 0.25*           | 0.18*| -0.23*  | -0.24*|         |
| Abeta42/Abeta40| 1.00| -0.09  | 0.38*           | -0.35*| -0.30*  |        |         |
| tTau   | 1.00    | -0.01   | 0.07            |      | 0.07    |      |         |
| CSF    |         |         |                 |      |         |      |         |
| Abeta42| 1.00    | -0.19*  | -0.14*          | 0.93*|         |      |         |
| tTau   | 1.00    | 0.93*   | 1.00            |      |         |      |         |
| 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.

* \( p < 0.01 \), ** \( p < 0.05 \).

Abeta = amyloid beta; CSF = cerebrospinal fluid; tTau = total tau; pTau181 = tau phosphorylated at threonine 181.
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 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.
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 e4 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 \( (HR = 1.74, 95\% \ CI = 1.19–2.56; \text{Abeta42/Abeta40 ratio: } HR = 2.31, 95\% \ CI = 1.55–3.43; \text{Fig 5}) \). Associations remained significant after correcting for age and sex \( (\text{Abeta42: } HR = 1.68, 95\% \ CI = 1.09–2.60; \text{Abeta42/Abeta40 ratio: } HR = 1.85, 95\% \ CI = 1.21–2.83) \). 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 e4 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
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

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**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.
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. 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. The complicated nature of their measurement methods, however, precludes immediate translation to a clinical setting. Two other studies used automated techniques, of which one study used the same analytical platform for plasma analysis as we did. 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. Our results show that a blood marker for Abeta becomes feasible, both in a trial

![FIGURE 4: Heat maps showing predicted probability of being cerebrospinal fluid (CSF)-amyloid abnormal based on plasma amyloid beta (Abeta) ratio and age when stratified for apolipoprotein E (APOE) ε4 carrierhip. Probabilities are presented as percentages. Red lines indicate the Youden cutoff of plasma Abeta/Abeta ratio. Plasma Abeta/Abeta 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) B(age) = 0.082, B(plasma Abeta/Abeta ratio) = −0.131, and B(APOE ε4 carrierhip) = 1.202. Age and plasma ratios were entered as continuous variables, and APOE ε4 carrierhip 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 Abeta (left) or plasma Abeta/Abeta ratio (right). cum = cumulative.](image-url)
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 t\(\text{t}^\text{au}\) was not altered in the CSF amyloid abnormal group compared to the amyloid normal group. Moreover, plasma t\(\text{t}^\text{au}\) levels were correlated with neither CSF t\(\text{t}^\text{au}\) nor CSF p\(\text{tau}\)181 levels. Former studies have shown diagnostic value of plasma t\(\text{t}^\text{au}\), 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 t\(\text{t}^\text{au}\) and p\(\text{tau}\) 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 t\(\text{t}^\text{au}\) 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 t\(\text{t}^\text{au}\) plasma analysis was performing least well compared to the analysis of the other 2 markers Abeta42 and Abeta40. Alternatively, it might be that plasma t\(\text{t}^\text{au}\) levels reflect AD pathology to a lesser extent\(^{38}\) than t\(\text{t}^\text{au}\) levels in CSF do. It might be more effective to measure specific tau isoforms in plasma, such as plasma p\(\text{tau}\)181.\(^{39}\) Combining t\(\text{t}^\text{au}\) 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 \(\varepsilon\)4 carrihership. 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.

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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.

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