Ultrasensitive Detection of Plasma Amyloid-β as a Biomarker for Cognitively Normal Elderly Individuals at Risk of Alzheimer's Disease

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Abstract

Introduction: Plasma amyloid-β (Aβ) is being investigated as a surrogate marker for brain Aβ deposition.

Methods: Plasma Aβ40 and Aβ42 concentrations were measured using the ultrasensitive Single Molecule Array (Simoa) assay in 95 cognitively normal elderly individuals, wherein all participants underwent positron emission tomography (PET) to assess brain Aβ deposition. Based on the standard uptake value ratios (SUVR) obtained from PET imaging, 32 participants were assessed to have low brain Aβ load (Aβ-, SUVR<1.35) and 63 were assessed to have high brain Aβ load (Aβ+, SUVR≥1.35).

Results: Plasma Aβ42/Aβ40 ratios were lower in the Aβ+ group compared to the Aβ- group. Plasma Aβ40 and Aβ42 levels were not significantly altered between Aβ- and Aβ+ groups, although a trend of higher plasma Aβ40 was observed in the Aβ+ group. Additionally, plasma Aβ42/Aβ40 ratios along with the AD risk factors, age and *APOE* ε4 status, resulted in an area under the receiver operating characteristic curve of 78% in distinguishing Aβ+ participants from Aβ- participants.

Conclusion: Observations from the current study indicate that plasma Aβ ratios are a potential biomarker for brain Aβ deposition and therefore, for preclinical AD, characterised by high brain Aβ load, prior to cognitive impairment. However, more sensitive and clinically feasible plasma Aβ measurement assays need to be developed to increase the accuracy of this potential Alzheimer's disease blood biomarker.

Introduction

Alzheimer's disease (AD) is the most common form of dementia. Presently, more than 50 million people worldwide are living with dementia and this statistic is expected to rise to over 150 million by 2050, if there is no medical breakthrough [1].

Given that the onset of aberrant amyloid-β (Aβ) deposition in the brain occurs about two decades prior to the manifestation of clinical symptoms [2, 3], brain Aβ load measured using positron emission tomography (PET) serves as a gold standard biomarker for preclinical and clinical AD. Preclinical AD diagnosis employing the gold standard markers are facilitating the recruitment of participants for clinical trials investigating potential drugs within the preclinical phase of AD, prior to extensive neuronal damage. Additionally, preclinical AD diagnosis may also encourage the implementation of protective lifestyle changes [4]. However, the uneconomical nature of PET makes its usage unfeasible for population wide screening and therefore, blood markers that reflect brain Aβ deposition are being investigated.

While several studies have investigated plasma Aβ in AD [5], two relatively recent studies reported that plasma Aβ ratios are significantly different between individuals with low brain Aβ load (Aβ-) versus those with high brain Aβ load (Aβ+) [6, 7]. The study by Ovod and colleagues reported that plasma Aβ42/Aβ40 ratios were lower in Aβ+ versus Aβ- participants and distinguished between Aβ+ versus Aβ- participants with approximately 88% accuracy [6]. Further, Nakamura and colleagues reported that plasma Aβ40/Aβ42 ratios were higher in Aβ+ versus Aβ- participants. Additionally, the composite scores they obtained from the amyloid precursor protein fragment, APP669-711, to Aβ1-42 ratio and the Aβ1-40 to Aβ1-42 ratio predicted Aβ+ versus Aβ- participants with approximately 90% accuracy [7]. However, both studies employed immunoprecipitation using monoclonal anti-Aβ antibodies (HJ5.1, anti-Aβ13–28 [6] and 6E10, anti-Aβ1-16 [7]) prior to a liquid chromatography coupled with mass-spectrometry approach which may be difficult to implement in most clinical settings. Additionally, both studies included participants with mild cognitive impairment and dementia in their Aβ- and Aβ+ groups.

Fandos and colleagues measured plasma Aβ in cognitively normal Aβ+ individuals compared to Aβ- individuals, utilising an enzyme linked immunosorbent assay (employing monoclonal antibody 1F3 specific to the Aβ N-terminal and polyclonal antibodies pAB002 and pAB031

specific to the C-terminal end of Aβ40 or Aβ42 respectively) and observed lower plasma Aβ42/Aβ40 ratios in the Aβ+ group [8]. Further, Verberk and colleagues utilised the Single Molecule Array (Simoa) technology and observed lower plasma Aβ42/Aβ40 ratios in cognitively normal individuals with subjective cognitive decline (SCD; referring to self-reported decline in cognitive performance [9]) carrying aberrant brain Aβ deposition (assessed by cerebrospinal Aβ4 levels ≤813pg/ml) compared to cognitively normal individuals with SCD carrying normal brain Aβ deposition [10]. Janelidze *et. al.* also reported that plasma Aβ42/Aβ40 ratios inversely correlated with brain Aβ load (assessed via PET) in cognitively normal individuals with SCD [11] (Supplementary table 1).

The current study aimed to validate the above studies utilising the ultra-sensitive Simoa technique to investigate whether plasma Aβ42/Aβ40 ratios are significantly different between Aβ- cognitively normal participants compared to Aβ+ cognitively normal participants assessed by PET in the Kerr Anglican Retirement Village Initiative in Ageing Health (KARVIAH) cohort. Additionally, the current study also evaluated the potential of plasma Aβ42/Aβ40 ratios in differentiating Aβ- and Aβ+ participants.

Methods

Participants

Study participants were from the KARVIAH cohort, at baseline. All cohort volunteers (N=206) were screened for the inclusion and exclusion criteria to be eligible. The inclusion criteria comprised an age range of 65-90 years, good general health, no known significant cerebral vascular disease, fluent in English, adequate/corrected vision and hearing to enable testing, and no objective cognitive impairment as screened by a Montreal Cognitive Assessment (MoCA) score ≥26. MoCA scores lying between 18-25 were assessed on a case by case basis by the study neuropsychologist following stratification of scores according to age and education [12]. The exclusion criteria comprised, the diagnosis of dementia based on the revised criteria from the National Institute on Aging - Alzheimer's Association [13], presence of acute functional psychiatric disorder (including lifetime history of schizophrenia or bipolar disorder), history of stroke, severe or extremely severe depression (based on the depression, anxiety, stress scales; DASS) and uncontrolled hypertension (systolic BP > 170 mm Hg or diastolic BP > 100 mm Hg).

While 134 volunteers met the inclusion/exclusion criteria, 105 participants underwent neuroimaging, neuropsychometric evaluation and blood collection since the remaining participants declined undergoing neuroimaging or withdrew from the study. Within these 105 participants, 100 participants were considered to have normal global cognition based on their Mini-Mental State Examination score [14] (MMSE ≥26). Both plasma Aβ40 and Aβ42 concentrations were measured in 95 of these 100 participants. Additionally, participants with a Memory Assessment Clinic - Questionnaire (MAC-Q) score between 25-35 were considered as subjective memory complainers (SMC, n=72; a specific form of SCD defined by self-reported memory complaints) while those with a MAC-Q score ≤24 were considered as non-complainers (n=23) (See Figure 1 for flowchart). All volunteers provided written informed consent prior to participation, and the Bellberry Human Research Ethics Committee, Australia, and the Macquarie University Human Research Ethics Committee provided approval for the study.

Evaluation of neocortical amyloid-β load via PET

All study participants were imaged within three months of blood collection wherein participants underwent PET using ligand ^{18}F-Florbetaben (FBB) at Macquarie Medical Imaging in Sydney. Participants were administered an intravenous bolus of FBB slowly over 30s, while in a rested position. Images were acquired over a 20 min scan, in 5 min acquisitions, beginning 50 min post injection. Brain (neocortical) amyloid-β load was calculated as the mean standard uptake value ratio (SUVR) of the frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions using the image processing software, CapAIBL [15, 16] to classify participants as Aβ- or Aβ+ using an SUVR cut-off =1.35 [17] within the current study.

Blood collection, measurement of plasma Aβ and APOE genotyping

All study participants fasted for a minimum of 10 hours overnight prior to blood withdraw employing standard serological methods and processing [17]. EDTA-plasma Aβ concentrations were measured employing the ultra-sensitive Single Molecule Array (Simoa, Quanterix) platform. Plasma samples were diluted eight times for Aβ40 and four times for Aβ42. For Aβ40, the quality control (QC) sample had a concentration of 219 pg/mL with repeatability 6.9 % and intermediate precision 7.9 %. For Aβ42, the QC sample had a concentration of 12.9 pg/mL with repeatability 2.4 % and intermediate precision 5.6 %.

Apolipoprotein E (*APOE)* genotype was determined from purified genomic DNA extracted from 0.5 ml whole blood as previously described [17].

Statistical analyses

Descriptive statistics including means and standard deviations were calculated for Aβ- and Aβ+ groups, with comparisons employing t-tests or Chi-square tests as appropriate. Linear models were employed to compare continuous variables between Aβ- and Aβ+ groups corrected for covariates age, gender and *APOE* ε4 carrier status. Plasma Aβ concentrations and their ratios were log transformed to better approximate normality and variance homogeneity as required. Logistic regression with Aβ-/+ as response was used to evaluate predictive models and receiver operating characteristic (ROC) curves constructed from the logistic scores. All analyses were carried out using IBM® SPSS® Version 23 and receiver operating characteristic curves were generated using the package Deducer on R (version 3.2.5).

Results

Cohort characteristics

Demographic characteristics of study participants have been presented in Table 1. No significant differences were observed in gender, age, body mass index, MMSE scores and the number of SMC between Aβ- and Aβ+ cohort participants. However, the *APOE* ε4 carriage frequency was significantly higher in the Aβ+ group compared to Aβ- group as expected [18] (Table 1).

Comparison of plasma Aβ40, Aβ42 and Aβ42/Aβ40 ratios in Aβ- versus Aβ+ participants

Plasma Aβ40 and Aβ42 concentrations and plasma Aβ42/Aβ40 ratios, measured in the study participants have been presented in Table 2. While no significant differences were observed in plasma Aβ40 and Aβ42 concentrations between the Aβ- and Aβ+ groups, significant differences in plasma Aβ42/Aβ40 ratios were observed between the two groups, wherein Aβ42/Aβ40 ratios were lower in the Aβ+ group compared to the Aβ- group with and without correcting for covariates age, gender and *APOE* ε4 status (Figure 2).

On stratifying study participants into subjective memory complainers (n=72) and non-complainers (n=23), plasma Aβ42/Aβ40 ratios continued to remain significantly lower in the Aβ+ SMC compared to Aβ- SMC with and without correcting for covariates age, gender and

APOE ε4 status (Table 2). However, no significant difference was observed in plasma Aβ42/Aβ40 ratios between Aβ+ and Aβ- non-SMC.

Evaluation of plasma Aβ42/Aβ40 ratio as predictor of brain Aβ status

Plasma Aβ42/Aβ40 ratios were evaluated as potential markers to predict Aβ+ status using logistic regression with Aβ+/- as response. A 'base' model incorporating the major risk factors for AD, namely age and *APOE* ε4 allele status, was generated and compared to the 'base+ Aβ42/Aβ40 ratio' model wherein plasma Aβ42/Aβ40 ratios were added to the base model (Figure 3). The area under the curve (AUC) of the 'base+ Aβ42/Aβ40 ratio' model (AUC=77.6%, specificity=67% at sensitivity=78%, 95% CI= 68-88%) outperformed the 'base' model (AUC=75.3%, specificity=56% at sensitivity=78%, 95% CI= 65-86%) in distinguishing Aβ+ from Aβ- participants.

Discussion

The current study found that while plasma Aβ40 and Aβ42 concentrations were not significantly altered between Aβ- and Aβ+ participants, the ratio of Aβ42/Aβ40 was significantly lower in Aβ+ participants compared to Aβ- participants. Further, on stratifying cohort participants into SMC and non-SMC, the ratio of Aβ42/Aβ40 was significantly lower in Aβ+ SMC compared to Aβ- SMC. While the mean of the ratio of Aβ42/Aβ40 was lower in Aβ+ non-SMC compared to Aβ- non-SMC, it did not reach statistical significance, which could be due to the small sample size following stratification based on self-reported memory complaints. Further, plasma Aβ42/Aβ40 ratios along with AD risk factors age and *APOE* ε4 status in all participants predicted Aβ+ individuals with approximately 78% accuracy. Interestingly, Nakamura *et al.* employed Aβ40/Aβ42 ratios to predict individuals with aberrant brain Aβ deposition while Ovod *et al.* employed Aβ42/Aβ40 ratios. Within the current study we observed similar AUCs for both ratios (Supplementary figure 1, Supplementary figure 2) [6, 7].

Two relatively recent studies also investigated plasma Aβ as a surrogate marker for abnormal brain Aβ deposition in cognitively normal individuals [8, 10]. Fandos and colleagues measured plasma Aβ levels using enzyme-linked immunosorbent assays (ELISA) (Araclon Biotech Ltd. Zaragoza, Spain) in individuals with normal and abnormal brain Aβ deposition classified by

PET and reported that plasma Aβ42/Aβ40 ratios were lower in individuals with abnormal brain Aβ deposition [8], which is in line with observations from the current study. Further, employing the ultra-sensitive Simoa assay (Quanterix) to measure plasma Aβ, Verberk and colleagues also observed significantly lower plasma Aβ42/Aβ40 ratios in individuals with abnormal brain Aβ deposition defined by cerebrospinal fluid (CSF) Aβ42 levels (≤813pg/ml) [10].

Along with AD risk factors, age and *APOE* ε4 carriage, Fandos *et al.* reported an AUC of 79% and Verberk *et al.* reported an AUC of 83% in distinguishing between individuals with abnormal brain Aβ deposition and those with normal brain Aβ deposition [8, 10]. However, only a trend of lower plasma Aβ42/Aβ40 ratios (p=.057) was observed in individuals with abnormal brain Aβ deposition (n=23) defined by PET, in the subset of participants that underwent PET (n=69) in the Verberk *et al.* study [10]. This observation could be attributed to the modest sample size of individuals who underwent PET and the multiple PET ligands employed in the study. Additionally, while the study by Fandos and colleagues accounted for employing multiple PET ligands using the "Before the Centiloid Kernel Transformation" (BeCKeT) scale, they employed a plasma Aβ measurement assay with a relatively lower sensitivity (lower limit of quantification, LOQ; Aβ40: 7.60 pg/ml, Aβ42: 3.60 pg/ml) [8, 19] compared to the Simoa assay used by Verberk and colleagues (LOQ; Aβ40: 0.16 pg/ml, Aβ42: 0.34 pg/ml) [10]. The current study utilised the ultrasensitive Simoa assay, to measure plasma Aβ concentrations, along with PET data (using a single ligand), to identify individuals with abnormal brain Aβ deposition, and validated findings from the above two studies wherein plasma Aβ42/Aβ40 ratios were lower in individuals at risk of AD (Aβ+).

Several previous studies have investigated plasma Aβ in AD, however findings have been inconsistent. For example, a number of studies reported that lower plasma Aβ42 and higher plasma Aβ40 were associated with increased AD or dementia risk [20, 21] while other studies did not observe any association of plasma Aβ42 or Aβ40 with AD [22, 23]. Further, several other studies also reported that lower plasma Aβ42/Aβ40 ratios were significantly associated with increased AD risk [24-27] although other studies did not observe these associations [28, 29]. These inconsistencies could be attributed to poorly characterised cohorts, non-sensitive plasma Aβ assays, variations between study designs (fasting bloods, time of blood collection and processing time) and inadequate sample sizes. While the current study endeavoured to address these issues by employing a highly characterised cohort that has undergone PET to measure brain Aβ deposition (with a single Aβ specific ligand), an ultra-sensitive plasma Aβ

measurement assay and a sample collection and processing design similar that used by Fandos and colleagues [8], it is also acknowledged that the study has its limitations of employing a modest sample size and a cross-sectional study design.

To conclude, while our current observations together with those of Fandos *et al.* and Verberk *et al.* validate that plasma Aβ ratios (Aβ42/Aβ40) are altered in cognitively normal individuals with aberrant brain Aβ deposition, the accuracy to identify aberrant brain Aβ deposition attained by the methods employed to measure plasma Aβ ratios by Ovod *et al.* and Nakamura *et al.* makes plasma Aβ ratios a promising marker [6-8, 10]. However, given that the assays employed by Ovod *et al.* and Nakamura *et al.* cannot readily be implemented in a clinical setting, more sensitive and clinically feasible assays to measure plasma Aβ are still required to be developed [6, 7].

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Tables

Table 1. Demographic characteristics of cohort participants. Baseline characteristics including gender, age, body mass index (BMI), *APOE ε4* status, mini mental state examination (MMSE) scores, subjective memory complainer status and brain Aβ load represented by the standard uptake value ratio (SUVR) of ligand ^{18}F-Florbetaben (FBB) in the neocortical region normalised with that in the cerebellum, have been compared between Aβ- (SUVR<1.35) and Aβ+ (SUVR≥1.35) study participants. Chi-square tests or linear models were employed as appropriate.

	Aβ -	**Aβ +**	**p**
Gender (M/F)	19/44	13/19	.308
Age (years, mean ±SD)	77.65±5.62	79.50±5.32	.126
BMI (mean ±SD)	27.54±4.46	27.62 ± 4.13	.931
n*APOE ε4* carriers (%)	5 (7.9)	13 (40.6)	<.0001
MMSE (mean ±SD)	28.51±1.15	28.72±1.11	.395
Subjective memory complainers (n)	49	23	.526
FBB-PET SUVR (mean ±SD)	1.16±0.09	1.73±0.27	-

Table 2. Comparison of plasma Aβ40, Aβ42 and Aβ42/40 ratios between Aβ- and Aβ+ participants. Plasma Aβ concentrations and their ratios were compared between cognitively normal individuals with low brain Aβ load (Aβ-) and high brain Aβ load (Aβ+) using linear models. All participants were further categorised into subjective memory complainers (SMC, n=72) and non-SMC (n=23). † represents p-values obtained from log transformed plasma Aβ concentrations and ratios to better approximate normality. p^a represents p-values adjusted for age, gender and *APOE* ε4 status.

	Aβ -	**(95% CI)**	**Aβ +**	**(95% CI)**	**p**	**p^a**
All participants	**n=63**		**n=32**			
Aβ40 (pg/mL, mean±SD)	307.44±54.16	(292.08-322.79)	332.82± 73.71	(311.28-354.37)	.087†	.095
Aβ42 (pg/mL, mean±SD))	16.01±3.74	(15.09-16.92)	15.71±3.48	(14.43-17.00)	.711	.741
Aβ42/40 ratio (mean±SD)	0.052±.008	(0.050-0.054)	0.047±0.005	(0.045-0.050)	**.004†**	**.025†**
SMC	**n=49**		**n=23**			
Aβ40 (pg/mL, mean±SD)	307.23±51.07	(290.45-324.00)	337.90±73.07	(313.41-362.38)	**.043**	.085
Aβ42 (pg/mL, mean±SD))	15.80±3.26	(14.86-16.75)	15.69±3.42	(14.32-17.07)	.898	.990
Aβ42/40 ratio (mean±SD)	0.052±.007	(0.050-0.054)	0.047±0.005	(0.044-0.050)	**.006**	**.040**
Non-SMC	**n=14**		**n=9**			
Aβ40 (pg/mL, mean±SD)	308.17±66.00	(268.78-347.57)	319.85±78.14	(270.72-368.98)	.704	.438
Aβ42 (pg/mL, mean±SD))	16.74±5.17	(14.12-19.36)	15.77±3.83	(12.51-19.03)	.635	.835
Aβ42/40 ratio (mean±SD)	0.054±.009	(0.049-0.058)	0.049±0.004	(0.044-0.055)	.201	.204

Figures

Figure 1. Flow chart representing the Kerr Anglican Retirement Village Initiative in Ageing Health (KARVIAH) cohort participants included within the current study. *MMSE: Mini-mental state examination score, SMC: subjective memory complainers*

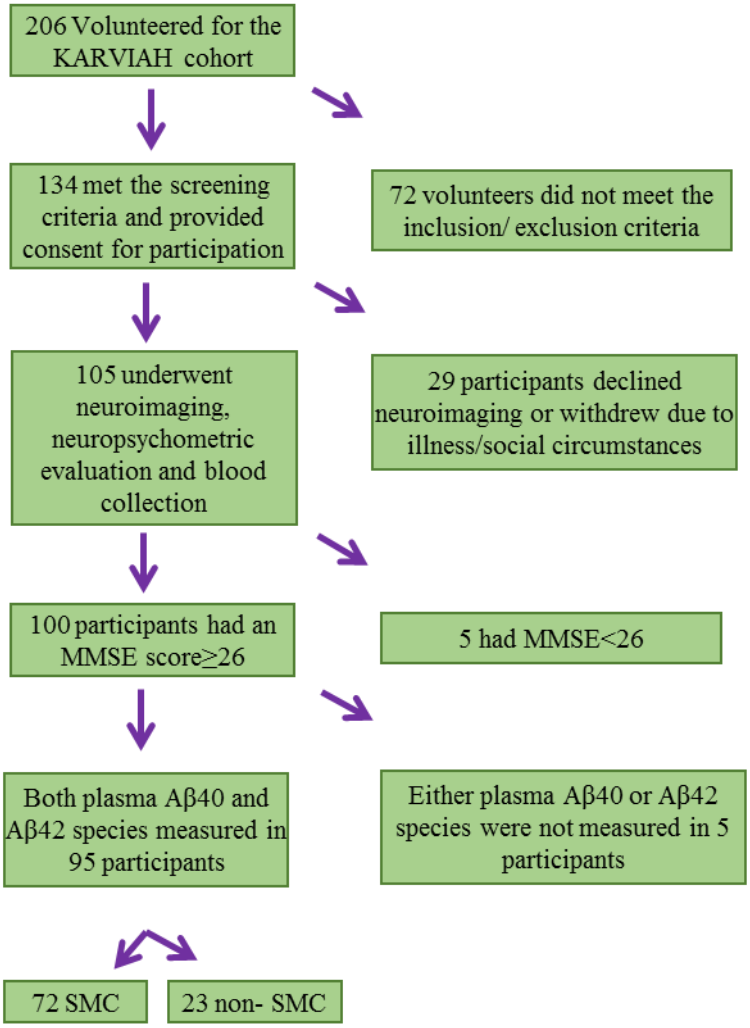


Figure 2. Comparison of plasma Aβ40, Aβ42 and Aβ42/Aβ40 ratios between Aβ- versus Aβ+ participants. Plasma Aβ concentrations (in pg/mL) and their ratios were compared between participants with neocortical amyloid-β load (assessed by the standard uptake value ratio observed via positron emission tomography using ligand ^{18}F-florbetaben) <1.35 (Aβ-) and ≥1.35 (Aβ+) using linear models. Plasma Aβ42/Aβ40 ratios were significantly lower in Aβ+ (N=32) participants compared to Aβ- (N=63) participants. The line segment within each jitter plot represents the median of the data and error bars in the graphs represent the data range for the Aβ- and Aβ+ groups. P-values were obtained from log transformed plasma Aβ concentrations and ratios to better approximate normality and variance homogeneity when required. * p<.005.

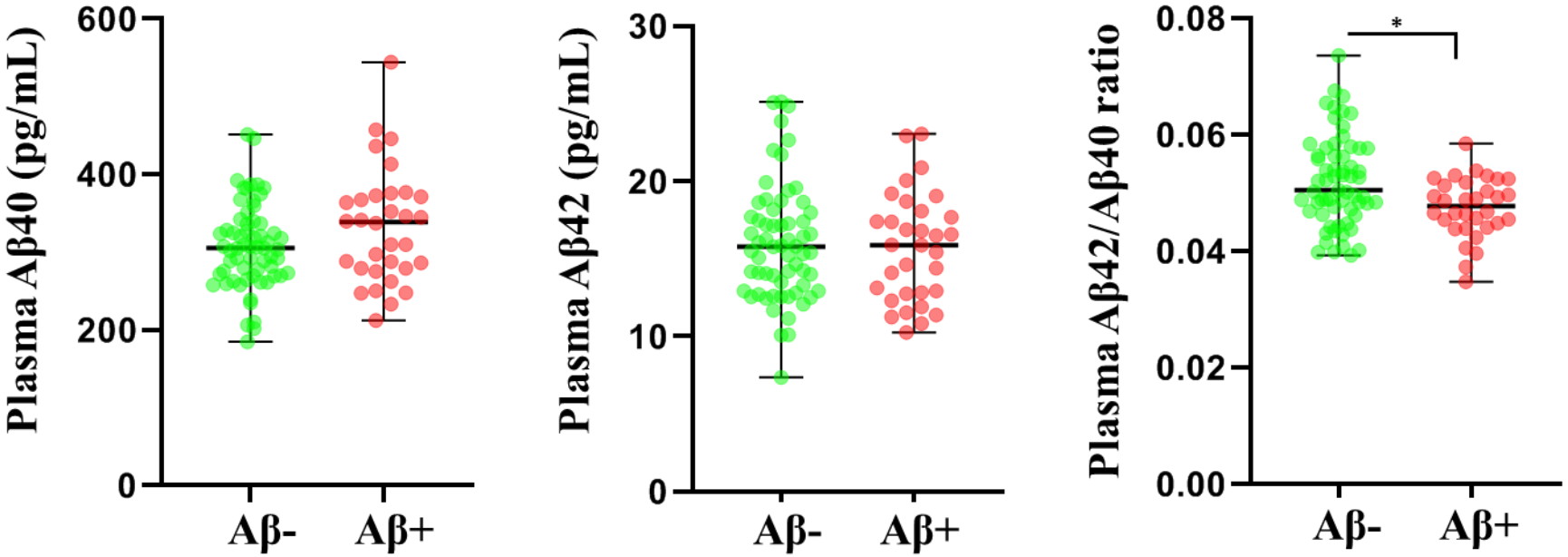
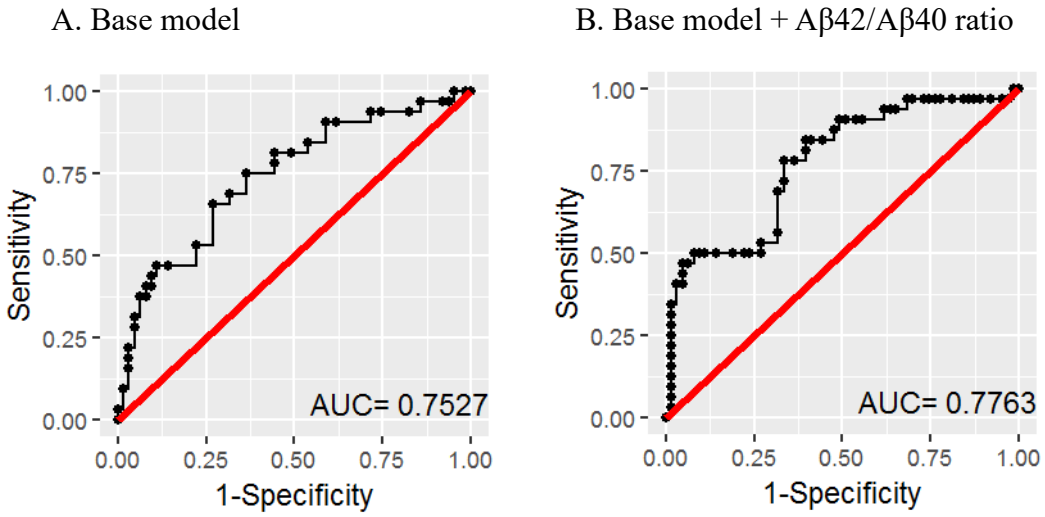
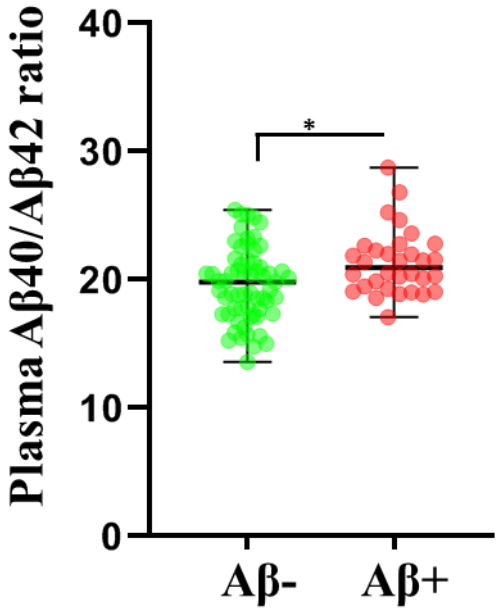


Figure 3. Receiver operating characteristic curves for the prediction of Aβ+ versus Aβ- participants. The 'base' model comprising major risk factors age and *APOE* ε4 allele status **(A)** was outperformed by the 'base + plasma Aβ42/Aβ40 ratio' model **(B)**. Logistic regression models were employed to perform the analyses. AUC: area under the curve. 95% CI for A= 65-86%, 95% CI for B= 68-88%.



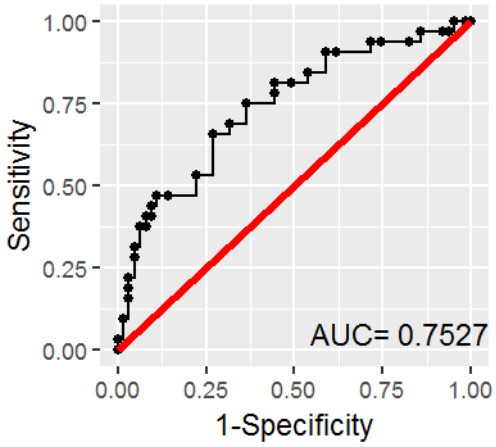
Supplementary material

Supplementary figure 1. Comparison of plasma Aβ40/Aβ42 ratios between Aβ- versus Aβ+ participants. Plasma Aβ40/Aβ42 ratios were compared between participants with neocortical amyloid-β load (assessed by the standard uptake value ratio observed via positron emission tomography using ligand ^{18}F-florbetaben) <1.35 (Aβ-) and ≥1.35 (Aβ+) using linear models. Plasma Aβ40/Aβ42 ratios were significantly higher Aβ+ (N=32) participants compared to Aβ- (N=63) participants. The line segment within each jitter plot represents the median of the data and error bars in the graphs represent the data range for the Aβ- and Aβ+ groups. The p-value was obtained from the log transformed values of plasma Aβ40/ Aβ42 ratios to better approximate normality and variance homogeneity. * p<.005.

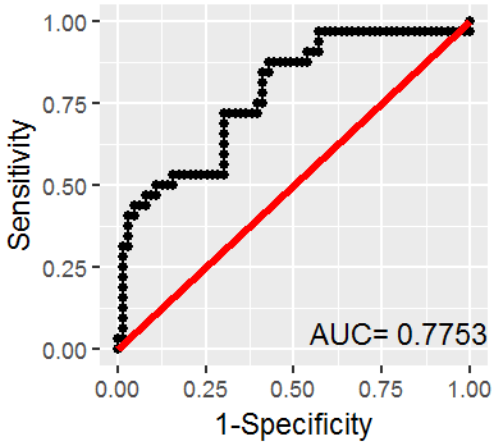


Supplementary figure 2. Receiver operating characteristic (ROC) curves for the prediction of Aβ+ versus Aβ- participants. The 'base' model comprising major risk factors age and *APOE* ε4 allele status **(A)** was outperformed by the 'base + plasma Aβ40/Aβ42 ratio' model **(B)**. Logistic regression models were employed to perform the analyses. AUC: area under the curve. 95% CI for A= 65-86%, 95% CI for B= 68-88%

A. Base model



B. Base model + Aβ40/Aβ42 ratio



Supplementary table 1: Comparison of studies that investigated plasma Aβ ratios between individuals with low (Aβ-) and high (Aβ+) brain Aβ burden described within the current manuscript text.

Author	Technology used	Clinical classification of participants within the Aβ-/+ groups	Brain Aβ burden (Aβ-/+) assessed by PET or CSF	Findings reported on plasma Aβ ratios between Aβ-/+ individuals
Ovod et. al., 2017	IP-MS	CN, MCI, AD	PET	• Lower plasma Aβ42/Aβ40 ratios in Aβ+ participants versus Aβ- participants. • Plasma Aβ42/Aβ40 ratios distinguished between Aβ+ and Aβ- participants with ~88% accuracy
Nakamura et. al., 2018	IP-MS	CN, MCI, AD	PET	• Higher plasma Aβ40/Aβ42 ratios in Aβ+ participants versus Aβ- participants. • Composite scores obtained from Aβ1-40/Aβ1-42 ratio and APP669-711/Aβ1-42 ratio distinguished between Aβ+ and Aβ- participants with over 90% accuracy in discovery and validation cohorts
Jandalidze et. al., 2016	Simoa	Non-SCD-CN, SCD-CN, MCI, AD	PET	• Inverse correlations between brain Aβ load and plasma Aβ42/Aβ40 ratio in the all participants within the study • Plasma Aβ42/Aβ40 ratio correlated with brain Aβ load in the SCD-CN group, but not in non-SCD-CN and MCI
Fandos et. al., 2017	ELISA	CN	PET	• Lower plasma Aβ42/Aβ40 ratios in Aβ+ participants versus Aβ- participants. • Plasma Aβ42/Aβ40 ratios, along with risk factors, age and *APOE*ε4 status, distinguished between Aβ+ and Aβ- participants with ~79% accuracy, based on the AUC under the ROC curve
Verberk et. al., 2018	Simoa	SCD-CN	CSF	• Lower plasma Aβ42/Aβ40 ratios in Aβ+ participants versus Aβ- participants. • Plasma Aβ42/Aβ40 ratios, along with risk factors, age and *APOE*ε4 status, distinguished between Aβ+ and Aβ- participants with ~83% accuracy, based on the AUC under the ROC curve
Current study	Simoa	CN (non-SMC-CN and SMC-CN combined, as well as in non-SMC-CN and SMC-CN independently stratified)	PET	• Lower plasma Aβ42/Aβ40 (higher Aβ40/Aβ42) ratios in Aβ+ participants versus Aβ- participants. • Plasma Aβ42/Aβ40 ratios, along with risk factors, age and *APOE*ε4 status distinguished between Aβ+ and Aβ- participants with ~78% accuracy, based on the area under the ROC curve in all participants.