Plasma Amyloid- β Oligomerization Assay as a Pre-Screening Test for Amyloid Status

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Abstract

Background: We assessed the performance of plasma amyloid oligomerization tendency (OA β) as a marker for abnormal amyloid status. Additionally, we examined long-term storage effects on plasma OA β .

Methods: We included 399 subjects regardless of clinical diagnosis from the Amsterdam Dementia Cohort and European Medical Information Framework for AD project (age:63.8 $\pm$ 6.6; 44% female). Amyloid status was determined by visual read on Positron Emission Tomography (PET; n_{abnormal}= 206). Plasma OA β was measured using the Multimer Detection System (MDS). Long-term storage effects on MDS-OA β were assessed using General Linear Models. Associations between plasma MDS-OA β and A β -PET status were assessed using logistic regression and receiver operating characteristics analyses. Correlations between plasma MDS-OA β and CSF biomarker levels were evaluated using Pearson correlation analyses

Results MDS-OA β was higher in individuals with abnormal amyloid, and it identified abnormal A β -PET with an Area Under the Curve (AUC) of 0.74 (95%CI: 0.67-0.81), especially in samples with short-term storage duration. Combining APOE ϵ 4 and age with plasma MDS-OA β revealed an AUC of 81% for abnormal amyloid PET status (95%CI:74%-87%). Plasma MDS-OA β correlated negatively with MMSE (r=-0.29,p<.01), and CSF A β 42 (r=-0.20,p<0.05), and positively with CSF t-Tau (r=0.20,p=0.01).

Conclusions: Plasma MDS-OA β combined with APOE ϵ 4 and age, accurately identifies brain amyloidosis in a large A β -confirmed population. Using plasma MDS-OA β as a screener reduced the costs and number of PET scans needed to screen for amyloidosis, which is relevant for clinical trials. Additionally, plasma MDS-OA β levels appeared affected by long-term storage duration, which could be of interest for others measuring plasma A β biomarkers.

Background

Accumulating evidence shows that small soluble Amyloid- β oligomers (A β Os), are the most toxic and pathogenic form of A β species in Alzheimer's Disease (AD) (Hayden and Teplow, 2013; Selkoe and Hardy, 2016). Many toxicities have been ascribed to A β Os including synaptic dysfunction, induction of tau pathology, neuroinflammation, impaired axonal transport, and neuronal death (Cline et al., 2018). In addition, A β Os have shown a better correlation with the presence and degree of cognitive symptoms than A β plaque counts (Haass and Selkoe, 2007), suggesting that A β Os might provide a more accurate reflection of clinical presentation than A β plaque load.

Currently, proxies of A β plaques are measured with high sensitivity and specificity with Positron Emission Tomography (PET) imaging or measurement of Cerebrospinal fluid (CSF) A β 42 concentrations. However, these methods are not available in routine laboratory settings, and often come with high costs or burden for the patient. Therefore, blood-based biomarkers are considered as low-cost and minimally invasive alternatives.

Plasma A β O concentrations or misfolded A β oligomeric assemblies have previously shown good diagnostic accuracies in identifying AD from controls (Area Under the Curve (AUC): 0.71–0.80) (Santos et al., 2008; Nabers et al., 2018). Using the Multimer Detection System (MDS) to measure plasma A β O levels has resulted in even higher diagnostic accuracies (AUC: 0.85–0.87) in discriminating AD from controls (An et al., 2017). However, the ability of plasma MDS-OA β to identify individuals with abnormal amyloid status has not yet been studied. This is relevant, because the definition of AD *in-vivo* is shifting to a biological construct and increasingly based on amyloid status (Jack et al., 2018). Therefore, we aimed to assess the performance of plasma MDS-OA β as a marker for abnormal amyloid status.

Methods

Subjects

We included 399 subjects from the Amsterdam Dementia Cohort (ADC) and the European Information Framework for AD (EMIF-AD) Preclinical AD project, regardless of clinical diagnosis. Inclusion criteria were met when amyloid PET results were available and the time between plasma sampling and PET scan did not exceed one year. During their visit, all subjects underwent comprehensive dementia screening including neurologic examination, laboratory tests, Magnetic Resonance Imaging (MRI), and electroencephalography (EEG) (Van Der Flier et al., 2014; van der Flier and Scheltens, 2018). Clinical diagnosis was established by consensus according to international consensus criteria (Román et al., 1993; Petersen et al., 1999; Rascovsky et al., 2011; McKeith et al., 2017; Jack et al., 2018), and included: Mild Cognitive Impairment (MCI; n = 42), AD (n = 164), non-AD dementia (n = 58) and other disorders (n = 61) including neuropsychiatric disorders, neurological disorders, or individuals with postponed diagnosis. Controls consisted of participants with subjective cognitive decline (SCD; n = 14), and normal controls (NC; n = 60). Normal controls in this study were included from the Preclinical AD study (Konijnenberg et al., 2018).

Amyloid status

Amyloid PET status was available in all subjects (n = 399). [18F]Florbetaben (n = 138), [18F]Florbetapir (n = 1), [18F]Flutemetamol (n = 138), or [11C]Pittsburgh compound B (PiB; n = 122) were used as radioactive amyloid tracers. A Medrad (Warrendale, PA) infusion system was used for tracer infusion. [18F]Florbetapir and [11C]PiB scans were acquired through 90-minute dynamic scanning using a PET/CT Ingenuity TF or Gemini TF [Philips Medical Systems, Best, the Netherlands] ([18F]Florbetapir), and ECAT EXACT HR + scanner [Siemens/CTI, Knoxville, TN]) ([11C]PiB). Scanning started simultaneously with tracer infusion at approximately 370 MBq [18F]florbetapir, and 351 MBq [11C]PiB. [18F]Florbetaben and [18F]flutemetamol scans were acquired through 20-minute static PET scanning using a PET/MR and Gemini TF-64 PET/CT scanner, Philips Medical Systems, respectively. Scanning started 90 minutes after tracer injection at approximately 250 MBq [18F]florbetaben, and 180 MBq [18F]flutemetamol. Amyloid status was defined as either abnormal or normal after visual assessment by either one (ADC) or three experienced nuclear medicine physicians (preclinical AD project) where majority vote ruled.

Cerebrospinal fluid analysis

CSF A β 42 was measured using two analytical methods: Innostest and Euroimmun ELISAs. Innostest ELISAs were used to measure levels of CSF amyloid beta 1–42, total Tau (t-Tau) and Tau phosphorylated at threonine 181 (p-Tau) for 268 subjects (Fuijirebio, Ghent, Belgium). CSF A β levels were corrected for the drift seen throughout CSF analysis years (Tijms et al., 2018). Euroimmun beta-amyloid ELISAs (Euroimmun, Lübeck, Germany/ADx Neurosciences, Ghent, Belgium) were used to measure levels of CSF A β 1–40 and 1–42 of normal controls (n = 60). All CSF samples were measured centrally at the Neurochemistry Laboratory of Amsterdam UMC.

Apolipoprotein E status

Apolipoprotein E (APOE) genotyping was performed using LightCycler ApoE mutation Detection Kit (Roche Diagnostics, GmbH, Mannheim, Germany), after isolation of genomic DNA from Ethylenediaminetetraacetic acid (EDTA) plasma (Qiagen, Venlo, the Netherlands). APOEe4 status was dichotomized into APOEe4 allele carriers (i.e. at least one APOEe4 allele), and non-carriers (i.e. no APOEe4 allele).

Plasma sampling and analysis

Blood plasma was collected in an EDTA vacutainer tube through venipuncture using standardized in-house protocols. EDTA plasma was centrifuged at 1,800 × g for 10 minutes at room temperature and stored in 0.5 ml polypropylene tubes at -80 °C in the Amsterdam UMC biobank. Prior to analysis, plasma aliquots were thawed at 37 °C for 15 minutes. Peoplebio inc. measured MDS-OA β levels in all plasma samples centrally using the Multimer Detection System. All samples were analyzed twice in singlet, on two consecutive days. Intra-assay variations were below 10%, and 2% of the samples (n = 8) showed interassay variations higher than 20% CV. All samples were above the lower limit of quantification (LLOQ; 0.239 ng/ml). Hemolytic samples (n = 15) were excluded, as hemoglobin might interfere with the MDS-OA β signal (Watt et al., 2012). The exact MDS method was described in detail previously (Youn et al., 2019).

Statistical analyses

SPSS version 24 (IBM, Armonk, NY) was used for all statistical analyses, and data were visualized using R version 3.5.2 ("eggshell igloo"). Subject characteristics were compared between amyloid normal and abnormal subjects, using students t-test, chi-squared test, and Man-Whitney U tests where appropriate. Plasma MDS-OA β was normalized using a 2-step transformation (Templeton, 2011). Upon visual inspection we recently observed a possible effect of long-term storage on plasma MDS-OA β levels (data not published). As plasma samples which are used for research purposes might have a long storage period after biobank retrieval, we additionally assessed the effect of long-term storage on plasma MDS-OA β levels by using General Linear Models (GLM) with factors sample storage period, PET amyloid status, and their 2-way interactions (full model shown in table e-1). In case of a significant 2-way interaction between storage period in years and PET amyloid status, we performed GLM for plasma MDS-

OAB stratified for storage period based on the median storage period (4 years). Differential expression of plasma MDS-OAB levels between normal amyloid and abnormal amyloid subjects was evaluated using GLM with plasma MDS-OAB as outcome measure, amyloid status as factor, and age, sex, APOEε4 status and cohort as covariates. Syndrome diagnosis was not a confounder, and therefore not included as a covariate. Pearson correlation analyses were used to assess correlations between plasma MDS-OAB and MMSE scores as measure of global cognition, or levels of CSF Aβ42, t-Tau and p-Tau measured by Innotech ELISA. The potential of the plasma Aβ oligomer assay to identify PET abnormal amyloid status was assessed by receiver operating characteristic (ROC) analysis using predicted values of binary logistic regression models. The Area Under the Curve (AUC), and corresponding sensitivities and specificities were calculated at an optimal cut-off for each model using Youden J's index (sensitivity + specificity - 1). The models contained 1) plasma MDS-OAB, 2) age and APOEε4 status, or 3) plasma MDS-OAB combined with age and APOEε4 status. AUC's between models were compared using DeLong analysis (DeLong, DeLong and Clarke-Pearson, 1988). Additionally, to assess the performance of the plasma MDS-OAB assay in early AD stages, analyses were repeated in a subgroup of pre-dementia subjects including controls and MCI subjects. Lastly, we investigated how the use of the plasma MDS-OAB marker can reduce the costs and number of PET scans needed to screen individuals for a hypothetical clinical trial which needs 100 abnormal amyloid individuals. For this analysis we used the sensitivity and specificity levels that corresponded with the highest Youden cut-off. Analyses were stratified for SCD, MCI and AD diagnosis as the prevalence of abnormal amyloid individuals differs per diagnosis (Jansen et al., 2015). We assumed an average cost of 5000 USD (Insel et al., 2016) per amyloid PET scan, and estimated 100 USD per plasma MDS-OAB sample. A p -value < 0.05 was considered statistically significant.

Results

Patient characteristics

The average age of the total population was, 63.8 ± 6.6 years old, 44% was female, and the average MMSE was 24 ± 5 . Abnormal amyloid status was found in 206 (52%) subjects which included more often AD dementia subjects, whereas normal amyloid subjects included more often controls. Individuals with abnormal amyloid had lower MMSE scores and were more often APOEε4 carrier compared to individuals with normal amyloid status (Table 1). An interaction with storage period was found ($p < 0.01$; Fig. 1, Table 2), after which the cohort was stratified based on the median storage period (4 years). The groups with storage period ≤ 4 years and > 4 years did not differ from each other in patient characteristics. Both had more abnormal amyloid subjects who had lower MMSE scores, and were more often APOEε4 carrier than amyloid normal subjects (Table 1).

Table 1
Subject characteristics

	Total cohort		Sample storage period (≤ 4 years)		Sample storage period (> 4 years)	
	PET based amyloid status (n = 399)		PET based amyloid status (n = 207)		PET based amyloid status (n = 192)	
	normal (n = 193)	abnormal (n = 206)	normal (n = 139)	abnormal (n = 68)	normal (n = 54)	abnormal (n = 138)
Age, yr	64.4 (6.5)	63.2 (6.6)	64.6 (6.2)	63.2 (6.5)	64.0 (7.3)	63.2 (6.7)
Female sex, n %	75 (39%)	101 (49%)*	54 (39%)	34 (50%)	21 (39%)	67 (49%)
MMSE (n = 393)	26 (4)	22 (5)***	27 (3.9)	22 (4.7)***	25 (3.7)	22 (4.6)***
Education, yr (n = 334)	10.7 (2.9)	11.4 (2.9)*	10.5 (2.9)	11.0 (2.6)	10.9 (3.0)	11.7 (3.0)
Diagnosis						
CN ^b /MCI/AD/ Non-AD/Other	70/15/11/ 49/48	4/27/153/*** 9/13	63/4/6/ 30/36	2/10/44/*** 3/9	7/11/5/ 19/12	2/17/109/*** 6/4
APOE ε4 carrier (n = 389)	59 (30%)	140 (70%)***	42 (31%)	46 (69%)***	17 (32%)	94 (72%)***
CSF aβ42 (pg/ml)						
Innotest (n = 268)	1033 (246)	629 (131) ***	1036 (284)	610 (182)***	1027 (184)	638 (102)***
Euroimmun (n = 60)	999 (309)	NA	999 (309)	NA	999 (309)	NA
Plasma MDS- OAβ assay ^a	0.80 (0.33)	0.97 (0.35) ***	0.76 (0.32)	1.03 (0.28)***	0.89 (0.35)	0.95 (0.37)

^a hemolytic samples excluded. ^b Controls include normal controls and SCD subjects. Non-AD dementia patients include possible AD, FTD, DLB, VaD and CBD, PSP. Other includes psychiatry, other neurological diseases, postponed diagnosis or PPA. Data are presented as mean (SD) unless otherwise specified. Independent t-test or chi-squared test were performed where appropriate. Abbreviations: NA; Not Available, MMSE; Mini Mental State Examination, MCI; Mild Cognitive Impairment, FTD; Frontotemporal Dementia, DLB; Dementia with Lewy Bodies, VaD; Vascular Dementia, PPA; Primary Progressive Aphasia,

AD; Alzheimer’s disease dementia, non-AD; non-Alzheimer’s disease dementia, aβ42; amyloid-β1–42, MDS-OAβ; Aβ oligomerization Tendency, PET: Positron Emission Tomography.

Table 2
Full model for storage period and amyloid PET status for plasma MDS-OA

β		
	β (se) ^b	p-value
Storage period, yrs	.03 (.01)	.02
Amyloid PET status ^a	.38 (.08)	< .001
Amyloid PET status*Storage period, yrs	– .04 (.01)	.003

We used General Linear Models (GLM) with factors storage period and PET amyloid status, and their 2-way interactions. ^a Reference is normal amyloid PET status. $p < 0.05$ is considered significant. ^b plasma MDS-OAβ was normalized using two-step transformation. Abbreviations: MDS-OAβ, Aβ oligomeric tendency; yrs, years; PET, Positron Emission Tomography.

Plasma MDS-OAβ performance in samples with a short-term storage duration

For samples with a storage period ≤ 4 years ($n = 207$), plasma MDS-OAβ levels corrected for age, sex, APOEε4 status and cohort, were higher in abnormal amyloid subjects compared to normal amyloid subjects ($\beta \pm se$: 0.17 ± 0.05 ; $p = 0.001$; Fig. 1). Plasma MDS-OAβ was negatively correlated with CSF Aβ42 levels ($r = -0.20$, $p = 0.035$) and MMSE scores ($r = -0.29$, $p < 0.01$), and positively correlated with CSF t-Tau ($r = 0.20$, $p = 0.01$) (Fig. 2). There was no correlation with CSF p-Tau levels ($r = 0.12$, $p > 0.05$). ROC analyses (Fig. 3) revealed that plasma MDS-OAβ could accurately identify individuals with abnormal amyloid PET (AUC = 0.74, 95% CI = 0.67–0.81), with a sensitivity of 76% and specificity of 67%. When combined with age and APOEε4 status the AUC increased to 0.81 (95% CI = 0.74–0.87), with a sensitivity and specificity of 58% and 89%, which performed better than age and APOEε4 genotype alone (AUC = 0.70, 95% CI = 0.63–0.78, $p = 0.01$).

Plasma MDS-OAβ performance in samples with a long-term storage duration

We repeated our analyses in samples ($n = 192$) that had been stored for a longer period (> 4 years), and observed no difference in plasma MDS-OAβ levels between abnormal and normal amyloid individuals ($\beta \pm se$: 0.04 ± 0.06 , $p > 0.05$, Fig. 2), nor could it discriminate between abnormal and normal amyloid status (AUC: 0.50, $p > 0.05$).

Plasma MDS-OAβ as an early predictor of amyloid status

Next, analyses were repeated in a pre-dementia subgroup including CN and MCI subjects (storage period ≤ 4 years; $n = 78$). Plasma MDS-OA β could identify individuals with abnormal amyloid PET with an AUC of 0.77 (95% CI = 0.60–0.93), and a sensitivity and specificity of 67% and 83%, respectively. When combined with age and APOE $\epsilon 4$ status the AUC increased to 0.86 (95% CI = 0.75–0.96), with a sensitivity and specificity of 75% and 83%, which performed better than age and APOE $\epsilon 4$ genotype on a trend level (AUC: 0.76, 95% CI: 0.62–0.89, $p = 0.10$).

Plasma MDS-OA β as a pre-screener

Lastly, we investigated how the use of plasma oligomers as a pre-screen could reduce costs to find 100 individuals with amyloid pathology on a PET scan among individuals with normal cognition, MCI, or AD dementia. Given an expected prevalence of amyloid pathology of 30% in CN, 50% in MCI and 70% in AD subjects (Jansen et al., 2015), the number of amyloid PET scans to find 100 amyloid positives within each group without pre-screening would be 333 (CN), 200 (MCI), and 143 (dementia). Assuming the sensitivity of 76% and specificity of 69% of the plasma MDS-OA β test (highest Youden cut-off: 0.45), MDS-OA β needed to be determined in 439 CN, 263 MCI and 188 AD subjects to identify 195 CN, 141 MCI and 118 AD subjects with an abnormal MDS-OA β test in order to find 100 abnormal amyloid PET cases within each clinical group. The plasma MDS-OA β assay as a pre-screener would reduce the number of PET scans with 138 (40%) in CN, 59 (30%) in MCI, and 25 (18%) in AD subjects. When considering plasma MDS-OA β costs, this would result in a cost reduction of 40% in CN, 30% in MCI, and 15% in AD compared to when the plasma MDS-OA β assay was not used.

Discussion

We showed that plasma MDS-OA β has good accuracy to pre-screen for brain amyloidosis in a memory clinic population, particularly when combined with APOE $\epsilon 4$ and age (AUC > 0.80). In addition, plasma MDS-OA β showed a negative correlation with CSF A β 42 and MMSE, and a positive correlation with CSF t-Tau. Using plasma MDS-OA β as a pre-screener resulted in reduced number of PET scans and lowered costs for amyloid screening up to 40%, which is highly beneficial for clinical trials. Of note, these results are only valid for relatively fresh samples, as a negative effect of long-term storage was found for plasma MDS-OA β concentrations.

To date, few studies have measured A β oligomers levels in blood plasma, as detecting crude oligomeric A β in plasma is challenging owing to its low concentration in blood. Using the MDS platform, we successfully measured increased plasma MDS-OA β levels in abnormal amyloid-PET individuals compared to individuals with normal amyloid-PET levels. This finding is in line with previous studies reporting increased levels of A β oligomers in brain tissue, CSF, and plasma of AD patients. (Georganopoulou *et al.*, 2005; Santos *et al.*, 2008; Xia *et al.*, 2009; Zhou *et al.*, 2012) This increase in A β oligomer levels is in contrast to monomeric A β levels, which show an evident decrease rather than increase in blood plasma. (Janelidze *et al.*, 2016; Ovod *et al.*, 2017; Nakamura *et al.*, 2018; Verberk *et al.*, 2018; Palmqvist *et al.*, 2019) This upregulation of A β oligomers could be explained by oligomerization of A β monomers, resulting in higher plasma A β oligomer levels and decreased monomeric A β levels. Our

results also showed a correlation between plasma MDS-OA β and CSF A β 42, t-Tau or MMSE scores, which is in line with previous plasma A β monomer studies. (Janelidze *et al.*, 2016; An *et al.*, 2017; Verberk *et al.*, 2018; Palmqvist *et al.*, 2019) However, these correlations were not strong, and an explanation for this could be the peripheral production of plasma A β , by platelets, skeletal muscle cells and other cell types (Roher *et al.*, 2009) that contribute to circulating A β levels resulting in a dilution of the relation with CNS processes.

This is the first study to report on plasma A β Os as a marker for brain amyloidosis in a large amyloid PET confirmed cohort. As the definition of AD is shifting from a syndrome to a biological construct, it is relevant to evaluate the performance of biomarkers in discriminating amyloid status (Jack *et al.*, 2018). One small-scale study did evaluate oligomeric assemblies of misfolded A β protein as a plasma marker for amyloid status between prodromal PET-positive individuals (n=36) and healthy elderly PET-negative individuals (n=37) (Nabers *et al.*, 2018). Using an immune-infrared sensor method they achieved an AUC of 0.78 (95% CI 0.68–0.88) (Nabers *et al.*, 2018). We showed a similar good accuracy of plasma MDS-OA β to screen for amyloid status (AUC: 0.81) in a large amyloid PET confirmed cohort when combined with APOE ϵ 4 and age. When restricting the analyses to individuals in pre-dementia stages (i.e. CN and MCI), the accuracy of plasma MDS-OA β combined with APOE ϵ 4 and age increased further to 0.86.

It is well known that preanalytical factors concerning sample handling and processing can influence the measured concentration of (plasma) biomarkers, therefore leading to variability in results, preventing establishment of a universal cutoffs and between-laboratory comparisons (Hansson *et al.*, 2018; Rózga *et al.*, 2019). We previously observed a negative effect of long-term storage on plasma MDS-OA β levels upon visual inspection (data not published), and therefore, decided to evaluate this in the current study. We found that with longer shelf-life plasma MDS-OA β levels no longer differed between normal and abnormal amyloid individuals. Our finding is not fully in line with one recent study that investigated the long term storage effect on plasma monomeric A β , and found stable plasma A β levels after long-term storage up to 5 years at -80°C (Chiu *et al.*, 2019). This discrepancy might be caused by the difference in storage length between the previous study (up to 5 years) and the current study (up to 19 years). It might also be caused by the difference in analytical methods (MDS vs. IMR) or the different A β species (MDS-OA β vs (in principle) monomeric A β 42). It could be the case that plasma MDS-OA β levels of normal amyloid increase over time and reach similar levels as plasma MDS-OA β levels of abnormal amyloid individuals, whereas in monomeric A β 42 this does not happen. It could be hypothesized that long-term storage might induce stress on the oligomeric A β 42 protein which results in perturbation of the protein and an increased aggregation tendency in normal amyloid individuals, which does not occur in abnormal amyloid individuals as they have already reached maximum oligomerization. A similar increase in protein aggregation induced by protein-stress has previously been reported after freeze-thawing (Carpenter, Manning and Randolph, 2002). The effect of long-term storage time implies that the plasma MDS-OA β assay cannot be used to perform research projects with samples that have been stored in biobanks for a long period. This novel finding could be of interest to other research groups interested in measuring plasma biomarkers of amyloid. Additional pre-analytical testing is needed to determine the precise

maximum storage period, and to compare the effect of long-term storage with other types of blood based A β biomarkers. Nonetheless, as in daily clinical routine fresh blood samples are used we do not expect this will present a problem for daily clinical practice.

Strengths and limitations

Our study has several strengths including our large well-defined amyloid PET confirmed memory clinic population. In addition, CSF and plasma collection follows a highly standardized protocol in our center, thus minimizing confounding effects in pre-analytical processing. Moreover, the oligomerization assay technique developed for this plasma MDS-OA β assay can potentially be employed for other proteinopathies as well, such as α -synuclein which is often seen in dementia with Lewy bodies. This might result in a screening panel of plasma biomarkers for different types of neurodegenerative disease. Among the limitations of our study is that the plasma MDS-OA β assay is not yet available on an automatic platform, thus enhancing the risk for analytical variation. However, automation is currently under development, further facilitating broad implementation and minimizing analytical variation. In addition, plasma MDS assays for other AD biomarkers, such as phosphorylated Tau, are currently under development to further capture the full pathological profile of AD (Jack *et al.*, 2018). Lastly, it would be interesting to study the association between plasma MDS-OA β with specific cognitive domains, through elaborate neuropsychological testing, to get an in-depth understanding of the association between plasma MDS-OA β and cognitive impairment.

Conclusions

In conclusion, plasma MDS-OA β has the potential to be used as a pre-screener for brain amyloidosis in large heterogeneous memory clinic populations. The advantages of the low-cost MDS-OA β blood test include the ease of blood collection over a lumbar puncture or a costly PET scan. Additionally, using plasma MDS-OA β as a pre-screener based on the results of this current study, reduced the number of amyloid PET scans needed and lowered total costs up to 40%, highlighting a potential use for clinical trials settings. In addition, the novel finding of long-term storage duration on plasma MDS-OA β levels could be of interest to other research groups interested in measuring plasma biomarkers of amyloid.

Abbreviations

PiB 11C]Pittsburgh compound B

AD Alzheimer's Disease

ADC Amsterdam Dementia Cohort

A β Os Amyloid- β oligomers

APOE Apolipoprotein E

AUC Area Under the Curve

CSF Cerebrospinal fluid

EEG electroencephalography

EDTA Ethylenediaminetetraacetic acid

EMIF-AD European Information Framework for AD

GLM General Linear Models

LLOQ lower limit of quantification

MRI Magnetic Resonance Imaging

MCI Mild Cognitive Impairment

MDS Multimer Detection System

PET Positron Emission Tomography

ROC receiver operating characteristic

SCD subjective cognitive decline

p-Tau Tau phosphorylated at threonine 181

t-Tau Total Tau

Declarations

Ethics approval and consent to participate

The research is in accordance with the ethical consent by the VU University and with the Helsinki Declaration (1991). For all patients written informed consent was available.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

Babapour Mofrad reports no competing interests. P. Scheltens has acquired grant support (for the institution) from GE Healthcare, Danone Research, Piramal, and Merck. In the past 2 years, he has received consultancy/speaker fees from Lilly, GE Healthcare, Novartis, Sanofi, Nutricia, Probiodrug, Biogen, Roche, Avraham, and EIP Pharma. Gerald Luscan is a Pfizer employee. All funding is paid to the institution. S. Kim reports no competing interests. S. Kang reports no competing interests. Y.C. Youn reports no competing interests. S.S.A. An reports no competing interests. J. Tomassen reports no competing interests. B.N.M. van Berckel received research support from ZON-MW, AVID radiopharmaceuticals, CTMM and Janssen Pharmaceuticals. He is a trainer for Piramal and GE. He receives no personal honoraria. P.J. Visser reports non-financial support from GE Healthcare, other from Eli-Lilly, other from Janssen Pharmaceutical, grants from Biogen, outside the submitted work. W.M. van der Flier holds the Pasman chair and performs contract research for Biogen and has research programs funded by the ZonMW, NWO, EU-FP7, Alzheimer Nederland, CardioVascular Onderzoek Nederland, Stichting Dioraphte, Gieskes-Strijbis Fonds, Boehringer Ingelheim, Piramal Neuroimaging, Roche BV, Janssen Stellar, and Combinostics. All funding is paid to her institution. C.E. Teunissen received grants from the European Commission, the Dutch Research Council (ZonMW), Association of Frontotemporal Dementia/Alzheimer's Drug Discovery Foundation, The Weston Brain Institute, Alzheimer Netherlands. She has functioned in advisory boards of Roche, received non-financial support in the form of research consumables from ADxNeurosciences and Euroimmun, performed contract research or received grants from Probiodrug, Biogen, Esai, Toyama, Janssen prevention center, Boehringer, Axon Neurosciences, EIP farma.

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Authors' contributions

Babapour Mofrad was involved with the design and conceptualized of the study; analyzed the data; interpreted the data, and was a major contributor in writing the manuscript. P. Scheltens interpreted the data and revised the manuscript for intellectual content. S.Y. Kim revised the manuscript for intellectual content. S. Kang performed sample analysis and revised the manuscript for intellectual content. Y.C. Youn revised the manuscript for intellectual content. S.S.A. An revised the manuscript for intellectual content. J. Tomassen was involved with sample selection and revised the manuscript for intellectual content. B. N.M. van Berckel rated all PET scans and revised the manuscript for intellectual content. P.J. Visser provided samples, interpreted the data, and revised the manuscript for intellectual content. W.M.

van der Flier interpreted the data; revised the manuscript for intellectual content. C.E. Teunissen interpreted the data; and was a major contributor in writing the manuscript.

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Not applicable

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Figures

Figure 1

Scatterplot presents the correlation between plasma MDS-OAβ and storage period in years. Blue dots represent normal amyloid PET individuals, and red dots represent abnormal amyloid PET individuals.

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Figure 2

Boxplots present higher levels of plasma MDS-OAβ in abnormal amyloid PET individuals in samples with ≤ 4 years storage (n= 207) (A), and no change in samples > 4 years storage (n= 192) (B). Scatterplots present the correlation in samples ≤ 4 years storage (n= 207) between plasma MDS-OAβ and CSF biomarker concentrations: CSF Aβ 42 (C), CSF Tau (D), CSF p-Tau (E), and the correlation between plasma MDS-OAβ and MMSE score (F). Blue dots represent normal amyloid PET individuals, and red dots represent abnormal amyloid PET individuals. *** represents a p-value <0.001.

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Figure 3

Receiver Operating Characteristic (ROC) curves discriminate abnormal amyloid from normal amyloid subjects as defined by amyloid PET scan based on plasma A β oligomer levels (blue line), age and APOE ϵ 4 genotype (purple line), and a multivariate model including APOE ϵ 4 genotype, age and plasma A β oligomer levels (yellow line).

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