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Plasma amyloid- β oligomerization assay as a pre-screening test for amyloid status



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Abstract

Objective: 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; $n_{\text{abnormal}} = 206$). 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 a storage duration < 4 years. 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 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.

Keywords: Blood-based biomarker, Plasma A β oligomer, Amyloid status, Multimer detection system, Long-term storage

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Background

Accumulating evidence shows that small soluble Amyloid- β oligomers (A β O β s) are the most toxic and pathogenic form of A β species in Alzheimer's disease (AD) [1, 2]. Many toxicities have been ascribed to A β O β s including synaptic dysfunction, induction of tau pathology, neuroinflammation, impaired axonal transport, and neuronal death [3]. In addition, A β O β s have shown a better correlation with the presence and degree of cognitive symptoms than A β plaque counts [4], suggesting that A β O β s 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 often come with high costs or burden for the patient. Therefore, blood-based biomarkers are considered low-cost and minimally invasive alternatives.

Plasma A β O β 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) [5, 6]. Using the multimer detection system (MDS) to measure plasma A β O β levels has resulted in even higher diagnostic accuracies (AUC, 0.85–0.87) in discriminating AD dementia patients from controls [7]. However, the ability of plasma amyloid oligomerization tendency measured by the multimer detection platform (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 [8]. 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 1 year. During their visit, all subjects underwent comprehensive dementia screening including neurologic examination, laboratory tests, magnetic resonance imaging (MRI), and electroencephalography (EEG) [9, 10]. Clinical diagnosis was established by consensus according to international consensus criteria [8, 11–14], and included mild cognitive impairment (MCI; $n = 42$), AD dementia ($n = 164$), 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 [15]. No known familial AD patients were included. CSF and PET results (below) were used to support the AD dementia diagnosis and to define the number of amyloid-positive subjects within each clinical diagnostic group.

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-min 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-min static PET scanning using a PET/MR and Gemini TF-64 PET/CT scanner, Philips Medical Systems, respectively. Scanning started 90 min 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 (Tau) and Tau phosphorylated at threonine 181 (pTau) for 268 subjects (Fujirebio, Ghent, Belgium). CSF A β levels were corrected for the drift seen throughout CSF analysis years [16]. 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

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). APOE ϵ 4 status was dichotomized into APOE ϵ 4 allele carriers (i.e., at

least one APOEε4 allele) and non-carriers (i.e., no APOEε4 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 1800×g for 10 min 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 min. Peoplebio Inc. measured MDS-OAβ levels in all plasma samples centrally using the multimer detection system, which is CE marked, approved by the Korean FDA and under commercialization for the Asian and European markets. The assay essentially is an ELISA assay, with the exception that samples and standards were mixed with a proprietary amyloid beta protein mixture before starting the sandwich procedure. Results are expressed as ratio of the concentration calculated in the standard curve for each sample over the average results obtained for two internal standards. All samples were analyzed twice in singlet, on 2 consecutive days. Intra-assay variations were below 10% and 2% of the samples (n = 8) showed interassay variations higher than 20% coefficient of variation (CV) (these samples were included in the analyses). 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-OAβ signal [17]. The exact MDS method was described in detail previously [18]. The reproducibility of MDS-OAβ test confirmed by testing the control materials with four concentrations (highly concentrated positive, medium concentrated positive, low concentrated positive and negative) with three different LOTs, two different instruments/site, three different testers for 5 days with five replicate per each sample. As a result, all 225 positive sample test results were positive and all 75 negative sample test results were negative, showing 100% reproducibility, and calculated CV% of MDS ratio for total and between LOT, tester, and instrument (site) was approximately 5%. There was no interference of albumin, hemoglobin, or bilirubin, nor cross reactivity of Mutant Aβ1-42 monomer peptide, Aβ4-42 peptide, Aβ9-42 peptide, and Aβ1-24 peptide with a concentration of 1.25 ng/ml.

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 Student's t-test, chi-squared test, and Mann-Whitney U tests where appropriate. Plasma MDS-OAβ was normalized using a 2-step transformation [19]. Upon

visual inspection, we recently observed a possible effect of long-term storage on plasma MDS-OAβ 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-OAβ levels by using general linear models (GLM) with factors sample storage period, PET amyloid status, and their 2-way interactions. In case of a significant 2-way interaction between storage period in years and PET amyloid status, we performed GLM for plasma MDS-OAβ stratified for storage period based on the median storage period (4 years). Differential expression of plasma MDS-OAβ levels between normal amyloid and abnormal amyloid subjects was evaluated using GLM with plasma MDS-OAβ 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-OAβ and Mini Mental State Examination (MMSE) scores as measure of global cognition, or levels of CSF Aβ42, Tau, and pTau 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-OAβ, (2) age and APOEε4 status, or (3) plasma MDS-OAβ combined with age and APOEε4 status. AUC's between models were compared using DeLong analysis [20]. Additionally, to assess the performance of the plasma MDS-OAβ 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-OAβ 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 dementia diagnosis as the prevalence of abnormal amyloid individuals differs per diagnosis [21]. We assumed an average cost of 5000 USD [22] per amyloid PET scan and estimated 100 USD per plasma MDS-OAβ 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

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, year	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, year (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/	70/15/11/	4/27/153/***	63/4/6/	2/10/44/***	7/11/5/	2/17/109/***
Non-AD/Other	49/48	9/13	30/36	3/9	19/12	6/4
APOE $\epsilon 4$ carrier (n = 389)	59 (30%)	140 (70%)***	42 (31%)	46 (69%)***	17 (32%)	94 (72%)***
CSF $a\beta 42$ (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-OA β 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)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ^aHemolytic samples excluded^bControls include normal controls and SCD subjects

Non-AD dementia patients include possible AD, FTD, DLB, VaD and CBD, and 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 was 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, $a\beta 42$ amyloid- $\beta 1-42$, MDS-OA β A β oligomerization tendency, PET positron emission tomography

(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 APOE $\epsilon 4$ 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 APOE $\epsilon 4$ carrier than amyloid normal subjects (Table 1).

Plasma MDS-OA β performance in samples with a storage duration < 4 years

For samples with a storage period ≤ 4 years (n = 207), plasma MDS-OA β levels corrected for age, sex, APOE $\epsilon 4$ 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-OA β was negatively correlated with CSF A $\beta 42$ levels ($r = -0.20$, $p = 0.035$) and MMSE scores ($r = -0.29$, $p < 0.01$) and positively correlated with CSF Tau ($r = 0.20$, $p = 0.01$) (Fig. 2). There was no correlation with CSF pTau levels ($r = 0.12$, $p > 0.05$). ROC analyses (Fig. 3) revealed that plasma MDS-OA β 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 APOE $\epsilon 4$ 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 APOE $\epsilon 4$ genotype alone (AUC = 0.70, 95% CI = 0.63–0.78, $p = 0.01$).

Plasma MDS-OA β 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-OA β 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-OA β as an early predictor of amyloid status and syndrome diagnosis

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 $\epsilon 4$

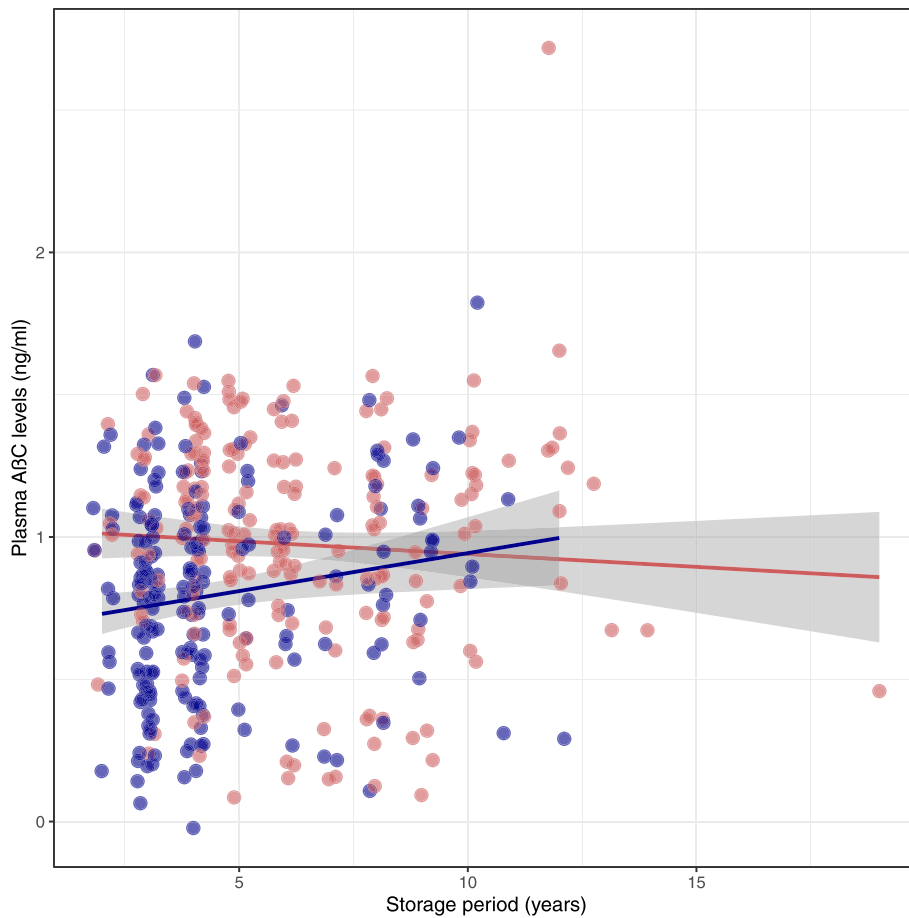


Fig. 1 Scatterplot presents the correlation between plasma MDS-OAβ and storage period in years. Blue dots represent normal amyloid PET individuals, and red dots represent abnormal amyloid PET individuals

genotype on a trend level (AUC, 0.76; 95% CI, 0.62–0.89, $p = 0.10$).

We next performed an exploratory analysis of the prediction of amyloid positivity in the non-AD and other diagnosis subgroups. The data showed largely similar results. For the comparison of abnormal amyloid PET vs normal amyloid PET, we observed an AUC of 0.72 in non-AD subgroup and an AUC of

0.75 in the other diagnosis subgroup, controlled for age and APOEε4 status.

Exploratory cost-evaluation for plasma MDS-OAβ as a pre-screener

Lastly, we explored how the use of plasma oligomers as a pre-screen could reduce costs to find 100 individuals with amyloid pathology on a PET scan in the total group of 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 dementia subjects [21], 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 67% of the plasma MDS-OAβ test (highest Youden cut-off, 0.45) in this total cohort, MDS-OAβ needed to be determined in 439 CN, 263 MCI, and 188 AD dementia subjects to identify 195 CN, 141 MCI, and 118 AD dementia subjects with an abnormal MDS-OAβ test in order to find 100 abnormal amyloid PET cases

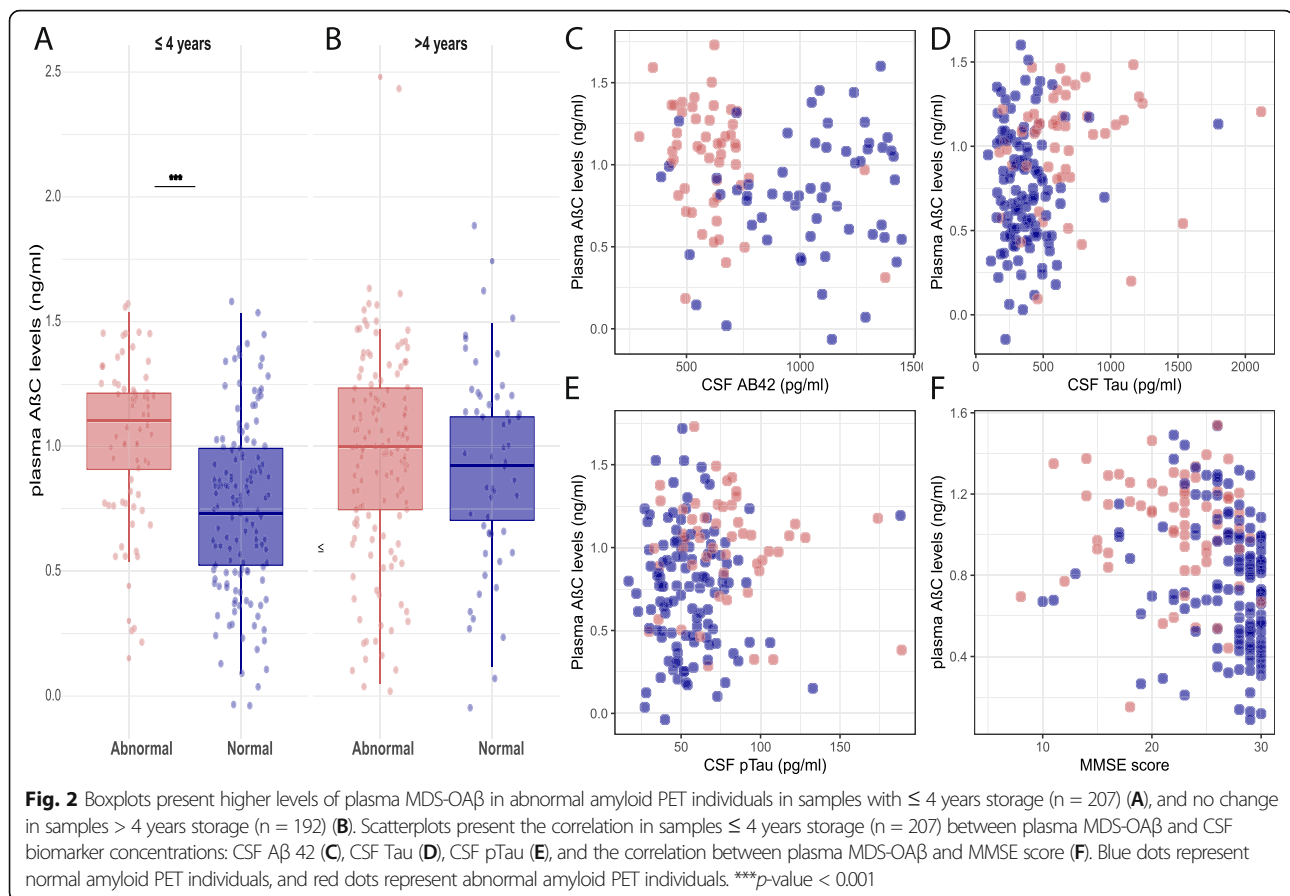
Table 2 Full model for storage period and amyloid PET status for plasma MDS-OAβ

	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

^aReference is normal amyloid PET status. $p < 0.05$ is considered significant

^bPlasma MDS-OAβ was normalized using two-step transformation MDS-OAβ Aβ oligomeric tendency, yrs years, PET positron emission tomography



within each clinical group. The plasma MDS-OA β assay as a pre-screener for amyloid PET analysis would thus reduce the number of PET scans with 138 (40%) in CN, 59 (30%) in MCI, and 25 (18%) in AD dementia subjects. Considering the costs for a PET analyses and MDS-OA β only, this would result in a cost reduction of 40% in CN, 30% in MCI, and 15% in AD dementia based on these figures.

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 ϵ 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 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 [5, 23–25]. 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 [26–30]. 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, Tau, or MMSE scores, which is in line with previous plasma A β monomer studies [7, 26, 27, 30]. 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 [31] 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

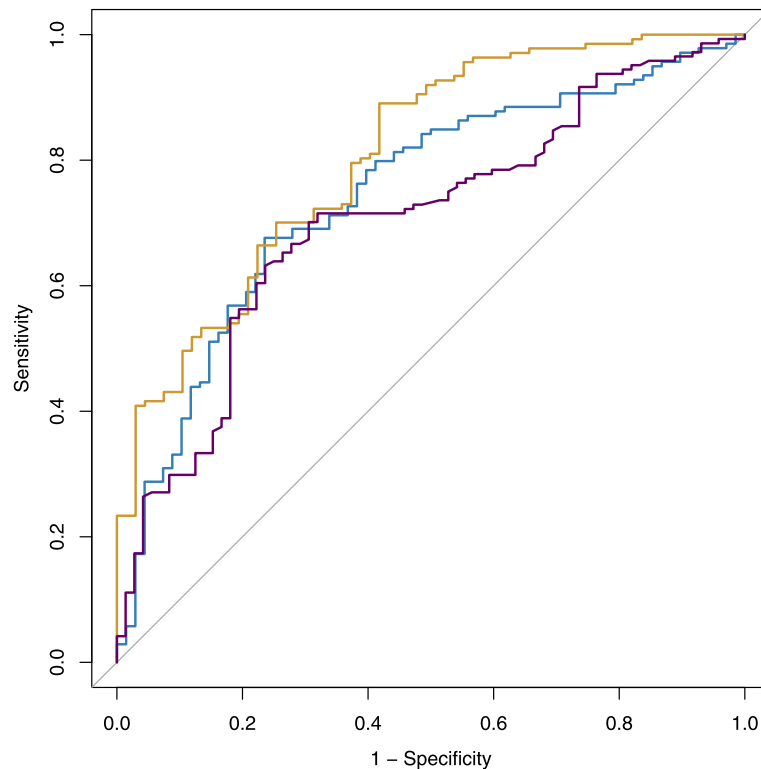


Fig. 3 Receiver operating characteristic (ROC) curves discriminate abnormal amyloid from normal amyloid subjects as defined by amyloid PET scan based on plasma A β oligomer levels (blue line), age and APOE ϵ 4 genotype (purple line), and a multivariate model including APOE ϵ 4 genotype, age, and plasma A β oligomer levels (yellow line)

to evaluate the performance of biomarkers in discriminating amyloid status [8]. 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 ($n = 37$) [6]. Using an immune-infrared sensor method, they achieved an AUC of 0.78 (95% CI 0.68–0.88) [6]. 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.

One of the suggested applications of a plasma A β biomarker is a screening test for brain amyloidosis in specialized memory clinics or for clinical trial inclusion [32]. Previous studies have been successful in identifying amyloid status with high accuracies (AUC, 0.79–0.97) using various types of plasma A β markers [26–30, 33] and plasma pTau isoforms [34–38]. The plasma MDS-OA β assay had similar or somewhat lower accuracies compared to these other plasma biomarker tests. While techniques used in some studies, such as

immunoprecipitation mass spectrometry [28, 29], are labor-intensive and time-consuming, the Simoa assays and the MDS method allow high-throughput analysis. The MDS method highly resembles an ELISA in simplicity and automation possibilities [7, 18, 39] and as such, allows broad implementation. Another added value of our study is that we have tested the plasma MDS-OA β assay in a heterogeneous cohort including other neurodegenerative or neuropsychiatric disorders besides the clinical AD spectrum, while previous plasma A β included primarily cohorts which contained the clinical AD spectrum (i.e., healthy controls, MCI, or AD dementia). The heterogeneity of the cohort used in this current study better resembles a memory clinic population, the setting where plasma biomarkers will likely be applied in the future to pre-screen for brain amyloidosis. Additionally, using plasma MDS-OA β as a pre-screener in a hypothetical clinical trial scenario lowered the number of PET scans up to 40% depending on clinical diagnosis. Therefore, plasma MDS-OA β could be beneficial for pre-screening in clinical trial settings, as it could potentially reduce costs.

It is well known that pre-analytical 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 [40, 41]. 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 for samples with a storage time > 4 years 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 $-80\text{ }^{\circ}\text{C}$ [42]. 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 [43]. 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. This is supported by recent results from a systematic study into pre-analytical stability, showing no effect of up to 2 weeks storage at either room temperature or $-20\text{ }^{\circ}\text{C}$ on the plasma MDS-OA β levels (manuscript under review).

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 [8]. 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

AD: Alzheimer's disease; ADC: Amsterdam Dementia Cohort; Amsterdam UMC: Amsterdam University Medical Centers; APOE ϵ 4: Apolipoprotein epsilon 4; AUC: Area under the curve; A β : Amyloid beta; A β 42: Amyloid Beta 1-42; CSF: Cerebrospinal fluid; CV: Coefficient of variation; EDTA: Ethylenediamine tetraacetic acid; EEG: Electroencephalography; ELISA: Enzyme-linked immunosorbent assay; EMIF-AD: The European Information Framework for AD; GLM: General linear models; MCI: Mild cognitive impairment; MDS: Multimer detection system; MDS-OA β : Amyloid oligomerization tendency measured by the multimer detection platform; MMSE: Mini Mental State Examination; MRI: Magnetic resonance imaging; NC: Normal controls; OA β : Amyloid oligomerization tendency; PET: Positron emission tomography; pTau: Tau phosphorylated at threonine 181; SCD: Subjective cognitive decline; Tau: Total Tau; USD: US Dollar

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Authors' contributions

RBM designed and conceptualized the study, analyzed the data, interpreted the data, and was a major contributor in writing the manuscript. PS interpreted the data and revised the manuscript for intellectual content. SYK and YCY revised the manuscript for intellectual content. SK performed the sample analysis and revised the manuscript for intellectual content. SSAA helped develop the multimer detection system platform and revised the manuscript for intellectual content. JT helped with the sample selection and revised the manuscript for intellectual content. BNMB rated all PET scans and

revised the manuscript for intellectual content. PJV provided samples, interpreted the data, and revised the manuscript for intellectual content. WMvdF interpreted the data and revised the manuscript for intellectual content. CET interpreted the data and was a major contributor in writing the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

Data are available from the authors upon reasonable request.

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

Competing interests

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