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Plasma Amyloid Concentration in Alzheimer's Disease: Performance of a High-Throughput Amyloid Assay in Distinguishing Alzheimer's Disease Cases from Controls

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Abstract.

Background: Collection of cerebrospinal fluid (CSF) for measurement of amyloid-β (Aβ) species is a gold standard in Alzheimer's disease (AD) diagnosis, but has risks. Thus, establishing a low-risk blood Aβ test with high AD sensitivity and specificity is of outmost interest.

Objective: We evaluated the ability of a commercially available plasma Aβ assay to distinguish AD patients from biomarker-healthy controls.

Method: In a case-control design, we examined plasma samples from 44 AD patients (A + N+) and 49 controls (A–N–) from a memory clinic. AD was diagnosed using a combination of neuropsychological examination, CSF biomarker analysis and brain imaging. Total $A\beta_{40}$ and total $A\beta_{42}$ in plasma were measured through enzyme-linked immunosorbent assay (ELISA) technology using ABtest40 and ABtest42 test kits (Araclon Biotech Ltd.). Receiver operating characteristic (ROC) analyses with outcome AD were performed, and sensitivity and specificity were calculated.

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Results: Plasma A$\beta_{42/40}$ was weakly positively correlated with CSF A$\beta_{42/40}$ (Spearman's rho 0.22; $p = 0.037$). Plasma A$\beta_{42/40}$ alone was not able to statistically significantly distinguish between AD patients and controls (AUC 0.58; 95% CI 0.46, 0.70). At a cut-point of 0.076 maximizing sensitivity and specificity, plasma A$\beta_{42/40}$ had a sensitivity of 61.2% and a specificity of 63.6%.

Conclusion: In this sample, the high-throughput blood Aβ assay was not able to distinguish well between AD patients and controls. Whether or not the assay may be useful in large-scale epidemiological settings remains to be seen.

Keywords: Alzheimer's disease, amyloid, diagnosis, high-throughput assay, plasma

INTRODUCTION

Alzheimer's disease (AD) is threatening global healthcare systems [1] and generates immense economic, medical, and societal costs [2]. As its neuropathological hallmark, AD is characterized by an accumulation of amyloid-β (Aβ) peptides in the brain and AD diagnosis largely depends on an estimation of brain Aβ burden. Aβ is derived through cleavage of the amyloid-β protein precursor (AβPP), a transmembrane protein, and aggregates as neurotoxic amyloid plaques ultimately impairing synaptic function [3], though whether or not Aβ causes AD or functions as a 'bystander' of AD pathogenesis is yet to be determined [4, 5].

Aβ antemortem is quantifiable via radioactive labelling of Aβ on positron emission tomography (PET) [6] and can also be estimated from Aβ concentrations in the cerebrospinal fluid (CSF) as a molecular biomarker [7]. Amyloid PET and CSF Aβ can be used interchangeably for clinical diagnosis [7, 8] and are increasingly relied upon in diagnostic frameworks [9, 10]. Both have also been shown to predict future cognitive decline [11–13]. Nonetheless, amyloid PET and CSF Aβ are used infrequently in clinical practice [14]. Amyloid PET is cost-intensive and dependent on radioactive tracers, and lumbar punctures to obtain CSF can cause minor complications such as back pain as well as more severe complications such as spinal hematoma [15], and they can cause psychological distress [16]. In contrast, blood collection is well tolerated, making measurement of blood Aβ for estimation of brain Aβ burden suitable for large-scale application in routine diagnostics. For instance, with sufficient sensitivity and specificity, analysis of blood Aβ it could serve as a first-step screening tool for selection of patients for more cost-intensive and high-risk diagnostic measures. Ultimately, blood Aβ analysis might have a comparable impact on diagnostic procedures as amyloid PET [6, 17] and CSF Aβ analysis [18, 19], and even more so due to a projected wider uptake.

However, measuring Aβ in blood is inherently difficult [20, 21]. Plasma concentrations of Aβare around 10-fold lower than in the CSF, whereas the total protein content is 10-fold higher [22], causing technical difficulties. Sophisticated methods for Aβ analysis have been developed in recent years, but results from the first diagnostic and epidemiological applications of these methods have been inconsistent. A number of studies have found an association of lower plasma Aβ concentrations (thought to reflect a greater brain Aβ burden) with more severe neuropsychological deficits [23, 24], with an increased risk of developing AD [25, 26], and with amyloid-positive PET [8, 27, 28] or amyloid-abnormal CSF [29, 30] as current gold standards for AD diagnosis. Others report results in the opposite direction [31–34] and null findings, too, are frequent [35–37]. It has been suggested that one reason for this inconsistency may lie in between-study differences in the cognitive profiles of study samples given that plasma Aβ levels follow a complex temporal trajectory: concentrations increase with age [22, 38] but, potentially due to brain Aβ aggregation, reduce in symptomatic stages of AD [22, 32]. The inconsistent research findings may additionally stem from variations in Aβ measurement methods. Of note, in-house methods with limited feasibility for upscaling are frequent [27] but hinder clinical application which is dependent on high-throughput methods.

Here, we determined the ability of plasma Aβ concentration to discriminate between AD patients and biomarker-healthy, non-diseased controls. We used a recently established, commercially available and high-throughput plasma Aβ assay that to our knowledge has never been evaluated independently of the manufacturer. We hypothesized that Aβ concentration was lower in the plasma of AD patients than in controls. The ratio of plasma A$\beta_{42/40}$ served as the main biomarker of interest as it reflects the more pathological of the amyloid species (Aβ_{42}) [39] with individual differences in overall Aβ production (Aβ_{40}) accounted for.

MATERIALS AND METHOD

Study design and sample size calculation

In a case-control study design, Aβ was measured in plasma samples previously stored at a biobank for AD patients and biomarker-healthy, non-diseased controls. With a two-tailed analysis and a power of 80%, 47 observations were required per group (total N = 94) to detect statistically significant group differences in plasma Aβ (expected effect size $d = 0.6$). To account for occasional technical difficulty in biomarker measurement, we arrived at a target sample size of N = 100. The study complied with the Declaration of Helsinki.

Study sample

Cases in our study included patients with AD, who were diagnosed during a visit of a memory clinic in Berlin, Germany, between 2014 and 2018. Controls were selected among individuals who presented to the clinic with memory concerns during the same time period, but who were otherwise neurobiologically healthy and consequently did not receive a diagnosis of AD or other forms of dementia. The memory clinic is part of the German Dementia Competence Network (DCN). AD patients and controls were not matched.

Clinical examinations

All participants underwent a thorough and identical clinical examination that included lumbar puncture for CSF collection and collection of blood. Participants were not required to fast. Plasma samples were stored at a biobank at –80°C for future analysis. CSF was collected into polypropylene tubes and frozen at –80°C according to standard operating procedures detailed elsewhere [40]. Total tau (t-tau), $A\beta_{40}$, and $A\beta_{42}$ in CSF were measured in Mesoscale System (MSD) immunoassays (Mesoscale Discovery, Gaithersburg, MD, USA) at a laboratory adjacent to the clinic site. For t-tau, the MSD MS6000 Phospho-, Total Tau Kit was used; for $A\beta_{40}$ and $A\beta_{42}$, the MSD MS6000 Human (6E10) Aβ3-Plex Kit was used [41]. The ratio $A\beta_{42/40}$ was calculated. Consenting participants were genotyped for apolipoprotein (*APOE*) status. '*APOE* ε4' was defined as presence of at least one ε4 allele. Participants additionally underwent computed tomography (CT) and/or magnetic resonance imaging (MRI) on a separate visit. Neuropsychological testing was mainly based on the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) recommendations. Tests included the Mini-Mental State Examination (MMSE) [42], Boston Naming, verbal fluency (category), figure copying, and word list recall. The battery was supplemented by the Clock Drawing test as a screening tool for dementia and Trail-Making Tests A and B (TMT-A; TMT-B) as measures of processing speed and executive function. The Logical Memory subtest of the Wechsler Memory Scale 4th edition assessed verbal memory and included immediate and delayed recall.

Clinical diagnosis of cases and controls

AD was diagnosed according to DSM-V criteria in a consensus conference involving psychiatrists, physicians and neuropsychologists from a combination of results from the neuropsychological examination, CSF biomarker analysis and brain imaging data. Diagnostic confidence was exceptionally high compared to non-specialized centers, as AD patients were selected for enrollment into clinical trials at the memory clinic. AD patients were thus considered both clinically and neurobiologically diseased, whereas the control group was considered biomarker-healthy. Plasma Aβ concentration was unknown at the time of diagnosis.

Aβ in plasma

Plasma samples were extracted from the biobank in 2018 and shipped to an analysis laboratory (Araclon Biotech Ltd., Zaragoza, Spain) for measurement of total $A\beta_{40}$ (referred to as $A\beta_{40}$ hereafter) and total $A\beta_{42}$ (referred to as $A\beta_{42}$ hereafter) through enzyme-linked immunosorbent assay (ELISA) technology using the ABtest40 and ABtest42 test kits (Araclon Biotech Ltd., Zaragoza, Spain) [43]. The laboratory was blinded to our research question and to patient characteristics. Of N = 100, the analysis produced data on $A\beta_{40}$ for $n = 97$ ($n = 50$ controls; $n = 47$ patients) and on $A\beta_{42}$ for $n = 93$ participants ($n = 49$ controls; $n = 44$ patients). Intra-assay coefficient of variation (CV) was 4.5% for $A\beta_{40}$ and 15.8% for $A\beta_{42}$. Inter-assay CV was 3.7% for $A\beta_{40}$ and 5.0% for $A\beta_{42}$. The ratio $A\beta_{42/40}$ was calculated for $n = 49$ controls and $n = 44$ patients, and served as the main plasma biomarker of interest.

Table 1
Sociodemographic, clinical, and cognitive characteristics in controls and AD patients

	Controls ($n = 50$)	AD ($n = 50$)	p
Age, years, mean ± SD	65.82 ± 8.96	71.30 ± 7.42	0.001
Female sex, *n* (%)	26 (52.0%)	25 (50.0%)	0.841
Years of education*, mean ± SD	14.27 ± 2.98	13.46 ± 2.96	0.187
≥1 *APOE* ε4 allele**, *n* (%)	11 (26.8%)	33 (71.7%)	<0.001

Results from *t*-tests, Mann-Whitney tests or χ^2 tests. *total $n = 94$. **total $n = 87$.

Table 2
Correlations of plasma Aβ with CSF Aβ

	CSF $A\beta_{40}$	CSF $A\beta_{42}$	CSF $A\beta_{42/40}$
Total sample			
Plasma $A\beta_{40}$	0.10 (0.350)	–0.05 (0.637)	–0.14 (0.171)
Plasma $A\beta_{42}$	0.24 (0.023)	0.26 (0.012)	0.13 (0.220)
Plasma $A\beta_{42/40}$	0.23 (0.029)	0.33 (0.001)	0.22 (0.037)
Controls			
Plasma $A\beta_{40}$	–0.05 (0.741)	–0.07 (0.636)	–0.11 (0.482)
Plasma $A\beta_{42}$	0.15 (0.332)	0.31 (0.043)	0.24 (0.112)
Plasma $A\beta_{42/40}$	0.15 (0.334)	0.34 (0.026)	0.29 (0.052)
AD			
Plasma $A\beta_{40}$	0.22 (0.131)	–0.01 (0.953)	–0.32 (0.025)
Plasma $A\beta_{42}$	0.27 (0.057)	0.23 (0.116)	–0.04 (0.761)
Plasma $A\beta_{42/40}$	0.26 (0.071)	0.28 (0.053)	0.07 (0.636)

Spearman's rho (*p*-value). CSF; cerebrospinal fluid. $n = 44$ to 97.

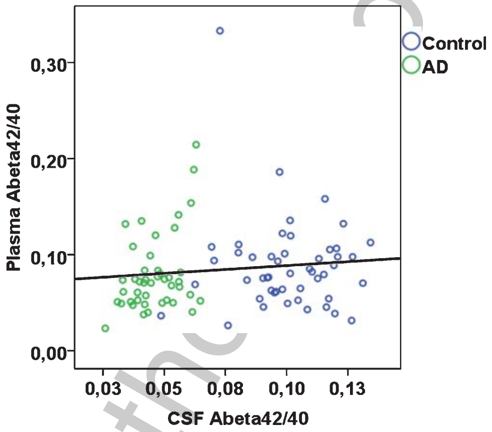

Fig. 1. Plasma $A\beta_{42/40}$ plotted against CSF $A\beta_{42/40}$ according to diagnostic group (rho = 0.22; $p = 0.037$ across total sample).

Statistical analysis

Differences between AD patients and controls in terms of sociodemographics, frequency of the *APOE* ε4 allele and CSF biomarkers were compared using independent samples *t*-tests, Mann-Whitney tests or χ^2 tests. In the total sample, associations of plasma $A\beta_{42}$, $A\beta_{40}$, and $A\beta_{42/40}$ with CSF $A\beta_{42}$, $A\beta_{40}$, and $A\beta_{42/40}$ were determined using univariate Spearman correlation analyses.

Plasma $A\beta_{42}$, $A\beta_{40}$, and $A\beta_{42/40}$ were compared between AD patients and controls and between carriers of the *APOE* ε4 allele (≥1 allele) and non-carriers using Mann-Whitney tests.

The diagnostic accuracy of plasma $A\beta_{40}$, plasma $A\beta_{42}$, and plasma $A\beta_{42/40}$ was determined in receiver operating characteristic (ROC) analyses to calculate areas under the curve (AUCs) with the outcome AD patients versus controls. ROC analyses were performed separately for age, *APOE* ε4, plasma $A\beta_{40}$, plasma $A\beta_{42}$, and plasma $A\beta_{42/40}$, and for selected combinations of these predictors. For plasma $A\beta_{42/40}$ as the main biomarker of interest, the optimal cut-off and associated sensitivity, specificity and Youden's index [44], as well as positive predictive value (PPV) and negative predictive value (NPV) were calculated. Analyses were performed in SPSS (Version 18, IBM SPSS, Chicago, Illinois) and R.

RESULTS

Sample characteristics

AD patients were statistically significantly older and were more likely to have at least one *APOE* ε4 allele compared with controls (Table 1). CSF $A\beta_{42}$, CSF $A\beta_{42/40}$, CSF t-tau, and neuropsychological test results were in line according to diagnostic group (data not shown).

Associations of plasma Aβ with CSF biomarkers

In the total sample, plasma $A\beta_{40}$ was significantly positively correlated with plasma $A\beta_{42}$ (Spearman's rho 0.46; $p < 0.001$). Plasma $A\beta_{42}$ and plasma $A\beta_{42/40}$ were each significantly, albeit weakly, positively correlated with CSF $A\beta_{40}$, $A\beta_{42}$, and $A\beta_{42/40}$ (Table 2, Fig. 1). Plasma $A\beta_{40}$ was not significantly correlated with CSF $A\beta_{40}$, $A\beta_{42}$, or $A\beta_{42/40}$. When stratified by case-control status, in controls, plasma $A\beta_{42}$ and $A\beta_{42/40}$ were significantly correlated with CSF$A\beta_{42}$, whereas none of the remaining correlations were statistically significant (Table 2). In AD patients, plasma $A\beta_{40}$ was significantly inversely correlated with CSF $A\beta_{42}/_{40}$, but none of the remaining

Table 3
Plasma Aβ concentration in controls and AD patients

	Controls			AD			Mann-Whitney
	Min	Max	Median (interquartile range)	Min	Max	Median (interquartile range)	p
Plasma $A\beta_{40}$ (pg/mL)	129	415	237 (212 – 264)	109	376	237 (216 – 267)	0.809
Plasma $A\beta_{42}$ (pg/mL)	5.8	88.2	19.9 (13.2 – 26.4)	4.8	53.3	17.4 (12.1 – 27.1)	0.404
Plasma $A\beta_{42/40}$	0.03	0.33	0.08 (0.06 – 0.10)	0.02	0.21	0.07 (0.05 – 0.08)	0.173

$n = 93$ to 97.

correlations were statistically significant. $A\beta_{40}$ and $A\beta_{42}$ concentrations were overall around 30-fold and 26-fold higher in CSF than in plasma respectively (CSF $A\beta_{40}$, median 6,727 pg/mL in controls and 7,345 pg/mL in AD patients; CSF $A\beta_{42}$, median 677 pg/mL in controls and 310 pg/mL in AD patients; plasma $A\beta_{40}$, median 237 pg/mL in controls and 237 pg/mL in AD patients; plasma $A\beta_{42}$, median 20 pg/mL in controls and 17 pg/mL in AD patients).

Plasma Aβ in AD patients and controls

Plasma $A\beta_{40}$, plasma $A\beta_{42}$, and plasma $A\beta_{42/40}$ were not significantly different between AD patients and controls (Table 3; Supplementary Figure 1).

In ROC analyses, the area under the curve (AUC) was 0.51 (95% CI 0.40, 0.63) for plasma $A\beta_{40}$, 0.55 (95% CI 0.43, 0.67) for plasma $A\beta_{42}$, and 0.58; (95% CI 0.46, 0.70) for plasma $A\beta_{42/40}$, indicating that the ability to discriminate between AD patients and controls based solely on these plasma markers is poor. In comparison, the AUCs based on age only or based on *APOE* ε4 only were 0.70 (95% CI 0.59, 0.80) and 0.73 (95% CI 0.62, 0.83) respectively. When plasma $A\beta_{42/40}$ as the main biomarker of interest was added to these models, the AUCs did not change (age and $A\beta_{42/40}$, AUC, 0.70; 95% CI 0.59, 0.80; *APOE* ε4 and $A\beta_{42/40}$, AUC, 0.76; 95% CI 0.65, 0.87). The AUC for a model that included age and *APOE* ε4 was 0.79 (95% CI 0.69, 0.89). When plasma $A\beta_{42/40}$ was added to this model, the AUC was 0.80 (95% CI 0.70, 0.91; see Fig. 2 for selected biomarker combinations). Taken together, these data show that plasma $A\beta_{42/40}$ did not contribute to the ability to discriminate between AD patients and controls.

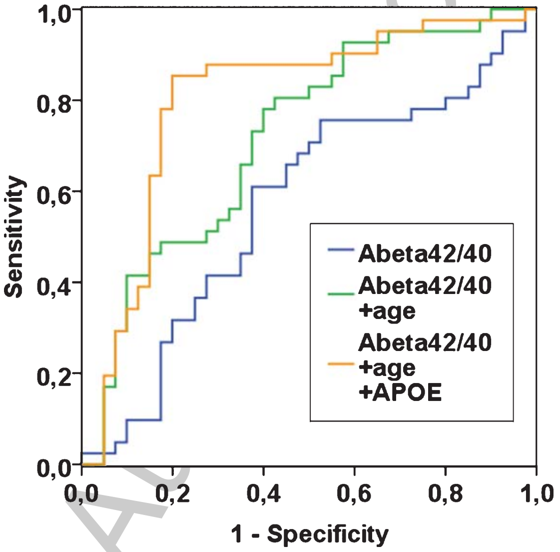

Fig. 2. ROC curves for plasma $A\beta_{42/40}$ alone, plasma $A\beta_{42/40}$ with age, and plasma $A\beta_{42/40}$ with age and *APOE* ε4, in $n = 81$ patients with complete data. Outcome is "AD" with reference "controls". To create a ROC curve *above* the reference line, $A\beta_{42/40}$ was transformed to "1- $A\beta_{42/40}$" (blue line).

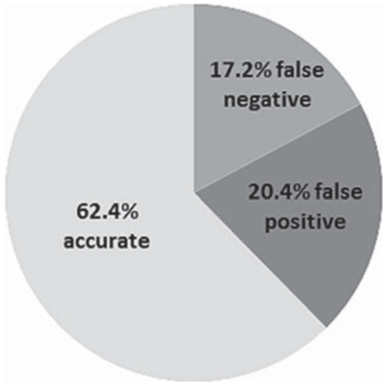

Fig. 3. Diagnostic accuracy of plasma $A\beta_{42/40}$ at optimal cut-point (total $n = 93$).

Based on the ROC analysis, we determined a cut-point of 0.076 for a plasma $A\beta_{42/40}$ concentration with a maximum in both sensitivity and specificity. However, at this cut-point, plasma $A\beta_{42/40}$ had low sensitivity (61.2%) and specificity (63.6%; Youden's index 0.25) and correctly identified 28 of the 44 AD patients and 30 of the 49 controls. Sixteen AD patients were misclassified as controls (false negatives) and 19 controls were misclassified as AD cases (false positives). Plasma $A\beta_{42/40}$ at this cut-point had a positive predictive value (PPV) of 59.6% and a negative predictive value (NPV) of 65.2%. We applied further experimental cut-points to plasma A$\beta_{42/40}$ in *post-hoc* analyses to reduce the number of false positives, but all resulted in low diagnostic accuracy (Supplementary Table 1).

Plasma Aβ in non-carriers and carriers of APOE ε4

Across the full study sample, plasma Aβ_{40} (median 238.1 pg/mL versus 228.9 pg/mL; $p = 0.466$), plasma Aβ_{42} (median 21.3 pg/mL versus 16.7 pg/mL; $p = 0.251$), and plasma A$\beta_{42/40}$ (median 0.08 versus 0.07; $p = 0.212$) were each not statistically different in non-carriers and in carriers of the *APOE* ε4 allele, respectively.

DISCUSSION

Blood-based biomarkers of AD have the potential to revolutionize AD diagnostic procedures. Here, in a unique case-control study with exceptionally detailed assessments that included a neuropsychological examination, CSF biomarker analysis and brain imaging as gold standards in AD diagnosis, we found that plasma Aβ was not able to satisfactorily distinguish AD patients well from biomarker-healthy, non-diseased controls. Sensitivity and specificity based on plasma A$\beta_{42/40}$ levels alone were low and not indicative of a diagnostic test with scope for clinical application. Only 64% of AD patients were correctly detected based on plasma A$\beta_{42/40}$.

Plasma A$\beta_{42/40}$ was only weakly but significantly correlated with CSF A$\beta_{42/40}$. This finding is in agreement with several previous investigations [27, 30, 45] though the strength of this correlation was markedly smaller in our sample. CSF Aβ itself comes with measurement difficulties [12, 41], but based on its established function as a gold-standard in AD diagnosis [7], combined with the fact that CSF Aβ was measured using the Mesoscale System (MSD) [41], our finding suggests that plasma Aβ reflected brain Aβ burden only to some limited extent. In contrast to several previous studies comparing AD patients and controls [22, 30, 33], we did not find evidence of reduced plasma Aβ in AD patients. Several reasons may underlie this null result. Firstly, plasma Aβ may in fact be unrelated to AD status. Secondly, due to the 'noise' associated with peripheral production and clearance of Aβ [7, 46], effect sizes may have been too small to detect differences in plasma Aβ between AD cases and controls. Thirdly, measurement error from varying time lapse before freezing, from varying storage time of plasma samples, uncontrolled fasting status and time of day, and/or plasma Aβ analysis itself may have affected results. To our knowledge, we provide the first application of a novel, high-throughput technique for plasma Aβ analysis [43] to a research study that was run independently of the manufacturer. The assay had previously been used in four studies [28, 45, 47, 48] of which one [47] used a subsample of an earlier investigation [45]. In sum, the studies found correlations of plasma A$\beta_{42/40}$ with CSF A$\beta_{42/40}$ [45], as well as associations of low plasma Aβ with presence of [28, 45, 47, 48] and an increased 3-year accumulation of Aβ burden on PET [28]. Further, a lower plasma A$\beta_{42/40}$ was reported in patients with mild cognitive impairment (MCI) compared with cognitively normal individuals; additionally MCI patients with lower plasma A$\beta_{42/40}$ were at increased risk of 2-year conversion to AD [45]. In two of the four studies sensitivity, specificity and AUC of plasma A$\beta_{42/40}$ for Aβ-positive PET were more promising [28, 47] compared with our own analysis. Yet, in one of these studies, when the full cohort was analyzed rather than a subsample, plasma A$\beta_{42/40}$ performed poorly in discriminating MCI from cognitively normal individuals [47], mirroring our own results comparing AD patients with controls. A cross-sectional analysis of people with subjective cognitive decline, too, found low AUC and low specificity of the compound alone for Aβ-positive PET [48]. The final, prospective study on 2-year conversion from MCI to AD only reported a fully adjusted model that included plasma Aβ as well as age, *APOE*, and education [45] so that the added benefit of plasma Aβ is difficult to evaluate.

The discrepancy of our results from many of the manufacturer-funded results remains unclear. AD diagnosis in our study was based on neuropsychological test scores, CSF biomarker analysis and brain imaging results, and so we are confident that we have been successful in selecting neurobiologically diseased AD patients and neurobiologically healthy controls. That, combined with recent reports of acceptable diagnostic performance of plasma Aβ when measured using different high-throughput techniques [8, 30], leads us to suggest that the assay may have failed to produce accurate plasma Aβ data. Technical issues in our study were also indicated by the fact that plasma Aβ relative to CSF Aβ was far lower than the expected 10-fold difference [22, 27]; our >20-fold difference mirrored that of another investigation which also found no association of plasma Aβ with cognitive status [36].

Aβ metabolism is strongly influenced by the *APOE* protein. *APOE* is involved in cholesterol transport in the brain as well as in Aβ production and clearance, and binds to Aβ in CSF [49]. The *APOE* gene occurs in three polymorphisms ($\varepsilon 2$; $\varepsilon 3$; $\varepsilon 4$) of which the *APOE* $\varepsilon 4$ allele is a strong predictor of late-onset AD [49]. Carriers of *APOE* $\varepsilon 4$ have greater brain Aβ burden imaged on PET [50, 51] and lower CSF Aβ compared with non-carriers [51–53]. Several population-based cohort studies also point to lower plasma Aβ in carriers [8, 54], but we and others that have used the same assay [48] found no such evidence. Effect sizes speak against low statistical power as the root cause, corroborating plasma Aβ—at least when measured using the present assay—as a peripheral biomarker with little scope for capturing AD-type neuropathological burden.

Detailed characterization of participants using genetic, CSF biomarker, and brain imaging data is a strength of our study, but some limitations must be considered. Due to small sample size, our analyses were underpowered to detect more subtle group differences in plasma Aβ. For instance, we only had a two-tailed power of around 30% to detect a small group difference. Nonetheless, the anticipated large effect had been reasonable given that we had selected distinct groups of neurobiologically confirmed AD patients and biomarker-healthy controls, and a large group difference is also a prerequisite for implementation of a diagnostic test in clinical settings, which is at the core of plasma Aβ research. Time interval between plasma collection and freezing, fasting status of participants, and time of day had not been fully standardized and this may have contributed to measurement error. However, recent evidence suggests that plasma Aβ concentration is relatively immune to these factors [55, 56]. Plasma samples had been stored for between 7 months and 4 years prior to extraction from the biobank for Aβ analysis. Though we are not aware of studies that have assessed an influence of storage time on plasma Aβ, we have no reason to believe it may be less stable compared with Aβ in frozen CSF for which we have previously demonstrated long-term stability [41]. Finally, we did not consider AD staging and included both early-onset and late-onset AD in our sample (6 AD patients were <65 years old). All of these factors may have played a role in generating a large range of plasma Aβ measurements thus may have contributed to our null findings. At the same time, with exception of storage time, they are all part of a real-world setting which any diagnostic test for AD must be able to withstand for implementation in the clinic.

In a recent analysis of a mostly cognitively unimpaired older cohort, baseline plasma Aβ measured with immunoprecipitation and liquid chromatography-mass spectrometry assay predicted conversion from amyloid-negative to amyloid-positive PET during a 4-year follow-up. Results indicated that implementing the plasma Aβ test in clinical practice would reduce PET scans by 62% [8]. Further studies in this direction are needed in spite of the present null findings to fully determine the diagnostic value of plasma Aβ and for head-to-head comparison with other biomarkers of brain Aβ burden. For instance, plasma t-tau [55] and plasma neurofilament light (NFL) [56] have recently been reported as predictive of AD in three independent cohorts. Serum NFL has also been shown to be elevated in patients with familial AD [57] and to predict their rate of cognitive decline [58]. A substantial proportion of patients with MCI or dementia with potential AD etiology appears to be misdiagnosed once followed up with amyloid PET [17], and plasma biomarkers could be evaluated as follow-up diagnostic tools. For Aβ in particular, different methods for measuring plasma concentration of the protein should be compared with one another and with a recently developed structure-based approach that measures misfolded Aβ [59]. The goal should be to standardize analysis methods across labs. Here, we did not create CSF/plasma Aβ ratios, because AD diagnosis was based in part on CSF Aβ so that such ratios would have led to circular arguments, but future studies could explore their usefulness (e.g., [36]) as well as ratios combining several plasma biomarkers (e.g., [60]).

Any biomarker of AD needs to offer scope for large-scale application. Here, we used a recently established, commercially available, high-throughput technique and found that plasma Aβ correlated weakly with CSF Aβ and was unable to distinguish between AD patients and biomarker-healthy, non-diseased controls. Plasma Aβ at the cut-point with maximum sensitivity and specificity identified 38% of our sample incorrectly as false positive or false negative. We thus deem its performance inacceptable. Diagnostic confidence for clinical AD diagnosis can be considered exceptionally high in our study because AD diagnosis was based on a combination of detailed diagnostic procedures that are not routinely applied in clinical practice outside of specialized memory clinics. Plasma Aβ measured with the present assay can therefore be expected to be even less adequate in more diverse, 'real-world' samples that include 'gray zones' and prodromal stages of AD. Nonetheless, plasma Aβ may well be a useful biomarker of age-related cognitive impairment in population-based epidemiological research studies aimed at risk stratification and elucidation of pathophysiological mechanisms underlying impairment. The utility of the blood Aβ assay presented here is yet to be determined in that context.

Overall, we conclude that as the evidence currently stands, the plasma Aβ concentration assay may have limited ability to distinguish between AD cases and biomarker-healthy, non-diseased controls. Its evaluation in larger samples and, potentially, a shift of focus toward other blood-based biomarkers and/or efforts for technological advancement of plasma Aβ measurement, which has recently gained momentum [30], are warranted.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-200046.

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