Plasma concentrations of free amyloid β cannot predict the development of Alzheimer's disease

Hugo Lövheim[1,*], Fredrik Elgh[2], Anders Johansson[3,4], Henrik Zetterberg[5,6], Kaj Blennow[5], Göran Hallmans[4], and Sture Eriksson[1,4]

[1]Department of Community Medicine and Rehabilitation, Geriatric Medicine, Umeå University, Umeå, Sweden

[2]Department of Clinical Microbiology, Virology, Umeå University, Umeå, Sweden

[3]Department of Odontology, Umeå University, Umeå, Sweden

[4]Departmenty of Biobank Research and Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, Umeå, Sweden

[5]Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[6]Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK

Corresponding Author:

H. Lövheim, Department of Community Medicine and Rehabilitation, Geriatric Medicine, Umeå University, SE-901 85 Umeå, Sweden.

Telephone: +46 90 785 88 59. Fax: +46 90 13 06 23. E-mail: hugo.lovheim@umu.se

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ABSTRACT

Background: Biomarkers that identify individuals at risk of Alzheimer's disease (AD) development would be highly valuable. Plasma concentration of amyloid β (Aβ) – central in the pathogenesis of AD – is a logical candidate, but studies to date have produced conflicting results on its utility.

Methods: Plasma samples from 339 preclinical AD cases (76.4% women, mean age 61.3 years) and 339 age- and sex-matched dementia-free controls, taken an average of 9.4 years before AD diagnosis, were analyzed using Luminex xMAP technology and INNOBIA plasma Aβ form assays to determine plasma concentrations of free $A\beta_{40}$ and $A\beta_{42}$.

Results: Plasma concentrations of free $A\beta_{40}$ and $A\beta_{42}$ did not differ between preclinical AD cases and dementia-free controls, in the full sample or in sub-groups defined according to sex and age group (<60 and ≥60 years). The interval between sampling and AD diagnosis did not affect the results. Aβ concentrations did not change in the years preceding AD diagnosis among individuals for whom longitudinal samples were available.

Conclusion: Plasma concentrations of free Aβ could not predict the development of clinical AD, and Aβ concentrations did not change in the years preceding AD diagnosis in this sample. These results indicate that free plasma Aβ is not a useful biomarker for the identification of individuals at risk of developing clinical AD.

Keywords: Plasma amyloid β; Aβ; Alzheimer's disease; Dementia; Preclinical Alzheimer's disease; Biomarker

Research in context

Systematic review

The Pubmed database was searched using relevant keywords to identify previous research. Previous research suggested a possibly predictive value of plasma Aβ measurement for the identification of individuals at risk of AD development, but results were conflicting.

Interpretation

Our results indicate that free plasma Aβ cannot serve as a biomarker for the identification of individuals at risk of developing clinical AD.

Future directions

As free plasma Aβ has a low predictive value, future research should seek to identify other plasma biomarkers for the risk of AD.

Introduction

Several possibly disease-modifying treatments for Alzheimer's disease (AD) are currently being tested in phase 3 clinical trials. The identification of persons at risk of developing AD before symptom onset may thus soon become clinically relevant [1].

Amyloid pathology is present several years before the clinical onset of AD [2]. The analysis of peptide amyloid β (Aβ) 1-42 in cerebrospinal fluid (CSF) has good diagnostic properties for AD in the clinical and prodromal disease stages, as has the measurements of amyloid burden with positron emission tomography (PET) [3, 4]. These two measures are highly concordant [4], and new AD criteria highlight their importance in directly reflecting amyloid pathology in AD [5]. However, neither CSF sampling through lumbar puncture nor amyloid PET investigation is feasible for screening to identify individuals at risk of developing AD in the general population. A blood test would be much practical in this context.

Plasma Aβ can be measured reliably using current assays, and given the firm relation between CSF Aβ and AD pathology, it has been investigated as a possible biomarker for AD [6-10]. Findings, however, are conflicting. Results of some studies have indicated that lower $A\beta_{42}$ concentrations or $A\beta_{42}$:$A\beta_{40}$ ratios are associated with a significantly increased risk of AD, whereas other studies have found the reverse relation or failed to identify any association [7-10]. Funnel plots from a meta-analysis suggest the presence of publication bias toward studies showing a relationship between $A\beta_{42}$ and AD [7].

The aim of the present nested case-control study was to investigate the association between free plasma Aβ and AD in a large sample of persons with preclinical AD and closely matched dementia-free controls, using plasma samples taken several years before AD diagnosis.

Methods

2.1 Participants

This nested case-control study is part of the Consortium on Health and Ageing: Network of Cohorts in Europe and the United States (CHANCES) project [11]. Participants were selected using a computerized procedure. Individuals diagnosed with AD at the University Hospital Memory Clinic, Umeå, Sweden, for whom stored EDTA plasma samples collected before clinical disease onset were available in the Medical Biobank of Umeå (The Northern Sweden Health and Disease Study Cohort [12] were identified. Samples were selected when suitable age-, sex-, cohort- and sampling date-matched dementia-free controls could be identified. Specificera pre-analytics (är det EDTA plasma, hur togs proverna [på morgonen eller närsomhelst, fastande eller inte], hur centrifugerades och alikvoterades proverna?)

2.2 Confirmation of AD diagnoses

All AD cases had been examined and diagnosed at the University Hospital Memory Clinic in Umeå, Sweden. These diagnoses were the result of regular clinical investigations and were not related to participation in any study cohort. The diagnosis of AD was supported by typical symptoms of progressive cognitive failure; physical examination findings; results of cognitive screening tests, such as the Mini-Mental State Examination [13]; results of standard blood tests; and findings of examination using at least one brain imaging technique (x-ray computed tomography, magnetic resonance tomography, ^{99m}Tc single-photon emission computed tomography and/or fluorodeoxyglucose PET). In many cases, the diagnoses were further supported by findings from neuropsychological examination and CSF analysis. An experienced specialist in psychogeriatric medicine assessed the accuracy of AD diagnoses by

thorough review of medical records before the final inclusion of cases in the data set. All AD cases were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition (DSM-IV) criteria [14], and clinical diagnoses were also compatible with the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [15].

The dementia-free status of matched controls was checked using Swedish diagnosis registries, and persons were excluded when the diagnosis of any dementia disorder was found. The Swedish Death Registry was used to confirm that all controls were alive on the date of AD diagnosis for corresponding cases.

2.3 Plasma analyses

Plasma $A\beta_{40}$ and $A\beta_{42}$ concentrations were measured using Luminex xMAP technology and the INNOBIA plasma Aβ forms assays (Innogenetics, Ghent, Belgium), as described previously [16]. As the measurements were performed on neat EDTA plasma without any pre-treatment, we measured the pool of Aβ for which epitopes were available to the antibodies (free Aβ). Plasma Aβ concentrations are presented in nanograms per liter. The measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data.

2.4 Statistical analysis

Conditional logistic regression was used to test whether differences in Aβ variables between AD cases and dementia-free controls were associated with AD, in the full sample and in the following sub-groups: men and women, participants aged ≥60 and <60 years at the time of

plasma sampling, and quartiles defined according to the interval between sampling and AD diagnosis. Multiple linear regression was used to investigate the effects of age and sex on plasma Aβ concentrations. A linear mixed model was used to analyze changes in Aβ level between first sample collection and AD diagnosis among cases. $P < 0.05$ was considered to be statistically significant. SPSS software (version 22.0 for Macintosh; IBM Corporation, Armonk, NY, USA) was used for statistical calculations.

Results

The selection procedure resulted in the identification of 339 pairs of AD cases and dementia-free controls (80 men and 259 women per group) that could be included in the present analysis. Matching was perfect with regard to sex and Medical Biobank sub-cohort. Samples from three Medical Biobank cohorts were included in the present study; the mammography screening cohort [n = 156 pairs (46.0%)], the Multinational MONItoring of trends and determinants in CArdiovascular disease (MONICA) project screening cohort [n = 3 pairs (0.9%)], and the Västerbotten Intervention Programme (VIP) cohort [n = 180 pairs (53.1%)] [12]. For some of the AD cases, additional plasma samples (n = 1, 89 persons; n = 2 , 45 persons; n = 3, 13 persons; $n \geq 4$, 3 persons) were available and were obtained from the Medical Biobank. These samples had been collected between the time of the first (matched) sample collection and the diagnosis date. A total of 570 plasma samples, including the first sample, were available for analysis from the AD cases.

Sampling dates ranged from 12 November 1986 to 2 March 2006, and AD diagnosis dates ranged from 1 August 1995 to 10 March 2010. The mean difference in age between cases and controls was 0.08 ± 0.81 years, and the mean difference in sampling date was 0.49 ± 53.4 days. Mean ages of cases and controls at the time of plasma sampling were 61.3 ± 5.6 and 61.2 ± 5.6 years respectively. Women were older than men in both groups (cases, 62.3 ± 5.3

vs. 58.1 ± 5.3 years; controls, 62.3 ± 5.3 *vs*. 57.9 ± 5.2 years; both $p < 0.001$). For AD cases, the mean age at diagnosis was 70.8 ± 6.4 years and the mean interval between plasma sampling and diagnosis was 9.4 ± 4.0 (range 0.2 to 20.7) years.

Plasma Aβ measurements for preclinical AD cases and dementia-free controls are shown in Table 1. Results of multiple logistic regressions analyses of the effects of age and sex on free Aβ concentrations are presented in Table 2. Among AD cases, greater age was associated significantly with higher concentrations of free plasma $A\beta_{42}$; among controls, women had significantly higher concentrations of free $A\beta_{40}$ than did men. Plasma concentrations of free $A\beta_{40}$ and $A\beta_{42}$ were correlated significantly among cases and controls (cases: 0.375, $p < 0.001$; controls: 0.492, $p < 0.001$).

Conditional logistic regression revealed no association between the difference in plasma concentrations of free Aβ between cases and controls and AD (Table 1). In addition, sub-group analyses showed no such association according to sex and age (Table 3).

No Aβ variable was correlated significantly with the interval between plasma sampling and AD diagnosis (Table 4). Analysis of quartiles defined according to this interval also showed no significant association with AD (Table 5).

The analysis of changes in plasma Aβ markers with linear mixed models included two or more samples from 150 AD cases. No significant change in markers in the years prior to diagnosis was detected ($A\beta_{40}$: +0.40 units/year, $p = 0.317$; $A\beta_{42}$: +0.11 units/year, $p = 0.420$).

Discussion

In this sample of persons with preclinical AD and closely matched dementia-free controls, plasma concentrations of free Aβ were not associated with the risk of AD development. Among those who developed AD, repeated plasma sampling samples in the years prior to diagnosis detected no changes in plasma concentrations of free Aβ.

The strengths of the present study include the analysis of a large sample of preclinical AD cases, identified by cross-referencing of a population-based biobank with subsequent memory clinic records, the high clinical diagnostic quality of AD diagnoses, and the use of closely matched dementia-free controls. Plasma samples were taken an average 9.4 years before the AD diagnosis.

This study adds further evidence that free plasma Aβ is not a reliable measure of AD risk, and thus that it should not be used as an AD biomarker. These findings are in line with previous reports of the low predictive value of plasma Aβ [6, 9, 17]. Previous studies have shown a low degree of correlation between plasma and CSF concentrations of Aβ [16], and have demonstrated that plasma Aβ does not reflect brain Aβ deposition [18]. These results may explain the lack of association found in this and other studies.

One possible explanation for the weak association between plasma concentration of free Aβ and AD is that the Aβ peptides measured in plasma primarily represent production of Aβ from extra-cerebral cell types. Platelets may be a significant source of plasma Aβ [19], and fibroblasts, for example, have been found to produce Aβ upon cytomegalovirus infection [20].

The current results are limited to the value of plasma concentrations of free Aβ to predict the development of AD when measured in a preclinical phase. The results therefore do not

exclude the possibility that certain fractions of blood or plasma Aβ, or plasma Aβ in combination with other markers, could have a predictive value. Furthermore, the results do not exclude the possibility that plasma Aβ might have a diagnostic value in symptomatic phases of AD. These limitations must be taken into account when interpreting the results.

Conclusion

Plasma concentrations of free Aβ could not predict the development of clinical AD, and Aβ concentrations did not change in the years preceding AD diagnosis in this sample. These results indicate that free plasma Aβ is not a useful biomarker for the identification of individuals at risk of developing clinical AD.

Table 1

Conditional logistic regression of plasma concentrations of free amyloid β and Alzheimer's disease

Group	Alzheimer's disease cases	Dementia free controls	Odds ratio	95% CI	*p*-value
$pA\beta_{42}$, mean ± SD, [ng/L]	43.6 ± 13.1	44.6 ± 12.5	0.994	0.982 – 1.006	0.316
$pA\beta_{40}$, mean ± SD, [ng/L]	142.3 ± 36.3	143.9 ± 41.0	0.999	0.994 – 1.003	0.525
$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.325 ± 0.131	0.331 ± 0.129	0.664	0.190 – 2.325	0.522

Abbreviations: CI, confidence interval; pAβ, free plasma amyloid β peptide; SD, standard deviation.

Table 2

Multiple linear regression of plasma concentrations of free amyloid β

	Free plasma amyloid β 1-42		Free plasma amyloid β 1-40	
	β	*p*-value	β	*p*-value
Alzheimer's disease cases				
Age (years)	0.275	0.041	0.341	0.358
Female sex	0.910	0.605	8.597	0.079
Dementia free controls				
Age (years)	0.128	0.317	0.357	0.400
Female sex	3.838	0.023	5.100	0.360

Table 3

Conditional logistic regression of plasma concentrations of free amyloid β and Alzheimer's disease in different subgroups

Group	Alzheimer's disease cases	Dementia free controls	Odds ratio	95% CI	*p*-value
		Men, n=80			
$pA\beta_{42}$, mean ± SD, [ng/L]	42.1 ± 14.2	41.3 ± 12.4	1.005	0.981 – 1.029	0.700
$pA\beta_{40}$, mean ± SD, [ng/L]	134.6 ± 34.0	138.8 ± 38.5	0.996	0.986 – 1.006	0.396
$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.334 ± 0.154	0.312 ± 0.097	3.896	0.291 – 52.199	0.304
		Women, n=259			
$pA\beta_{42}$, mean ± SD, [ng/L]	44.1 ± 12.7	45.7 ± 12.3	0.990	0.977 –1.004	0.167
$pA\beta_{40}$, mean ± SD, [ng/L]	144.6 ± 36.7	145.5 ± 41.7	0.999	0.994 – 1.004	0.774
$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.322 ± 0.123	0.337 ± 0.136	0.343	0.075 – 1.571	0.168
		Age < 60 years, n=123			
$pA\beta_{42}$, mean ± SD, [ng/L]	41.2 ± 13.9	43.8 ± 13.7	0.987	0.969 – 1.005	0.160
$pA\beta_{40}$, mean ± SD, [ng/L]	135.5 ± 35.6	140.5 ± 39.5	0.996	0.988 – 1.003	0.253
$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.324 ± 0.137	0.333 ± 0.142	0.606	0.095 –3.865	0.596
		Age ≥ 60 years, n=216			
$pA\beta_{42}$, mean ± SD, [ng/L]	45.0 ± 12.4	45.1 ± 11.7	1.000	0.984 – 1.016	0.962
$pA\beta_{40}$, mean ± SD, [ng/L]	146.1 ± 36.2	145.9 ± 41.8	1.000	0.995 –1.006	0.950

$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.326 ± 0.127	0.330 ± 0.120	0.719	0.130 –3.958	0.719

Abbreviations: CI, confidence interval; pAβ, free plasma amyloid β peptide; SD, standard deviation.

Table 4

Correlation between length of time between plasma sampling and diagnosis and plasma concentrations of free amyloid β

	Correlation with length of time between plasma sampling and diagnosis	
	Correlation	*p*-value
Alzheimer's disease cases		
$pA\beta_{42}$	-0.027	0.623
$pA\beta_{40}$	-0.062	0.256
$pA\beta_{42}$:$A\beta_{40}$ ratio	0.037	0.492
Dementia free controls		
$pA\beta_{42}$	-0.061	0.260
$pA\beta_{40}$	-0.049	0.370
$pA\beta_{42}$:$A\beta_{40}$ ratio	-0.019	0.734

Abbreviations: pAβ, free plasma amyloid β peptide.

Table 5

Conditional logistic regression of plasma concentrations of free amyloid β and Alzheimer's disease, time between sampling and diagnosis in quartiles

Group	Alzheimer's disease cases	Dementia free controls	Odds ratio	95% CI	*p*-value
First quartile (78 to 2370 days), n=84					
$pA\beta_{42}$, mean ± SD, [ng/L]	42.6 ± 12.5	44.5 ± 14.3	0.988	0.964 – 1.012	0.326
$pA\beta_{40}$, mean ± SD, [ng/L]	144.2 ± 37.2	141.9 ± 37.6	1.002	0.993 – 1.011	0.662
$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.313 ± 0.131	0.331 ± 0.121	0.309	0.026 – 3.619	0.350
Second quartile (2371 to 3565 days), n=85					
$pA\beta_{42}$, mean ± SD, [ng/L]	44.1 ±12.1	46.5 ± 12.6	0.987	0.966 – 1.010	0.263
$pA\beta_{40}$, mean ± SD, [ng/L]	144.9 ± 36.9	153.8 ± 48.3	0.994	0.986 – 1.002	0.147
$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.324 ± 0.121	0.328 ± 0.137	0.679	0.040 – 11.459	0.788
Third quartile (3566 to 4461 days), n=85					
$pA\beta_{42}$, mean ± SD, [ng/L]	44.5 ± 14.1	45.1 ± 10.1	0.995	0.969 – 1.021	0.702
$pA\beta_{40}$, mean ± SD, [ng/L]	140.3 ± 36.6	144.0 ± 32.8	0.996	0.986 –1.006	0.423
$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.330 ± 0.111	0.333 ± 0.141	0.846	0.067 – 10.739	0.897
Fourth quartile (4462 to 7574 days), n=85					
$pA\beta_{42}$, mean ± SD, [ng/L]	43.3 ± 13.6	42.3 ± 12.5	1.006	0.983 – 1.030	0.595
$pA\beta_{40}$, mean ± SD, [ng/L]	139.6 ± 34.7	136.0 ± 42.3	1.003	0.994 – 1.013	0.472

$pA\beta_{42}$:$A\beta_{40}$ ratio, mean ± SD	0.334 ± 0.156	0.332 ± 0.114	1.085	0.107 – 10.956	0.945

Abbreviations: CI, confidence interval; pAβ, free plasma amyloid β peptide; SD, standard deviation.

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