Plasma Aβ42 and Aβ40 as markers of cognitive change in follow-up: a prospective, longitudinal, population-based cohort study

T T Seppälä, S-K Herukka, T Hänninen, S Tervo, M Hallikainen, H Soininen, T Pirttilä

Department of Neurology, Kuopio University Hospital, University of Eastern Finland, Kuopio, Finland

Correspondence to
Dr Tuula Pirttilä, Department of Neurology, Bldg 5, Kuopio University Hospital POB 1777, 70211, Kuopio, Finland; tuula.pirttila@kuh.fi

ABSTRACT

Background Single measurements of plasma Aβ are not useful in the diagnostics of Alzheimer's disease (AD). However, changes in plasma Aβ levels during repeated testing may be helpful in the prediction and evaluation of progression of the incipient AD or mild cognitive impairment.

Objective To examine the relation of baseline and serial plasma Aβ levels to cognitive change in follow-up.

Methods 269 subjects (52 cognitively impaired and 217 controls) from a population-based cohort were clinically followed up from 3 to 6 years. Serial plasma samples were available from 70 subjects who were followed up for 3 years and 43 subjects followed for 6 years. The plasma Aβ levels were measured using ELISA.

Results Subjects who declined cognitively during the follow-up had lower levels of plasma Aβ42 at the baseline. Plasma Aβ42 and the Aβ42/Aβ40 ratio decreased (−2.4 pg/ml for Aβ42 in 6 years) in those who declined in follow-up, whereas Aβ42 and the Aβ42/Aβ40 ratio increased in the subjects who remained cognitively stable or improved in follow-up. Subjects using acetylsalicylic acid, dipyridamole, antidiabetic or anticoagulant drugs as well as subjects with coronary heart disease had higher levels of Aβ40.

Conclusions Low or decreasing plasma Aβ42 during the follow-up is associated with cognitive decline. Serial measurement of plasma Aβ42 may be useful in the detection of the subjects who are at risk for cognitive decline.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia. At present, its diagnosis is based on clinical criteria and the exclusion of other causes. However, objective biomarkers for the early diagnosis and monitoring of the disease process are clearly needed because symptomatic treatments are available, and disease-modifying drugs are already in phase III trials. The presence of amyloid β deposition in senile plaques is one pathological hallmark of Alzheimer's disease (AD) together with neurofibrillar tangles.[1] The amyloid β is a peptide secreted by neurons[1] and platelets,[2] derived from amyloid precursor protein APP via the activity of proteases β and γ secretase.[3] Most of the Aβ deposited in the brain is composed of 42 amino acids (Aβ42) form.[4] Aβ42 has also been shown to be first amyloid form to accumulate with Aβ40 being deposited later in the process of AD pathogenesis.[5] The level of Aβ42 in cerebrospinal fluid (CSF) is reduced in patients with mild cognitive impairment (MCI) and AD,[6][7] and a combination test of CSF Aβ42 and τ or phospho-tau has been claimed to be helpful in the early diagnosis of AD.[8]

Aβ is present in plasma, but it is still unknown whether it originates from peripheral sources or from the brain. In Tg2576 transgenic mouse, plasma Aβ levels decline in parallel with their increasing accumulation in the brain.[9] Since Aβ can be transported bidirectionally across the blood—brain barrier, it has been hypothesised that there may be an equilibrium between CSF and plasma pools of Aβ.[10][11] Seeing that it is well established that CSF Aβ42 levels decrease in conjunction with the cognitive decline, it has been postulated that plasma Aβ42 may decrease similarly.[12] If so, plasma Aβ would offer a straightforward, non-invasive and economical biomarker for AD. However, patients with known mutations in chromosome 21 causing early-onset familial AD as well as patients with trisomy 21 have increased plasma Aβ42 levels which are detectable before the onset of the symptoms of dementia.[13][14] Also, the first-degree relatives of late-onset AD patients exhibit elevated Aβ levels measured in plasma.[15]

Previous studies have suggested that the levels of plasma Aβ40 are increased before the onset of sporadic AD.[16][17] However, one recent study concluded that low plasma Aβ40 level predicted AD in older men.[18] Other studies have found elevated Aβ42 levels in patients who later develop dementia,[19] particularly in MCI amnestic type. (aMCI)[20] Finally, several studies have not been able to detect any significant difference of Aβ levels between AD converters and cognitively stable controls.[21][22]

It seems that a single measurement of plasma Aβ is not useful, whereas the change in plasma Aβ levels observed in repeated testing may be of help in the prediction and evaluation of progression of incipient AD or MCI.[23] However, only a limited number of longitudinal studies have been performed.[24–26] Our aim was to examine whether the change in plasma Aβ levels during follow-up would be more predictive of cognitive decline than straightforward baseline plasma Aβ levels in a population-based cohort of MCI and cognitively intact controls.

SUBJECTS AND METHODS
Subjects

Study subjects were participants in the population-based study (n=806, aged 60—76 years) examining the risk factors and predictors of dementia in older people[27][28] (table 1). At baseline (years 1997—1998), 52 subjects were cognitively impaired (Clinical Dementia rating (CDR) 0.5 n=51 and CDR 1 n=1). Group 1 of this study included all of the cognitively impaired subjects from the original cohort who

Table 1 Baseline demographic information of the subjects

	Population-based cohort	Group 1 (cognitive follow-up)		Group 2 (plasma follow-up)	
n	806	269		70	
Age	68 (60–76)	70 (60–77)	$p > 0.1$	71 (61–77)	$p > 0.1$
Men/women	321/485 47%/53%	121/148 45%/55%	$p > 0.1$	25/45 36%/64%	$p > 0.1$
APOE $\epsilon4$ −/+	414/207* 67%/33%	169/99† 63%/37%	$p > 0.1$	37/33 53%/47%	$p > 0.05$
Mini-Mental State Examination	26 (7–30)	27 (13–30)		26 (17–30)	
CDR=0	731	217		59	
CDR=0.5	70	51		11	
CDR=1‡	3	1		0	

Data are given as median values (range) or as number of subjects with the percentage of all subjects in the group.
*APOE data missing from 185 subjects.
†APOE data missing from one subject.
‡One subject had CDR 2. Data missing from one subject.
CDR, Clinical Dementia rating.

provided a plasma sample (n=52). For each of them, we randomly selected 4–5 cognitively unimpaired (n=217) age- and sex-matched controls from the same cohort. These subjects were clinically re-evaluated after 3 years (n=197) and 6 years (n=60).

The longitudinal marker group (group 2) included 70 subjects of the original cohort of 269 subjects who provided a 3-year follow-up plasma sample and 43 of them a 6-year follow-up sample. Group 2 included 11 cognitively impaired (at baseline) non-demented subjects (CDR 0.5) who provided at least one follow-up plasma sample and 59 cognitively intact (at baseline) age- and sex-matched controls with at least one follow-up plasma sample (figure 1). Drop-outs occurred mainly due to a refusal of the participants to continue the study. Written informed consent was obtained from all the subjects, and the study was approved by the local Ethical Committee.

Clinical evaluation

The evaluation included a structured detailed interview including demographic information, medical history, medication, smoking habits and alcohol consumption, and a subjective assessment of memory disturbances and depression. The evaluation also included clinical examination as well as an assessment of cognitive impairment by applying the CDR and using a battery of neuropsychological tests: Memory: Visual Reproduction Test from Wechsler Memory Scale,[29] Word List Recall from the CERAD Neuropsychological Assessment Battery,[30] Logical Memory Test from Wechsler Memory Scale—Revised,[31] NYU Paragraph Recall,[32] Delayed Recall of the Constructional Praxis from CERAD;[30] Language: vocabulary subtest from the

Wechsler Adult Intelligence Scale-Revised,[33] Abbreviated (15 items) Boston Naming Test;[34] Attention and executive function: Trail Making Test[35] parts A and B, Verbal Fluency Test;[36 37] Visuospatial skills: Block Design from the WAIS-R,[33] Constructional Praxis from CERAD;[30] Global functioning: Mini-Mental State Examination[38] (MMSE) Clock Drawing Test.[30]

Cognitive decline was defined by the CDR change from 0 to 0.5 or 0.5 to 1.

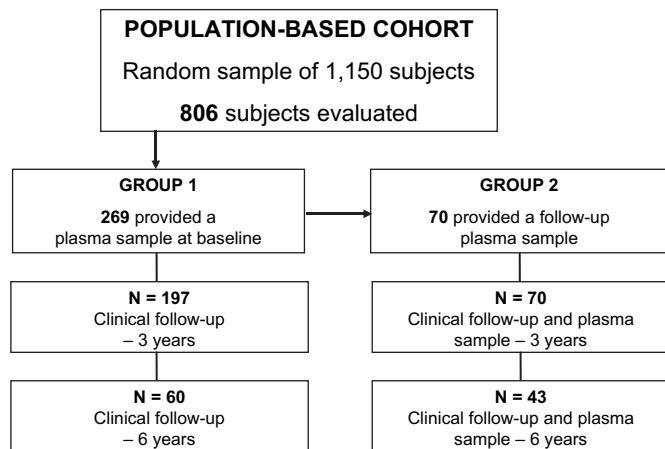### Measurement of Aβ40 and Aβ42

The 269 baseline samples (group 1) were measured in year 2002–2003. After completing the 6-year follow-up, we reanalysed the baseline samples of 70 subjects (group 2) together with their follow-up samples in year 2006–2007 (group 2).

A venous blood sample was obtained into heparin tubes, and plasma was separated using standard methods. The samples were aliquotted and stored in polypropylene tubes at −70°C until analyses. Aβ40 was measured by the ELISA method modified from a well-established method.[16] The capture antibody was 6E10 (Sigma, St Louis, Missouri), and the detection antibody was a biotin-labelled G2-10 antibody (The Genetics Company, Schlieren, Switzerland). The synthetic $A\beta_{1-40}$ peptide (Bachem, Bubendorf, Switzerland) was used as the standard. Before the analyses, 0.05% Tween 20–0.5% BSA was added to the samples. Aβ42 was measured by a high-sensitivity method of a commercially available ELISA (Innogenetics, Gent, Belgium) which we modified to be suitable for the measurements of concentrations higher than 7 pg/ml. Before the analyses, 0.5 M guanidine chloride was added to the standards and samples. The detergents were used to avoid coagulation of samples and to release Aβ peptides from plasma proteins.

In a longitudinal analysis, baseline and follow-up samples from one individual were placed on the same plate to prevent interassay variation. Thus, we measured baseline samples from 70 subjects twice (4 years apart). The absolute concentrations differed between these two measurements; median level for Aβ40 174.5 (year 2003) and 198 pg/ml (74) (year 2007), and Aβ42 17 (year 2003) and 49 pg/ml (110) (year 2007). However, there was a moderately good correlation between these measurements (Aβ40 $r=0.674$, $p<0.001$ and Aβ42 $r=0.824$, $p<0.001$). The correlation figure is presented in the supplemental data (available at http://www.jnnp.com).

The interassay variation for the Aβ40 assay was 23.8% and for the Aβ42 assay 19.1%. The inter-CVs were measured using reference samples of medium concentration (∼250 pg/ml for Aβ40 and ∼400 pg/ml for Aβ42). The intra-assay variations for Aβ40 were 0.71% for high (∼1200 pg/ml), 0.95% for medium and 5.9% for low concentrations (∼150 pg/ml). The intra-assay

POPULATION-BASED COHORT
Random sample of 1,150 subjects
806 subjects evaluated

GROUP 1
269 provided a plasma sample at baseline

GROUP 2
70 provided a follow-up plasma sample

N = 197
Clinical follow-up – 3 years

N = 70
Clinical follow-up and plasma sample – 3 years

N = 60
Clinical follow-up – 6 years

N = 43
Clinical follow-up and plasma sample – 6 years

Figure 1 Formation of the study population.

CVs (median) for Aβ42 were 1.6% (∼1000 pg/ml), 2.5% and 9.8% (∼15 pg/ml), respectively.

APOE genotyping

The APOE allele genotyping was done by a PCR-based method.[39] The subjects were subdivided into the APOE ε4 negative and APOE ε4 positive subjects.

Statistics

The statistical analyses were conducted using SPSS for Windows release 14.0.1 (SPSS, Chicago, Illinois). Due to the non-normal distribution of data, Kruskal Wallis, Mann–Whitney U and Spearman correlation tests were used. The categorical data were analysed by the χ^2 test. The ORs for cognitive decline of patients in different groups were calculated by logistic regression analysis. We fitted a linear regression slope by Microsoft Excel to analyse the alteration trend of Aβ levels.

RESULTS

Table 1 presents the demographic information about the subjects. The baseline Aβ40 and Aβ42 levels of 269 individuals were generally low, although some subjects exhibited extremely high Aβ42 levels. The limit for the 90th percentile was 101 pg/ml, but the highest measured level was 1341 pg/ml. The Aβ40 levels showed a weak correlation with age (r=0.186, p=0.002), but this was not the case with the Aβ42 levels. There were no differences in Aβ40 and Aβ42 levels between the sexes or between the APOE ε4 carriers and non-carriers. Aβ42 levels did not correlate with Aβ40 concentrations.

Baseline Aβ levels and cognitive decline during the follow-up

No significant differences were found in plasma Aβ40, Aβ42 or the Aβ42/Aβ40 ratio between cognitively impaired (n=52) and cognitively intact subjects (n=217) at baseline.

However, 197 of these subjects were clinically assessed after 3 years, and 60 were clinically assessed after 6 years. The baseline Aβ42 levels were significantly lower in the subjects who showed cognitive decline after 3 years of follow-up (cognitively stable, n=147: 19 pg/ml (0–1341), cognitive decline, n=50: 12 pg/ml (0–276), p=0.001). Baseline Aβ42 levels were also lower in subjects who had declined cognitively after 6 years (10 pg/ml, n=36) compared with those who remained cognitively stable (18 pg/ml, n=24), p=0.013.

Subjects who had baseline Aβ42 levels in the lowest quartile displayed an OR of 3.12 (95% CI 1.25 to 7.79, p=0.015) for cognitive decline after 3 years and 4.77 (95% CI 1.14 to 19.98,

p=0.033) after 6 years in comparison with subjects who had Aβ42 levels in the highest quartile. Similarly, subjects who had an Aβ42/Aβ40 ratio in the lowest quartile had an OR of 3.26 (1.31 to 8.11, p=0.011) for cognitive decline after 3 years and 8.40 (1.83 to 83.568, p=0.006) after 6 years of follow-up when compared with the subjects in the highest quartile.

Relationship between changing plasma Aβ levels and cognitive decline

The follow-up plasma samples were available from 70 subjects after 3 years and 43 subjects after 6 years of follow-up. The median levels of Aβ42 did not change or decreased in subjects with cognitive decline (n=27 after 3 years and n=14 after 6 years), whereas they increased in those who remained cognitively stable (table 2). No statistically significant changes were found in Aβ40 levels. The Aβ42/Aβ40 ratio decreased significantly in the subjects who experienced a cognitive decline.

A trend analysis was undertaken by calculating a slope for each subject, and this was used to assess the change of Aβ42 between cognitively stable and cognitively declining subjects. During the follow-up of 3–6 years, the cognition of 28 out of the total 70 subjects had declined, and there was a decreasing trend in the level of Aβ42 in 24 subjects during the follow-up. The Aβ42 level remained the same in the declining subjects and increased in the cognitively stable subjects (0.0 (4.9) pg/ml/year and 2.1 (21) pg/ml/year; p=0.009 for slope difference). The corresponding changes of Aβ42/Aβ40 ratio were also significant (0.0 (0.029) per year and 0.0036 (0.055) per year; p=0.02 for slope difference).

Plasma Aβ and general health

Table 3 shows the relationship between medication as well as certain diseases on the plasma Aβ levels. The Aβ40 levels were not associated with the use of lipid-lowering drugs or non-steroidal anti-inflammatory drugs (NSAIDs) at baseline. Hormone-replacement therapy was not related to Aβ values in women. The Aβ40 levels were higher in those subjects using acetylsalicylic acid (ASA) (n=62, p=0.004) or dipyridamole (n=12, p=0.017). The Aβ40 values were lowest in subjects using neither of the drugs, intermediate in subjects using either ASA or dipyridamole and highest in the subjects taking both drugs. The Aβ40 levels were also higher in subjects using insulin alone (n=5, p=0.009) or insulin in combination with oral antidiabetic drugs (n=13, p=0.003). The Aβ42 levels were not associated with the use of any of the drugs. Coronary heart disease was associated with a high plasma Aβ40 level (p=0.035). There was

Table 2 Cognition and changes of Aβ between baseline and follow-ups in subjects

Baseline	Cognitively healthy		Cognitively impaired	
N	59		11	
Aβ40	195 (88)		201 (60)	
Aβ42	50 (121)		46 (92)	
Aβ42/Aβ40	0.236 (0.464)		0.291 (0.487)	
	Cognitively stable or improved		Cognitive decline	
Follow-up	3 years	6 years	3 years	6 years
N	43†	29‡	27	14
Change of Aβ40	18 (55)	33 (45)	21 (24)	33 (75)
Change of Aβ42	3.7 (29)	12 (54)	0.0 (14)*	−2.4(50)*
Change of Aβ42/Aβ40	0.0093 (0.169)	0.0166 (0.451)	−0.0027 (0.084)*	−0.0486 (0.251)*

Data are given as medians (IQR). The values of p reflect the significance against the cognitively stable subgroup.
*p<0.05 against 'stable or improved.'
†Aβ data of one subject is from a plasma sample of 4 years of follow-up.
‡Cognition data of two subjects is from the previous year.

Table 3 Relation of Aβ40 level to medication at baseline

	Group 1 n	Aβ40		pg/ml Users	pg/ml Non-users
Sex, female	148	−			
Smoking	21	−			
Coronary heart disease	61	↑	p=0.035	188 (105−360)	176 (0−780)
Diabetes	22	−			
Anticoagulants	16	↑	p=0.038	198 (144−360)	176 (0−780)
Non-steroidal anti-inflammatory drugs	22	−			
Acetylsalicylic acid	62	↑	p=0.004	194 (0−278)	175 (0−780)
Dipyridamole	12	↑	p=0.016	208 (156−278)	176 (0−780)
Antidiabetics*	13	↑	p=0.003	219 (161−426)	179 (0−780)
Lipid-lowering agents	24	−			

*Insulin and oral antidiabetics.

no statistically significant difference in medication use between the cognitively stable and the cognitive decliners.

DISCUSSION

Our results indicated that low or decreasing plasma Aβ42 levels and Aβ42/Aβ40 ratio were related with cognitive decline during the follow-up. Moreover, a possible age-related increase in plasma Aβ42 levels in serial measurements was associated with stable cognitive performance.

We found no differences in plasma Aβ levels between cognitively intact and impaired subjects in the cross-sectional analysis at baseline. Previous cross-sectional studies comparing plasma Aβ levels in patients with sporadic AD and controls have reported contradictory results. Elevations of plasma Aβ40[16] or Aβ42[19] have been described, whereas other studies have found no differences between AD and controls.[21] One recent study found increased levels of plasma Aβ42 in women with amnestic MCI compared with healthy controls or affected men.[40] However, low Aβ42 levels at baseline were associated with a cognitive decline occurring during the follow-up. In line with our results, a recent study detected an association between a low Aβ42/Aβ40 ratio and cognitive decline.[12] Respectively, a prospective three-city study of 257 dementia patients found an association of high Aβ42/Aβ40 ratio with a lower risk of dementia in follow-up.[41] Another population-based case-cohort study claimed that individuals with a combination of low Aβ42 and high Aβ40 measured from plasma at baseline had more than a 10-fold risk of dementia but found no association between the Aβ42 or Aβ40 levels alone with cognitive decline.[17] Other studies have reported different results. The VITA study found no association between baseline Aβ levels and cognitive decline during the follow-up. Other studies have found elevated concentrations of Aβ42 at the baseline in subjects who developed AD during the follow-up,[19 42] although plasma Aβ levels were not associated with AD in the fully adjusted multivariate model.[42]

Differences in study cohorts, for example timing with respect to cognitive decline, assessment of cognitive functioning (different tests) and presence of confounding factors such as medication and other diseases, are all factors that can influence the results. The outcome in some studies has been conversion to dementia,[17 19] whereas the outcome in our study as well as in some other studies[12] was cognitive decline. The difference in the selected outcome and the timing of the sample collection may partially explain differences in the results.

Single measurement of plasma Aβ may not be a suitable marker for AD due to many confounding factors. The timing of the Aβ measurement in terms of the natural history of AD may be critical. Experimental studies on transgenic animals suggest that plasma Aβ levels decrease at the time when accumulation of Aβ begins in the brains.[9] It is possible that the increased plasma Aβ concentration is related to the development of AD as suggested by the findings of elevated plasma Aβ levels in AD gene mutation carriers[43] and in the first-degree relatives of the patients with late-onset AD.[15] However, since amyloid pathology in the brain begins years before the appearance of the first symptoms, the possible increase in plasma Aβ may not be detected in the symptomatic individuals. This hypothesis can only be addressed in longitudinal studies. In line with our results, previous studies have suggested that decreasing Aβ levels are associated with cognitive decline,[19 24] and Aβ42 levels were lower in patients diagnosed having AD than in those with MCI.[44] Many studies have shown that plasma Aβ levels increase with age, as was found in the cognitively intact subjects in our study.[19 25 42] In one study, the age-related increase was smallest in those subjects who converted to AD from MCI.[25] It is possible that age-related changes of plasma levels of Aβ40 and Aβ42 differ in subjects with AD. In this respect, the Aβ42/Aβ40 ratio may be a better predictor for AD than the single markers.

Differences in study cohorts make the comparison between different studies difficult. There are many confounding factors that may influence plasma Aβ levels. Renal dysfunction may increase plasma, since plasma Aβ is excreted through the kidneys.[45] Many studies have suggested an association between vascular disease and plasma Aβ levels.[46−48] The levels of plasma homocysteine, a possible marker for vascular disease, correlate positively with plasma Aβ40 and Aβ42 levels.[49] Previous studies have also suggested that certain drugs may influence plasma Aβ.[50] We found elevated levels of Aβ40 in the subjects who were using ASA and dipyridamole, that is drugs that directly influence platelet function and activation, and subjects who used anticoagulation and antidiabetic drugs, whereas there was no association between the use of NSAIDs or lipid-lowering agents and plasma Aβ levels. The association between ASA and plasma Aβ40 was seen also in subjects without cardiovascular diseases. In line with previous studies,[50 51] no relationship was found between Aβ levels and lipid-lowering agents in previous studies.

Differences in methodology and experimental conditions may also influence results. Erythrocytes and plasma proteins, for example albumin and lipoproteins, bind Aβ and denaturating conditions liberate Aβ into the free pool of plasma.[52] Also, the different antibodies used in the immunological assays may detect different fractions of Aβ. Previous studies suggested that the absolute levels of Aβ vary across different ELISA batches.[53] We also noticed a difference between absolute levels in 70 samples that were measured twice 4 years apart. Because of these methodological difficulties, the diagnostic value of a single measurement is limited.

The significance of the standardisation of the conditions in storing and handling the samples is reported in a study by Vanderstichele *et al*.[22] To better utilise these analyses, the assays used should be commercially available, well standardised and thoroughly validated.

We conclude that plasma Aβ is not a diagnostic marker for AD, but the decreasing levels of Aβ42 in serial measurements may be associated with cognitive decline and indicate the development of AD.

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