Plasma β Amyloid and the Risk of Alzheimer Disease and Dementia in Elderly Men

A Prospective, Population-Based Cohort Study

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Background: β Amyloid (Aβ) protein accumulates in the brains of individuals with Alzheimer disease (AD) and is detectable in cerebrospinal fluid and plasma.

Objective: To examine plasma levels of Aβ peptides Aβ40 and Aβ42 as predictors of incident AD and other types of dementia.

Design: Prospective, population-based cohort study.

Setting: The Uppsala Longitudinal Study of Adult Men.

Participants: Plasma Aβ40 and Aβ42 levels were analyzed as predictors of incident AD in 1045 men at age 70 years and 680 men at age 77 years using Cox proportional hazards analyses. Alzheimer disease and other types of dementia were diagnosed by standardization screening, clinical evaluation, and medical record review.

Main Outcome Measures: Hazard ratios of AD (primary outcome) and vascular dementia or other dementia (secondary outcomes) according to baseline levels of plasma Aβ40 and Aβ42.

Results: From the age of 77 years at baseline, 46 individuals developed AD at follow-up (median, 5.3 years). A low plasma Aβ40 level at age 77 years was associated with higher incidence of AD. The multivariate-adjusted hazard ratio was 4.87 (95% confidence interval, 1.63-14.6) for the lowest Aβ40 tertile compared with the highest tertile. On follow-up from age 70 years at baseline (median, 11.2 years), 82 individuals developed AD. Plasma Aβ40 and Aβ42 levels measured at age 70 years were not significantly associated with incident AD.

Conclusions: Low plasma Aβ40 levels predicted incident AD in elderly men independently of potential confounders. Plasma Aβ42 levels were not significantly associated with AD incidence. The clinical value of Aβ measurement in plasma remains to be established in future studies.

Arch Neurol. 2008;65(2):256-263

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STUDY POPULATION

The Uppsala Longitudinal Study of Adult Men (ULSAM) was initiated in 1970 when all 50-year-old men living in Uppsala, Sweden, were invited to participate in a health survey, initially focused on identifying factors for cardiovascular disease (described in detail at http://www.pubcare.uu.se/ULSAM). Our analyses are based on the second and third reinvestigations of the ULSAM cohort, when the participants were aged approximately 70 (1990-1994, n=1221) and 77 (1998-2001, n=838) years.

Plasma Aβ40 and Aβ42 levels were measured in 1082 (88.6%) participants at age 70 years and in 733 (87.5%) at age 77 years. For our study, all participants with a diagnosis of any type of dementia at baseline (3 at age 70 years and 33 at age 77 years) and those who did not agree to have their medical records reviewed (34 at age 70 years and 20 at age 77 years) were excluded. Thus, our study samples comprise 1045 participants at age 70 years and 680 participants at age 77 years. Two serial samples (at ages 70 and 77 years) were available from 630 individuals. Fifty participants provided samples only at age 77 years. The study was approved by the regional ethical committee at Uppsala University. Informed consent from all participants was obtained.

BASELINE MEASUREMENTS AND DEFINITIONS

Information on definite and possible risk factors for dementia were collected from examinations at ages 70 and 77 years. Fasting serum cholesterol concentrations were assayed by enzymatic techniques. The apolipoprotein E gene (APOE) was genotyped by minisequencing. Smoking status (current smokers or nonsmokers) was assessed by questionnaires, and alcohol consumption was assessed by interviews at age 72 years. Education level was stratified as low (elementary school only, 6-7 years), medium (high school), or high (college studies). Systolic and diastolic blood pressure was measured in the supine position after a 10-minute rest to the nearest 2 mm Hg. Hypertension at baseline was defined as systolic blood pressure at or above 140 mm Hg, diastolic blood pressure at or above 90 mm Hg, and/or use of antihypertensive medication. The presence of diabetes at baseline was defined according to the Chui criteria. Other dementia disorders included Parkinson disease with dementia and frontotemporal dementia. Cases of dementia with insufficient recorded medical data were diagnosed as dementia not otherwise specified. Mild cognitive impairment was defined as subjective and objective evidence of a cognitive decline that did not interfere with activities of daily life. All cases were validated according to current guidelines.

STUDY DESIGN

Analysis plans were defined a priori. Separate analyses were performed at 70 years and 77 years to explore the association between plasma Aβ40 and Aβ42 levels and AD incidence at different points in the same cohort. Kaplan-Meier curves with log-rank tests were used to examine the AD-free survival by cohort-specific tertiles of Aβ40 and Aβ42 levels, as well as the Aβ42/Aβ40 ratio. Cox proportional hazard regression models were used to estimate crude and multivariate-adjusted estimates of the hazard ratio (HR) of AD according to Aβ levels (Stata, version 8; Stata Corp, College Station, Texas). To control for confounders, and at the same time to avoid over-fitted models, forward selection was applied to identify covariates associated with AD to include in the full models. Candidate covariates (APOE ε4 allele status, hypertension, diabetes, body mass index, serum cholesterol, serum creatinine, smoking, and education level) were incorporated into Cox models by forward selection (P<.05, by likelihood ratio test for retention). For both the 70-year-old cohort and the 77-year-old cohort, only age and APOE genotype (coded as presence or absence of APOE ε4 allele) remained significant and were included in the multivariate models of Aβ level. We then tested whether introducing dummy variables for tertiles of Aβ40 or Aβ42 levels were significant in the optimal forward selection model by using likelihood ratio tests. To determine if there was residual confounding, we compared the β coefficient of the Aβ40 and Aβ42 variables in the final forward selection model with that of the maximal model incorporating all covariates. Observations were censored at death, date of migration/emigration, date of diagnosis of AD or any other type of dementia, or end of follow-up (December 31, 2005). Dates of deaths...
The clinical characteristics of the participants at baseline for the 70-year-old and the 77-year-old cohorts are presented in Table 1. In the 70-year-old cohort, the participants were followed up for a median time of 11.2 years (maximum of 14.4 years), totaling 10,208 person-years at risk. Seventy-four participants developed AD and 25 had diagnosed VaD. Participants in the 77-year-old cohort had a median follow-up of 3.3 years and a maximum follow-up of 7.9 years, totaling 3,420 person-years at risk. Seventy-four participants developed any type of dementia, of whom 46 had diagnosed AD and 10 had diagnosed VaD (Table 2). Presence of the APOE ε4 allele was significantly associated with AD in the 70-year-old (HR, 2.8; 95% confidence interval [CI], 1.7-4.7) and the 77-year-old (HR, 2.1; 95% CI, 1.1-3.8) cohorts. Kaplan-Meier curves on the cumulative HR of AD by tertiles of plasma Aβ40 and Aβ42 levels in the 2 cohorts are shown in the Figure. In unadjusted Cox proportional hazards analyses in the 77-year-old cohort, low Aβ40 levels were significantly associated with higher AD incidence, both as a continuous variable and when split into tertiles (model A, Table 3). Men in the lowest tertile of Aβ40 level had a 5-fold higher risk of AD relative to the highest tertile (95% CI, 1.63-14.6; P = .006). A low plasma Aβ40 level remained significantly associated with increased risk of AD when adjusting for age and APOE genotype (model B, Table 3). There was a similar trend for Aβ42 level and risk of AD. In the unadjusted models, the lowest tertile of Aβ42 level was significantly associated with increased risk of AD (P = .03). In the adjusted models, men in the lowest tertile of Aβ42 level had a more than 2-fold increase of AD risk relative to the highest tertile, which did not reach significance (95% CI, 0.95-5.56; P = .06). In the 70-year-old cohort, neither Aβ40 nor Aβ42 level was associated with risk of AD (Table 4). Thus, there appears to be a temporal trend in the influence of Aβ40 level and even Aβ42 level in AD risk; Aβ40 and Aβ42 levels are not associated with AD at age 70 years, but low levels tend to be associated with AD at age 77 years. There was no association between a change in Aβ40 and Aβ42 levels between ages 70 and 77 years and AD risk among the 630 individuals with 2 plasma samples (data not shown). At age 70 years, the middle but not the lowest tertile of the Aβ42:Aβ40 ratio was significantly associated with reduced risk of AD incidence. The incidence rates of VaD and all types of dementia according to baseline plasma Aβ levels at ages 70 and 77 years are presented in Table 3 and Table 4. In adjusted models in the 70-year-old cohort, a 1 SD increase in Aβ42:Aβ40 ratio was associated with increased risk of VaD (HR, 1.78; 95% CI, 1.18-2.94;
The association between A\(\beta\) level and risk of VaD was borderline significant \((P = .06)\). In the 77-year-old cohort, the lowest tertile of A\(\beta\) level was significantly associated with increased risk of all type of dementia \((HR, 2.40; 95\%\ CI, 1.24-4.50; P = .008)\).

To rule out the possibility of residual confounding, HRs from the maximal model of all covariates (education and vascular risk factors) were compared with those from the association between A\(\beta\) level and incident AD in the 77-year-old cohort; the association of the A\(\beta\)\(_{42}:\)A\(\beta\)\(_{40}\) ratio with

\[A\beta_{42}\text{ Level in the 70-Year-Old Cohort} \quad A\beta_{42}\text{ Level in the 77-Year-Old Cohort} \]

\[A\beta_{40} \text{ Level in the 70-Year-Old Cohort} \quad A\beta_{40} \text{ Level in the 77-Year-Old Cohort} \]

\[A\beta_{42}:A\beta_{40}\text{ Ratio in the 70-Year-Old Cohort} \quad A\beta_{42}:A\beta_{40}\text{ Ratio in the 77-Year-Old Cohort} \]

\[\text{Alzheimer Disease–free Survival} \quad \text{No. at Risk} \quad \text{Lowest tertile} \quad \text{Middle tertile} \quad \text{Highest tertile} \]

\[\begin{array}{cccc}
\text{No. at Risk} & \text{Lowest tertile} & \text{Middle tertile} & \text{Highest tertile} \\
\text{348} & 329 & 276 & 130 \\
\text{348} & 327 & 276 & 122 \\
\text{349} & 285 & 213 & 100 \\
\end{array} \]

\[\begin{array}{cccc}
\text{No. at Risk} & \text{Lowest tertile} & \text{Middle tertile} & \text{Highest tertile} \\
\text{227} & 174 & 20 \\
\text{227} & 190 & 26 \\
\text{224} & 187 & 32 \\
\end{array} \]

\[\begin{array}{cccc}
\text{No. at Risk} & \text{Lowest tertile} & \text{Middle tertile} & \text{Highest tertile} \\
\text{348} & 321 & 271 & 130 \\
\text{348} & 322 & 270 & 128 \\
\text{348} & 288 & 224 & 96 \\
\end{array} \]

\[\begin{array}{cccc}
\text{No. at Risk} & \text{Lowest tertile} & \text{Middle tertile} & \text{Highest tertile} \\
\text{227} & 182 & 31 \\
\text{227} & 185 & 19 \\
\text{224} & 186 & 28 \\
\end{array} \]

\[\begin{array}{cccc}
\text{No. at Risk} & \text{Lowest tertile} & \text{Middle tertile} & \text{Highest tertile} \\
\text{348} & 315 & 255 & 117 \\
\text{348} & 319 & 268 & 133 \\
\text{348} & 307 & 242 & 102 \\
\end{array} \]

\[\begin{array}{cccc}
\text{No. at Risk} & \text{Lowest tertile} & \text{Middle tertile} & \text{Highest tertile} \\
\text{227} & 183 & 32 \\
\text{227} & 188 & 22 \\
\text{224} & 182 & 24 \\
\end{array} \]

**Figure.** Alzheimer disease–free survival analysis by tertiles of levels of plasma \(\beta\) amyloid peptides 40 \((A\beta_{40})\) and 42 \((A\beta_{42})\) in the 70-year-old and 77-year-old cohorts. Number at risk represents those individuals who have been followed up for that length of time and have remained cognitively healthy.
VaD remained significant. None of the results were affected when excluding cases diagnosed within 2 years after baseline or cases of mild cognitive impairment at baseline, after adjustments for participation or exclusion of participants who died and who might have had undiagnosed AD. Interactions between Aβ42 level and the covariates APOE ε4 allele status and age were investigated in the 70-year-old and 77-year-old cohorts and no significant interactions were found.

In this study, low plasma Aβ42 levels at approximately 77 years of age were associated with an increased risk of incident AD, independent of age and APOE genotype. Furthermore, low plasma Aβ42 level was associated with AD incidence, but this association did not remain statistically significant after adjustments for APOE genotype. There was no link between plasma Aβ concentrations (measured 7 years earlier when the participant was aged approximately 70 years) and risk of AD, indicating that this association is only present in older ages. The middle, but not the lowest, tertile of the Aβ42:Aβ40 ratio in the 70-year-old cohort was significantly associated with AD incidence. Furthermore, in the tertile analysis of the participants in the 77-year-old cohort, the data suggest a threshold effect for Aβ42 level and a dose effect for Aβ42. It is unclear whether this reflects true differences in the biology of these proteins, the particular parameterization in the survival models, or the sensitivity of the assay at very low levels of Aβ.

There are 3 previous longitudinal studies on plasma Aβ levels and risk of AD. In one study, van Oijen et al20 recently reported an association between dementia and a high level of plasma Aβ40 and a low Aβ42:Aβ40 ratio, but not a high Aβ42 level, in the case-cohort Rotterdam Study. This study had a mean follow-up time similar to ours (8.6 years), but its participants were about 9 years younger than the participants in the 77-year-old cohort in ULSAM. Graff-Radford et al21 also report an association between a low Aβ42:Aβ40 ratio and AD and MCI incidence. Mayeux et al19,32 found that those who developed AD during follow-up (mean, 5 years) had significantly higher Aβ42 but not Aβ40 levels at baseline. Although the results of these studies appear inconsistent, their differences may be related to the temporal changes in plasma Aβ levels associated with age and timing relative to incident AD. Plasma Aβ levels appear to decline in individuals who...
Table 4. Incidence Rate of Alzheimer Disease, Vascular Dementia, and All Types of Dementia According to Baseline Plasma Aβ Levels in the 70-Year-Old Cohort

<table>
<thead>
<tr>
<th>Plasma Aβ Levels</th>
<th>Alzheimer Disease</th>
<th>Vascular Dementia</th>
<th>All Types of Dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Events/No. at Risk</td>
<td>Unadjusted HR (95% CI)</td>
<td>Multivariate HR (95% CI)</td>
</tr>
<tr>
<td>Per 1-SD increase</td>
<td>82/1045</td>
<td>0.88 (0.70-1.10)</td>
<td>0.89 (0.66-1.20)</td>
</tr>
<tr>
<td>Lowest tertile, 28.1-63.2 pmol/L</td>
<td>34/348</td>
<td>1.64 (0.90-2.98)</td>
<td>1.40 (0.73-2.70)</td>
</tr>
<tr>
<td>Middle tertile, 63.5-78.5 pmol/L</td>
<td>31/348</td>
<td>1.43 (0.77-2.70)</td>
<td>1.16 (0.60-2.26)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma Aβ42:Aβ40 Ratio</th>
<th>Alzheimer Disease</th>
<th>Vascular Dementia</th>
<th>All Types of Dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per 1-SD increase</td>
<td>82/1044</td>
<td>1.03 (0.82-1.28)</td>
<td>1.06 (0.81-1.39)</td>
</tr>
<tr>
<td>Lowest tertile, 2.30-17.5 pmol/L</td>
<td>25/348</td>
<td>0.85 (0.48-1.50)</td>
<td>0.84 (0.44-1.60)</td>
</tr>
<tr>
<td>Middle tertile, 14.7-31.1 pmol/L</td>
<td>34/348</td>
<td>1.13 (0.65-1.94)</td>
<td>1.03 (0.56-1.90)</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ, β amyloid; CI, confidence interval; HR, hazard ratio.
a Adjusted for age.
b Adjusted for age and APOE genotype.
c Adjusted for age, APOE genotype, and diabetes.
d P < .05.

are likely to develop AD. Plasma Aβ levels may be higher years before the AD diagnosis, reflecting either genetic predisposition or the balance between Aβ production and clearance, but decrease closer to diagnosis. In addition, plasma Aβ has been analyzed with different methods, and these studies may be measuring different Aβ species depending on the antibody affinity. Assays also vary in their ability to detect N-terminally truncated Aβ or Aβ incorporated into soluble oligomers. The assay we employed detects free and protein-bound monomeric, but not oligomeric, Aβ. Third, misclassification is an issue for any clinical study of dementia but is likely to be random with respect to Aβ levels, and false negatives due to misclassification would probably drive results toward the null hypothesis. Results were unchanged after excluding all those who died (who might have had undiagnosed AD), minimizing the possibility of competing risks. Adjustments for potential confounders did not influence the results. Associations between plasma Aβ levels and AD incidence may also differ in different racial subpopulations.

The mechanism, whether peripheral and/or central, by which low Aβ levels in plasma are associated with a higher risk of AD remains to be clarified. It is now well established that AD is associated with a decline of Aβ levels in CSF. We believe that low plasma Aβ levels mirror a decline of Aβ in CSF owing to increased aggregation in the brain. In the Tg2576 transgenic mouse model of AD, progressive amyloid deposition in the brain was associated with parallel declines of Aβ levels in CSF and plasma. In humans, previous studies have reported no association between CSF and plasma levels in manifest AD. However, a case-control study of ours suggests a correlation between Aβ42 and Aβ40 in CSF and plasma in healthy individuals but not in people with AD, indicating that plasma levels of Aβ42 and Aβ40 might mirror the aggregation process in the central nervous system before clinically manifest AD. It should be noted that it is not possible to establish causality in the present study. The associations between brain, CSF, and plasma levels of Aβ in those with AD and healthy elderly individuals need further study.

Interestingly, plasma Aβ levels seem to be associated not only with AD but with other types of dementia. This is the first prospective longitudinal study reporting a significant association between the plasma Aβ42:Aβ40 ratio and risk of VaD. This is in contrast with the Rotterdam Study, in which high levels of Aβ40 increased the risk of VaD. Owing to the small number of VaD cases, this association needs to be further investigated in other longitudinal studies.

The strengths of our study include its prospective longitudinal design, population-based setting, large
In conclusion, this study shows that low plasma Aβ42 levels are associated with a higher risk of developing AD in elderly men. The association between low plasma Aβ and incidence of AD might be a reflection of an increased deposition of Aβ in the central nervous system prior to the onset of clinically manifest cognitive decline. This is also the first prospective longitudinal population-based study to report a significant association between a high Aβ42:Aβ40 ratio and risk of VaD. Further prospective longitudinal studies of different types of dementia and with repeated measurements of Aβ levels in both plasma and CSF are needed to clarify the role of plasma Aβ as a predictor of different types of dementia.

Accepted for Publication: April 27, 2007.
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Financial Disclosure: Dr Basun worked for AstraZeneca when the study was performed.

Funding/Support: This study was supported by Wallenberg Consortium North, Härfonden, Bertil Hällstens forskningsstiftelse, Alzheimerfonden, grant 2003-5546 from the Swedish Research Council, the European Union 6th Consortium APOPIS (contract No. LSHM-CT-2003-503330), Stiftelsen Gamla Tjanarinnor, Capios Forskningsstiftelse, Gun och Bertil Stohnes forskningsstiftelse, Swedish Lions Research Foundation, grant 1T32NS048005-01 from the National Institutes of Health, grant AG05134 from the Massachusetts Alzheimer Disease Research Center, AFAR Beeson Award (Dr Irizarry), J.D. French Alzheimer’s Foundation, and an unrestricted grant from AstraZeneca.

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