ORIGINAL CONTRIBUTION

Cerebrospinal Fluid β-Amyloid$_{(1-42)}$ in Alzheimer Disease

Differences Between Early- and Late-Onset Alzheimer Disease and Stability During the Course of Disease

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Objectives: To study the diagnostic potential of the 42 amino acid form of β-amyloid (β-amyloid$_{(1-42)}$) in cerebrospinal fluid (CSF) as a biochemical marker for Alzheimer disease (AD), the intra-individual biological variation of CSF-β-amyloid$_{(1-42)}$ level in patients with AD, and the possible effects of differential binding between β-amyloid and apolipoprotein E isoforms on CSF-β-amyloid$_{(1-42)}$ levels.

Design: A 20-month prospective follow-up study.

Setting: Community population-based sample of consecutive patients with AD referred to the Piteå River Valley Hospital, Piteå, Sweden.

Patients: Fifty-three patients with AD (mean ± SD age, 71.4 ± 7.4 years) diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria and 21 healthy, age-matched (mean ± SD age, 68.8 ± 8.0 years) control subjects.

Main Outcome Measures: Cerebrospinal fluid β-amyloid$_{(1-42)}$ level—analyzed using enzyme-linked immunosorbent assay—and severity of dementia—analyzed using the Mini–Mental State Examination.

Results: Mean ± SD levels of CSF-β-amyloid$_{(1-42)}$ were decreased ($P<.001$) in patients with AD (709 ± 304 pg/mL) compared with controls (1678 ± 436 pg/mL). Most patients with AD (49 [92%] of 53 patients) had reduced levels (<1130 pg/mL). A highly significant correlation ($r = 0.90$; $P<.001$) between baseline and 1-year follow-up CSF-β-amyloid$_{(1-42)}$ levels was found. There were no significant correlations between CSF-β-amyloid$_{(1-42)}$ level and duration ($r = –0.16$) or severity ($r = –0.02$) of dementia. Low levels were also found in patients with mild dementia (Mini–Mental State Examination score, >25).

Conclusions: The sensitivity of CSF-β-amyloid$_{(1-42)}$ level as a diagnostic marker for AD is high. The intra-individual biological variation in CSF-β-amyloid$_{(1-42)}$ level is low. Low CSF-β-amyloid$_{(1-42)}$ levels are also found in the earlier stages of dementia in patients with AD. These findings suggest that CSF-β-amyloid$_{(1-42)}$ analyses may be of value in the clinical diagnosis of AD, especially in the early course of the disease, when drug therapy may have the greatest potential of being effective but clinical diagnosis is particularly difficult.

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ALZHEIMER disease (AD) is the most common form of dementia. Although rare familial forms of AD exist, most patients have no clear family history and are classified as having sporadic AD. Today, the diagnosis of sporadic AD is based on relatively vague clinical criteria[1]; diagnosis is definite only with an autopsy examination. Therefore, in clinical routine and especially in view of existing (acetylcholine esterase inhibitors) and emerging (eg, neuroprotective) therapeutic compounds, there is a pressing need for biochemical diagnostic markers of AD. Such biochemical markers would be especially helpful in the early course of the disease, when drug administration may have the greatest potential of being effective but clinical diagnosis is particularly difficult. Moreover, biochemical markers might also be useful to monitor the effect of new potential therapeutic compounds during treatment trials.

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Because intercellular space in the brain is in direct contact with cerebrospinal fluid (CSF), biochemical changes in the

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PATIENTS, MATERIALS, AND METHODS

STUDY POPULATION

The AD group consisted of 53 patients, 16 men and 37 women, with a mean ± SD age of 71.4 ± 7.4 years. All patients underwent a thorough clinical investigation, which included a medical history; physical, neurologic, and psychiatric examinations; screening laboratory tests; an electrocardiogram; a chest radiograph; an electroencephalogram; and a computed tomographic scan of the brain.

The diagnosis of probable AD was made according to the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria.[1] In short, probable AD was diagnosed in patients with (1) progressive dementia with deficits in 2 or more areas of cognition, (2) no disturbance of consciousness, (3) onset between ages 40 and 90 years, and (4) absence of systemic disorders or other brain diseases that alone could account for progressive deficits in memory and cognition, such as multiple infarcts, depression, normal-pressure hydrocephalus, and metabolic disturbances. No patient with AD had a family history of dementia suggestive of autosomal-dominant AD. Mild or moderate white matter lesions, or leukoaraiosis, defined as computed tomographic findings of periventricularly decreased attenuation, mainly around the frontal and occipital horns of the lateral ventricles, was not regarded as exclusion criteria for the diagnosis of AD,[30,31] but no patient had a history or symptoms of transient ischemic attack or stroke episodes or computed tomographic findings of lacunas or infarcts. All clinical diagnoses were made individually by 1 of us (N.A.) and were established without knowledge of the results of biochemical analyses.

The severity of dementia was evaluated using the Mini–Mental State Examination (MMSE) according to Folstein and coworkers.[32] The mean ± SD MMSE score was 22.8 ± 5.2 in the AD group.

The total probable AD group was subdivided into early-onset AD (EAD), with onset of symptoms before age 65 years (n = 17; mean ± SD age, 64.1 ± 6.8 years), and late-onset AD (LAD), with onset of symptoms at or after age 65 years (n = 36; mean ± SD age, 74.9 ± 4.6 years). Mean MMSE score did not significantly differ between the EAD (23.6 ± 4.0) and LAD (22.4 ± 5.6) subgroups.

The control group consisted of 21 individuals, 8 men and 13 women (mean ± SD age, 68.8 ± 8.0 years), without a history, symptoms, or signs of psychiatric or neurologic disease, malignant disease, or systemic disorders (eg, rheumatoid arthritis or infectious disease). Cognitive status was examined using the MMSE,[32] and individuals with scores below 28 were not included as controls. Mean age did not significantly differ between the AD and control groups.

The study was approved by the ethics committees of the universities of Göteborg, Lund, and Umeå, Sweden. All patients (or their nearest relatives) and controls gave informed consent to participate in the study, which was conducted in accordance with the provisions of the Helsinki Declaration. Ethical approval included longitudinal examinations, including lumbar punctures.

CSF ANALYSES

In the AD and control groups, CSF samples were obtained by lumbar puncture in the L3/L4 or L4/L5 interspace. The first 12 mL of CSF was collected in plastic (polypropylene) tubes to avoid absorbance of β-amyloid into the test tube walls.[22] All CSF samples were gently mixed to avoid possible gradient effects. No CSF sample contained more than 500 erythrocytes per microliter. The CSF samples were centrifuged at 2.000 × 1*g* for 10 minutes to eliminate cells and other insoluble material and were then frozen and stored at −80°C pending biochemical analyses without being thawed and refrozen.

To study the stability of CSF-β-amyloid$_{(1-42)}$ over time, CSF samples were collected on 2 occasions from all patients with AD: at baseline (first admission for medical

brain may be reflected by CSF analyses. Cerebrospinal fluid biochemical markers should reflect the central pathogenic processes in AD, of which 1 is the deposition of β-amyloid, Aβ or β/A4 protein, in the form of senile, or neuritic, plaques (SPs). The SP cores are made up of primarily aggregated forms of β-amyloid,[2,3] which is a proteolytic product from the amyloid precursor protein.[4]

β-amyloid is generated continuously as a soluble protein during normal cellular metabolism and is secreted into the extracellular space and biological fluids and thus, into the CSF.[5,6] However, results of studies of total CSF-β-amyloid levels in patients with AD are contradictory, finding no significant change,[7-10] a slight decrease,[11-13] or a slight increase,[14] but with large overlaps between patients with AD and control subjects.

There are 2 major C-terminal variants of β-amyloid: a shorter form ending at Val-39 (β-amyloid$_{(1-39)}$) or Val-40 (β-amyloid$_{(1-40)}$) and a longer form ending at Ala-42 (β-amyloid$_{(1-42)}$). The different forms of amyloid deposits contain different C-terminally truncated forms of β-amyloid, with β-amyloid$_{(1-42)}$ predominating in diffuse plaques,[15,16] SP cores,[17] and cortical homogenate[18]; and shorter forms predominating in vascular amyloid[17,19] and CSF.[20]

Using enzyme-linked immunosorbent assays (ELISAs) that specifically recognize β-amyloid$_{(1-42)}$, a marked decrease has been found in patients with AD.[8,21,22] Also, using Western blotting, a less marked decrease in CSF-β-amyloid levels was found in patients with AD.[23] However, these studies were biased by including only patients seen at research centers. We further examined the sensitivity of CSF-β-amyloid$_{(1-42)}$ level as a biochemical marker for AD. In the Piteå River Valley in Sweden, all individuals with memory disturbances must be admitted for medical examination to the Piteå River Valley Hospital.[24] Such a patient cohort provides a unique epidemiological opportunity to study the diagnostic potential of a biochemical marker for AD in the general population.

In addition, although a decrease in CSF-β-amyloid$_{(1-42)}$ level has been found in several cross-sectional studies, none has examined longitudinal CSF samples from individual patients. Thus, our aim was also to examine whether CSF-β-amyloid$_{(1-42)}$ level changes over time during the disease process by analyzing paired samples at baseline and at 1-year

examination) and at follow-up after approximately 1 year. The average (± SD) time for collection of the follow-up sample was 10.1 ± 5.7 months. In 10 patients with AD, a third CSF sample was also collected. The average time for this follow-up sample collection was 20.0 ± 8.6 months from the first examination.

To determine whether test tubes of different materials affect the level of CSF-β-amyloid$_{(1-42)}$, lumbar puncture was performed on 7 patients with AD, and CSF was collected in polyetene tubes. Thereafter, fresh CSF was pipetted into 3 different types of tubes (polypropylene, polystyrene, and glass), incubated at 37°C for 3 hours, and finally assayed for CSF-β-amyloid$_{(1-42)}$. Cerebrospinal fluid neuron-specific enolase level was also determined as a reference protein using a sandwich ELISA.[33]

Cerebrospinal fluid β-amyloid$_{(1-42)}$ level was determined using a sandwich ELISA (INNOTEST β-amyloid$_{(1-42)}$, Innogenetics NV, Ghent, Belgium) constructed to specifically measure β-amyloid$_{(1-42)}$, as described previously.[22] The monoclonal antibody 21F12—which is highly specific for the C-terminus of the β-amyloid peptide—was used as capturing antibody, and the biotinylated monoclonal antibody 3D6—specific to the N-terminus—was used as detector. Characteristics of the antibodies were described previously.[34,35] No cross-reactivity was observed with β-amyloid$_{(1-40)}$ or shorter peptides, and β-amyloid$_{(1-43)}$ was detectable only with a 40-fold lower affinity.[22] High-performance liquid chromatography–purified β-amyloid$_{(1-42)}$ (Bachem, Bubendorf, Switzerland) was used as standard. Intra-assay variability was less than 5% (n = 501) and interassay variability was less than 10% (n = 11). Sensitivity for the ELISA for CSF samples was 50 pg/mL (5 SD above the absorbance level in the blank). The assay was linear in the range of 200 to 1500 pg/mL.

In brief, 21F12 antibody was suspended in Tris and sodium chloride (10 mmol of each) and coated onto Nunc Maxisorp (Nunc, Roskilde, Denmark) microtiter plates overnight at +4°C. After 1 wash step, plates were blocked for 2 hours at 25°C with phosphate-buffered saline solution containing 0.1% casein. To prepare the standards, a 0.1-mg/mL solution of a pool of different batches of β-amyloid$_{(1-42)}$ peptides dissolved in 0.1% ammonia were stored at −70°C and further diluted with a phosphate-buffered saline solution containing Triton X-705 (0.286% vol/vol), sodium chloride (138 mmol), potassium chloride (2.68 mmol), sodium hydrogen phosphate (8 mmol), potassium hydrogen phosphate (1.47 mmol), and casein (0.1%). After decanting, biotinylated 3D6 (75 µL) was incubated simultaneously with 25 µL of CSF or standard for 1 hour at 25°C. After washing, the amount of bound antibody was determined by adding horseradish peroxidase and streptavidine, 100 µL (RDI; Reserc Diagnostic Inc, Flanders, NJ). Incubation was continued for 30 minutes at 25°C, after which 100 µL of 0.42-mmol 3,5,3',5'-tetramethylbenzidine was added as peroxidase substrate. The reaction was stopped after 30 minutes with 0.9N sulfuric acid (50 µL). All analyses were run on the same batch of antibodies and ELISA plates.

DETERMINATION OF apoE ISOFORMS

Determination of apoE alleles was performed by isoelectric focusing of isolated apolipoproteins, followed by Western blotting[36] or by polymerase chain reaction, followed by reverse DNA hybridization using an apoE kit (INNO-LiPA; Innogenetics N.V., Ghent), depending on the sample material available. Serum or whole blood samples were attainable from 52 of 53 patients with AD and all 21 controls.

STATISTICAL ANALYSIS

For group comparisons of clinical and biochemical variables, analysis of variance with post hoc comparisons (Tukey) was used. The Spearman coefficient was used for correlation. The reference limit was estimated as the 0.95 fractile of the control values using a rank-based method.[37]

Data are given as mean ± SD.

follow-up, and by studying the relation between CSF-β-amyloid$_{(1-42)}$ level and disease duration. Our interest was to see whether low CSF-β-amyloid$_{(1-42)}$ levels are present early in the disease process and consequently whether this analysis may be of use as an early biochemical marker of the disease in patients with AD. Similarly, it is still an open question of whether CSF-β-amyloid$_{(1-42)}$ level changes with increasing severity of dementia. Therefore, we also studied the relation between CSF-β-amyloid$_{(1-42)}$ level and severity of dementia.

Finally, apolipoprotein E (apoE) has been found to be involved in the pathogenesis of AD. A higher frequency of the apoE allele ε4 is found in patients with AD than in the general population.[25] However, the pathogenic mechanism of apoE4 in AD is still unknown. Apolipoprotein E has been shown to bind to β-amyloid in vitro,[26] but the binding avidity between β-amyloid and the apoE isoforms differs depending on the experimental procedure, ranging from higher avidity for apoE E4 than E3[27] to lower avidity for apoE E4 than E3[28] to no difference.[29] It is possible that differences in binding between β-amyloid and the apoE isoforms may affect the CSF levels of β-amyloid. Therefore, we also studied whether the CSF-β-amyloid$_{(1-42)}$ level differs between patients with AD who do or do not possess the apoE ε4 allele.

RESULTS

There was a significant positive correlation between age and CSF-β-amyloid$_{(1-42)}$ level in the AD group ($r = 0.46$; $P < .001$), whereas no such correlation was found in the control group ($r = -0.15$).

Cerebrospinal fluid β-amyloid$_{(1-42)}$ levels were significantly ($P < .001$) decreased in the AD group (709 ± 304 pg/mL) compared with the control group (1678 ± 436 pg/mL). The individual values are shown in **Figure 1**. The reference limit was 1130 pg/mL. Using this cutoff level, 49 of 53 patients had low CSF-β-amyloid$_{(1-42)}$ levels, ie, a sensitivity of 92% (95% confidence interval, 82%-98%).

When comparing the AD subgroups (Figure 1), the CSF-β-amyloid$_{(1-42)}$ level was significantly ($P < .001$) lower in the EAD group (422 ± 170 pg/mL) compared with the LAD group (845 ± 255 pg/mL).

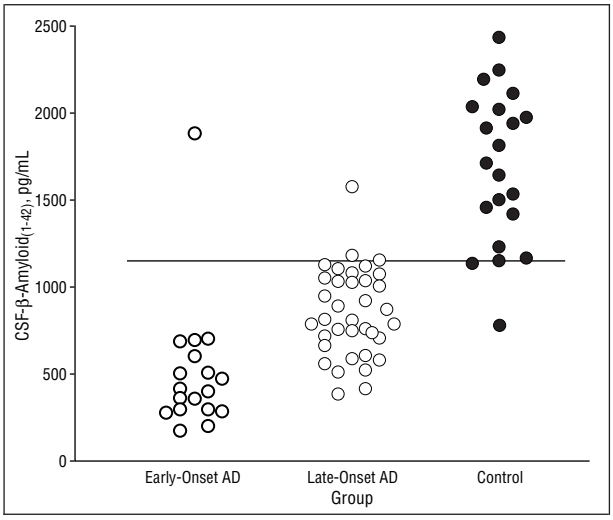

Figure 1. *Individual cerebrospinal fluid (CSF) β-amyloid(1-42) levels in a community-based patient sample. The horizontal line represents the cutoff level in healthy controls (1130 pg/mL). The total Alzheimer disease (AD) group vs the control group is significant at P<.001, and early-onset AD vs late-onset AD is significant at P<.001.*

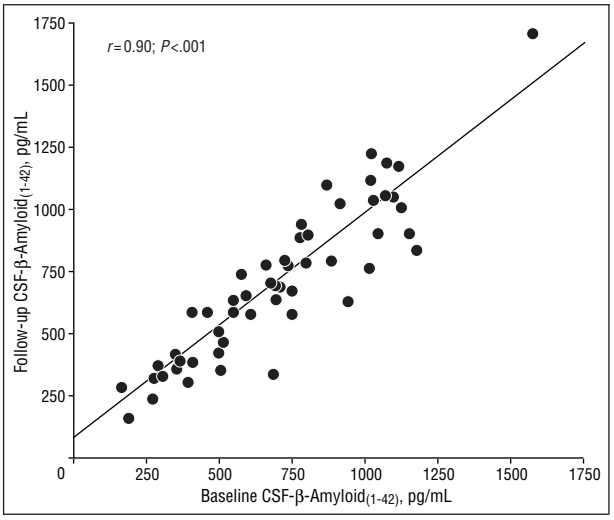

Figure 2. *Cerebrospinal fluid (CSF) β-amyloid(1-42) level at baseline and follow-up in patients with Alzheimer disease. The diagonal line represents the regression line for the correlation (r = 0.90; P<.001).*

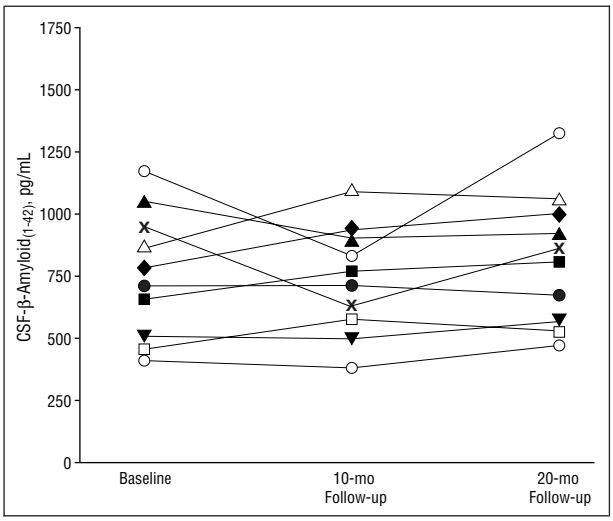

Figure 3. *Cerebrospinal fluid (CSF) β-amyloid(1-42) level at baseline and at follow-up 10 and 20 months later in 10 patients with Alzheimer disease.*

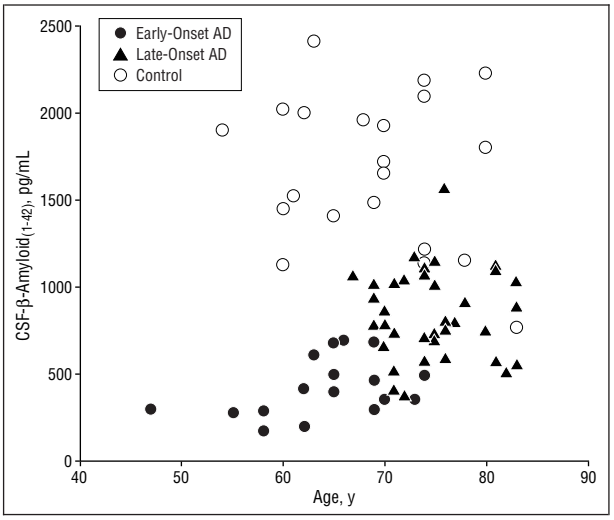

Figure 4. *Relation between age and cerebrospinal fluid (CSF) β-amyloid(1-42) level in patients with Alzheimer disease (AD) and healthy controls. Spearman r = 0.46 (P<.001) in the AD group and r = -0.15 (P = .30) in the control group.*

We then studied whether CSF-β-amyloid$_{(1-42)}$ level changes during the course of disease. In the AD group, CSF-β-amyloid$_{(1-42)}$ levels did not significantly differ between baseline (709 ± 304 pg/mL) and the first follow-up investigation at approximately 10 months (701 ± 309 pg/mL). The corresponding values were 422 ± 170 pg/mL at baseline and 406 ± 145 pg/mL at the first follow-up visit in the EAD subgroup, and 845 ± 255 pg/mL at baseline and 840 ± 265 pg/mL at the first follow-up visit in the LAD subgroup. There were also highly significant correlations between baseline and first follow-up visit CSF-β-amyloid$_{(1-42)}$ levels ($r = 0.90$; $P<.001$) (**Figure 2**), and the coefficient of variation between baseline and follow-up levels was low (10.8%). The corresponding correlations were $r = 0.80$ ($P<.001$) in the EAD subgroup and $r = 0.81$ ($P<.001$) in the LAD subgroup.

In 10 patients with AD, 3 longitudinal CSF samples were collected (at baseline and at 10- and 20-month follow-up visits). The individual values are shown in **Figure 3**. Also in these patients, CSF-β-amyloid$_{(1-42)}$ levels showed no consistent change, and most patients showed stable levels over time.

Incubation experiments in different test tubes showed that the level of CSF-β-amyloid$_{(1-42)}$ fell from the start (set to 100%) to 91.6% ± 2.6% ($P = .73$) in polypropylene, 63.4% ± 14.4% ($P<.001$) in polystyrene, and 66.7% ± 12.2% ($P<.001$) in glass tubes. In contrast, the level of neuron-specific enolase did not significantly change after incubation in polypropylene (101.8% ± 9.4%), polystyrene (102.4% ± 5.4%), or glass (99.9% ± 2.8%) tubes.

There was no significant correlation ($r = –0.16$) between duration of dementia and level of CSF-β-amyloid$_{(1-42)}$ in the total AD group (**Figure 4**). Also, there were no significant correlations between duration of dementia and level of CSF-β-amyloid$_{(1-42)}$ in the EAD ($r = 0.35$) or LAD ($r = 0.08$) subgroups. There was no significant correlation

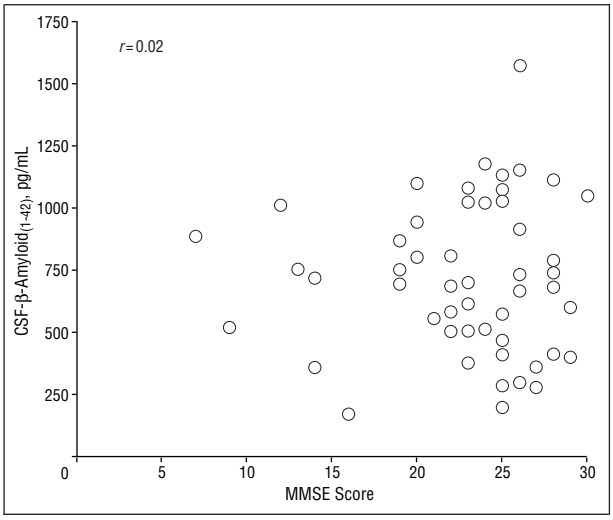

Figure 5. *Relation between severity of dementia and cerebrospinal fluid (CSF) β-amyloid$_{(1-42)}$ level in patients with Alzheimer disease. Spearman r = -0.02 (P = .87). MMSE indicates Mini–Mental State Examination.*[32]

(r = –0.02) between severity of dementia, estimated using the MMSE, and CSF-β-amyloid$_{(1-42)}$ level in the AD group (**Figure 5**) or between MMSE score and CSF-β-amyloid$_{(1-42)}$ level in the EAD (r = –0.14) or LAD (r = 0.13) subgroups.

We then studied the rate of progression of dementia in patients with AD, measured as the change in MMSE score between baseline and follow-up investigations, and the CSF-β-amyloid$_{(1-42)}$ level. The MMSE score fell from 22.8 ± 52.0 at baseline to 18.3 ± 6.7 at follow-up (mean, 4.5 points). However, there were no significant correlations between rate of progression of dementia and CSF-β-amyloid$_{(1-42)}$ levels in the total AD group (r = 0.08) or in the EAD (r = –0.24) or LAD (r = –0.15) subgroups.

Within the AD group, there were no significant differences between patients with and without the apoE ϵ4 allele regarding age (71.1 ± 7.1 vs 71.7 ± 7.6 years), duration of dementia (48.0 ± 31.2 vs 54.7 ± 32.5 months), or severity of dementia (MMSE score, 22.6 ± 4.9 vs 22.9 ± 5.4). The apoE ϵ4 allele frequency was 41% in the AD group and 19% in the control group. In comparing CSF-β-amyloid$_{(1-42)}$ levels between individuals with and without the apoE ϵ4 allele, no significant differences were found in the AD group (n = 16 without ϵ4; CSF-β-amyloid$_{(1-42)}$, 731 ± 393 pg/mL vs n = 36 with ϵ4; CSF-β-amyloid$_{(1-42)}$, 690 ± 259 pg/mL) or in the control group (n = 13 without ϵ4; CSF-β-amyloid$_{(1-42)}$, 1702 ± 339 pg/mL vs n = 8 with ϵ4; CSF-amyloid$_{(1-42)}$, 1641 ± 587 pg/mL).

COMMENT

In the present study, we found a marked decrease in CSF-β-amyloid$_{(1-42)}$ levels in patients with AD compared with healthy, age-matched controls. Using the lower 0.95 fractile of the control values as a reference limit, the sensitivity of the assay for diagnosis of AD was high (92%). Thus, we were able to confirm previous findings of reduced CSF levels of β-amyloid$_{(1-42)}$ in patients with AD.[8,21-23] The high sensitivity of CSF-β-amyloid$_{(1-42)}$ in the present study compared with previous studies has many possible explanations. First, we studied healthy elderly individuals as controls, whereas some previous studies[21] used patients with neurologic disorders without cognitive impairment.[20] Alternate explanations include differences in selection and characteristics of patients and controls and in CSF sampling or analytic methods. Incubation experiments in test tubes of different materials showed that the level of neuron-specific enolase—a cytosolic hydrophilic enzyme—did not significantly differ when incubated in different test tubes. In contrast, CSF-β-amyloid$_{(1-42)}$ levels showed a 33% reduction in glass tubes; a 36% reduction in polystyrene tubes, commonly used in laboratories; and no change in polypropylene tubes. Therefore, we collected all CSF samples in polypropylene test tubes, to which β-amyloid does not adhere. If such confounding factors are taken into account, the high sensitivity suggests that CSF-β-amyloid$_{(1-42)}$ level may be useful as a biochemical marker for AD, especially to differentiate AD from normal aging. However, the specificity of CSF-β-amyloid$_{(1-42)}$ must be further evaluated.

Although the interindividual variation in CSF-β-amyloid$_{(1-42)}$ level was low within each diagnostic group, 2 patients with AD had deviating CSF-β-amyloid$_{(1-42)}$ levels (Figure 1). The patient with LAD (CSF-β-amyloid$_{(1-42)}$ level = 1569 pg/mL) had a typical history and clinical findings of AD. However, review of the medical records of the patient with EAD with a high CSF-β-amyloid$_{(1-42)}$ level (1876 pg/mL) revealed that this patient had a history of repeated head trauma, when practicing his profession as a construction worker and as a "head-specialist" elite soccer player. At age 45 years, he experienced concentration difficulties and reduced short-term memory, and his wife also reported changes in his personality such as aggressiveness and suspiciousness. He was given a diagnosis of AD but so far has not shown any progress during 18 months of follow-up. Thus, this patient had atypical AD, suggestive of a variant of dementia pugilistica.

The mechanism(s) leading to a reduction in CSF-β-amyloid$_{(1-42)}$ level in patients with AD is still unclear. One possible explanation is that reduction is secondary to the progressive degeneration of neurons. However, after acute ischemic stroke, there is a marked increase in CSF-tau within 1 to 2 days that peaks after 2 to 3 weeks and returns to normal values after 3 to 4 months, whereas the level of CSF-β-amyloid$_{(1-42)}$ remains unchanged (C. Hesse, L. Rosengren, MD, PhD, H. Vanmechelen, PhD, P. Davidsson, PhD, K. Blennow MD, PhD, unpublished data, 1998). These data support the hypothesis that the level of CSF-τ reflects neuronal damage and degeneration, whereas the level of CSF-β-amyloid$_{(1-42)}$ does not seem to simply be a marker for neurodegeneration.

β-Amyloid is secreted to the extracellular space, which is continuous with CSF. In AD and control brains, β-amyloid exists in a water-soluble form,[38] whereas in AD, a portion of β-amyloid aggregates and is incorporated into highly insoluble fibrils in the plaques. These amyloid deposits consist primarily of β-amyloid$_{(1-42)}$.[16,17,39] Because β-amyloid$_{(1-42)}$ is more hydrophobic than shorter variants of β-amyloid,[40] it is possible that this form is more prone to aggregate in SP. Thus, alternatively, a reduction of CSF-β-amyloid$_{(1-42)}$ level in patients with AD may be secondary to an aggrega-

tion in the amyloid deposits and SP, decreasing the amount of β-amyloid$_{(1-42)}$ that can be secreted to extracellular space and thereby resulting in lower levels remaining in CSF, as suggested previously.[38] However, alternative explanations include reduced production or secretion of β-amyloid in AD brains.

In the present study, the difference in CSF-β-amyloid$_{(1-42)}$ levels between the control and AD groups was 1678 – 709 = 969 pg/mL. Assuming that (1) this amount of β-amyloid is deposited in SPs; (2) average CSF production is approximately 500 mL per 24 hours[41]; (3) the mean (preclinical and clinical) duration of AD is about 30 years[42]; and (4) the intensity of the degenerative process, including the rate of SP formation, is relatively constant during the disease process, then 969 pg/mL × 500 mL = 484 ng β-amyloid$_{(1-42)}$ would be deposited per day, 484 ng × 365 days = 177 μg β-amyloid$_{(1-42)}$ would be deposited per year, and 177 μg × 30 years = 5.3 mg of β-amyloid$_{(1-42)}$ would be deposited in the brain throughout the course of AD. A direct comparison between CSF and the brain is difficult because data on biochemical quantification of β-amyloid in the brain is sparse. However, a study[43] using a dot-blot assay to quantify biochemically the amount of sodium dodecyl sulfate–insoluble total β-amyloid in the temporal cortex found a level of approximately 100 pmol (approximately 4 μg) per 400 mg of tissue. Assuming that (1) the amount of deposited β-amyloid is fairly similar in other affected brain regions and (2) the weight of affected cortical tissue is about 100 g, the amount of deposited β-amyloid is approximately 4 μg/0.4 g × 100 g = 1 mg. The amount of total β-amyloid (also sodium dodecyl sulfate soluble from diffuse plaques) is probably several times higher. Thus, although these types of calculations are hazardous because of several uncertain assumptions, we consider our value of approximately 5 mg of β-amyloid$_{(1-42)}$ to be reasonable.

We also studied whether CSF-β-amyloid$_{(1-42)}$ level changes over time during the disease process. In the present study, CSF-β-amyloid$_{(1-42)}$ levels were stable between baseline and follow-up investigations, and there were no correlations between CSF-β-amyloid$_{(1-42)}$ level and duration of dementia. These findings show that CSF-β-amyloid$_{(1-42)}$ levels are stable for each patient, ie, that the summarized biological and methodological variations for CSF-β-amyloid$_{(1-42)}$ level are low. Furthermore, these findings suggest that low CSF-β-amyloid$_{(1-42)}$ levels are present during the earlier stages of the disease, but because relatively few patients with MMSE scores below 15 were included, we cannot exclude that CSF-β-amyloid$_{(1-42)}$ level changes late in the course of the disease. Nonetheless, CSF-β-amyloid$_{(1-42)}$ level may also be useful as a diagnostic marker early in the disease process, when diagnosis is most difficult. This is of significance in selecting patients with early memory disturbances for treatment and for clinical drug trials. Studies to resolve this question are in progress.

We then investigated whether CSF-β-amyloid$_{(1-42)}$ level varies with severity of dementia or rate of progression of the disease. In the EAD group, there was a tendency for a correlation between CSF-β-amyloid$_{(1-42)}$ level and rate of progression of dementia; lower levels of CSF-β-amyloid$_{(1-42)}$ correlated with faster progression. However, we did not find any correlation between CSF-β-amyloid$_{(1-42)}$ level and either severity or rate of progression of dementia. Two previous studies[8,23] also were not able to find any significant correlation between CSF-β-amyloid$_{(1-42)}$ level and severity of disease or MMSE scores. However, the MMSE is a relatively insensitive instrument for reflecting the course of the disease, and the 1-year follow-up is relatively short. Thus, further studies are needed to clarify these issues.

Finally, we studied whether CSF-β-amyloid$_{(1-42)}$ level varies between patients who do or do not possess the apoE ε4 allele. Several lines of evidence suggest a link between apoE and β-amyloid. First, apoE immunoreactivity is found in SP.[26,44] Second, results of in vitro studies show that apoE binds to β-amyloid. However, results regarding different binding affinity between β-amyloid and apoE isoforms are controversial and seem mainly to depend on the experimental procedure.[27-29] In the present study, CSF-β-amyloid$_{(1-42)}$ levels did not differ between patients with AD, with and without the apoE4 allele. These results also agree with those from a previous CSF study[8] and suggest that possible differential binding affinity between the different apoE isoforms and β-amyloid does not affect the CSF level of β-amyloid.

Although the duration and severity of dementia did not significantly differ between the EAD and LAD subgroups, the decrease in CSF-β-amyloid$_{(1-42)}$ level was more pronounced in the EAD subgroup. This finding further supports the hypothesis that the current clinical criteria for probable AD delimits a heterogeneous group of patients. The term "Alzheimer disease" (AD) was originally reserved for dementia in patients with presenile (before age 65 years) onset of symptoms, whereas the term "senile dementia" was used when the onset was at or after age 65 years. However, since the 1960s, largely based on the histopathologic observations that neurofibrillary tangles and SP are found in the brains of patients with EAD and LAD, EAD and LAD have been held to represent a single, homogeneous entity. Evidence from several reports[45-48] suggests that the degree of neuronal and synaptic[49] degeneration, degree of neurotransmitter disturbances,[50] and density of neurofibrillary tangles and SP in the cortex[46,48,51-55] are more severe in patients with EAD than in those with LAD. In contrast, the degree of concomitant cerebrovascular pathologic findings, especially white matter lesions, or leukoaraiosis, is more severe in patients with LAD than in those with EAD.[30,56-58] Therefore, several investigators suggested a multifactorial origin of dementia in LAD[45,47,48,59] and that 2 subgroups of probable AD can be delimited—one with "pure" EAD and another with LAD in which a combination of age-related changes, less severe Alzheimer encephalopathy, and concomitant cerebrovascular changes together produces the dementia.[60,61]

In summary, the results of the present study confirm previous findings that CSF-β-amyloid$_{(1-42)}$ level is decreased in patients with AD. The sensitivity of the assay to identify AD is high, and the test has good reproducibility and low intra-individual biological variation. Low levels of CSF-β-amyloid$_{(1-42)}$ are found throughout the course of AD. In evaluating patients with suspected AD, lumbar puncture has only a minimal risk for complications such as headache.[62] Thus, CSF-β-amyloid$_{(1-42)}$

level may be a useful tool in the routine clinical diagnosis of AD, especially to discriminate between incipient AD and normal aging, and also early in the course of the disease, when drug therapy has the greatest potential of being effective.

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