Decreased β-Amyloid$_{1-42}$ and Increased Tau Levels in Cerebrospinal Fluid of Patients With Alzheimer Disease

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Context Alzheimer disease (AD) is characterized by pathological results at autopsy of amyloid plaques and tau-associated neurofibrillary tangles, but the clinical diagnosis of AD is determined on the basis of medical history, cognitive symptoms, and exclusionary criteria. The search for antemortem biomarkers is intense and has focused on cerebrospinal fluid (CSF) β-amyloid$_{1-42}$ and tau proteins.

Objectives To compare CSF β-amyloid and tau levels in a new population of AD patients and controls. To perform a meta-analysis of studies of CSF β-amyloid and tau levels in AD patients and controls.

Design Cross-sectional study of the comparison of baseline CSF β-amyloid$_{1-42}$ and tau levels in AD patients and controls. Meta-analysis involved 17 studies of CSF β-amyloid and 34 studies of CSF tau.

Setting Clinical research unit of the National Institute of Mental Health, Bethesda, Md.

Patients The Geriatric Psychiatry Branch evaluated AD patients as inpatients at the National Institutes of Health Clinical Center between May 1985 and January 2001. A total of 203 patients participated in this study (131 with AD and 72 controls). None had other serious illnesses, and 31 of 131 AD cases had AD confirmed at autopsy. Meta-analysis provided an additional 3133 AD patients and 1481 controls.

Main Outcome Measures Levels of CSF β-amyloid$_{1-42}$ were measured by a sandwich enzyme-linked immunoabsorbent assay with a polyclonal capture antibody and a monoclonal detection antibody. Levels of CSF tau were measured with a standard commercial immunoassay.

Results Levels of CSF β-amyloid$_{1-42}$ were significantly lower in the AD patients vs controls (mean [SD], 183 [121] pg/mL vs 491 [245] pg/mL; $P<.001$). Levels of CSF tau were significantly higher in AD patients (mean [SD], 587 [365] pg/mL vs 244 [156] pg/mL; $P<.001$). The cutpoints of 444 pg/mL for CSF β-amyloid$_{1-42}$ and 195 pg/mL for CSF tau gave a sensitivity and specificity of 92% and 89%, respectively, to distinguish AD patients from controls, which is comparable with rates with clinical diagnosis. Meta-analyses of studies comparing CSF β-amyloid and tau levels in AD participants and controls confirmed an overall difference between levels in these 2 groups.

Conclusions Alzheimer disease is associated with a significant decrease in CSF β-amyloid$_{1-42}$ levels along with an increase in CSF tau levels. These findings suggest that the 2 measures are biological markers of AD pathophysiology. While these CSF measures may have a potential clinical utility as biomarkers of disease, the preliminary and retrospective nature of the findings, the absence of assay standardization, and the lack of comparison patient populations must be addressed in future studies testing the usefulness of these CSF measures for predictive, diagnostic, or treatment evaluation purposes.

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While the exact biological cascade associated with Alzheimer disease (AD) is only partially understood, many potential biomarkers of this disease process are known.[1] Two of the most obvious candidates are β-amyloid$_{1-42}$ and tau proteins, as they are intimately related to the pathognomonic features of amyloid plaques and neurofibrillary tangles in the AD brain.[2,3] Multiple previous studies have reported decreases in cerebrospinal fluid (CSF) measures of β-amyloid.[4-7] Similarly, CSF measures of tau have routinely showed considerable elevations of this peptide in AD cases worldwide.[7-12] Some authors have reported that these 2 measures alone can accurately differentiate clinically diagnosed AD cases from controls more than 85% of the time.[7,13]

Studies of CSF in AD patients have used widely varying methods and nomenclature for assessing and describ-

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ing CSF β-amyloid$_{1-42}$.[4,6,7,14,15] Furthermore, while the majority of studies report decreases in CSF β-amyloid levels in AD patients, some studies show no significant change or even a slight increase in CSF β-amyloid levels in AD patients when compared with controls.[16,17] Finally, the majority of CSF β-amyloid reports include a paucity of participants. We assessed CSF β-amyloid and tau levels in the largest cohort of AD patients and controls evaluated to date, to our knowledge. In addition, we performed a meta-analysis of CSF β-amyloid$_{1-42}$ and tau to help clarify whether consistent trends emerged.

METHODS

Participants

A total of 208 participants (136 with AD and 72 controls) were evaluated as part of an ongoing study of AD. (Five patients were excluded after autopsy showed they did not have AD. See "Autopsy" section.) Patients with AD were referred by their primary physician, and controls were self-referred, in response to local advertisements, as unpaid volunteers. Participants signed informed consent documents for this prospective study after approval of the National Institute of Mental Health institutional review board (protocols 82-M-123 and 95-M-96). For AD patients (n=136), signed informed consent was obtained from the patient and from the individual's durable power of attorney for research decisions, as previously described.[18,19] All AD patients were given both written and verbal explanations of the study procedures involved, and repeated assent was required before any individual procedure could proceed. For the control participants, written informed consent was obtained in a routine fashion.

Clinical Evaluation

The Geriatric Psychiatry Branch evaluated AD patients as inpatients at the National Institutes of Health Clinical Center between May 1985 and January 2001. Evaluation included thorough medical screenings, neurocognitive profiling, magnetic resonance imaging scan, lumbar puncture for CSF examination, and behavioral observations for 1 to 2 weeks. Individuals were participating in a longitudinal study of biological changes that occur over time in AD (protocols 82-M-123 and 95-M-96). Medical evaluations for all participants included a physical examination, a routine electrocardiogram, and blood tests (eg, venereal disease research laboratory test for syphilis, complete blood cell count, vitamin B_{12} levels, and thyroid function tests) to eliminate other known contributors to memory impairment. Routine computed tomography scans or magnetic resonance imaging scans (1.5 tesla) also were performed to exclude the possibility of overt cerebrovascular disease. Controls underwent medical evaluations to exclude serious medical illnesses (eg, type 1 diabetes mellitus, significant hypertension, or cardiovascular disease).

Clinical Assessment

Trained inpatient staff administered to all participants several global clinical rating instruments, including the Clinical Dementia Rating (CDR),[20] Global Deterioration Scale (GDS),[21] and the Mini-Mental State Examination (MMSE).[22] These well-established rating instruments were given within 1 month of the lumbar punctures. Patients with AD were diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, and NINCDS-ADRDA criteria.[23,24]

Lumbar Puncture

Lumbar punctures were performed at the start of the morning. Participants were inpatients at the National Institutes of Health Clinical Center and were kept at bedrest and had nothing by mouth until the procedure was completed. While the patient was in the lateral decubitus or sitting position, lumbar punctures were performed with a 20- or 22-gauge needle after application of local anesthesia with 1% to 2% lidocaine. Headache rates following lumbar punctures were less than 10% (ranging from mild discomfort to severe headaches requiring follow-up care with a blood patch). Approximately 30 mL of CSF was withdrawn during the lumbar puncture; the CSF was aliquoted into individual polypropylene tubes without preservative and frozen at the bedside on dry ice within minutes of withdrawal. Samples were then transferred to −70°C freezers. The low-temperature freezers are monitored daily for temperature control, and they have auxiliary liquid carbon dioxide backup in case of mechanical or electrical failures.

CSF Assays

Assays were performed on the CSF samples from AD patients that had been stored undisturbed at −70°C for variable lengths of time, ranging from less than 6 months to longer than 15 years. Since the time samples were stored in the freezer before CSF assay (shelf life) differed significantly by diagnostic category (mean [SD] for controls, 3.1 [2.9] years vs for AD patients, 7.1 [3.4] years; $P < .001$), we tested whether shelf life was associated with CSF β-amyloid$_{1-42}$ or tau levels. Within the control group, neither CSF β-amyloid$_{1-42}$ nor tau levels were significantly correlated with duration of shelf life ($r=-0.03$, $P=.78$ and $r=0.02$, $P=.81$, respectively). Similarly, neither CSF β-amyloid$_{1-42}$ nor tau levels were significantly associated with duration of shelf life in the AD group ($r=-0.07$, $P=.33$ and $r=0.02$, $P=.79$, respectively).

CSF β-Amyloid$_{1-42}$ Levels. β-Amyloid$_{1-42}$ was measured with a 1-step sandwich enzyme-linked immunoabsorbent assay (IGEN International Inc, Gaithersburg, Md) using a polyclonal antibody specific for β-amyloid$_{1-42}$ used as the capture antibody and a monoclonal antibody, 4G8, used as the detection antibody. This assay is designed specifically to measure the human β-amyloid 1-42 peptide in CSF in a 10× excess background of 1-40. The monoclonal antibody (catalog No. 4G8 240-10; Senetek, Napa, Calif) was supplied in the form of purified IgG at 1 mg/mL and ruthinylated at 7:1 ratio. The β-amyloid polyclonal antisera (cata-

log No. 44-344; QCB Division of BioSource International, Hopkinton, Mass) was supplied as 0.530 mg/mL and biotinylated at a 20:1 ratio. β-Amyloid peptide (catalog No. 03-111 [1-42] and catalog No. 03-136[1-40]; QCB) was supplied as 1 mg and as trifluoroacetate salt. Solubilization was performed in 1 mg/mL of dimethylsulfoxide and snap frozen on dry ice. Samples of CSF (200 µL) or standards (200 µL) were added to 50 µL of antibody (4 µg/mL of monoclonal antibody 4G8 and 3 µg/mL of polyclonal antibody) and 25 µL of streptavidin M-280 paramagnetic beads (IGEN International Inc) at 600 µg/mL, which was prepared in phosphate buffered saline with 15% bovine serum albumin. This mixture was incubated at room temperature for 3 hours with shaking followed by the addition of 300 µL of phosphate buffered saline at the end of the incubation period.

The immune complexes were quantitated by measurement of electrochemiluminescent signal using an ORIGEN 1.5 Analyzer (IGEN International Inc) with 5 standard concentrations from 125 to 2000 pg/mL. The intra-assay variability was assessed by calculating the percent coefficient variation for replicates of the individual samples on the same assay and then averaging those values for each assay. Using a standard curve, the inter-assay variability was determined by calculating the percent coefficient variation for the quality control samples across all of the assays. Intra-assay and inter-assay variability measures were 3.5% and 5.2%, respectively.

CSF Tau Protein Levels. Tau was measured using a commercial enzyme immunoassay (Innotest Inc, Ghent, Belgium). In this assay, the wells of polystyrene microtiter plates were coated with the solid phase anti–human tau monoclonal antibody (AT120). The test samples were incubated in these wells along with 2 separate biotinylated tau monoclonal antibodies (H57 and BT2) that recognize different tau epitopes. Samples were rinsed with an assay buffer and then incubated with peroxidase-labeled streptavidin. Samples were then incubated with tetramethylbenzidine and 0.006% hydrogen peroxide per manufacturer's instructions. The reaction was stopped with diluted sulfuric acid and optical density measurements read using a Molecular Devices Spectramax Plus plate reader. Intra-assay and inter-assay variability measures were 5.6% and 8.1%, respectively.

Autopsy. The original clinical population consisted of 136 patients with "probable" AD. Of those patients, 36 have since died and undergone autopsy. The diagnosis of AD was confirmed in 31 of 36 patients (86% accuracy) according to standard neuropathologic criteria[25,26] without knowledge of CSF data. Autopsy results revealed that 5 cases of clinical AD were found to have other neuropathological diagnoses: 2 with Lewy body dementia, 2 with cerebrovascular dementia, and 1 with thalamic dementia. These cases were excluded from the dataset for the CSF analysis. The final AD group (n=131) consists of 100 patients with "probable" AD who are still alive and 31 patients with autopsy-proven AD.

Statistical Analysis

Parametric comparisons between the AD and control groups were performed using unpaired *t* tests. Satterthwaite adjusted *t* tests and degrees of freedom are reported when the group variances were unequal. Pearson correlation coefficient was used for exploratory correlations within groups. When our key outcome measures (ie, CSF β-amyloid$_{1-42}$ and tau) were significantly correlated with the baseline variables ($P<.05$), factorial designs were applied to the data with sex and age as grouping variables.[27] Data are expressed as mean (SD) unless otherwise specified. All analyses were performed using the software packages of SAS version 8.02 (SAS Institute, Cary, NC), CART version 3.6 (Salford Systems, San Diego, Calif), and NCSS 2001 (Kaysville, Utah).

A classification and regression tree (CART) is a nonparametric, binary decision tree method of analysis (an "if-then" scenario) similar to the diagnostic decision trees used in differential diagnosis in medicine,[28] especially when looking for relationships between a small number of variables.[29] The CART approach allows for variables to be tested simultaneously for diagnostic classification without relying on classical statistical assumptions, such as the normality of the data and homogeneity of variance. CART also was used to estimate objective bivariate cutpoints for the CSF variables to determine maximal sensitivity and specificity associated with the clinical diagnosis of AD. In this analysis, the lead variable was CSF β-amyloid$_{1-42}$ followed by CSF tau.

A meta-analysis of CSF β-amyloid$_{1-42}$ comparisons in AD and control participants was performed by calculating and combining the effect sizes and *t* test scores across 17 CSF studies.[30] These studies were chosen from 188 articles that resulted from PubMed and MEDLINE literature searches from August 1989 to March 2003 using key words *Alzheimer's* and *CSF* and *beta-amyloid*, or *amyloid beta* in titles and abstracts. Studies were sorted according to relevance and were excluded if they were not in the English language, did not provide data for controls, did not provide diagnostic criteria, or failed to distinguish total CSF β-amyloid from its components (40 and 42 residue chains). Studies with fewer than 25 total participants or those that did not report the SD of the mean CSF β-amyloid level also were excluded. If identical or overlapping population samples were used in 2 separate articles, a judgment was made to include the more complete article in the meta-analysis. After application of these criteria, a total of 17 studies were included in the meta-analysis. In this meta-analysis, studies were weighted according to sample size.[27] Effect size was calculated by dividing the difference of the means for the outcome variable by the pooled SD.[31]

A meta-analysis using the same procedures also was performed for articles reporting CSF tau levels across the same time period as CSF β-amyloid$_{1-42}$ levels. Again, articles were excluded from further consideration if they included previously reported data, did not include control participants, had poorly de-

scribed methods, included mixed diagnostic populations, had missing SDs for mean CSF tau levels, or had fewer than 25 participants. The initial list of 200 articles was reduced to a final count of 34 relevant studies for the meta-analysis focusing on CSF tau levels.

Because the underlying units of measurement varied from study to study, all units were converted to picograms per milliliter using a commonly available calculator program (http://molbiol.ru/eng/scripts/01_04.html). The overall effect size and *t* test with the associated *P* value were calculated for this meta-analysis.[32] The *t* tests were calculated with unequal variances using the Satterthwaite adjusted degrees of freedom rounded to the nearest integer.

RESULTS

Baseline and Global Measures

The 131 AD participants (mean [SD] age, 68.1 [9.1] years; range, 44-88 years) had a mean (SD) age of dementia onset of 64.4 (9.4) years and duration of illness of 3.6 (2.4) years. The AD participants were mildly to moderately impaired with mean (SD) MMSE scores of 19.7 (6.7). The control participants (n=72; mean [SD] age, 59.4 [8.5] years; range, 45-86 years) were significantly younger and more educated than the AD patients (TABLE 1).

CSF Measures of β-Amyloid$_{1-42}$ and Tau

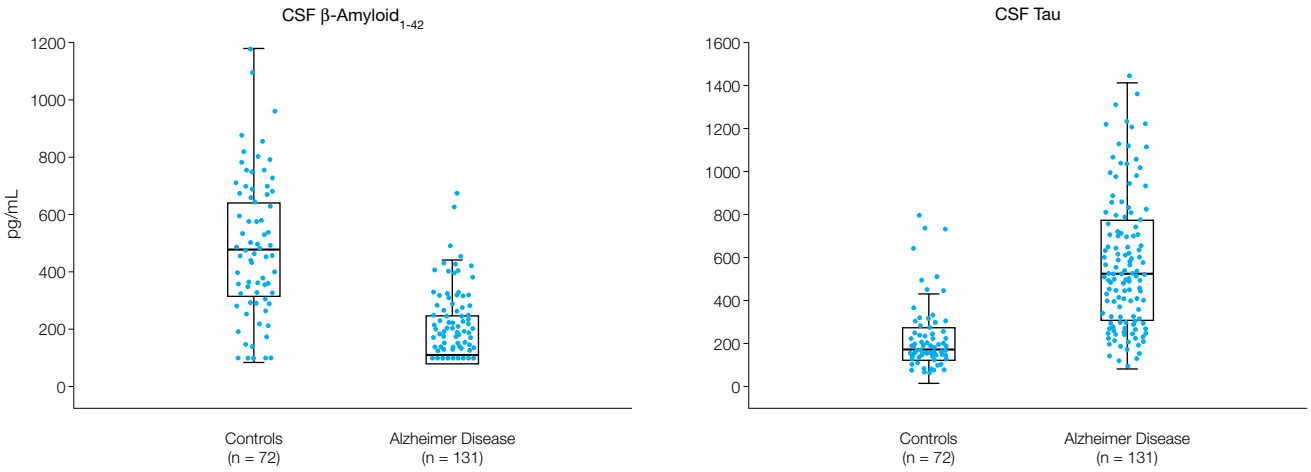
Mean (SD) CSF β-amyloid$_{1-42}$ were significantly lower in the AD patients compared with the controls (183 [121] pg/mL vs 491 [245] pg/mL; $P < .001$). Despite the statistically significant differences between groups, the data showed considerable variance, resulting in significant overlap between groups (FIGURE 1). Marked differences in mean CSF tau levels between AD patients and controls also were observed (587 [365] pg/mL vs 224 [156] pg/mL; $P < .001$). For CSF tau, tau concentration was significantly associated with age of controls ($r=0.43$, $P<.001$) but not age of AD patients ($r=0.012$, $P=.90$). Conversely, there was a significant sex effect with mean (SD) CSF tau in the AD patients (men, 506 [258] pg/mL vs women, 652 [424] pg/mL; $t_{120}=2.43$ [unequal variance]; $P=.02$) but not in the controls ($t_{70}=0.21$, $P=.84$). Because of these exploratory findings, we performed a more definitive 3-way factorial analysis of variance (ANOVA) (diagnosis × sex × age) with age stratified into 3 levels (40-60 years, 60-70 years, and older than 70 years). As expected, the results of the ANOVA revealed a significant difference by diagnosis of AD ($F_{1,191}=40.9$, $P<.001$) but no main level or interaction effects for the sex or age group variables (3-way ANOVA, $F_{2,191}=0.30$, $P=.74$). A similar 3-way ANOVA for CSF β-amyloid$_{1-42}$ also revealed a strong overall effect of AD diagnosis ($F_{1,191}=106.36$, $P<.001$) but no main

Table 1. Demographic Characteristics and Baseline Measures for Controls and Patients With Alzheimer Disease (AD)

	Controls (n = 72)	AD Participants (n = 131)	*t* (*df*)
Men/women	27/45	59/72	
Age, mean (SD), y	59.4 (8.5)	68.1 (9.1)	6.6 (201)
Education, mean (SD), y	16.7 (2.3)	14.7 (3.3)	–5.0 (189)*
MMSE score (0-30), mean (SD)	29.2 (1.1)	19.7 (6.7)	–15.2 (130)*
CDR score (0-3), mean (SD)	0	1.4 (0.7)	25.0 (130)*
GDS score (1-7), mean (SD)	1.0	4.3 (1.0)	35.3 (136)*
Duration of illness, mean (SD), y	NA	3.6 (2.4)	

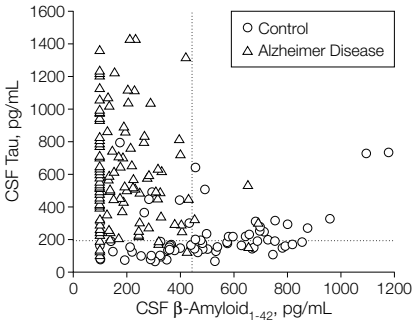
Abbreviations: CDR, Clinical Dementia Rating; GDS, Global Deterioration Scale; MMSE, Mini-Mental State Examination; NA, not applicable.
*Satterthwaite adjusted *t* test scores of unequal variance. $P<.001$ for all *t* scores.

Figure 1. Scattergraphs of β-Amyloid$_{1-42}$ and Tau Levels in Cerebrospinal Fluid (CSF) for Controls and Patients With Alzheimer Disease



Boxes represent 25th, 50th, and 75th percentiles of the data. The length of the box is the interquartile range. The lower and upper whiskers represent the 25th and 75th percentile plus or minus 1.5 times the interquartile range, respectively.

Figure 2. Specificity and Sensitivity Cutpoints for Measures of β-Amyloid$_{1-42}$ and Tau Levels in Cerebrospinal Fluid (CSF) Classifying Controls (n=72) and Patients With Alzheimer Disease (AD) (n=131)



Cutpoints are chosen for maximum separation of participant groups: upper left quadrant includes 120 participants with AD and 8 controls; upper right quadrant includes 3 participants with AD and 20 controls; lower left quadrant includes 7 participants with AD and 24 controls; and lower right quadrant includes 1 participant with AD and 20 controls. The cutpoints of 444 pg/mL for CSF β-amyloid$_{1-42}$ and 195 pg/mL for CSF tau generate a sensitivity of 92% and a specificity of 89%.

level or interaction effects for the sex or age group variables (3-way ANOVA, $F_{2,191}=0.57$, $P=.57$).

Neither CSF biomarker differed significantly by whether AD was confirmed by autopsy or clinically diagnosed (for mean [SD] CSF β-amyloid$_{1-42}$, 170 [115] pg/mL vs 187 [123] pg/mL, $t_{129}=0.68$, $P=.50$, respectively, and for mean [SD] CSF tau, 677 [250] pg/mL vs 559 [391] pg/mL, $t_{79}=-1.99$, $P=.06$, respectively). Therefore, the results of the 2 groups were combined.

Across all AD patients and controls, the CSF β-amyloid$_{1-42}$ and tau levels were significantly negatively correlated ($r=-0.30$, $P<.001$), perhaps a function of the number of AD patients with high CSF tau and low CSF β-amyloid$_{1-42}$ levels combined with the number of controls showing the opposite pattern. However, for the controls alone, the correlation between these 2 measures was positively correlated ($r=0.29$, $P=.02$), while for AD cases, the 2 measures were not correlated ($r=-0.08$, $P=.35$).

To determine whether changes in CSF β-amyloid$_{1-42}$ and CSF tau levels occur early in the onset of the disease, we tested for correlation between CSF β-amyloid$_{1-42}$ and tau measures and severity and duration of illness. Measures of CSF tau were significantly correlated with the CDR rating ($r=0.19$, $P=.03$) and MMSE score ($r=-0.20$, $P=.03$) and revealed a trend with the GDS score ($r=0.17$, $P=.053$), while CSF β-amyloid$_{1-42}$ levels showed a trend relationship with MMSE only ($r=0.16$, $P=.09$). Interestingly, years of education were associated with lower CSF tau levels in the overall AD group ($r=-0.19$, $P=.03$). Within the AD group, CSF β-amyloid$_{1-42}$ levels were not associated with age, age of onset, or duration of illness ($r=-0.14$, $P=.11$; $r=0.12$, $P=.18$; and $r=0.08$, $P=.35$, respectively). Similarly, CSF tau levels were not associated with age, age of onset, or duration of illness in the AD group ($r=-0.01$, $P=.90$; $r=0.01$, $P=.87$; and $r=-0.01$, $P=.90$, respectively). When patients with moderate and severe AD (CDR score of 2 or 3; n=56) were compared with controls, for both mean (SD) CSF β-amyloid$_{1-42}$ (175 [99] pg/mL vs 491 [245] pg/mL, $t_{99}=9.94$ [unequal variance], $P<.001$) and tau levels (660 [396] pg/mL vs 224 [156] pg/mL; $t_{68}=7.79$ [unequal variance], $P<.001$) were significantly different. When only patients with mild AD (CDR score of 0.5 or 1; n=75) were included, both mean (SD) CSF β-amyloid$_{1-42}$ (189 [135] pg/mL vs 491 [245] pg/mL; $t_{100}=9.21$ [unequal variance], $P<.001$) and tau levels (532 [333] pg/mL vs 224 [156] pg/mL; $t_{106}=7.22$ [unequal variance], $P<.001$) remained significantly different. While this analysis was exploratory, it suggests that changes in CSF β-amyloid$_{1-42}$ tau levels may be present early in the disease process.

CART Analysis

A CART analysis was performed to evaluate the combined contributions of CSF β-amyloid$_{1-42}$ and CSF tau to differentiate AD patients from controls. Cutpoints of 444 pg/mL for CSF β-amyloid$_{1-42}$ and 195 pg/mL for CSF tau maximized sensitivity to 92% and specificity to 82% in this analysis (FIGURE 2). These CART-defined cutpoints maximize the specificity and sensitivity for this particular group of participants, and would be lower in a new population.

Meta-Analysis

The meta-analysis of the literature on CSF β-amyloid$_{1-42}$ involved 17 studies that met criteria for inclusion (TABLE 2).[4-7,13,14,16,33-42] Other studies were reviewed[17,43-48] but they did not meet our criteria for the meta-analysis. Fourteen[4-7,13,14,16,33-39] of the 17 studies in the meta-analysis showed clear reductions in CSF β-amyloid$_{1-42}$ levels in AD vs control participants while 2 studies were equivocal and another reported changes in the opposite direction (Table 2 and FIGURE 3). The overall effect size (CSF β-amyloid$_{1-42}$ level difference between AD and control participants) of the meta-analysis with the previously published studies was 1.53 (95% confidence interval [CI], 1.39-1.69) (Figure 3). In the current study, the effect size was 1.76 (95% CI, 1.42-2.10). When the data from the current study are added to the meta-analysis (for a total of 18 studies), the effect size is 1.56 (95% CI, 1.43-1.69).

A similar meta-analysis was performed for 34 studies on CSF tau in the literature search (TABLE 3 and FIGURE 4).[4,5,7,10,11,14,36,40,4149-72] All the studies report a significant difference in CSF tau levels between AD participants and controls. For the combined series of previously published studies, the overall effect size was 1.31 (95% CI, 1.23-1.39). The overall effect size of the current study was 1.18 (95% CI, 0.87-1.49). When all 35 studies are included in the meta-analysis (ie, 34 studies and the current study), the effect size remains 1.31 (95% CI, 1.23-1.39).

COMMENT

The idea that CSF β-amyloid$_{1-42}$ and tau levels could be useful in diagnosing AD is not new. Numerous authors have documented the changes of these biomarkers in AD patients vs controls,[4-6,13,33] but not without controversy, especially with respect to CSF β-amyloid$_{1-42}$ levels. While most studies show a decrease of CSF

Table 2. Meta-analysis of 17 Studies With Participants With Alzheimer Disease (AD) and Controls Compared With the Current Study for Cerebrospinal Fluid (CSF) Measures of β-Amyloid$_{1-42}$ Levels

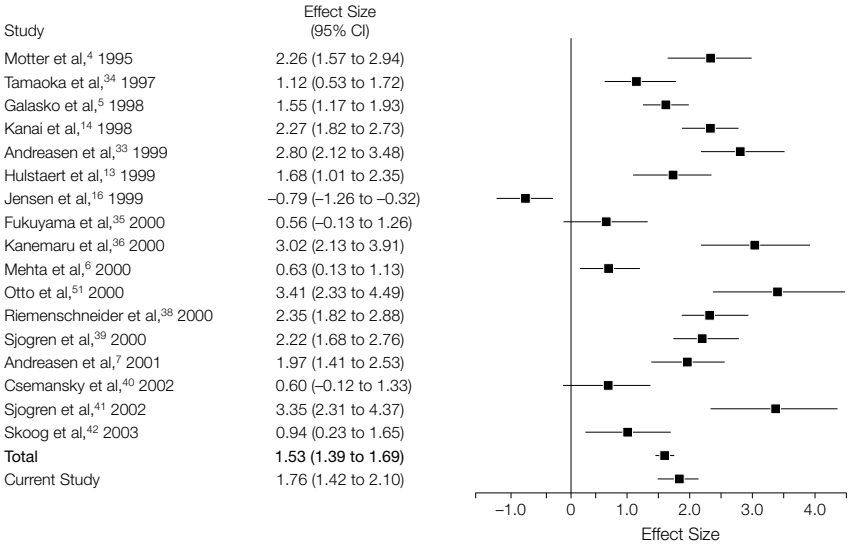
	AD Group		Control Group			
Study	No. of Study Participants	CSF β-Amyloid, Mean (SD), pg/mL	No. of Study Controls	CSF β-Amyloid, Mean (SD), pg/mL	*t* (*df*)*	*P* Value
Motter et al,[4] 1995	37	383 (76)	20	632 (156)	6.72 (24)	<.001
Tamaoka et al,[34] 1997	20	738 (374)	34	1450 (743)	4.67 (51)	<.001
Galasko et al,[5] 1998	82	833 (379)	60	1485 (473)	8.81 (110)	<.001
Kanai et al,[14] 1998	93	495 (164)	41	1090 (405)	9.08 (46)	<.001
Andreasen et al,[33] 1999	53	709 (304)	21	1678 (436)	9.33 (28)	<.001
Hulstaert et al,[13] 1999	84	522 (197)	11	874 (293)	3.87 (11)	.003
Jensen et al,[16] 1999	80	536 (284)	24	333 (135)	–4.83 (82)	<.001
Fukuyama et al,[35] 2000	23	331 (188)	13	626 (909)	1.16 (13)	.27
Kanemaru et al,[36] 2000	24	284 (92)	19	714 (188)	9.14 (25)	<.001
Mehta et al,[6] 2000	36	60 (78)	29	147 (188)	2.34 (36)	.02
Otto et al,[37] 2000	14	361 (153)	20	903 (163)	9.89 (29)	<.001
Riemenschneider et al,[38] 2000	75	455 (210)	30	916 (160)	12.14 (70)	<.001
Sjogren et al,[39] 2000	60	381 (127)	32	772 (244)	8.47 (40)	<.001
Andreasen et al,[7] 2001	105	523 (180)	18	897 (242)	6.27 (20)	<.001
Csernanaksy et al,[40] 2002	32	1777 (1055)	10	2400 (1030)	1.68 (15)	.11
Sjogren et al,[41] 2002	19	411 (99)	17	853 (161)	9.78 (26)	<.001
Skoog et al,[42] 2003	12	389 (161)	28	657 (320)	3.44 (36)	.002
Total No.	**849**	**554 (300)†**	**427**	**979 (419)†**		
Current study	131	183 (121)	72	491 (245)	10.02 (90)	<.001

*Weighted average and variance across all studies.
†Satterthwaite adjusted *t* test scores of unequal variance.

β-amyloid$_{1-42}$ levels in AD patients vs controls, a small number of studies have shown no changes or even elevations of the protein levels.[16,17,43,73] Given the range of methods used and the variable sample sizes of the studies, this result is perhaps not surprising, but it has left open the question of whether and when changes in CSF β-amyloid$_{1-42}$ levels are manifest in AD. To our knowledge, our study is the largest to confirm the decrease of CSF β-amyloid$_{1-42}$ and an increase in tau levels in AD participants. In addition, these differences appear to be found in patients with mild AD as well in patients with moderate to severe AD.

Two possible confounding factors within the current study deserve further explanation. First, the age of the controls is significantly lower than that of the AD patients. While this factor was not significantly associated with CSF β-amyloid$_{1-42}$ levels for either AD patients or controls, an age effect was found within the controls for CSF tau. However, after controlling for age, CSF tau levels remained significantly higher in the AD group vs controls. Second, freezer shelf life might be a factor in the assay values, as the CSF samples from AD patients had been frozen longer than the samples from controls. However, no relationship was found between CSF β-amyloid$_{1-42}$ or tau

Figure 3. Effect Sizes of Results from 17 Studies of Meta-analysis of Measures of β-Amyloid$_{1-42}$ in Cerebrospinal Fluid (CSF) for Controls and Participants With Alzheimer Disease (AD)



CI indicates confidence interval. Effect size is the difference in means between AD patients and controls divided by the pooled SD.

levels and shelf life for either the AD patients or controls.

CSF Tau and β-Amyloid$_{1-42}$ in AD

Some authors have found a modest correlation between CSF tau and baseline clinical measures,[55,74,75] while others, including our own group, have reported no significant relationship.[4,12,57] The lack of correlation may have been due in part to the relatively restricted range of dementia severity in some of these studies. In our current study, with a much larger sample population of AD patients, we found a small but statistically significant correlation between CSF tau levels and several of the global severity measures, including the CDR, GDS, and MMSE ratings. Also of interest, CSF tau levels were inversely correlated with education level, suggesting a possible protective factor. Conversely, analysis of CSF β-amyloid$_{1-42}$ levels revealed only a modest trend for a correlation with MMSE score in the AD patients, and no significant relationship was found with age, other severity measures of dementia, or CSF tau levels. While a significant correlation exists between CSF β-amyloid$_{1-42}$ and tau levels across all participants tested, this correlation did not persist within the AD population alone, perhaps reflecting the

Table 3. Meta-analysis of 34 Studies With Participants With Alzheimer Disease (AD) and Controls Compared With the Current Study for Cerebrospinal Fluid (CSF) Measures of Tau Protein Levels

	AD Group		Control Group			
Study	No. of Study Participants	CSF Tau, Mean (SD), pg/mL	No. of Study Controls	CSF Tau, Mean (SD), pg/mL	*t* (*df*)*	*P* Value
Vandermeeren et al,[49] 1993	27	10.9 (4.9)	51	0.1 (0.5)	11.42 (26)	<.001
Arai et al,[50] 1995	70	77 (46)	19	9 (5)	12.11 (75)	<.001
Blennow et al,[51] 1995	44	524 (280)	31	185 (50)	7.86 (47)	<.001
Mori et al,[52] 1995	14	820 (90)	36	380 (120)	14.07 (32)	<.001
Munroe et al,[53] 1995	24	1430 (739)	14	816 (355)	3.45 (35)	.002
Motter et al,[4] 1995	37	407 (241)	20	212 (102)	4.27 (53)	<.001
Skoog et al,[54] 1995	11	254 (113)	36	171 (78)	2.28 (13)	.04
Tato et al,[55] 1995	23	279 (100)	23	26 (11)	12.06 (23)	<.001
Vigo-Pelfrey et al,[56] 1995	71	361 (166)	26	190 (80)	6.79 (88)	<.001
Arai et al,[57] 1997	17	95 (44)	15	19 (15)	6.69 (20)	<.001
Golombowski et al,[58] 1997	19	53 (39)	12	31 (17)	2.16 (27)	.04
Andreasen et al,[59] 1998	43	796 (382)	18	190 (57)	10.14 (46)	<.001
Arai et al,[60] 1998	69	90 (45)	17	20 (13)	11.17 (82)	<.001
Galasko et al,[5] 1998	82	663 (481)	60	387 (167)	4.81 (106)	<.001
Kanai et al,[14] 1998	93	489 (298)	41	217 (128)	7.4 (132)	<.001
Kurz et al,[61] 1998	40	697 (447)	36	169 (64)	7.39 (41)	<.001
Mecocci et al,[62] 1998	29	436 (360)	23	212 (200)	2.84 (45)	.007
Nishimura et al,[63] 1998	163	426 (234)	65	188 (103)	10.65 (224)	<.001
Shoji et al,[64] 1998	55	467 (285)	34	218 (139)	5.51 (83)	<.001
Andreasen et al,[65] 1999	274	690 (341)	65	227 (101)	19.2 (324)	<.001
Burger et al,[66] 1999	38	580 (370)	28	273 (203)	4.31 (60)	<.001
Green et al,[67] 1999	17	802 (381)	9	198 (49)	6.44 (17)	<.001
Hampel et al,[68] 1999	25	566 (329)	19	245 (154)	4.3 (36)	<.001
Molina et al,[69] 1999	83	522 (290)	8	216 (150)	4.95 (13)	<.001
Kahle et al,[70] 2000	30	840 (560)	16	340 (230)	4.26 (42)	<.001
Kanemaru et al,[36] 2000	24	460 (301)	19	115 (76)	5.4 (27)	<.001
Sjoegren et al,[39] 2000	60	743 (503)	32	307 (168)	6.11 (80)	<.001
Andreasen et al,[7] 2001	105	759 (417)	18	264 (102)	10.47 (108)	<.001
Hampel et al,[71] 2001	17	496 (205)	12	312 (98)	3.22 (24)	.004
Itoh et al,[10] 2001	236	450 (252)	95	149 (107)	15.25 (328)	<.001
Roesler et al,[72] 2001	27	761 (407)	17	224 (81)	6.65 (29)	<.001
Shoji et al,[11] 2002	366	482 (271)	113	186 (107)	17.03 (452)	<.001
Sjogren et al,[41] 2002	19	919 (349)	17	342 (116)	6.80 (22)	<.001
Csernansky et al,[40] 2002	32	1260 (460)	10	800 (260)	3.98 (28)	<.001
Total No.	**2284**	**534 (317)†**	**1054**	**212 (122)†**		
Current study	131	587 (365)	72	224 (156)	9.8 (192)	<.001

*Weighted average and variance across all studies.
†Satterthwaite adjusted *t* test scores of unequal variance.

restricted range of the CSF values in the AD patients in this study.

Meta-analysis

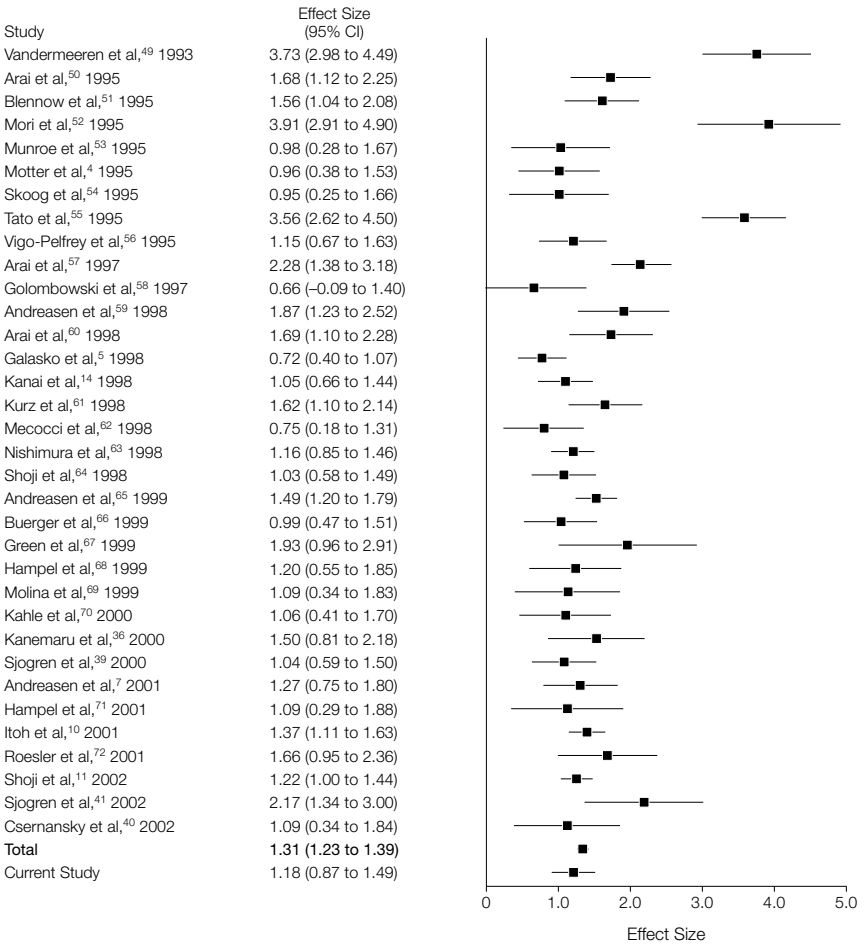
A meta-analysis was performed with published studies of CSF β-amyloid$_{1-42}$ in AD to elucidate the overall trends in the data and to determine the effect size of group differences. Not all the available studies were amenable to the meta-analysis method, but we did find 17 studies that met the criteria (Table 2 and Figure 3). The considerable variability in mean values among the studies highlights the lack of standardization of assay methods among centers and is of some concern. However, with 14 of the 17 studies showing significant reductions in CSF β-amyloid$_{1-42}$ levels in the AD vs control participants, the result of the meta-analysis was unequivocal and the effect size quite large, with AD participants showing a lower CSF β-amyloid$_{1-42}$ level. The direction and effect size of our current study was consistent with the meta-analysis, indicating a similar trend. For the meta-analysis of studies on CSF tau, the data are even more unequivocal (Table 3 and Figure 4). Despite differences in baseline levels across studies, the pattern of change is uniform; all previous studies report AD patients having higher CSF tau levels than that found with controls.

With the general consistency in the literature for CSF β-amyloid$_{1-42}$ and tau, it is not surprising that several companies have initiated commercial tests of these measures for clinical use with individual patients (Athena Diagnostics, Worcester, Mass; Innogenetics, Ghent, Belgium; and ABETA GmbH, Heidelberg, Germany). However, the claims regarding sensitivity and specificity from the commercial concerns and our own CART analysis are derived from clinically diagnosed AD cases in which the diagnostic accuracy already approximates 85% when validated by the standard pathologic diagnosis at autopsy.[25,76,77] Furthermore, the cutpoints for most sensitivity and specificity assessments are chosen to maximize the specificity and sensitivity results with those measures. Our data are fairly representative of the literature and clearly show considerable variance in the data that provides room for misclassification by an individual biomarker, whether the marker is CSF β-amyloid$_{1-42}$ or CSF tau (Figure 1).

Approach to Clinical Application

Given the overlap between AD and control groups, it is evident that the diagnostic sensitivity and specificity of these individual CSF β-amyloid$_{1-42}$ and tau assays is simply not sufficient to warrant general clinical use of these biomarkers for individual use. Nonetheless, when the CSF β-amyloid$_{1-42}$ and tau data are combined into 1 statistical analysis, the overlap with clinical diagnoses generally improves.[4,5,7] While the differences in CSF levels between AD populations and controls are indeed impressive, the interpretations of this data are limited because they result from contrasts of starkly different populations (ie, AD vs healthy, self-selected controls). This type of artificial contrast is not representative of realistic clinical comparisons and is considered phase 1 diagnostic testing.[78] A truer test of any suggested diagnostic marker would include comparisons among populations with different types of dementia, including vascular, Lewy body dementia, and other neurological disorders, or cases with very early cognitive impairment. It is likely

Figure 4. Effect Sizes of Results from 34 Studies of Meta-analysis of Measures of Tau Protein in Cerebrospinal Fluid for Controls and Participants With Alzheimer Disease (AD)



CI indicates confidence interval. Effect size is the difference in means between AD patients and controls divided by the pooled SD.

that this phase 2 testing with populations with mixed diagnoses will show poorer sensitivity and specificity results, and this approach has previously been attempted with only modest success.[7] Thus, while it can be said that these research diagnostic techniques are indeed improving, a great deal of developmental testing is still in order at the interface of clinical medicine and research methodology.[78]

Perhaps the biggest future challenge to the research in AD will be to standardize these CSF measures across numerous centers and then apply them as part of a prospective clinical evaluation of participants who are at risk for developing AD. Currently, a clinical criterion standard is not available to help with the early diagnosis of AD. As a result, analysis of CSF β-amyloid$_{1-42}$ and tau levels are likely to be most useful diagnostically when they are used in conjunction with other biomarkers, including structural magnetic resonance imaging, genetic markers, and positron-emission tomography scans when a tracer for β-amyloid burden is more readily available. This approach is the focus of an ongoing longitudinal, prospective study at the National Institute of Mental Health with a cohort of older controls (protocol 95-M-96).

Conclusion

The study of CSF biomarkers, such as β-amyloid$_{1-42}$ and tau, in AD participants is emerging as an important but still nascent field.[8] The cross-sectional data clearly show group differences between AD participants and controls, both in this large study and in a meta-analysis of the literature. However, these studies represent the early development of diagnostic measures. Additional studies are required to establish methodologic standardization in the CSF assays across centers and to see if a specificity for AD exists over other forms of dementia, which have substantial overlap with AD at postmortem (ie, Lewy body dementia or cerebrovascular dementia). Furthermore, the mixed findings in the literature relating these CSF biomarkers to clinical severity measures of dementia suggest the need for larger sample sizes to establish statistical significance. The reason for the varying data may be because single biomarkers may not be able to provide an accurate reflection of the pathologic process across the entire span of the illness. Rather, individual biomarkers may be correlated to the prominent pathophysiology of a particular stage of the illness but not to the pathophysiology of earlier or later stages.

Perhaps the most important future use for such biomarkers is in the prospective study of participants at risk for developing AD. However, much work is needed with the standardization of assay methods before prognostic significance can be attributed to these biomarkers. Once an individual's normal levels of the biomarkers are well established, it is possible that gradual changes in these levels (eg, CSF levels of β-amyloid$_{1-42}$ or tau) could eventually be interpreted as suggestive evidence of incipient AD. To test this hypothesis, longitudinal prospective studies of controls and early AD participants are necessary and are currently ongoing.

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