

# Cerebrospinal Fluid $\beta$ -Amyloid 42 and Tau Proteins as Biomarkers of Alzheimer-Type Pathologic Changes in the Brain

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**Background:** There is a clear need to develop an objective diagnostic test for Alzheimer disease (AD). Changes in the levels of cerebrospinal fluid (CSF) tau protein and  $\beta$ -amyloid 42 (A $\beta$ 42) peptide in patients with AD have been well documented, but the relationship between these biomarkers and neuropathologic changes in the brain is not established.

**Objective:** To study the relationship between antemortem CSF biomarker levels and Alzheimer-type neuropathologic changes in the brain.

**Design:** Cross-sectional study to correlate levels of CSF A $\beta$ 42, total tau, and phosphorylated tau protein with neuropathologic changes in the brain.

**Setting:** Academic research.

**Patients:** The study included 123 patients (79 with clinically diagnosed AD, 29 with other dementia, and 15 with other neurologic disease). All underwent clinical evaluation and provided antemortem lumbar CSF samples, and neuropathologic data were collected from September 11, 1990, to March 13, 2003, in the Department of Neuroscience and Neurology, University of Kuopio, Kuopio, Finland.

**Main Outcome Measures:** Levels of CSF A $\beta$ 42, total tau, and phosphorylated tau protein were measured using standard commercial immunoassays. Neuropathologic

evaluations included the classic silver impregnation method and immunohistochemistry for A $\beta$ , hyperphosphorylated tau, and  $\alpha$ -synuclein.

**Results:** Cerebrospinal fluid A $\beta$ 42 and tau protein levels were related to amyloid load and the presence of neurofibrillary pathologic abnormalities in the brain. Cerebrospinal fluid A $\beta$ 42 level correlated inversely with total A $\beta$  load in the brain, and CSF tau level correlated with results of immunohistochemistry for hyperphosphorylated tau and with the presence of neocortical neurofibrillary tangles. In multivariate logistic regression analysis, the number of neuritic plaques in the brain remained a significant predictor of decreased CSF A $\beta$ 42 level and of increased CSF tau level. Based on the ratio of phosphorylated tau level to A $\beta$ 42 level, sensitivity was 91.6%, and specificity was 85.7%, with an overall accuracy of 90.2% for the presence of pathologic neuritic plaque in the brain.

**Conclusions:** Cerebrospinal fluid A $\beta$ 42 and tau proteins are biomarkers of AD-associated pathologic changes in the brain. The combination of abnormally low CSF A $\beta$ 42 level and abnormally high CSF tau level predicted the presence of AD pathologic features with high accuracy. This combination assay may be helpful in diagnosing the presence of AD pathologic changes in the brain.

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**A**LZHEIMER DISEASE (AD) IS the leading cause of dementia. There is an increasing need to develop objective tests to aid in the diagnosis of early AD and to assess the efficacy of therapies. Furthermore, follow-up of the pathologic progression in patients with AD or in persons at risk of developing the disease is impossible without specific biologic markers.

The ideal biomarker should detect a fundamental neuropathologic feature of AD early in the course of the disease with high sensitivity and specificity.<sup>1</sup> The most promising biomarkers are cerebrospinal fluid (CSF) tau proteins and

$\beta$ -amyloid 42 (A $\beta$ 42) peptides. A decreased A $\beta$ 42 level in conjunction with an elevated tau or phosphorylated tau (P-tau) level differentiates patients with AD from control subjects or from patients with other neurologic conditions with high accuracy.<sup>2</sup> Results of recent studies<sup>3,4</sup> indicate that these changes can be found in patients with mild cognitive impairment who later will develop AD. One study<sup>5</sup> demonstrated that low postmortem A $\beta$ 42 levels in ventricular CSF reflect amyloid pathologic changes in the brain and cerebral vessels. Another study<sup>6</sup> showed that antemortem CSF tau levels were elevated in patients with AD pathologic features, while CSF

**Table 1. Demographic Characteristics of the Patients**

Characteristic	Clinical Diagnosis		
	AD (n = 79)	Other Dementia (n = 29)	Other Neurologic Disease (n = 15)
Clinicopathologic AD diagnosis, No. (%) <sup>a</sup>	74 (93.7)	19 (65.5)	2 (13.3)
Age, median (IQR), y	77 (12)	75 (22)	71 (21)
Female to male ratio	67:12	22:7	8:7
MMSE score, median (IQR)	5 (17)	14 (18.5)	25 (7)
APOE ε4 positive, No. (%)	55 (69.6)	10 (34.5)	8 (53.3)
Duration of disease at the time of CSF sample, median (IQR), y	6 (5)	3 (2.5)	2 (2.75)
Follow-up period from the time of CSF sample to death, median (IQR), mo	27 (29)	13 (35)	34 (36)

Abbreviations: AD, Alzheimer disease; CSF, cerebrospinal fluid; IQR, interquartile range; MMSE, Mini-Mental State Examination.

<sup>a</sup>Based on the National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease.<sup>7</sup>

Aβ42 levels were reduced in patients having dementia with Lewy bodies or prion disease.

We hypothesized that CSF Aβ42 and tau represent biomarkers of AD-related pathologic changes in the brain irrespective of the clinical phenotype and can be used in assessing subjects with various clinical symptoms. This hypothesis was tested in 123 patients who had provided a CSF sample during their clinical workup and who underwent a postmortem neuropathologic examination. We examined the relationships among clinical diagnoses, changes in CSF biomarker levels, and Alzheimer-type pathologic changes in the brain.

## METHODS

### PATIENTS

The study included 123 patients examined between September 11, 1990, and March 13, 2003, in the Department of Neuroscience and Neurology, University of Kuopio, Kuopio, Finland, who had consented to participate in an ongoing biomarker study and who underwent a detailed neuropathologic examination after death. The patients or their caregivers gave written informed consent, and the study was approved by the local ethics committee.

The subjects were participants in a longitudinal AD research project (n=55) or were referred to the Department of Neurology, Kuopio University Hospital (n=68). A summary of the demographic characteristics of the patients is given in **Table 1**. All subjects underwent a clinical examination. One of us (T.P.) retrospectively evaluated all patient information and confirmed the diagnoses of dementing diseases by applying the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer Disease and Related Disorders Association criteria for AD,<sup>8</sup> consensus criteria for dementia with Lewy bodies,<sup>9</sup> DSM-IV criteria for vascular dementia,<sup>10</sup> and the Lund-Manchester criteria for frontotemporal dementia.<sup>11</sup> Patients with symptoms of 2 different conditions (such as AD with Lewy body features and AD with significant cerebrovascular disease) were also registered and classified as having possible AD. The clinical diagnoses were assigned independently and blinded to CSF biomarker data.

### CSF SAMPLES AND MEASUREMENTS

The CSF samples were collected by lumbar puncture during the baseline visit and were stored in polypropylene tubes at

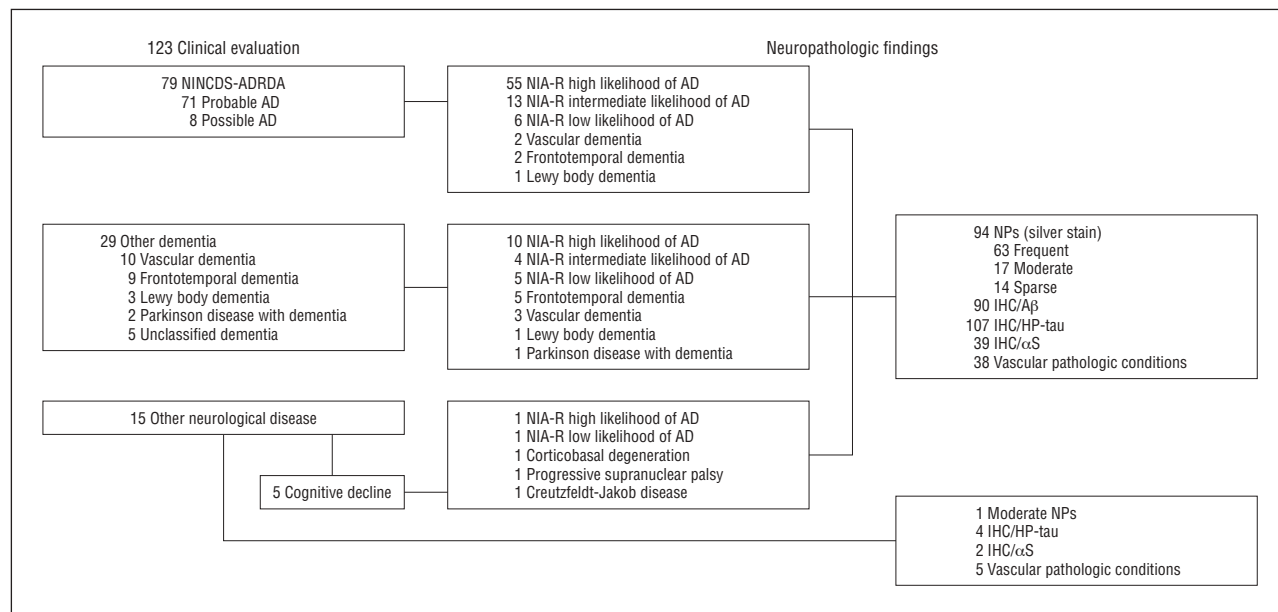
–70°C until analysis to ensure the stability of the CSF biomarker levels during the storage period.<sup>12</sup> Measurement of CSF Aβ42, total tau, and P-tau<sub>181</sub> was performed using commercially available enzyme-linked immunosorbent assays (Inno-genetics, Ghent, Belgium) according to the manufacturer's protocol and blinded to clinical and neuropathologic diagnoses and to apolipoprotein E (APOE) (OMIM 107741) genotype of the studied subjects.

## NEUROPATHOLOGIC ASSESSMENT

The neuropathologic examinations were performed by one of us (I.A.). Brain specimens procured and embedded in paraffin were obtained from the following 15 cortical and subcortical regions: striatum, gyrus cinguli, midbrain (including substantia nigra), pons (including locus ceruleus, medulla, vermis, and cerebellar cortex), basal forebrain (including amygdala, thalamus, and posterior hippocampus), and frontal (Brodmann area 9), temporal (Brodmann area 22), parietal (Brodmann area 39), precentral, and occipital cortices. Seven-micrometer-thick serial sections were obtained, and all sections were stained with hematoxylin-eosin. The modified Bielschowsky silver impregnation method was used on 4 sections (frontal, temporal, and parietal cortices and hippocampus). In addition, immunohistochemistry (IHC) for hyperphosphorylated tau (HP-tau) and Aβ was performed in frontal, temporal, and parietal cortices and hippocampus, and IHC for α-synuclein was performed in 10 brain regions as described by Parkkinen et al.<sup>13</sup>

Neuritic plaques (NPs) (ie, plaques with silver-stained thickened neuritis) were semiquantitatively assessed in Bielschowsky silver-impregnated sections as recommended by the Consortium to Establish a Registry for Alzheimer's Disease and were graded as 0 (none), 1 (sparse), 2 (moderate), or 3 (frequent).<sup>14</sup> The regional distribution of neurofibrillary pathologic abnormalities was assessed in HP-tau-stained sections on IHC and was staged as transentorhinal (Braak stages 1–2), limbic (Braak stages 3–4), or isocortical (Braak stages 5–6) following the recent recommendations by Braak et al.<sup>15</sup> The extent of HP-tau immunoreactivity (IR) on IHC in each individual section was assessed using the following scale: 0 (no IR is found), 1 (IR lesions have to be sought), 2 (IR lesions can be easily seen), or 3 (IR lesions are noted without the use of a microscope). A general estimate of HP-tau IR load on IHC in each individual case was calculated and is presented as the sum of HP-tau IR on IHC in 4 sections (ie, frontal, temporal, and parietal cortices and hippocampus).

The quantification of Aβ aggregates was performed as described previously.<sup>16</sup> The Aβ load was estimated in frontal, temporal, and parietal cortices within the total thickness of gray



**Figure 1.** Clinicopathologic findings in the patients. Aβ indicates β-amyloid; AD, Alzheimer disease; αS, α-synuclein; HP-tau, hyperphosphorylated tau; IHC, immunohistochemistry; NIA-R, National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease<sup>7</sup>; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer Disease and Related Disorders Association<sup>8</sup>; and NPs, neuritic plaques.

matter on 3 fields selected by chance, with the Aβ load being reported as the fraction of stained area.

α-Synuclein labeling was assessed in all stained sections. Cases with dominant pathologic findings restricted to and below the midbrain were categorized as brainstem predominant (ie, Braak stages 1-3). Cases with additional pathologic features in the transtentorial region were categorized as limbic (ie, Braak stages 4-5) and cases with notable cortical spread to a neocortical region as Braak stage 6.

In all cases, the extent of vascular lesions was assessed grossly by naked eye inspection at dissection and histologically in 15 hematoxylin-eosin-stained slides. The vascular pathologic findings were then graded on a 3-step scale as 0 (no macroscopic or microscopic lesions found), 1 (only microscopic lesions found), or 2 (both macroscopic and microscopic lesions found).<sup>16</sup>

For the clinicopathologic diagnoses, international consensus criteria were followed. For AD, the Neuropathological Assessment of Alzheimer's Disease consensus recommendation was used,<sup>7</sup> and for Parkinson disease or dementia with Lewy bodies, the Consortium on Dementia With Lewy Bodies recommendations were applied.<sup>9</sup> The clinicopathologic findings in the patients are shown in **Figure 1**.

## APOE GENOTYPING

APOE genotyping was performed using a method of polymerase chain reaction as described previously.<sup>17</sup> Subjects were classified according to their APOE genotype as APOE ε4-negative subjects (ε2/3 and ε3/3), subjects with 1 APOE ε4 allele (ε2/4 or ε3/4), or subjects with 2 APOE ε4 alleles (ε4/4).

## STATISTICAL ANALYSIS

All statistical analyses were performed using commercially available software (SPSS for Windows, release 11.5.1; SPSS Inc, Chicago, Illinois). Comparisons between groups of subjects were performed using nonparametric Kruskal-Wallis and Mann-Whitney tests. For categorical data, comparisons between groups were performed using χ<sup>2</sup> tests. Correlations between variables

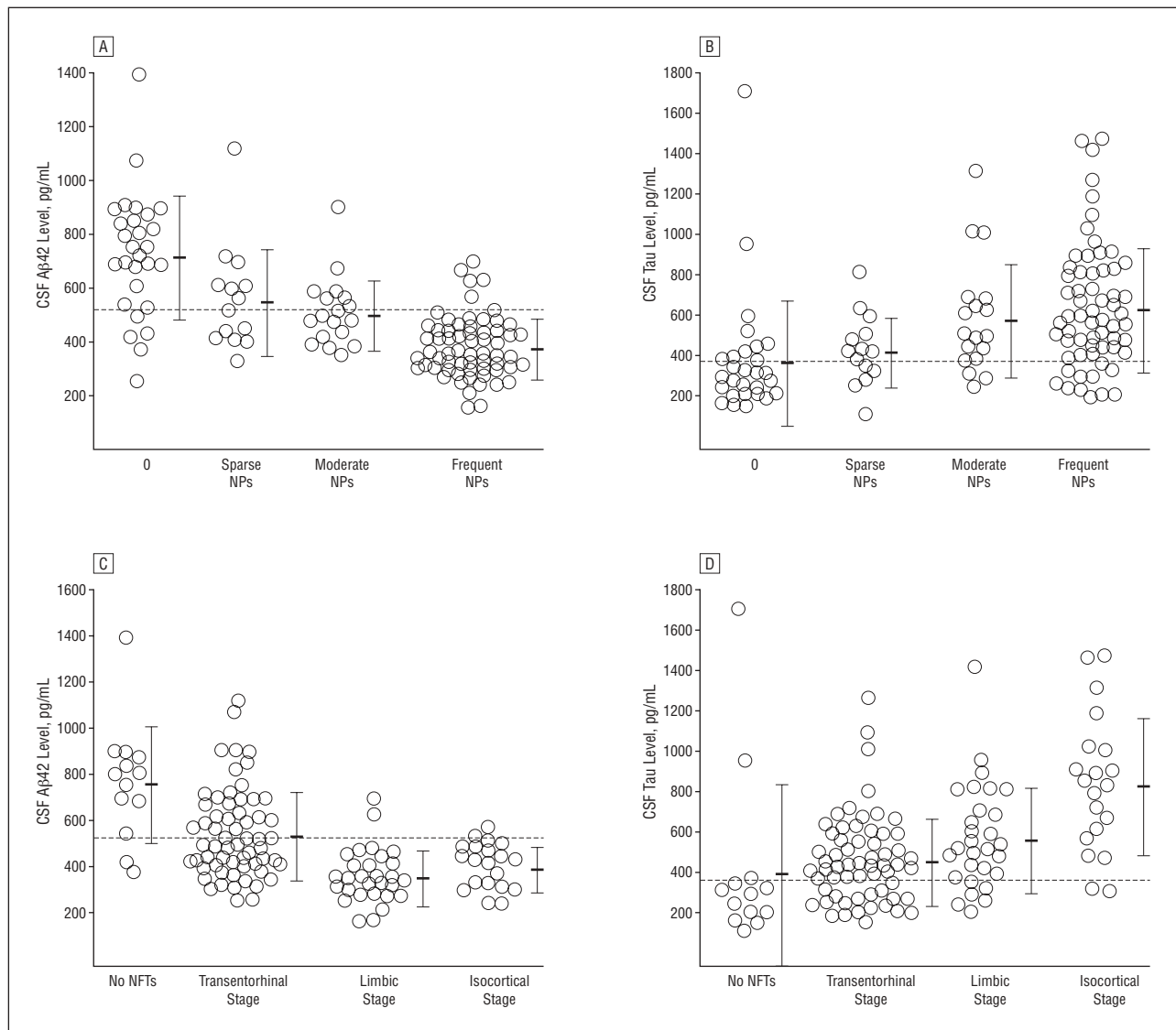
were calculated using Spearman rank correlation tests. Results of the analyses were considered significant at  $P \leq .05$ . The odds ratios (ORs) for different factors that might contribute to the levels of CSF biomarkers were determined using logistic regression analysis. The backward Wald method was used in multivariate logistic regression analysis. Receiver operating characteristic curve analysis was used to determine the best cutoff values for the measured biomarkers. The best cutoff value was defined as the highest sum of sensitivity and specificity.

## RESULTS

### CSF BIOMARKER LEVELS AND AMYLOID PATHOLOGIC CHANGES IN THE BRAIN

There was a significant relationship between quantities of NPs and levels of CSF biomarkers (**Figure 2A**) and an inverse correlation between total Aβ load and CSF Aβ42 level in the brain (**Figure 3**). Five cases had a high Aβ load in the brain (stained area fraction >2) but sparse or no NPs. In this cohort, CSF Aβ42 level was also lower than that in subjects without Aβ (data not shown). The presence of α-synuclein or vascular pathologic conditions in patients with concomitant pathologic NPs did not affect CSF Aβ42 levels (**Table 2** and **Table 3**). The levels of Aβ42 in patients with pathologic conditions other than NPs were similar to those found in patients without pathologic changes in the brain.

Receiver operating characteristic curve analysis showed that the optimum Aβ42 cutoff for pathologic NPs was 515 pg/mL (**Table 4**). This value exhibited a positive likelihood ratio (LR) of 4.48 (95% confidence interval [CI], 2.01-9.98) and a negative LR of 0.24 (0.16-0.38) for pathologic NPs. Five patients without NPs (3 with frontotemporal dementia and 2 with dementia and vascular pathologic findings) had low Aβ42 levels.

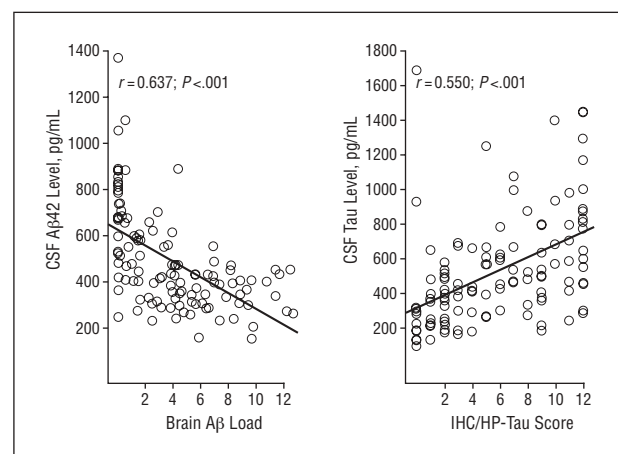


**Figure 2.** Relationship between cerebrospinal fluid (CSF) biomarkers and Alzheimer-type pathologic changes in the brain. The quantity of neuritic plaques (NPs) was assessed semiquantitatively in Bielschowsky silver-impregnated sections according to Consortium to Establish a Registry for Alzheimer's Disease recommendations<sup>14</sup> (A and B). The regional neurofibrillary pathologic abnormalities were categorized as Braak stages (C and D). Dashed lines represent the cutoff values of CSF  $\beta$ -amyloid 42 (A $\beta$ 42) (515 pg/mL) and tau (350 pg/mL). NFTs indicates neurofibrillary tangles.

There was a significant relationship between CSF tau level and quantity of NPs ( $P < .001$ ) (Figure 2B) and the total A $\beta$  load in the brain. In univariate logistic regression analysis, there were several significant predictors of low A $\beta$ 42 levels (**Table 5**), but, in multivariate logistic regression analysis (the entered variables were age, CSF storage time, number of APOE  $\epsilon$ 4 alleles, NP count, and Braak stage), NPs remained the only significant predictor of low CSF A $\beta$ 42 level (OR, 7.229; 95% CI, 1.866-27.995 for moderate NPs; and 53.360; 14.108-201.827, respectively, for frequent NPs).

#### CSF BIOMARKER LEVELS AND NEUROFIBRILLARY PATHOLOGIC ABNORMALITIES IN THE BRAIN

There was a significant relationship between CSF tau level and regional distribution of pathologic neurofibrillary tangles (NFTs) (ie, Braak stage) ( $P < .001$ ) (Figure 2D)



**Figure 3.** Correlation between cerebrospinal fluid (CSF) biomarkers and total  $\beta$ -amyloid (A $\beta$ ) load in the brain (stained area fraction) and hyperphosphorylated tau immunoreactivity on immunohistochemistry (IHC/HP-tau semiquantitative assessment scores).



**Table 2. Cerebrospinal Fluid Biomarker Levels in Patients With and Without Pathologic Neuritic Plaques (NPs)**

Biomarker	Median (IQR), pg/mL					
	NPs <sup>a</sup>			NPs <sup>b</sup>		
	All (n = 95)	$\alpha$ S (n = 35)	Vascular <sup>c</sup> (n = 33)	All (n = 28)	$\alpha$ S (n = 6)	Vascular <sup>c</sup> (n = 10)
A $\beta$ 42	404 (172)	418 (157)	425 (169)	722 (305)	766 (229)	761 (220)
Tau	521 (326)	541 (409)	484 (299)	283 (204)	334 (173)	245 (236)
P-tau	65 (30)	64 (26)	67 (30)	43.5 (31)	51.5 (24)	44 (23)

Abbreviations: A $\beta$ 42,  $\beta$ -amyloid 42;  $\alpha$ S, concomitant  $\alpha$ -synuclein pathologic finding; IQR, interquartile range; P-tau, phosphorylated tau.

<sup>a</sup>Fifteen patients had both  $\alpha$ S and vascular pathologic findings.

<sup>b</sup>Two patients had both  $\alpha$ S and vascular pathologic findings.

<sup>c</sup>Concomitant vascular pathologic finding.

and HP-tau IR on IHC (Table 3 and Figure 3). Five cases that had high HP-tau IR on IHC but no amyloid deposits had low CSF tau levels (data not shown). The presence of  $\alpha$ -synuclein on IHC or vascular pathologic conditions was not significantly related to CS tau or P-tau levels.

Receiver operating characteristic curve analysis showed that the optimum tau cutoff was 350 pg/mL for the presence of at least the transentorhinal (1-2) Braak stage, with a positive LR of 3.27 (95% CI, 1.21-8.87) and a negative LR of 0.32 (0.20-0.50) (Table 4). Three of 13 patients without NFTs (1 with Creutzfeldt-Jakob disease, 1 with spinocerebellar ataxia, and 1 with severe vascular pathologic conditions) had abnormal CSF tau levels. The optimum P-tau cutoff was 52.5 pg/mL for the presence of at least the transentorhinal Braak stage, with a positive LR of 4.49 (95% CI, 1.25-16.17) and a negative LR of 0.37 (0.25-0.53).

There was a significant relationship between CSF A $\beta$ 42 level and Braak stage ( $P < .001$ ) (Figure 2C) and HP-tau IR on IHC and the number of neocortical NFTs. Univariate logistic regression analysis showed many significant predictors of high CSF tau level (Table 5). However, on multivariate logistic regression analysis (the entered variables were age, CSF storage time, number of APOE  $\epsilon$ 4 alleles, NP count, and Braak stage), NPs remained the only significant predictor of high CSF tau levels (OR, 9.000; 95% CI, 2.088-38.787 for moderate NPs; and 8.509; 3.098-23.396, respectively, for frequent NPs).

### COMBINATION OF A $\beta$ 42 AND TAU

The combination of abnormal A $\beta$ 42 level and abnormal tau level resulted in a sensitivity of 65.3% and a specificity of 96.4% for pathologic NPs. The positive LR was 18.27 (95% CI, 2.65-125.93) and the negative LR was 0.36 (0.27-0.48). The corresponding values for pathologic NFTs (limbic or isocortical Braak stages) were a sensitivity of 75.5% and a specificity of 64.9%. The positive LR was 2.15 (95% CI, 1.52-3.04), and the negative LR was 0.38 (0.22-0.63). However, the ratio of P-tau level to A $\beta$ 42 level provided the best discrimination for Braak stage and for NPs (Table 4).

The combination of A $\beta$ 42 level and tau level was abnormal in 55% of the subjects with dementia. One of 10 subjects without dementia had an abnormal combina-

tion, and this subject had a moderate quantity of NPs in the brain. When we compared the subjects having high probability of AD ( $n=66$ ) with those having low probability or other pathologic features ( $n=39$ ), the combination of CSF A $\beta$ 42 level and tau level resulted in a sensitivity of 74.2% and a specificity of 89.7% for AD. The positive LR was 7.24 (95% CI, 2.83-18.52) and the negative LR was 0.29 (0.19-0.44). Three patients with low probability of AD and 1 patient with other pathologic features exhibited an AD-type profile in CSF. Three of these patients had a sparse quantity of NPs, and 1 patient with vascular dementia had no NPs but had an extensive A $\beta$  load on IHC.

### COMMENT

Few studies have analyzed CSF biomarker levels in neuropathologically examined patients. The strength of our study is that we were able to examine the relationship between antemortem lumbar CSF samples and different quantitatively assessed pathologic features in the brain, including AD-associated,  $\alpha$ -synuclein-positive, and vascular conditions. The results showed that the combination of low A $\beta$ 42 level and high tau level in CSF predicted the presence of AD-associated pathologic changes with high accuracy and strongly supported the probability of AD. The ratio of P-tau level to A $\beta$ 42 level exhibited the best sensitivity and the highest specificity, supporting the findings in recent clinical study.<sup>4</sup>

One study<sup>5</sup> reported an inverse correlation between ventricular CSF A $\beta$ 42 level and the number of neocortical NPs in patients with AD and in subjects without dementia. Our results revealed that A $\beta$ 42 levels in lumbar CSF samples collected a mean of 3 years before death correlated inversely with the presence of neocortical NPs and with the A $\beta$  load on IHC in the brain. These results support the hypothesis that CSF A $\beta$ 42 is a biomarker of pathologic NPs in the brain. Further support for the validity of CSF A $\beta$ 42 as a biomarker of amyloidosis in the brain has emerged from molecular imaging investigations. Reduced CSF A $\beta$  levels are associated with increased binding of Pittsburgh Compound B in the brain.<sup>18</sup>

Previously, a moderate correlation was found between CSF tau levels and pathologic NFTs in patients with AD.<sup>19</sup> A recent study<sup>20</sup> of 24 end-stage AD cases showed

**Table 3. Spearman Rank Correlations**

Variable	No. of Subjects	Aβ42	Tau	P-tau
		Median (IQR)		
Female to male ratio	97:26	430 (248)/450 (397)	484 (337)/341 (249) <sup>a</sup>	65 (33)/51 (30) <sup>a</sup>
APOE ε4 positive/negative	73/50	422 (213)/477 (279) <sup>a</sup>	523 (439)/370.5 (287) <sup>b</sup>	64 (37)/55.5 (37) <sup>a</sup>
		Spearman Rank <i>r</i>		
Age at the time of CSF sample	123	−0.113	0.104	0.102
Duration of disease at the time of CSF sample	106	−0.364 <sup>b</sup>	0.175	0.077
MMSE score	117	0.514 <sup>b</sup>	−0.156	−0.063
CSF storage time	108	−0.083	0.041	0.012
Aβ load in the brain	113	−0.637 <sup>b</sup>	0.492 <sup>b</sup>	0.360 <sup>b</sup>
IHC/HP-tau	123	−0.532 <sup>b</sup>	0.550 <sup>b</sup>	0.466 <sup>b</sup>
Silver stain <sup>c</sup>	123	−0.616 <sup>b</sup>	0.511 <sup>b</sup>	0.357 <sup>b</sup>

Abbreviations: A $\beta$ 42,  $\beta$ -amyloid 42; CSF, cerebrospinal fluid; IHC/HP-tau, hyperphosphorylated tau immunoreactivity on immunohistochemistry; IQR, interquartile range; MMSE, Mini-Mental State Examination; P-tau, phosphorylated tau.

<sup>a</sup>  $P < .05$ .

<sup>b</sup>  $P < .001$ .

<sup>c</sup> Bielschowsky silver impregnation of neocortical neurofibrillary tangle load given as semiquantitative scores.

**Table 4. Receiver Operator Characteristic (ROC) Curve Analysis**

Variable <sup>a</sup>	Optimum Cutoff	AUC (95% CI)	%		
			Sensitivity <sup>b</sup>	Specificity <sup>b</sup>	Accuracy
A $\beta$ 42, NPs	515 pg/mL	0.866 (0.778-0.953)	80.0	82.1	80.5
Tau, Braak	350 pg/mL	0.756 (0.581-0.931)	75.5	76.9	75.6
P-tau, Braak	52.5 pg/mL	0.816 (0.680-0.953)	69.1	84.6	70.7
P-tau to A $\beta$ 42 ratio, Braak	>0.0867	0.936 (0.983-0.999)	82.7	100.0	84.6
Tau to A $\beta$ 42 ratio, Braak	>0.6769	0.866 (0.738-0.994)	79.1	92.3	80.5
P-tau to A $\beta$ 42 ratio, NPs	>0.0867	0.909 (0.844-0.975)	91.6	85.7	90.2
Tau to A $\beta$ 42 ratio, NPs	>0.7121	0.899 (0.829-0.970)	84.2	89.3	85.4

Abbreviations: A $\beta$ 42,  $\beta$ -amyloid 42; AUC, area under the ROC curve; CI, confidence interval; NPs, neuritic plaques; P-tau, phosphorylated tau.

<sup>a</sup> Indicates the 2 variables included in the ROC analysis.

<sup>b</sup> Sensitivities and specificities of the biomarkers for the presence of NPs or at least transentorhinal (1-2) Braak stage (Braak).

that CSF P-tau<sub>231</sub> levels correlated with the levels of HP-tau in neocortex. The results of the present study indicated that the CSF tau level correlated significantly with the number of neocortical NFTs and with Braak stage. Phosphorylated tau levels showed higher specificity but lower sensitivity than tau levels in the detection of AD-associated pathologic changes in the brain. Cerebrospinal fluid A $\beta$ 42 levels were more closely related to AD-associated pathologic changes in the brain than tau levels, but the best discrimination was found for the ratio of P-tau level to A $\beta$ 42 level.

With 1 exception, results of clinicopathologic studies (including the present study) suggest that CSF biomarker levels reflect neuropathologic changes in the brain. Investigators in the exceptional study<sup>21</sup> examined 50 autopsy-confirmed AD cases and found no association between CSF biomarker levels and spread of plaques and NFT stage. The study included mainly patients with moderate to severe AD, and the mean interval between CSF sampling and autopsy was 1 year. The discrepancies in correlation between CSF and tangle or NP burden cannot be explained by the use of different staging methods for pathologic changes in the brain.

However, subjects in the study by Engelborghs et al<sup>21</sup> may provide an explanation. For example, differences in CSF tau levels are clearly apparent between Braak stages 1 and 2 vs the other Braak stages, but a few outliers may have had unduly affected the findings. Our results showed that the correlation between the biomarkers and pathologic changes in the brain became even stronger when assessed by semiquantitative IHC instead of by staging.

Most biomarker studies have used samples from clinically diagnosed patients. It is difficult to clinically exclude the presence of AD pathologic changes in cognitively intact control subjects or in patients with a clinical phenotype of "other type of dementia." Changes in CSF A $\beta$ 42 and tau levels can be detected in patients having progressive mild cognitive impairment who progress to a diagnosis of AD during the follow-up period.<sup>3,4</sup> One study<sup>22</sup> showed that CSF A $\beta$ 42 levels were decreased in asymptomatic subjects who progressed to dementia during a 3-year follow-up period. It is obvious that at least some of the false-positive cases in clinically based studies are not true false positives but may reflect the presence of AD pathologic changes in the brain. The short-

**Table 5. Univariate Logistic Regression Analysis<sup>a</sup>**

Variable	Aβ42 <515 pg/mL		Tau >350 pg/mL	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Female sex	...	NS	3.042 (1.241-7.455)	.02
Age at the time of CSF sample	...	NS	1.044 (1.007-1.082)	.02
MMSE score	0.875 (0.830-0.922)	<.001	...	NS
CSF storage time	...	NS	...	NS
Duration of disease at the time of CSF sample	1.232 (1.080-1.405)	.002	...	NS
APOE ε4 positive				
1 Allele	...	NS	...	NS
2 Alleles	13.630 (1.677-110.795)	.02	5.431 (1.120-26.333)	.04
NPs				
Sparse	4.600 (1.105-9.141)	.04	...	NS
Moderate	7.229 (1.866-27.995)	.004	9.000 (2.088-38.787)	.003
Frequent	53.360 (14.108-201.827)	<.001	8.509 (3.098-23.369)	<.001
Braak stage				
Transentorhinal, 1-2	6.926 (1.414-33.932)	.02	7.368 (1.818-29.862)	.005
Limbic, 3-4	74.250 (9.261-595.269)	<.001	12.778 (2.652-61.555)	.001
Isocortical, 5-6	49.500 (6.070-403.657)	<.001	30.000 (4.272-210.659)	.001

Abbreviations: Aβ42, β-amyloid 42; CI, confidence interval; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; NPs, neuritic plaques; NS, not significant; OR, odds ratio.

<sup>a</sup>Only the statistically significant results are given in the table.

coming of the combination of these 2 CSF variables (Aβ42 and tau levels) is their low sensitivity. It is unclear why extensive pathologic changes in the brain are not reflected in the CSF in many patients. It is probable that the dynamics of CSF turnover during pathologic evolution in the brain vary among patients.

Our study has limitations. The patients represent individuals from a specialty referral clinic and a single prospective clinicopathologic study cohort. This may be the reason for the high frequency of AD pathologic changes. Because of the limited number of patients, it is impossible to draw definite conclusions about the behavior of CSF biomarkers in association with other pathologic changes in the brain. However, the presence of α-synuclein or vascular pathologic conditions did not seem to influence the CSF biomarker levels in our patients. Furthermore, the cutoff values defined in this study are valid only in this study group, and they should be cross-validated in an independent population of patients. Other limitations are the few subjects without cognitive impairment and the complete lack of neurologically healthy subjects. These do not pose a differential diagnostic problem, but it is possible that the reference values of the biomarkers do not represent those that would be found in a neurologically healthy population. Although it is difficult to make comparisons among results from different laboratories, the mean values of our control subjects are close to those reported in subjects with intact cognition in another study.<sup>23</sup>

The recently proposed revised research criteria for AD have incorporated CSF biomarker levels as supportive tests for the disease.<sup>24</sup> Our results confirm that CSF Aβ42 and tau proteins are biomarkers of the presence of AD-associated pathologic changes in the brain and seem to be useful as diagnostic biomarkers, whereas their usefulness in monitoring the disease progression remains unclear.

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**Author Contributions:** Drs Tapiola and Pirttilä and Ms Herukka had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Soininen and Pirttilä. *Acquisition of data:* Tapiola, Alafuzoff, Hartikainen, and Pirttilä. *Analysis and interpretation of data:* Tapiola, Alafuzoff, Herukka, Parkkinen, and Pirttilä. *Drafting of the manuscript:* Tapiola and Pirttilä. *Critical revision of the manuscript for important intellectual content:* Alafuzoff, Herukka, Parkkinen, Hartikainen, and Soininen. *Statistical analysis:* Tapiola, Herukka, and Pirttilä. *Obtained funding:* Soininen and Pirttilä. *Study supervision:* Soininen and Pirttilä.

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