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Assessment of CSF levels of tau protein in mildly demented patients with Alzheimer's disease

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Article abstract—CSF levels of tau protein are increased in many patients with Alzheimer's disease (AD). Studies disagree on whether the increase is found in moderate or severe AD to a greater extent than in mild AD, and in two reports there was an inverse correlation between tau levels and cognitive scores. To readdress this question, we measured CSF tau in a group of mildly impaired patients with AD (Mini-Mental State Examination [MMSE] scores ≥20/30) and compared their tau levels with those in age-comparable normal and neurologic controls. We found that the mean level of CSF tau was significantly increased in the AD group compared with the controls, and 29 of 36 patients with AD had levels that exceeded a cutoff determined in a previous study. CSF tau levels did not correlate with MMSE scores. These findings and those of previous studies show that elevated CSF tau levels are found in most patients with AD, occur early in the course of dementia, and may be useful in supporting the diagnosis of AD.

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The accuracy of the clinical diagnosis of Alzheimer's disease (AD) at centers specializing in the evaluation and treatment of dementia is over 80%, as shown by several prospective studies with autopsy follow-up.[1-5] This degree of accuracy was achieved by extensively evaluating patients and following them to demonstrate the progression of dementia over time and to obtain autopsies. However, the diagnosis may be more difficult in a more typical clinical setting, especially in individuals in the early stages of AD who may have memory complaints that could be compatible with aging alone or in patients who have not been observed longitudinally. To assist in the diagnosis of AD, biological markers have been sought systemically and in CSF. An important question for any potential biological marker is whether its use helps to identify patients early in the course of AD.

Eleven studies, involving over a thousand subjects, have all concluded that CSF levels of the microtubule associated protein tau are significantly increased in subjects with AD compared with nondemented controls or with patients with other neurologic diseases or dementias.[6-16] Tau is the major constituent of neurofibrillary tangles (NFTs), which are markers of neuronal pathology in AD. In clinical-pathologic studies, counts of NFTs correlate with the severity of dementia in AD. The published reports on CSF tau do not agree on whether tau levels correlate with the level of cognitive impairment. If CSF tau elevation identifies mainly mid- to late-stage AD patients, as suggested by two reports in which tau levels correlated inversely with Mini-Mental State Examination (MMSE) scores,[10,13] its usefulness is restricted to this group of patients. Conversely, if increased CSF tau levels are present in very early stages of AD, the clinical utility should be much higher. Since each previous study included relatively few subjects with mild levels of dementia, we analyzed CSF tau levels in a group of patients with AD with MMSE scores of 20 (out of 30) or higher.

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Table *Clinical and CSF tau data in patients with mild AD and in controls*

	Alzheimer's disease (AD)	Normal controls (NC)	Other dementia (OD)	Neurologic disorders (ND)	*p*
Number	36	14	9	10	
Age (yr)	70.8 ± 5.5	70.3 ± 6.9	67.2 ± 10.0	66.3 ± 7.9	0.22
Sex (M:F)	22:14	6:8	6:3	8:2	
MMSE	23.8 ± 2.4	28.7 ± 2.1	19.1 ± 8.8	28.2 ± 2.8	<0.0001
Duration (yr)	4.3 ± 2.6				
CSF tau (pg/mL)	509 ± 255	177 ± 82	166 ± 43	160 ± 69	<0.0001

Values are mean ± SD.
Duration is from onset of dementia to lumbar puncture.
p values are for two-tailed ANOVA. For MMSE, there were significant post hoc differences (Scheffe's test, $p < 0.01$) between OD versus NC or ND, AD versus NC. For tau, there were significant post hoc differences (Scheffe's test, $p < 0.005$) for AD versus NC, ND, or OD. Similar results were obtained using nonparametric tests (Mann-Whitney U).

MMSE = Mini-Mental State Examination.

Methods. Subjects were recruited from neurology clinics at four centers as part of a previously reported research protocol,[8] and additional CSF samples were obtained from 22 patients with AD who were part of other research studies undertaken at the University of California, San Diego. All subjects or their guardians provided informed consent to undergo lumbar puncture. All subjects were aged 50 years or older, underwent a detailed diagnostic evaluation by neurologists that included appropriate laboratory tests and neuroimaging studies if dementia was suspected, and were tested with the MMSE within 3 months of the lumbar puncture. The diagnosis of AD was made according to the NINCDS-ADRDA guidelines[17] for probable AD. Among 12 patients with MMSE scores of 25 or higher at the time of the lumbar puncture, a few carried an initial diagnosis of very mild or questionable AD, and all developed clear progression of dementia on follow-up, consistent with probable AD. Control groups consisted of both cognitively normal subjects and patients with non-AD neurologic diagnoses. Cognitively normal elderly controls had no symptoms of cognitive or functional decline or of significant neurologic illness at the time of lumbar puncture or on follow-up 1 year later. Other neurologic disorders were diagnosed according to the neurologists' best clinical judgment.

Routine measures of protein, glucose, and cell counts were performed on all CSF samples. Samples were excluded from the present study if more than 15 RBC per mL were present, indicating potential contamination of CSF with blood. Aliquots of CSF were frozen and stored at −80 °C until tau was measured. The tau assay, a sandwich ELISA using two monoclonal antibodies, recognizes all forms of tau whether phosphorylated or not, and has been described.[7]

Results. This study comprised 36 AD subjects, 14 neurologically normal controls, 10 subjects with other neurologic disorders (cerebellar ataxia [3], Parkinson's disease [2], progressive supranuclear palsy [2], head trauma, seizure, Bell's palsy), and 9 with other dementing conditions (frontal lobe dementia [3], vascular dementia, progressive aphasia, normal pressure hydrocephalus, Parkinson's disease with dementia, cortical-basal ganglia degeneration, postanoxic amnesia). Demographic and clinical features of the subjects are shown in the table. The patients with AD and control groups did not differ significantly regarding age. CSF tau was significantly increased in AD patients compared with the groups of normal controls, other dementias, and neurologic controls (see table and figure). In a previous study[8] we found that CSF tau levels above a cutoff point of 312 pg/mL provided the optimal specificity for AD. Applying this cutoff point to the present study, we found that 29 of the 36 AD patients had increased CSF tau levels. Among the patients with AD whose MMSE scores were 25 or higher, 9 of 12 had tau levels >312 pg/mL.

The estimated duration of cognitive symptoms from onset of dementia until the lumbar puncture ranged from 1 to 12 years. For patients with MMSE scores ≥25, the mean duration of symptoms was 2.7 years, with a range of 1 to 6 years. Among the patients with AD, CSF tau levels did not correlate significantly with duration of symptoms, MMSE score, age, or sex. When we combined the present study and that of Motter et al,[8] there were 69 patients with

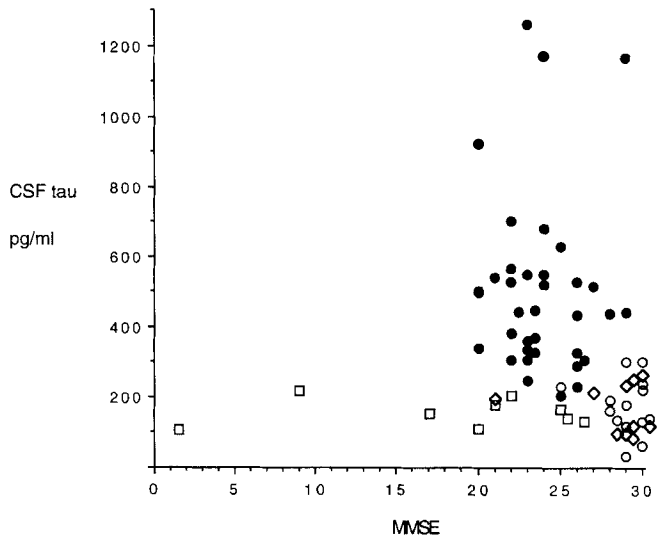

Figure. CSF tau levels versus Mini-Mental State Examination scores. Patients with AD are shown as solid circles, normal controls as open circles, neurologic controls as open diamonds, and subjects with other dementias as open squares. To improve the visual display of overlapping data points, MMSE scores have been adjusted slightly.

AD whose MMSE scores ranged from 0 to 29; once again CSF tau did not correlate significantly with MMSE score over this wide range of scores.

To assess the extent of test-retest variation, we have measured CSF tau in six patients with AD who underwent two serial lumbar punctures over 6 or 16 weeks; for each patient the two data points varied by 3 to 18% and correlated strongly (Pearson $R^2 = 0.82$). Tau levels in CSF thus remain stable over short periods.

Discussion. This study extends the findings of published reports that have shown CSF tau elevation in AD by finding that CSF tau is elevated early in the course of AD. Our results suggest that the increase in tau can be detected even in patients with very mild impairment and short duration of symptoms. Disagreement among previous studies about the value of CSF tau early in the course of AD may have arisen because of the relatively small sample of mildly demented patients in each study.

The ranges of CSF tau levels reported in previous studies have varied widely, and cutoff points range from 1 to 600 pg/mL. The major reason for these disparities is that laboratories have used different tau standards obtained from different preparations of tau (purified from human brain or from recombinant tau) and differ in technical aspects of ELISA. The general finding that the majority of patients with AD have increased levels of tau is consistent across studies. CSF samples can be stored frozen or undergo a freeze-thaw cycle without significantly affecting assay results, and tau levels do not seem to be influenced by a caudal-cranial concentration gradient in lumbar CSF.

It is not clear how soluble forms of tau reach the CSF in AD. One hypothesis is that tau release into the CSF originates from degenerating neurons,[9] which may be simultaneously forming neurofibrillary tangles and inappropriately metabolizing or processing tau. Tangle formation, and thus tau release, would be expected to occur throughout the course of the disease. Cross-sectionally, CSF studies have shown tau elevation in patients with AD over a wide range of severity of dementia,[7-9] suggesting that neurodegeneration continues at a sufficiently high tempo throughout the course of AD to sustain increases in CSF tau. To determine whether levels of CSF tau in AD are related to the rate of cognitive decline and to neurodegeneration as reflected by tangle formation requires longitudinal studies with clinical-pathologic correlation; these are currently in progress.

Not all clinically diagnosed patients with AD show increased CSF tau. Among published studies, assays and tau standards, cutoff points, diagnostic criteria, and the extent of clinical evaluation have varied, which may explain the varying percentages of patients with AD whose CSF tau exceeded the cutoff. The present study re-applied normative data from the cohort studied by Motter et al[8] because the same tau assay was used and all controls and subjects were rigorously diagnosed, reviewed, and followed. In that study, 59% of AD patients showed elevation of CSF tau above the cutoff, as did 80% of patients with mild AD in the present study. Although an increased level of CSF tau has positive predictive value in supporting the diagnosis of AD, a level that falls in the normal range does not exclude AD. It is not clear why some patients with AD have normal CSF tau values. Factors such as age, gender, or apolipoprotein E genotype have not provided an explanation. Autopsy follow-up may help by clarifying the relationship between CSF tau and the severity of neuropathologic markers such as NFTs or neuritic plaques.

Several neurologic and degenerative conditions besides AD may occasionally produce elevations in CSF tau, though none does so consistently. Many of these are not germane to the differential diagnosis of AD (e.g., meningoencephalitis,[6,9] Guillain-Barré syndrome,[6] or amyotrophic lateral sclerosis[6,7]). Increased CSF tau was reported in a minority of patients with vascular dementia and frontal lobe dementia, conditions whose clinical diagnosis is less accurate than that of AD, as well as in Creutzfeldt-Jakob disease and normal pressure hydrocephalus.[9,12,15] Unless autopsy correlation is obtained, it is unclear in what proportion of patients high CSF tau represents a false positive, in which tau is elevated due to neuronal or axonal damage, or whether AD is present as a contributory diagnosis. To illustrate this problem, a recent autopsy study of progressive supranuclear palsy[18] found that 7 of 13 patients had the additional pathology of AD.

How much does CSF tau add to the clinical diagnosis? In the hands of expert neurologists, research diagnoses of AD are already highly accurate. As noted, studies that achieved high concordance with autopsy included extensive evaluation (often with detailed psychometric testing) and follow-up that exceeded typical clinical practice. The practical value of CSF tau and its place in the dementia work-up will vary according to the clinician's expertise and the specific clinical question. The present study focuses on and supports one application of CSF tau, namely in patients in whom very mild or early dementia is suspected. In patients with more than one cause of dementia, for example, AD accompanied by stroke, alcohol abuse, or depression, elevated CSF tau increases the probability that AD is contributing to dementia, and in patients with unusual dementia syndromes, high CSF tau points toward AD as the diagnosis. Nevertheless, the finding of high CSF tau does not obviate the clinician from needing to search for other—potentially treatable—factors contributing to dementia.

An important question related to the present study is whether CSF tau increases presymptomatically. This is plausible since neuropathologic markers of AD appear to precede the onset of clinically detectable dementia.[19] The rarity of elevated CSF tau in elderly non-AD subjects studied to date argues

against high tau levels preceding symptoms of dementia by a lengthy period (e.g., decades). It would be of great interest to examine CSF tau levels in elderly subjects with symptoms of mild cognitive impairment who do not meet criteria for AD, and are then followed longitudinally, to determine if tau elevation predicts the later development of AD. Although there is no treatment that slows the progression of AD, a variety of medications with this potential are currently under development. If such an effect is demonstrated, it will be essential to make the diagnosis of AD as early as possible to obtain the maximal benefit from therapy. Biological measures such as CSF tau may help the clinician to achieve that goal.

Note added in proof: Similar findings have been reported by Riemenschneider M, Buch K, Schmolke M, Kurz A, Guder WG. Cerebrospinal protein tau is elevated in early Alzheimer's disease. Neurosci Lett 1996;212:209–211.

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