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Enzymes and Protein Markers

Tau and Aβ42 in Cerebrospinal Fluid from Healthy Adults 21–93 Years of Age: Establishment of Reference Values

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Background: **Tau protein and the 42-amino acid form of β-amyloid (Aβ42) measured in cerebrospinal fluid (CSF) have been proposed as potential biochemical diagnostic markers for Alzheimer disease. For the introduction of these assays in clinical practice, adequate reference values are of importance.**

Methods: **CSF samples were obtained from 231 neurologically and psychiatrically healthy individuals, 21–93 years of age, all with a MiniMental State examination score of 28 or above. Standardized ELISAs were used to measure tau and Aβ42 in CSF. Following IFCC recommendations, we used a rank-based method; the 0.90 and 0.10 fractiles were estimated to establish reference values for CSF-tau and CSF-Aβ42, respectively. Putative confounding factors, such as the influence of the passage of proteins from peripheral blood to CSF, influence of dysfunction of the blood-brain barrier, and freezing and thawing of CSF, were investigated.**

Results: **A correlation with age was found for CSF-tau ($r = 0.60$; $P < 0.001$). Therefore, separate reference values for different age groups were established for CSF-tau: <300 ng/L in the group 21–50 years of age, <450 ng/L in the group 51–70 years of age, and <500 ng/L in the group 71–93 years of age. CSF-Aβ42 did not correlate with age ($r = -0.045$), and the reference value was set to >500 ng/L. No correlation was found between blood-brain barrier function and CSF-tau or CSF-Aβ42.**

Conclusions: **These reference values can be applied when using CSF-tau and CSF-Aβ42 in clinical practice.**

The diagnosis of primary degenerative dementia disorders such as Alzheimer disease (AD)[10] is made largely by excluding other causes of dementia *(1)*. The search for biochemical diagnostic markers that could be used for an early diagnosis of AD has led to the suggestion that the concentrations of tau and the 42-amino acid form of β-amyloid (Aβ42) in cerebrospinal fluid (CSF) have a diagnostic value *(2, 3)*.

Tau is a normal axonal protein, which by binding to tubulin in microtubules promotes their assembly and stability *(4)*. Both normal and hyperphosphorylated tau are dispersed to the CSF *(5)*, and measurement of the CSF concentration of total tau is possible through the use of ELISA techniques *(6, 7)*. An increase in CSF-tau in AD has been found in numerous studies *(6–15)*, which probably reflects the neuronal and axonal degeneration *(7, 15)*, or possibly the successive accumulation of neurofibrillary tangles in AD *(16)*. The sensitivity of CSF-tau for AD in several studies has been high, often ~80–90% *(14, 17, 18)*. The specificity has also been relatively high because most patients with other dementias, chronic neurologic disorders (e.g., Parkinson disease), or psychiatric diagnoses

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[10] Nonstandard abbreviations: AD, Alzheimer disease; Aβ42, the 42-amino acid form of β-amyloid; CSF, cerebrospinal fluid; MAb, monoclonal antibody; and Aβ, β-amyloid.

(e.g., depressive pseudo-dementia) have physiologic CSF-tau values *(7, 19, 20)*.

Aβ42 has been implicated in the pathogenesis of AD and is the core peptide that accumulates in senile plaques *(21)*. Measurement of CSF-Aβ42 by ELISA is also possible, and several studies have found that CSF-Aβ42 is decreased in AD *(17, 19, 22, 23)*. A high sensitivity (~80–90%) for CSF-Aβ42 as a marker for AD has been found *(17, 22)*, whereas the specificity has to be investigated further. Some investigators, for example, have found decreased CSF-Aβ42 in Creutzfeldt-Jakob disease *(24)*. Concomitant measurements of CSF-tau and CSF-Aβ42 have been suggested to increase the diagnostic precision of AD *(2, 17)*. As part of the clinical routine, these markers have been found to be highly sensitive and specific *(3, 18)*.

Because CSF-tau and CSF-Aβ42 may be used as biochemical diagnostic markers of AD in clinical practice, it is of importance to establish adequate reference values for the analyses. In the present study, we present data on CSF-tau and CSF-Aβ42 obtained in a large (n = 231) sample of neurologically and psychiatrically healthy individuals with a large age range (age range, 21–93 years). We also examined the influence of some putative confounding factors, including passage of the proteins from the blood to the CSF across the blood-brain barrier and the effect of handling of CSF.

Materials and Methods

NEUROLOGICALLY AND PSYCHIATRICALLY HEALTHY PARTICIPANTS

Two hundred and thirty-one individuals (177 women and 54 men; age range, 21–93 years; mean age, 61.3 ± 18 years) participated in the study. None had symptoms or signs of psychiatric or neurologic disease. In individuals 60 years of age or older, the cognitive status was examined using the MiniMental State examination *(25)*; scores of 28–30 were inclusion criteria. Of the participants, 71 were from the Memory Clinic, Institute of Clinical Neuroscience, Göteborg University; 23 were from the Department of Neurology, Sahlgrenska University Hospital, Göteborg; 9 were from the Stockholm Gerontology Research Center, Division of Geriatric Medicine, Neurotec, Stockholm; 37 were from the Piteå River Valley Hospital, Piteå; 26 were from the Institute of Geriatric Medicine, Health University, Linköping; and 65 were from the H70 study at Göteborg University.

CSF SAMPLES FROM PATIENTS WITH BLOOD-BRAIN BARRIER DAMAGE

Sixteen patients with neurologic disorders associated with damaged blood-brain barrier were included in a separate study on serum concentrations of tau. One of these patients had multiinfarct dementia, two had epilepsy, four had brain tumors, one had a hereditary demyelinating disorder, and eight had suffered from stroke. Serum and CSF were collected during the active phase of the disorders.

ETHICS

The local Ethics Committees approved the study. Informed consent was obtained from all participants after the nature of the procedures had been fully explained to them. The study was conducted in accordance with the provisions of the Helsinki Declaration of 1975, as revised in 1996. The funding organizations listed in the acknowledgements have no right to approve or disapprove of publication of this report.

CSF, SERUM, AND PLASMA ANALYSES

Lumbar puncture was performed in the morning, under standard conditions, at the L3/L4 or L4/L5 interspace. The first 12 mL of CSF was collected in one polypropylene tube and gently mixed to avoid gradient effects *(26)*. Blood samples were taken at the same time to obtain serum and plasma. A cell count was performed in each sample, and CSF samples containing >500 erythrocytes/μL were discarded. After cell counting, the samples were centrifuged at 2000*g* for 10 min to eliminate cells and other insoluble material and were stored at −80 °C pending biochemical analyses.

Quantitative determination of albumin in serum and CSF was performed by nephelometry with the Behring Nephelometer Analyzer (Behringwerke AG). The CSF/serum (S) albumin ratio *(27)* was calculated as [CSF-albumin (mg/L)/S-albumin (g/L)] and was used as a measure of blood-brain barrier function.

CSF-tau was determined by a sandwich ELISA (Innotest hTAU-Ag; Innogenetics, Ghent, Belgium) constructed to measure total tau (both normal tau and phosphorylated tau), as described previously in detail *(7)*. In this ELISA, the monoclonal antibody (MAb) AT120 was used as capture antibody and two biotinylated MAbs (HT7 and BT2) were used as detection antibodies. MAb AT120 reacts equally well with normal and hyperphosphorylated human tau protein *(6)* as does MAb HT7, whereas MAb BT2 preferentially recognizes normal tau *(28)*. Affinity-purified tau protein was used as the calibrator and prepared as described previously *(29)*. Tau in serum and plasma was investigated accordingly.

CSF-Aβ42 was determined by a sandwich ELISA (Innotest β-amyloid$_{1-42}$; Innogenetics) constructed to specifically measure Aβ1–42 *(22, 30)*. In this ELISA, the MAb 21F12 (specific to Aβx–42) was used as capture antibody and biotinylated MAb 3D6 (specific to Aβ1–5) as detection antibody *(31)*.

Plasma Aβ42 was determined by a slightly modified protocol, as described previously *(31)*. In short, antigen and detector antibodies were incubated separately. Immunocoated plates were incubated with 100 μL of sample or calibrator for 3 h at 25 °C. After several wash steps, biotinylated 3D6 was added to the plates and incubated for 1 h. The last part of the assay protocol was identical to the CSF test *(31)*.

Table 1. Demographics, CSF-tau, CSF-Aβ42, and albumin ratio for 231 neurologically and psychiatrically healthy individuals.[a]

	n	Age, years	MMSE[b] score	CSF-tau, ng/L	CSF-Aβ42, ng/L	Albumin ratio[c]
Total	231	61 ± 18	29.2 ± 0.92	263 ± 164	794 ± 218	5.44 ± 2.16
Men	54	52 ± 20	29.3 ± 0.93	252 ± 178	821 ± 202	5.75 ± 2.49
Women	177	64 ± 16	29.1 ± 0.92	266 ± 160	786 ± 222	5.34 ± 2.04

[a] All values are expressed as mean ± SD.
[b] MMSE, MiniMental State examination score.
[c] The albumin ratio is CSF-albumin (mg/L)/S-albumin (g/L).

STATISTICAL ANALYSIS

CSF-Aβ42 followed a gaussian distribution, but CSF-tau had to be log-transformed to adjust to a gaussian distribution. The Shapiro–Wilk *W*-test was used to test for gaussian distribution. The significance was set to 0.05. Parametric statistics were consequently used in all analyses. The Pearson product–moment correlation coefficient was used for correlations.

As recommended by the IFCC *(32)*, a rank-based method was used; the 0.90 and 0.10 fractiles were estimated to establish the upper reference limit for CSF-tau and the lower reference limit for CSF-Aβ42, respectively. The results were also evaluated and compared with previously suggested reference limits *(2)*.

Results

There were no significant differences in age, CSF-tau, CSF-Aβ42, or albumin ratio between men and women. The demographics are shown in Table 1. There was no significant correlation between the albumin ratio and either CSF-tau ($r = 0.066$) or CSF-Aβ42 ($r = 0.005$).

There was a significant correlation between age and CSF-tau ($r = 0.60$; $P < 0.001$). This correlation remained significant ($r = 0.53$; $P < 0.001$) after exclusion of the individuals with the highest CSF-tau concentrations (CSF-tau >600 ng/L; n = 11). In contrast, there was no significant correlation between age and CSF-Aβ42 ($r = -0.045$). There was a weak but statistically significant correlation between age and albumin ratio ($r = 0.25$; $P < 0.001$).

Because CSF-tau correlated with age, we calculated separate reference intervals for different age categories. Division of the sample into the age groups 21–50 years, 51–70 years, and >71 years yielded large differences in mean CSF-tau concentrations, with highly significant differences between age categories (Table 2). The reference values for CSF-tau were <300 ng/L in the group 21–50 years of age, <450 ng/L in the group 51–70 years of age, and <500 ng/L in the group 71–93 years of age (Fig. 1).

Because there was no correlation between age and CSF-Aβ42 and no significant differences were found when the sample was divided into different age groups (Table 2), only one reference value for CSF-Aβ42 was set. This was >500 ng/L (Fig. 2).

The concentrations of both tau and Aβ42 were below the detection limit in serum from the neurologically and psychiatrically healthy participants. However, it was possible to detect tau (but not Aβ42) in serum from 7 of the 16 patients with neurologic disorders associated with damage to the blood-brain barrier (S-tau, 185 ± 332 ng/L; CSF-tau, 541 ± 595 ng/L). All 16 had a markedly increased albumin ratio (43.9 ± 40.0). The correlation between tau in serum and CSF was not significant ($r = 0.32$; *P*, not significant) in this group.

Although it was not possible to determine the S-Aβ42 concentrations, plasma Aβ42 could be measured. In 12 neurologically and psychiatrically healthy participants, the mean plasma Aβ42 was 144.1 ± 104.4 ng/L and the mean CSF-Aβ42 was 1040 ± 213 ng/L. All 12 had a normal albumin ratio (5.1 ± 1.8). The difference between plasma Aβ42 and CSF-Aβ42 was highly significant ($P < 0.0001$). No significant correlation between plasma Aβ42 and CSF-Aβ42 was found ($r = 0.27$; *P*, not significant).

The effects of freezing and thawing on the CSF concentrations of tau and Aβ42 were also investigated in eight neurologically and psychiatrically healthy participants. The mean CSF-tau concentration was 702 ± 222 ng/L in fresh CSF and 721 ± 345 ng/L in frozen/thawed CSF; the difference was not significant. The mean Aβ42 concentra-

Table 2. CSF-tau and CSF-Aβ42 in age groups of neurologically and psychiatrically healthy individuals.[a]

	n	CSF-tau, ng/L	CSF-Aβ42, ng/L	Significance
Total	231	263 ± 164	794 ± 218	
21–50 years	56	136 ± 89	792 ± 182	vs 51–70 years: tau, $P < 0.0001$; Aβ42, $P = 0.99$
				vs >71 years: tau, $P < 0.0001$; Aβ42, $P = 0.99$
51–70 years	67	243 ± 127	790 ± 228	vs >71 years: tau, $P < 0.0001$; Aβ42, $P = 0.98$
>71 years	108	341 ± 171	797 ± 230	

[a] All values are expressed as mean ± SD.

tion was 929 ± 540 ng/L in fresh CSF and 898 ± 531 ng/L in frozen/thawed CSF; the difference was not significant.

Discussion

To the best of our knowledge, this is the first report presenting reference values for CSF-tau and CSF-Aβ42 based on a large sample of neurologically and psychiatrically healthy individuals with a wide age range. Increased knowledge of these CSF markers in healthy individuals will be useful in future studies of the same markers in patients with neurodegenerative disorders.

We found a clear correlation between age and CSF-tau, making it necessary to determine separate reference intervals for different age categories. Such a correlation was also found in a previous study with a smaller number of participants *(33)*. A previous study also investigated the CSF concentrations of tau and Aβ42 in a group of 100 controls *(2)* without finding any age dependency for either CSF-tau or CSF-Aβ42, probably because most of the controls were elderly.

In the present study, the reference values for CSF-tau were <300 ng/L in the group 21–50 years of age, <450 ng/L in the group 51–70 years of age, and <500 ng/L in the group 71–93 years of age; the reference value for CSF-Aβ42 was >500 ng/L and was not age-dependent. We believe that these reference limits are appropriate and suggest that they be applied when using the Innotest hTAU-Ag and the Innotest β-amyloid$_{(1-42)}$ ELISAs in clinical practice.

Increased CSF-tau probably reflects neuronal, preferentially axonal, damage or degeneration *(7, 34)*. Neuronal loss with aging in several neocortical regions and in the hippocampus has been found in numerous studies *(35–41)*. The increase in CSF-tau with aging found in the present study, as well as the age-related increase in CSF neuron-specific enolase *(42)*, another neuronal marker, may reflect mild progressive neuronal degeneration. In neurodegenerative disorders such as AD, high CSF-tau concentrations are present during the whole course of the disease *(7, 18)*. One study also found increased CSF-tau in patients with mild cognitive impairment who later developed AD *(43)*. Therefore, one may speculate on whether older individuals with high CSF-tau concentrations, e.g., >600 ng/L, have preclinical AD *(33)*. Follow-up studies aimed at resolving this question are under way.

The influences of confounding factors that may reduce the clinical utility of these assays have been investigated in previous studies. One of these factors is the possible passage of tau and Aβ42 across the blood-brain barrier. If a protein measured in the CSF is also present in significant quantities in the serum, the CSF concentration, which should reflect changes in the central nervous system, will also be influenced by changes in the periphery because the protein partly emanates from serum that has crossed the blood-brain barrier *(44)*. This may cause problems, which was the case when the CSF concentration of neuronal thread protein was analyzed in one study *(7)*. In the present study, no correlation was found between any of the markers and the albumin ratio, which suggests that tau and Aβ42 do not cross the blood-brain barrier in neurologically and psychiatrically healthy individuals.

Neither tau nor Aβ42 could be determined in the serum of the neurologically and psychiatrically healthy participants. However, the results of the study performed on patients with neurologic disorders associated with a damaged blood-brain barrier suggest that tau, but not Aβ42, could be measured in serum when the integrity of the blood-brain barrier is markedly disrupted. The presence of tau in the serum of these patients may reflect a reverse passage of tau from the central nervous system to the serum across a damaged blood-brain barrier. CSF-tau is thought to reflect neuronal damage or degeneration. Indeed, after acute ischemic stroke, there is a marked increase in CSF-tau within 1–2 weeks, which peaks after 2–3 weeks, and there is a return to normal after 3–4

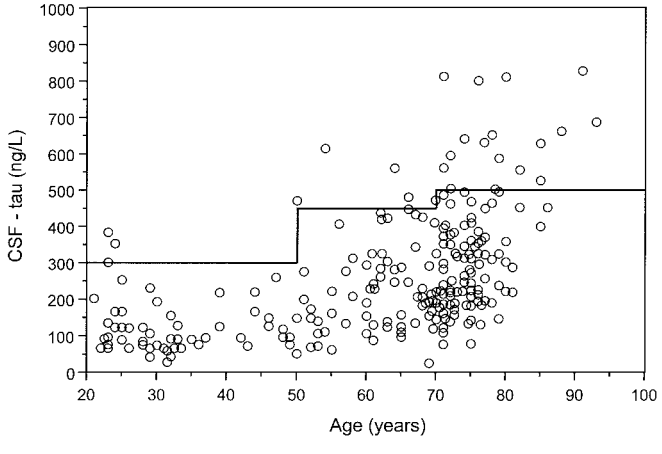

Fig. 1. Relationship between age and CSF-tau in 231 neurologically and psychiatrically healthy individuals.

The *solid line* represents the upper reference limits for CSF-tau: 300 ng/L in the group 21–50 years of age, 450 ng/L in the group 51–70 years of age, and 500 ng/L in the group 71–93 years of age.

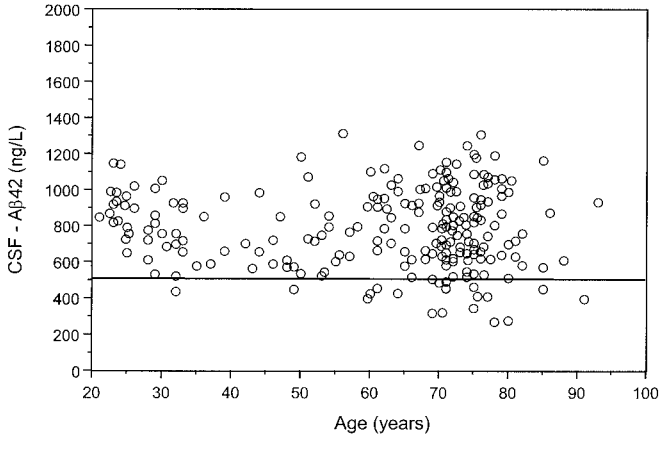

Fig. 2. Relationship between age and CSF-Aβ42 in 231 neurologically and psychiatrically healthy individuals.

The *solid line* represents the lower reference limit for CSF-Aβ42 (500 ng/L).

months *(45)*. This increase probably reflects leakage of tau from damaged neurons to the CSF.

The concentration of Aβ42 in serum was below the detection limit, but plasma Aβ42 could be determined after minor modifications of the ELISA. The reason that Aβ42 is found in plasma but not in serum is unknown and has to be investigated further. Plasma Aβ42 was clearly lower than CSF-Aβ42, and no correlation was found between them, suggesting that the main production of Aβ42 is within the central nervous system.

There is a concentration gradient along the spinal cord for some CSF proteins *(26, 46)*. To avoid getting values that depend on the gradient effect, it may be necessary to analyze a specified portion of the CSF. This is essential when investigating certain neurotransmitters, for example, the monoamine metabolites homovanillic acid and 5-hydroxy-indoleacetic acid, for which marked concentration gradients have been found *(47)*, but also when investigating proteins such as albumin and immunoglobulins *(26)*. In a previous investigation, we performed serial sampling of lumbar CSF, where the first 60 mL was drawn in five portions. No significant gradients between the portions were found for CSF-tau. This suggests that it does not matter what volume or portion of CSF is analyzed when CSF-tau is determined. This facilitates the introduction of CSF-tau measurements into the clinical routine, where the volume of CSF collected often differs for various reasons.

Previous investigations have shown that the material of which the test tubes are constructed may affect the results obtained for tau and Aβ42. When the CSF was collected in plastic (polypropylene) tubes, the measured concentrations of tau and Aβ42 were unchanged, but the measured values decreased by 40% when glass tubes were used [Ref. *(22)* and Sjögren, unpublished data]. When CSF was collected in polystyrene tubes, Aβ42 but not tau decreased. Because of this decrease, which is probably attributable to hydrophobic adsorption of tau and Aβ42 to the test tubes, tubes with nonadsorbing plastic material (polypropylene) should be used for analyses of CSF-tau and CSF-Aβ42.

The influence of freezing and storage on CSF-tau was also investigated in a previous study *(30)*. This study found no significant differences in tau concentrations between fresh samples of CSF and the same samples after storage for 1 month at −80 °C. We confirmed this finding and also showed that freezing and thawing do not affect CSF-Aβ42. Consequently, CSF samples can be frozen and stored without loss or degradation of tau or Aβ42.

In conclusion, we established reference values for CSF-tau and CSF-Aβ42 based on a large sample of neurologically and psychiatrically healthy individuals with a wide age range. A clear correlation between age and CSF-tau was found, making it necessary to determine separate reference values for different age categories, whereas no age dependency was found for CSF-Aβ42. Nonadsorbing test tubes should be used for collecting and handling CSF because some tube materials may affect CSF-tau and CSF-Aβ42. Neither CSF-tau nor CSF-Aβ42 is otherwise affected by confounding factors such as blood-brain barrier damage, freezing-thawing effects, or concentration gradients. This facilitates their establishment in clinical practice.

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