

## Tau protein in cerebrospinal fluid (CSF): a blood–CSF barrier related evaluation in patients with various neurological diseases

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Received 8 December 2000; received in revised form 16 January 2001; accepted 17 January 2001

### Abstract

Tau protein (tau) is primarily localised in neurons, and after brain parenchymal damage its release into cerebrospinal fluid (CSF) is increased. The particular influences of blood–CSF barrier function and of disease topography on CSF tau levels have not been studied yet. CSF tau concentrations determined by enzyme-immunoassay in various neurological diseases ( $n = 61$ ) were not dependent upon blood–CSF barrier dysfunction. Significant elevation of tau levels in patients with meningoencephalitis and cerebral hemorrhage indicates brain parenchymal damage. In contrast, tau levels remained normal in patients with bacterial meningitis if encephalitic complications did not occur. In patients with Guillain–Barré syndrome tau levels were low. Increased tau levels in active multiple sclerosis compared to clinically nonactive states indicate axonal pathology in active disease. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Tau protein; Cerebrospinal fluid; Brain specific proteins; Blood–cerebrospinal fluid barrier function

Tau protein (tau) is a phosphorylated microtubule-associated protein primarily localised in neuronal axons where it promotes microtubule assembly and stability [1,6]. After neuronal damage, tau is released into extracellular space and may be increased in the cerebrospinal fluid (CSF) [2]. Elevated CSF levels of tau occur in parenchymal diseases, including neurodegenerative as well as vascular or inflammatory diseases [4,5,10,18–20]. So far, it has not been evaluated whether an increase of CSF tau is specific for brain parenchymal diseases only or whether damage to meninges and nerve roots within the CSF space may lead to an increase of tau as well. Furthermore, it is known that age per se has some influence on CSF tau levels [4,9,14]. Studies on blood-derived CSF proteins revealed that blood–CSF barrier dysfunction and distance between brain lesions and ventricular CSF space influence their concentration [3,7,11,13]. Brain-derived proteins in CSF may also be influenced by the above mentioned factors as shown for the beta-trace protein [12,17]. To what extent the blood–CSF barrier dysfunction may contribute to CSF tau elevation is still unknown. Therefore, the aim of this study was to

evaluate the role of the blood–CSF barrier function on CSF tau elevation in various neurological diseases, and secondly, to re-evaluate the specificity of CSF tau for brain parenchymal diseases with different etiologies.

The disease groups consisted of 61 patients with Guillain–Barré syndrome (GBS;  $n = 5$ ), neuroborreliosis (with peripheral signs only;  $n = 10$ ), bacterial meningitis ( $n = 7$ ), viral encephalitis (HSV,  $n = 2$ ; VZV,  $n = 2$ ; other  $n = 3$ ), multiple sclerosis (MS;  $n = 17$ ), stroke (cerebral ischemia,  $n = 3$ ; intracerebral bleeding,  $n = 6$ ), and spinal canal stenosis comprising disk prolapses and spinal tumors without signs of inflammation ( $n = 6$ ). The samples were frozen at  $-20^{\circ}\text{C}$  until assayed. All samples were analysed in the Neurochemistry Laboratory of the University of Göttingen.

Neuroborreliosis was diagnosed by clinical criteria and CSF analysis with determination of the intrathecal *Borrelia*-specific antibody synthesis, with CSF cell counts between 46 and 619/ $\mu\text{l}$  and increased CSF/serum ratios of albumin ( $Q_{\text{Alb}}$ ) ( $10 \times 10^{-3}$  to  $41 \times 10^{-3}$ ). Patients with bacterial meningitis had CSF cell counts between 100 and 11 093/ $\mu\text{l}$ , elevated CSF lactate ( $>3.5$  mmol/l) and increased  $Q_{\text{Alb}}$  values ( $10 \times 10^{-3}$  to  $112 \times 10^{-3}$ ). Viral encephalitis was diagnosed by clinical criteria, magnetic resonance imaging (MRI) and CSF findings with determination of intrathecal specific antibody synthesis. The diagnosis of GBS, spinal canal stenosis, stroke and MS was based on clinical criteria,

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MRI or CT, neurophysiological findings and CSF analysis. Analysis of normal controls were omitted in this study since the specific performance characteristics of the assay as well as age-dependent control values have been described in detail by several other studies [4,9,14,19] including a report from our laboratory [10].

CSF tau levels were determined using a sandwich ELISA based on the original method of Vandermeeren et al. [19] (INNOTEST hTAU, Innogenetics, Zwijndrecht, Belgium). The assay was performed according to the protocol supplied with the kit, and CSF tau concentrations of the samples were estimated from standard curves made for each assay.

In four CSF samples with GBS the tau concentration was below the detection limit of the kit (59 ng/l). In these cases we assumed a CSF tau concentration of 50 ng/l for calculation of the median. We compared our results with the tau ranges of normal individuals given in the protocol. Statistical evaluation was done by Wilcoxon–Mann–Whitney *U*-test.

Fig. 1 shows the tau protein concentration in CSF of patients with different diseases including GBS, neuroborreliosis, and spinal canal stenosis associated with blood–CSF barrier dysfunction (elevated  $Q_{\text{Alb}}$ ) and without brain parenchymal damage. There was no correlation between  $Q_{\text{Alb}}$  and the tau level indicating that tau level in CSF is not influenced by the blood–CSF barrier dysfunction. The tau concentration with a median of 149 ng/l (range 132–237 ng/l) in CSF of patients with spinal canal stenosis was within the reported normal range. In this disease group,

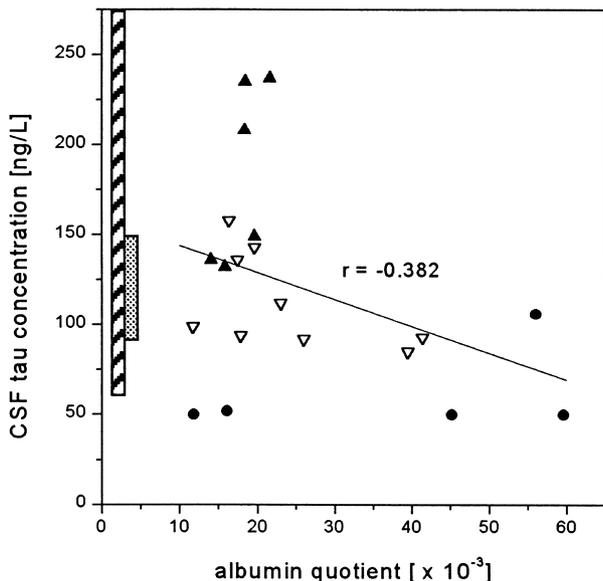


Fig. 1. CSF tau level plotted against albumin concentration quotient ( $Q_{\text{Alb}}$ ) in patients with spinal canal stenosis (▲), neuroborreliosis (▽), GBS (●). Tau levels remain invariant to increasing  $Q_{\text{Alb}}$ . Consistently decreased tau levels are seen in patients with GBS. Regression line is characterised by a slope of  $-1.49$  and a correlation coefficient of  $r = -0.382$ . Bars represent age-related reference ranges; grey,  $<60$  years; striped:  $>60$  years.

age-dependent concentration changes were seen as described in the literature [4,9,14]. All five patients with GBS showed the lowest CSF tau concentrations (values  $< 59$  and  $106$  ng/l), even in cases with moderate to severe blood–CSF barrier dysfunction. Tau concentrations in all patients with peripheral neuroborreliosis were within the normal range (median  $99$  ng/l), independent of duration of symptoms. In order to investigate whether CSF tau concentration changes over disease course, we examined the CSF tau level of two patients in a control lumbar puncture 11 and 28 days after the first one. In both cases, we found a slight increase of  $23.5$  and  $47\%$  of CSF tau which were still within the normal range.

CSF samples from patients with brain parenchymal disorders due to inflammatory or to vascular origin were analysed. As shown in Fig. 2, there were remarkable differences in the levels of tau in different inflammatory diseases of the central nervous system (CNS). If diagnostic lumbar puncture was done in the early stage of bacterial meningitis within the first 2 days, tau levels appeared normal (median  $95$  ng/l; range between  $60$  and  $180$  ng/l). All these patients received early antibiotic treatment and recovered completely without neurological deficits. One patient with a moderate CSF tau increase of  $310$  ng/l had an additional ischemia of the cerebellum 18 days prior to lumbar puncture. Another patient with a CSF tau concentration of  $624$  ng/l on the fourth day of symptoms had a meningoencephalitic complication. No correlation was found between tau and CSF cell counts or the CSF lactate level in these cases (not shown). In contrast to bacterial meningitis, patients with damage of the brain parenchyma as in viral encephalitis showed a clear

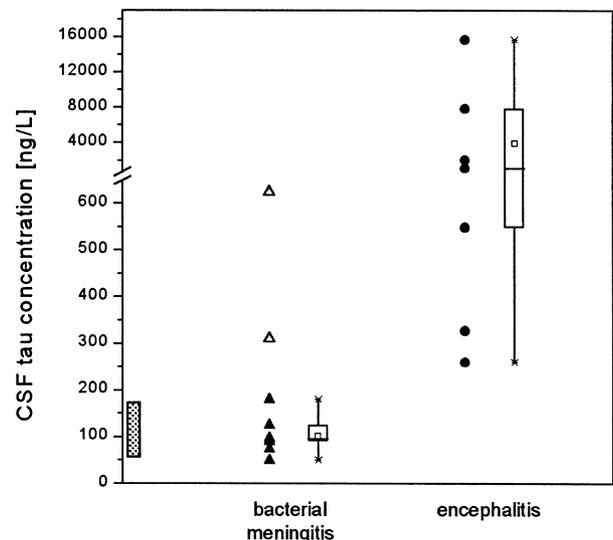


Fig. 2. Combined scatter and box and whiskers plots of CSF tau levels in early bacterial meningitis (▲) and encephalitis (●). The box represents the 25th–75th quartile, divided horizontally by the median, the whiskers represent the range, and the square indicates the mean. Elevated tau levels in bacterial meningitis with an encephalopathic complication (△). Grey vertical bar, age-related reference range.

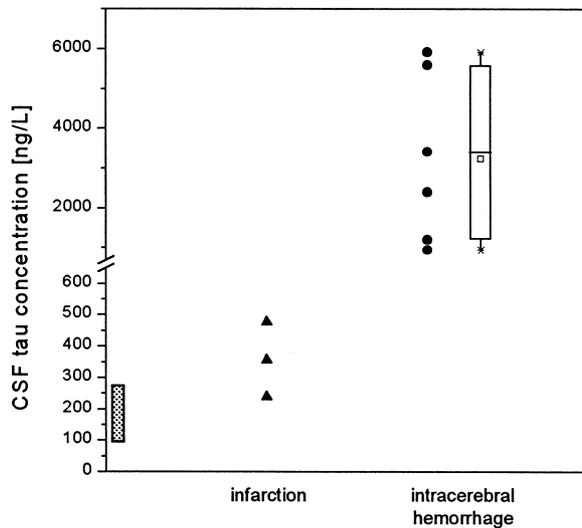


Fig. 3. Scatter and boxplot of CSF concentrations of tau in brain infarctions ( $\blacktriangle$ ) and intracerebral bleeding ( $\bullet$ ), indicated as described in Fig. 2. Grey vertical bar, age-related reference range.

CSF tau elevation ( $P < 0.05$ ;  $U$ -test). Increased tau values ranged from 327 and 548 ng/l (both Varicella zoster encephalitis) up to 15 653 ng/l (Herpes simplex encephalitis). The tau concentration showed no obvious correlation to the individual outcome.

As shown in Fig. 3, CSF tau levels in patients with parenchymal damage due to brain infarction or intracerebral bleeding (ICB) were increased. Tau levels reached significantly higher values in patients with ICB as compared to patients with brain infarctions ( $P < 0.05$ ,  $U$ -test).

In MS, CSF tau levels ranged between 80 and 321 ng/l (Fig. 4). Ten out of 17 patients showed CSF tau levels above the normal range. Analysing CSF tau levels in the MS group by differentiating patients according to clinically active vs. inactive stages, we found a significant elevation in CSF tau in the first group (median 188 ng/l mean age,  $41.1 \pm 12.5$ , and 109 ng/l mean age,  $50.5 \pm 16.2$ , respectively;  $P < 0.05$ ,  $U$ -test).

To our knowledge this is the first report on the relationship between CSF tau concentration and the blood–CSF barrier function. Analysing CSF tau in GBS, neuroborreliosis, and spinal canal stenosis, we found no evidence for a correlation between tau level and  $Q_{Alb}$ . In contrast to CSF proteins originating mainly from meningeal and spinal cord structures [12,17], CSF proteins from brain parenchyma such as tau are not influenced by the blood–CSF barrier dysfunction.

Patients with spinal canal stenosis as well as patients with neuroborreliosis showed normal CSF tau levels, indicating that damage to the nerve roots does not lead to an increase of CSF tau levels. Our results could not confirm CSF tau elevation in GBS, as reported in a previous study with elevated tau in 5 out of 11 GBS patients [19]. The extremely low levels of CSF tau in our GBS patients, even in cases with

severe blood–CSF barrier dysfunction, could be the result of increased elimination or consumption of tau in some way. Moreover, it is possible that CSF factors induce conformational changes of tau protein. Since Vandermeeren et al. [19] used the same assay for tau detection, these findings are somewhat surprising, but CSF tau levels could depend on the severity and stage of disease, co-morbidity and type of the therapy.

Previous studies also reported elevated CSF tau levels in different neurological diseases. However, in most studies it remained unclear whether an elevated CSF tau level is diagnostically useful or whether it is to be used only as a surrogate marker in such different diseases as stroke, multisystem atrophy, epilepsy, or different forms of encephalitis [4,14]. We have found increased CSF tau levels in brain parenchymal diseases independent of etiologic origin, except for patients with bacterial meningitis. This is consistent with the localisation of the pathology in the early stage of bacterial meningitis confined to the meninges. Since in all these patients early antibiotic intervention was performed and encephalitic complications were prevented, tau levels remained within the normal range. We therefore suggest that elevated CSF tau in bacterial meningitis may be a marker for brain parenchymal complications. Consequently, we found elevated tau levels in diseases with viral meningoencephalitis. Compared to patients with HSV encephalitis, patients with VZV encephalitis showed only slightly increased CSF tau levels. This might be explained by hemorrhagic effects in HSV encephalitis, since patients with intracerebral hemorrhage had the highest CSF tau levels.

In the present study we found increased tau levels in CSF

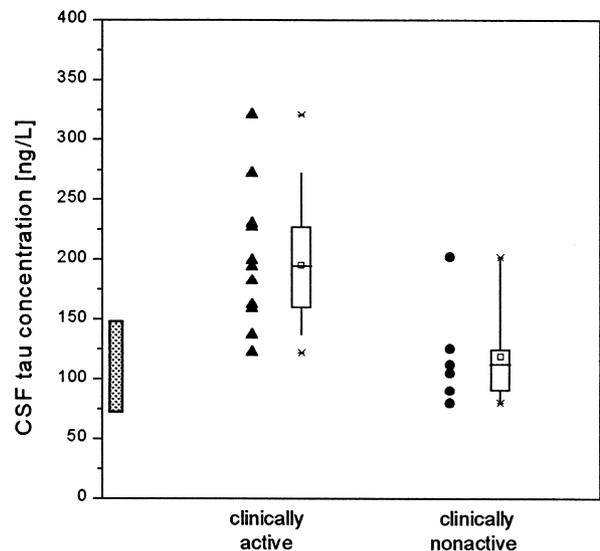


Fig. 4. Combined scatter and boxplot of CSF tau levels from patients with MS, subdivided into clinically active stages ( $\blacktriangle$ ) with significantly elevated tau concentrations vs. clinically nonactive stages ( $\bullet$ ) with normal tau levels ( $P < 0.05$ ,  $U$ -test). Grey vertical bar, age-related reference range.

of some patients with MS. Interestingly, higher tau concentrations were detected during acute relapses in contrast to patients with clinically non-active stages. Kapaki et al. [5] recently reported similar results with increased tau protein levels in MS, but they found significant tau elevations in both the relapsing-remitting and the progressive subtypes. Since it is known that in MS acute axonal injury and brain atrophy continuously go on even in clinically silent phases [15,16], it is possible that tau levels generally reflect the ongoing axonal damage. Since it is likely that human oligodendrocytes contain tau as well [8], it appears possible that CSF tau elevation in MS is an effect of axonal injury and/or oligodendrocyte damage.

In summary, the findings of this study demonstrate: (1) that CSF tau level is not dependent upon blood–CSF barrier dysfunction; (2) that elevation of CSF tau is specific for brain parenchymal damage; (3) that in patients with bacterial meningitis elevation of CSF tau indicates encephalitic complications; (4) that elevation of CSF tau level is associated with clinically active MS.

- [1] Binder, L.I., Frankfurter, A. and Rebhun, L.I., The distribution of tau in the mammalian central nervous system, *J. Cell Biol.*, 101 (1985) 1371–1378.
- [2] Delacourte, A., Pathological tau proteins of Alzheimer's disease as a biochemical marker of neurofibrillary degeneration, *Biomed. Pharmacother.*, 48 (1994) 287–295.
- [3] Felgenhauer, K., The filtration concept for the blood–CSF barrier as basis for the differentiation of CSF proteins, In J. Greenwood, D.J. Begley and M.B. Segal (Eds.), *New Concepts of a Blood–Brain Barrier*, Plenum Press, New York, 1995, pp. 209–217.
- [4] Jensen, M., Basun, H. and Lannfelt, L., Increased cerebrospinal fluid tau in patients with Alzheimer's disease, *Neurosci. Lett.*, 186 (1995) 189–191.
- [5] Kapaki, E., Paraskevas, G.P., Michalopoulou, M. and Kilidireas, K., Increased cerebrospinal fluid tau protein in multiple sclerosis, *Eur. Neurol.*, 43 (2000) 228–232.
- [6] Kosik, K.S. and Finch, E.A., MAP2 and tau segregate into dendritic and axonal domains after the elaboration of morphologically distinct neurites, *J. Neurosci.*, 7 (1987) 3142–3153.
- [7] Lewczuk, P., Reiber, H. and Tumani, H., Intercellular adhesion molecule-1 in cerebrospinal fluid—the evaluation of blood-derived and brain-derived fractions in neurological diseases, *J. Neuroimmunol.*, 87 (1998) 156–161.
- [8] Richter-Landsberg, C., The oligodendroglia cytoskeleton in health and disease, *J. Neurosci. Res.*, 59 (2000) 11–18.
- [9] Mecocci, P., Cherubini, A., Bregnocchi, M., Chionne, F., Cecchetti, R., Lowenthal, D.T. and Senin, U., Tau protein in cerebrospinal fluid: a new diagnostic and prognostic marker in Alzheimer's disease? *Alzheimer Dis. Assoc. Disord.*, 12 (1998) 211–214.
- [10] Otto, M., Wiltfang, J., Tumani, H., Zerr, I., Lantsch, M., Kornhuber, J., Weber, T., Kretzschmar, H.A. and Poser, S., Elevated levels of tau-protein in cerebrospinal fluid of patients with Creutzfeldt–Jakob disease, *Neurosci. Lett.*, 225 (1997) 210–212.
- [11] Reiber, H., Flow rate of cerebrospinal fluid (CSF)—a concept common to normal blood–CSF barrier function and to dysfunction in neurological diseases, *J. Neurol. Sci.*, 122 (1994) 189–203.
- [12] Reiber, H., CSF Flow—its influence on CSF concentration of brain-derived and blood-derived proteins, In A. Teelken and J. Korf (Eds.), *Neurochemistry*, Plenum Press, New York, 1997, pp. 423–432.
- [13] Rieckmann, P., Altenhofen, B., Riegel, A., Baudewig, J. and Felgenhauer, K., Soluble adhesion molecules (sVCAM-1 and sICAM-1) in cerebrospinal fluid and serum correlate with MRI activity in multiple sclerosis, *Ann. Neurol.*, 41 (1997) 326–333.
- [14] Rösler, I., Wichart, N., Bancher, C. and Jellinger, K.A., Tau protein and apolipoprotein E in CSF diagnostics of Alzheimer's disease: impact on non Alzheimer's dementia? *J. Neural Transm. [Suppl]*, 47 (1996) 259–266.
- [15] Rudick, R.A., Fisher, E., Lee, J.C., Simon, J. and Jacobs, L., Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group, *Neurology*, 53 (1999) 1698–1704.
- [16] Jagust, W.J. and Noseworthy, J.H., Brain atrophy as a surrogate marker in MS: faster, simpler, better? *Neurology*, 54 (2000) 807–812.
- [17] Tumani, H., Nau, R. and Felgenhauer, K., Beta-trace protein in cerebrospinal fluid: a blood–CSF barrier-related evaluation in neurological diseases, *Ann. Neurol.*, 44 (1998) 882–889.
- [18] Urakami, K., Mori, M., Wada, K., Kowa, H., Takeshima, T., Arai, H., Sasaki, H., Kanai, M., Shoji, M., Ikemoto, K., Morimatsu, M., Hikasa, C. and Nakashima, K., A comparison of tau protein in cerebrospinal fluid between corticobasal degeneration and progressive supranuclear palsy, *Neurosci. Lett.*, 259 (1999) 127–129.
- [19] Vandermeeren, M., Mercken, M., Vanmechelen, E., Six, J., Van de Voorde, A., Martin, J.-J. and Cras, P., Detection of tau proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme-linked immunosorbent assay, *J. Neurochem.*, 61 (1993) 1828–1834.
- [20] Vigo-Pelfrey, C., Seubert, P., Barbour, R., Blomquist, C., Lee, M., Lee, D., Coria, F., Chang, L., Miller, B., Lieberburg, I. and Schenk, D., Evaluation of microtubule-associated protein tau in the cerebrospinal fluid of patients with Alzheimer's disease, *Neurology*, 45 (1995) 788–793.