Phosphorylated tau in cerebrospinal fluid as a marker for Creutzfeldt–Jakob disease

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**Objective:** To determine the concentrations of microtubule associated protein tau and multiple phosphorylated tau epitopes in the cerebrospinal fluid of patients with sporadic Creutzfeldt–Jakob disease (sCJD), dementias, and controls, in order to evaluate their diagnostic use and clinical relevance.

**Methods:** The CSF concentrations of total tau and phosphorylated tau at epitope 181 were determined by enzyme linked immunosorbent assay in 66 definite and nine probable sCJD patients, and in 97 controls. Other phosphorylated tau epitopes were investigated by western blot.

**Results:** In the sCJD population, determination of 14-3-3 protein and total tau protein concentrations was of the highest diagnostic value, with a sensitivity of 96% and 92%, respectively, and a specificity of 94% and 97%. Two distinct subgroups could be identified among the 75 sCJD patients based on the detection of phosphorylated tau at threonine 181 and serines 199, 202, and 404. A high phosphorylated tau concentration was clinically correlated with a significantly shorter disease duration, early onset of akinetic mutism, and a higher rate of typical EEGs (p < 0.05).

**Conclusions:** Although the determination of phosphorylated tau levels cannot be used as a diagnostic biomarker, it may prove useful for estimating the prognosis of an sCJD patient. These experiments reconfirm that sCJD is a disease with a complex pathology.
When formalin fixed, paraffin embedded brain tissue was available, neurofibrillary tangles, prion deposition, and amyloid β plaques were detected immunohistochemically using AT8 (Innogenetics Inc), 3F4 (Senetec, St Louis, Missouri, USA), and 4G8 (Senetec) monoclonal antibodies, respectively. The prion protein codon 129 polymorphism and the prion protein strain were determined as described previously.

**Statistics**

For all statistical analyses, a cut off probability (p) value of 0.05 was used to determine statistical significance. The results of the ELISA and western blot determinations were examined using the Kolmogorov–Smirnov test (InStat software package), which showed that the results obtained did not have a normal distribution (p < 0.05). Therefore, all results were expressed as medians and centiles, and all analyses were performed using the Mann–Whitney U test. The correlation between groups was calculated using the Pearson product moment coefficient of correlation. The analysis of the different features between the two CJD subgroups was done using Fisher’s exact test except for duration and age of onset (Mann–Whitney U test). Significant values are indicated in bold.

**RESULTS**

In sCJD patients, 14-3-3 and the total tau concentration resulted in the highest sensitivity and specificity. Positive 14-3-3 results (> 1350 pg/ml) were identified in 69 patients at all four centres. Upon investigating the clinical features of the two sCJD subgroups, akinetic mutism and a typical EEG were significantly more common in the sCJDhigh subgroup (table 1) than in the sCJDlow subgroup. Furthermore, disease duration was found to be significantly shorter in the sCJDhigh patients, although no difference was observed with respect to the age of onset (table 1). Possible differences between the two sCJD groups were further analysed (table 1). The sCJDlow group was found to have significantly lower tau levels than the sCJDhigh subgroup. No difference was found between the two sCJD subgroups with respect to 14-3-3 protein and median Aβ42 concentration (table 1). Finally, we identified patients with or without neurofibrillary tangles and amyloid β plaques in both subgroups (table 1). No difference was observed when comparing the neuropathological lesions between the patients formerly classified as MM1 patients who were found to be either sCJDhigh or sCJDlow (table 1).

The analysis of phospho-tau epitopes by immunoblot showed an increased signal for sCJDhigh in some but not all investigated epitopes (fig 1). A significant difference was observed between the sCJDlow, Alzheimer’s disease, and non-dementia controls for the pT181, pS199–202, and pS404 phospho tau epitopes.

**DISCUSSION**

We investigated the concentration of tau and phospho tau in CSF from sCJD and control patients using ELISA and western blot. Two significantly different sCJD subgroups (sCJDhigh and sCJDlow) were identified, based on their p181T phospho-tau concentration. The sCJDhigh subgroup was characterised by an extremely high concentration of p181T phospho-tau compared with the sCJDlow and controls. All sCJDlow patients were associated with early akinetic mutism and short disease duration (maximum four months).

The sCJDhigh group showed increased values of certain phospho-tau epitopes—indeed of all known disease modifiers but associated with duration of the disease—pointing to a difference in the rate of disease progression. In these “acute” CJD cases neurodegeneration must progress at an increased rate, releasing both tau and phospho-tau in the extracellular space. We hypothesise that in sCJDhigh patients, oxidative stress activates the specific kinases, which results in increased levels of phospho-tau. These pathways might lead to the additional (extra-)cellular phosphorylation of tau, retrieved in the CSF. In our series, only the epitopes pT181, pS199–202, and pS404 were found to be significantly hyperphosphorylated. Previous experiments have shown that epitope specific phosphorylation is induced by certain kinases, and the glycogen synthase kinase 3B specifically phosphorylates the epitopes found in our study. Conversely, reduced activity of phosphatases or greater resistance to dephosphorylation could also contribute to the observed effect. Whether the difference in
phosphorylation reflects mainly kinase or mainly phosphatase activity and whether it plays a physiologically significant role must be addressed in future studies.

Although the results we obtained in this study indicate that determination of the p181T phospho-tau concentration cannot be employed as a diagnostic biomarker, it could be useful for estimating prognosis (suspected disease duration) of an sCJD patient. For use in a clinical setting, however, the test validity should be examined prospectively. Finally, the determination of the p181T phospho-tau concentration could conceivably be used in future clinical trials to measure the effect of a drug on disease progression.

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NEUROLOGICAL STAMP

Constantin von Economo (1876–1931)

B aron Constantin von Economo was the first Austrian to obtain a pilot’s diploma. He served aviation with distinction and supported preparations for the International Aviation Congress held in Vienna. Economo, of Greek parentage, was brought up in Austrian Trieste.

He enrolled in engineering school, but after two years began his medical training in Vienna and received his medical degree in 1901. In 1906, Economo became an assistant to Julius Wagner Jauregg, psychiatrist in Vienna. Wagner Jauregg received the Nobel Prize in 1927 for his use of malaria inoculation to induce fever in patients with syphilitic dementia paralytica.

In his early studies, Economo concentrated on the anatomy and physiology of the midbrain, pons, and trigeminal nerve pathway. In 1930 he described (with L Horn) the upper temporal lobe on the left as usually larger than on the right. A major contribution of his was The Cytarchitectures of the Cerebral Cortex in Adult Man published in 1925 but he is eponymously known for his description of encephalitis lethargica, also known as Von Economo’s Disease. ‘As well as the clinical features, Economo also discussed the pathology and histology. The disease first appeared in Romania in 1915 and raging globally until 1927. Encephalitis lethargica was once a major cause of post-encephalitic Parkinson’s Disease, but there have been no new cases for years. The last reported case was in 1940, though the clinical sequelae were seen for years after that.

Economo was a man of independent means. He rejected the chair of psychiatry when von Jauregg retired in 1928. In 1976 on the centenary of Economo’s birth a stamp was issued by Austria to honour him. (Stanley Gibbons no. 1765; Scott no. 1040). Austria also philately honoured Wagner Jauregg, in 1957 on the centenary of his birth.

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