Dissociation between CSF total tau and tau protein phosphorylated at threonine 231 in Creutzfeld–Jakob disease

Katharina Buerger a, *, Markus Otto b,1, Stefan J. Teipel a, Raymond Zinkowski c,2, Kaj Blennow d,3, John DeBernardis c, Daniel Kerkman c, Johannes Schröder e,4, Peter Schönknecht e, Lukas Cepek b, Cheryl McCulloch c, Hans-Jurgen Möller a, Jens Wiltfang e,5, Hans Kretzschmar f,6, Harald Hampel a

a Dementia Research Section and Memory Clinic, Alzheimer Memorial Center and Geriatric Psychiatry Branch, Department of Psychiatry, Ludwig-Maximilian University, Nussbaumstrasse 7, 80336 Munich, Germany
b Department of Neurology, University of Goettingen, Robert-Koch Str. 40, 37073 Goettingen, Germany
c Applied NeuroSolutions Inc. (formerly Molecular Geriatrics Corp.), 50 Lakeview Pkwy, Vernon Hills, IL 60061, USA
d Department of Clinical Neuroscience, Unit of Neurochemistry, University of Goteborg, Sahlgrens’ University Hospital, Malmo, Sweden
e Section of Gerontopsychiatry, University of Heidelberg, Voss-Str. 4, D-69115 Heidelberg, Germany
f Department of Psychiatry, Friedrich-Alexander University Erlangen-Nurnberg, Schwabachanlage 6, 91054 Erlangen, Germany
g Institute of Neuropathology, Ludwig-Maximilian University Munich, Feodor Lynen Strasse 21, 81377 München, Germany

Received 13 July 2004; received in revised form 26 August 2004; accepted 1 December 2004

Abstract

To study the potential diagnostic value of abnormally phosphorylated tau protein in Creutzfeldt–Jakob disease (CJD) compared to Alzheimer’s disease (AD), we determined levels of tau phosphorylated at threonine 231 (p-tau231) and of total tau (t-tau) in cerebrospinal fluid (CSF) of CJD patients, AD patients, and healthy controls (HC). CJD patients showed excessively high t-tau levels but relatively low p-tau231 concentrations compared to the AD group. t-tau alone yielded the best diagnostic accuracy to differentiate between CJD and AD patients, when compared to p-tau231 and the p-tau231/t-tau ratio (97, 78, and 95% correctly allocated cases, respectively). Our findings indicate a dissociation in the direction of change in CSF levels of t-tau and p-tau231 in CJD when compared to AD.

Keywords: Creutzfeldt–Jakob disease (CJD); Alzheimer’s disease (AD); Diagnosis; Differential diagnosis; Cerebrospinal fluid (CSF); Biological marker; Tau protein; Phosphorylated tau protein

Abbreviations: CJD, Creutzfeldt–Jakob disease; AD, Alzheimer’s disease; CSF, cerebrospinal fluid; t-tau, total tau protein; p-tau231, tau protein phosphorylated at threonine 231; HC, healthy control; ELISA, enzyme linked immunosorbent assay; ROC, receiver operating characteristics; AUC, area under the curve; CAC, correctly allocated cases; MMSE, Mini Mental State Examination; M–W–U, Mann–Whitney U test

E-mail addresses: katharina.buerger@med.uni-muenchen.de, hampel@med.uni-muenchen.de (K. Buerger), motto@gwdg.de (M. Otto), zinkowski@appns.com (R. Zinkowski), kaj.blennow@neuro.gu.se (K. Blennow), johannes.schroeder@med.uni-heidelberg.de (J. Schröder), jens.wiltfang@psych.imed.uni-erlangen.de (J. Wiltfang), hans.kretzschmar@med.uni-muenchen.de (H. Kretzschmar).

© 2005 Elsevier Inc. All rights reserved.

1. Introduction

Major efforts are under way to define biological markers of neurodegenerative disorders like Creutzfeldt–Jakob (CJD) and Alzheimer’s disease (AD). Moreover, a biomarker to support differential diagnosis between CJD and AD would be clinically meaningful. In cerebrospinal fluid (CSF), determination of total tau protein (t-tau) concentration is of high diagnostic value for CJD with sensitivity and specificity levels above 90% when distinguishing CJD from non-demented and demented neurological controls and AD [11,15,20,21]. T-tau levels are elevated both in CJD and AD relative to controls, but the increase is several magnitudes higher in CJD compared to AD. Extreme concentrations of t-tau in the CSF of CJD patients most likely reflect the high acuity and magnitude of neuronal destruction.

In AD, abnormal phosphorylation of tau protein is associated with the development of neurofibrillary tangles (NFT), a hallmark of AD neuropathology which is usually absent in sporadic CJD. Abnormally phosphorylated tau protein (p-tau) can be detected in vivo in the CSF. Using monoclonal antibodies specific for different phosphorylated epitopes of tau, enzyme-linked immunosorbent assays (ELISA) have been developed that sensitively measure concentrations of CSF p-tau. Highly significant increases in CSF p-tau in AD patients have recently been demonstrated in independent studies, mainly using three different immunoassays specific for the phosphorylated epitopes threonine 231 (p-tau231) [12], threonine 181 (p-tau181) [22], and serine 199 (p-tau199) [10]. Evidence from these studies indicates that quantification of tau phosphorylated at these specific sites may improve early detection (as a prognostic and diagnostic marker) [2], differential diagnosis (as a classificatory marker) [3,4,7,18], and tracking of disease progression in AD (for review see [1,8]). Therefore, CSF measurement of p-tau proteins comes closest to fulfilling criteria of a core feasible biomarker of AD [5].

In a recent study, a subgroup of patients with sporadic CJD showed high CSF levels of tau protein normally phosphorylated at threonine 181 (p-tau181) [20]. Measurement of CSF p-tau181, however, did not improve diagnostic power between CJD and AD compared to measurement of t-tau [20]. Another study showed an overlap of CSF p-tau181 concentrations between AD and CJD patients and suggested the p-tau181/t-tau ratio as a potential biomarker to differentiate CJD from AD [16].

Phosphorylation of tau protein at threonine 231 (p-tau231) has been hypothesized to be specific for AD [23]. To further explore the diagnostic value of phosphorylated tau protein in CJD and AD, we investigated p-tau231 in the CSF using a recently developed assay [12]. We hypothesized that in contrast to AD, CJD patients would present a dissociation between increased levels of t-tau indicating a high rate of general neuronal loss, and relatively low or normal levels of p-Tau231.

2. Materials and methods

CSF was obtained from 21 patients with CJD (12 women; age: 64.2 ± 8.4 years). All patients were seen by a member of the national CJD surveillance team. Since June 1993, suspected cases of CJD in Germany have been reported to the CJD surveillance unit at the Department of Neuropathology at Georg-August-University in Göttingen. Each patient was visited by a research physician and examined using a standardized protocol. According to the clinical criteria, all suspected cases of CJD were classified as “probable”, “possible”, or “other” cases [15]. Before clinical staging into “probable” or “possible”, all patients underwent at least one cranial CT or MRI, or both, to exclude ischemic stroke, hemorrhage, or space-occupying lesions as a cause of the illness.

The diagnostic criteria for the clinical CJD staging are as follows:

- **Probable**: patients with a rapidly progressive dementia of less than 2 years duration, periodic sharp-wave complexes in the EEG, and two of the following:
  - Myoclonus.
  - Visual or cerebellar symptoms.
  - Pyramidal or extrapyramidal signs.
  - Akinetic mutism.

- **Possible**: patients fulfilling the preceding criteria but without typical EEG abnormalities.

- **Other**: patients not fulfilling the criteria for possible or probable CJD.

Definite cases have been hypothesized to be specific for AD [23]. To further explore the diagnostic value of phosphorylated tau protein in CJD and AD, we investigated p-tau231 in the CSF using a recently developed assay [12]. We hypothesized that in contrast to AD, CJD patients would present a dissociation between increased levels of t-tau indicating a high rate of general neuronal loss, and relatively low or normal levels of p-Tau231.
Differences in age between groups were assessed with the Mann–Whitney-U-test. Group differences in the gender distribution were tested through the H-test. Since sample sizes are less than $N = 100$, we tested for normal distribution with the Kolmogorov–Smirnov-test. Tau proteins were normally distributed in the AD and HC groups, but not in CJD patients. Therefore, we used non-parametric approaches in our statistical analyses. Differences in mean CSF levels of the t-tau and p-tau231 between groups were assessed with the Kruskal–Wallis test. Pairwise comparisons between groups were performed with the Mann–Whitney-U-test. Correlation between t-tau and p-tau231 was assessed with Spearman’s rank correlation. For t-tau, p-tau231, and the p-tau231/t-tau ratio, sensitivity and specificity levels and numbers of correctly allocated cases were derived from receiver operating characteristic (ROC) curve analysis when the sum of specificity and sensitivity was maximized. Areas under the ROC curves (AUC) as measures of diagnostic accuracy were compared between t-tau, p-tau231, and the p-tau231/t-tau ratio using the algorithm of Hanley [9].

3. Results

Mean CSF p-tau231 levels in CJD were $324.9 \pm 345.8$ pg/ml, $613.6 \pm 337.5$ pg/ml in AD, and $92.9 \pm 104.8$ pg/ml in HC (Fig. 1A). Levels of CSF total tau were $9406.6 \pm 7796.7$ pg/ml in CJD, $609.0 \pm 258.8$ pg/ml in AD, and $340.9 \pm 117.7$ pg/ml in HC (Fig. 1B). Only in the AD group, t-tau and p-tau231 were correlated (Spearman’s rho 0.627, $p < 0.001$), but not in the CJD group (Spearman’s rho $-0.118$, $p = 0.61$), and the HC subjects (Spearman’s rho 0.018, $p = 0.96$) (Fig. 2A–C). The lack of correlation between tau proteins in the CJD group remained stable after exclusion of five CJD patients with extremely high t-tau concentrations (Spearman’s rho $-0.147$, $p = 0.59$). CSF p-tau231 as well as t-tau levels were significantly different between groups ($p < 0.001$). p-tau231 levels were higher in AD versus CJD ($p < 0.01$), AD versus HC ($p < 0.001$), and in CJD versus HC ($p < 0.01$). CSF t-tau was increased in CJD versus AD ($p < 0.001$), CJD versus HC ($p < 0.01$), and in AD versus HC ($p < 0.01$).

The p-tau231/t-tau ratio was significantly different between groups ($p < 0.001$) and lowest in patients with CJD, followed by controls and AD patients (CJD versus HC: $p < 0.01$; CJD versus AD: $p < 0.001$; HC versus AD: $p < 0.001$). Results remained stable after exclusion of five CJD patients with extremely high t-tau values ($p < 0.001$ over all groups; CJD versus HC: $p = 0.012$; CJD versus AD: $p < 0.001$; HC versus AD: $p < 0.001$).

Discriminative power between CJD and AD was highest for t-tau alone with a sensitivity of 90%, a specificity of 100%, and 97% correctly allocated cases. Using the p-tau231/t-tau ratio, sensitivity was 91% at a specificity of 97% and 95% correctly allocated cases. CSF p-tau231 yielded a sensitivity of 81%, a specificity of 76% and correctly allocated 78% of cases. The area under the ROC curve was 0.75 for p-tau231, 0.99 for t-tau, and 0.93 for the p-tau231/t-tau ratio (Fig. 3A). Comparison of AUCs yielded significant differences between p-tau231 compared to t-tau ($p < 0.001$) as well as compared to the p-tau231/t-tau ratio ($p < 0.001$) with lower values for p-tau231. Between t-tau and the p-tau231/t-tau ratio, AUC was not significantly different ($p = 0.99$).
4. Discussion

Our group studied p-tau231 and t-tau in the CSF of patients with CJD, in AD patients, and in healthy controls. In agreement with earlier studies, we found excessively high levels of t-tau in CJD patients compared to the AD and the age-matched HC groups [15,20,21]. CSF p-tau231 concentrations, however, were significantly lower in CJD patients compared to the AD group, indicating an opposite direction of change in t-tau and p-tau231 expression in the CSF of CJD patients.

The small number of controls enrolled in our study limits power to preclude a type II error, i.e. to assume that there is no difference between groups despite a true difference of markers in the population. Our results, however, showing significantly lower levels of CSF p-tau231 and t-tau in HC subjects compared to AD patients as well as extremely high t-tau values in CJD compared to other diagnostic entities are in accordance with the literature [2,3,4,7,11,15]. The main findings of our study refer to the CJD and the AD groups, i.e. a dissociation between CSF p-tau231 and t-tau in CJD patients and the diagnostic accuracy to differentiate CJD from AD using t-tau, p-tau231, and the p-tau231/t-tau ratio. Therefore, the relatively small HC group enrolled in the study is unlikely to affect the main findings of the study.

We found no improvement of diagnostic accuracy for the differentiation between CJD and AD patients through the p-tau231/t-tau ratio compared to t-tau alone. In a recent study, the ratio of tau protein phosphorylated at threonine 181 and t-tau had been suggested as a useful measurement to identify patients with CJD [16]. In agreement with that study, we found that the p-tau/t-tau ratio was lowest in the CJD group compared to the other groups studied. The previously reported results, however, did not include an estimate of the diagnostic accuracy of p-tau, t-tau, or the p-tau/t-tau ratio.

We found significant differences in CSF p-tau231 levels between the groups enrolled in our study. Van Everbroeck et al. [20] did not report a difference in CSF p-tau231 between...
CJD and AD patients, as well as HC subjects. This finding might be due to differences between the methods used (Everbroeck et al. determined CSF p-tau231 by western blot using polyclonal rabbit antibodies).

The elevation of CSF p-tau231 in some of our CJD patients compared to HC subjects may not be merely an effect of neuronal damage and increased release of intracellular proteins, since there is no correlation between t-tau reflecting neuronal damage and p-tau231 in CJD. Increased CSF p-tau231 in CJD might partly be due to underlying AD pathology and could partly account for an elevation of CSF p-tau231 in our CJD patients compared to the HC subjects. Twenty-three percent of our CJD patients had high p-tau231 levels whereas in the age between 60 and 69, a prevalence of AD of only 0.3% has been reported [17]. Coexisting AD pathology has been described in CJD. In an Austrian neuropathological longitudinal study of 110 CJD patients, coexisting AD pathological changes have been described in 11% of cases of which 20% showed NFT pathology [6]. Another study reported NFT pathology in 19% of 65 sporadic CJD patients [19]. An increase of p-tau231 in a subgroup of CJD patients, however, was not related to coexisting AD neuropathological features [20]. The increase of p-tau231 in CJD might partly be due to induced (extra)cellular phosphorylation of tau protein or release of phosphorylated tau protein into the CSF in a subgroup of CJD patients. The role of differences in phosphorylation in CJD therefore should be addressed in future studies.

Acknowledgements

This work was supported by grants from the Volkswagen-Foundation, Hannover, Germany (H.H.); the Hemliga, e.V. (H.H., K.B.); from the Medical Faculty of the Ludwig-Maximilian University, Munich (K.B., S.J.T., H.H.); from the German Competency Network on Dementias.
References


