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BRIEF COMMUNICATION

CSF nonphosphorylated Tau as a biomarker for the discrimination of AD from CJD

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Abstract

Creutzfeldt–Jakob disease and Alzheimer's disease are characterized by the presence of elevated total-Tau cerebrospinal fluid concentrations while the presence of hyperphosphorylated Tau forms in the cerebrospinal fluid is rather a hallmark of Alzheimer's disease. Here we aimed to investigate potential contribution of nonphospho-Tau epitopes (non-P-Tau) in the discrimination between both diseases. Non-P-Tau cerebrospinal fluid concentration was highly increased in Creutzfeldt–Jakob disease (n = 57, 3683 ± 3599 pg/mL) compared to Alzheimer's disease (n = 41, 148 ± 219 pg/mL) and neurological controls (n = 56, 62 ± 40 pg/mL), and significantly improved the proportion of correctly classified patients (99%) compared to that achieved by total-Tau (90%), P-Tau (62%) and 14-3-3 (91%).

Introduction

Creutzfeldt–Jakob disease (CJD) and Alzheimer's disease (AD) are neurodegenerative dementias with different etiologies displaying a partial overlap on clinical and biomarkers' presentation. CSF total-Tau (measuring nonphosphorylated and phosphorylated Tau) and P-Tau (measuring phosphorylated Tau forms) quantification are used to support the clinical diagnosis of both diseases. Highest CSF total-Tau concentrations are reported in CJD and concentrations above 1300 pg/mL are indicative of CJD.[1,2] Increased total-Tau is also detected in CSF in AD, but levels are usually lower than in CJD.[3,4] In contrast, CSF P-Tau concentrations are higher in AD, mirroring the hyperphosphorylation of Tau in the brain tissue.[3,4] Marginal to slightly elevated P-Tau concentrations are also reported in CJD,[5] most likely reflecting basal phosphorylation of Tau molecules released into the CSF as a consequence of neuronal damage.

The overlap of CSF total-Tau and P-Tau concentrations between both diseases results in a decrease in the specificity of the tests, impairing their discriminatory power in the differential diagnostic context. To overcome this problem, we aimed to investigate if the newly developed Non-P-Tau assay[6] presented and improved diagnostic performance in discriminating CJD from AD cases. To this goal we comparatively evaluated the diagnostic performance of total-Tau, P-Tau, and Non-P-Tau, as well as 14-3-3 protein, a classical CSF biomarker of prion disease,[7,8] in discriminating CJD from AD and control cases.

Methods

Ethics

The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines and approved by the Ethical Committee of the University of Göttingen. Informed consent for this study was given by all study participants or their legal next of kin.

Study population

The study included a total of 154 patients recruited at Clinical Dementia Center Göttingen and at the National Reference Center for CJD Surveillance at the Department of Neurology of the University Medical Center of Göttingen, Germany (Table 1). Patients diagnosed with probable or definite sporadic Creutzfeldt-Jakob disease (CJD) ($n = 57$)[2] as well as patients diagnosed with AD ($n = 41$) according to the Dubois criteria[9] were considered for inclusion in the study. The control group (neurological controls, $n = 56$) was composed of patients with either clinically or pathologically defined neurological disease with non-neurodegenerative etiology (psychiatric disorders, epilepsy, autoimmune diseases, alcohol abuse disorder, headache, vertigo, pain syndromes, and alternative neurologic conditions). Control cases with Aβ42 < 450 pg/mL were excluded to avoid the presence of cases with an underlying AD-related pathology. Lumbar punctures were performed at time of first routine diagnostic work up.

Table 1. Demographic characteristics of the cohort analyzed in this study.

	Controls ($n = 56$)	sCJD ($n = 57$)	AD $n = (41)$
Gender (number, f/m)	32/24	30/27	22/19
Age (mean ± SD, years)	66 ± 12	68 ± 11	68 ± 10
Tau (mean ± SD, pg/mL)	258 ± 225	6704 ± 6651	775 ± 561
P-Tau (mean ± SD, pg/mL)	36 ± 14	59 ± 21[1]	86 ± 63
Non P-Tau (mean ± SD, pg/mL)	62 ± 40	3683 ± 3599	148 ± 219
Amyloid β42 (mean ± SD, pg/mL)	796 ± 257	788 ± 322[2]	420 ± 175
14-3-3 (number, positive/trace/negative)	NA	53/2/2	4/3/29[3]

The total number of cases, gender distribution, and age is indicated. Total-Tau, P-Tau, Non-P-Tau, Amyloid β42 concentrations and 14-3-3 analysis are reported. F = female, M = male. SD = Standard Deviation. No statistical differences in gender distribution and age were detected among groups ($P > 0.05$).

[1]For 1 case, p-tau value was not available.

[2]For 5 cases, Amyloid β42 values were not available.

[3]For 5 cases, 14-3-3 data were not available.

CSF tests

CSF total-Tau (Fujirebio-Europe, Ghent, Belgium), phosphorylated Tau (P-Tau) (Fujirebio-Europe), Non-P-Tau (capturing molecules unphosphorylated at Thr-175/Thr-181) (AJ Roboscreen, Leipzig, Germany), and amyloid β 1-42 (Aβ1-42) (Fujirebio-Europe) concentrations were analyzed using commercially available ELISA kits according to the manufacturer's instructions. All analyses were performed in duplicates. The presence of 14-3-3 protein was analyzed by western blot as described previously.[7]

Statistical analysis

Kruskal–Wallis test followed by Dunns posttest was applied in multiple comparisons. To test biomarker's diagnostic accuracy, receiver operating characteristic (ROC) curve analyses were carried out and the areas under the curves (AUC) with their 95% confidence

intervals were compared with DeLong test. McNemar paired proportion test was used to compare the accuracies of the assays. The best cut-off value was estimated at the maximized Youden index. Spearman rank correlation was used to test association between biomarker concentrations. A $P < 0.05$ was considered significant. Logistic regression was performed to model probability of CDJ diagnosis (versus AD) as functions of total-Tau (M1), 14-3-3 (M2), and Non-P-Tau (M3) adjusted for age and sex of the individuals, and the models were compared with Akaike Information Criterion (AIC). Analyses were performed with Stata 14.2 (StataCorp, College Station, TX, USA).

Results

The quantification of CSF total-Tau revealed increased concentrations in CJD and AD cases compared to controls, as well as in CJD compared to AD cases ($P < 0.001$) (Table 1, Fig. 1A). P-Tau concentrations were increased in CJD and AD cases compared to controls ($P < 0.001$) and in AD compared to CJD ($P < 0.05$) (Table 1, Fig. 1B). Non-P-Tau concentrations were increased in CJD ($P < 0.001$) and AD ($P < 0.01$) cases compared to controls, as well as in CJD compared to AD cases ($P < 0.001$) (Table 1, Fig. 1C).

To determine the diagnostic accuracy of the three biomarkers in the discrimination of CJD from AD cases, AUC derived from ROC analysis were calculated (Fig. 1D). ROC AUC was the largest for Non-P-Tau (0.9923 ± 0.0079), followed by total-Tau (0.9709 ± 0.0130), and P-Tau (0.6866 ± 0.0530) (Fig. 1E). The difference between the total-Tau and Non-P-Tau ROC AUC separating AD from CJD was insignificant but with a clear tendency toward larger AUC for Non-P-Tau ($P = 0.096$). AUC for Non-P-Tau and total-Tau tests were significantly larger than for P-Tau ($P < 0.001$).

Diagnostic discrimination power was better for Non-P-Tau than total-Tau: at the cut-off of 637 pg/mL, Non-P-Tau reached sensitivity of 98% and specificity of 100% discriminating CJD from AD cases. Instead, total-Tau, at the cut-off of 2277 pg/mL, showed 100% sensitivity and 88% specificity in the discrimination of both diseases. The proportion of the correctly classified AD and CJD patients was significantly higher in case of Non-P-Tau test (99%) compared to total-Tau (90%, $P = 0.012$) and to 14-3-3 (available in 93 subjects, 91%, $P = 0.008$). The proportions of the correctly classified patients were comparable with total-Tau and 14-3-3 tests ($P = 0.8$).

Of the three statistical models considered here, the M3 (Non-P-Tau) showed the lowest AIC (24.7), followed by M1 (total-Tau, 50.2) and M2 (14-3-3, 59.5), strongly suggesting Non-P-Tau as the best explanatory variable. In none of the models, the effects of either age or sex were significant.

We observed a strong, highly significant correlation between total-Tau and Non-P-Tau ($rs = 0.8577$, $P < 0.001$), but only weak, though significant, correlation between P-Tau and Non-P-Tau ($rs = -0.21$, $P = 0.043$).

Recently, it has been reported that total-Tau concentrations correlate with disease duration in CJD cases.[10] When allowing for nonlinear associations between t-Tau and Non-P-Tau concentrations and disease duration (time between disease onset and death of the patient), Non-P-Tau was able to explain more of the variability in disease duration ($r^2 = 0.19$) than total-Tau ($r^2 = 0.11$) in 47 CJD cases were information about disease duration was available. Thus, our data indicate that Non-P-Tau harbors a higher prognostic value than total-Tau.

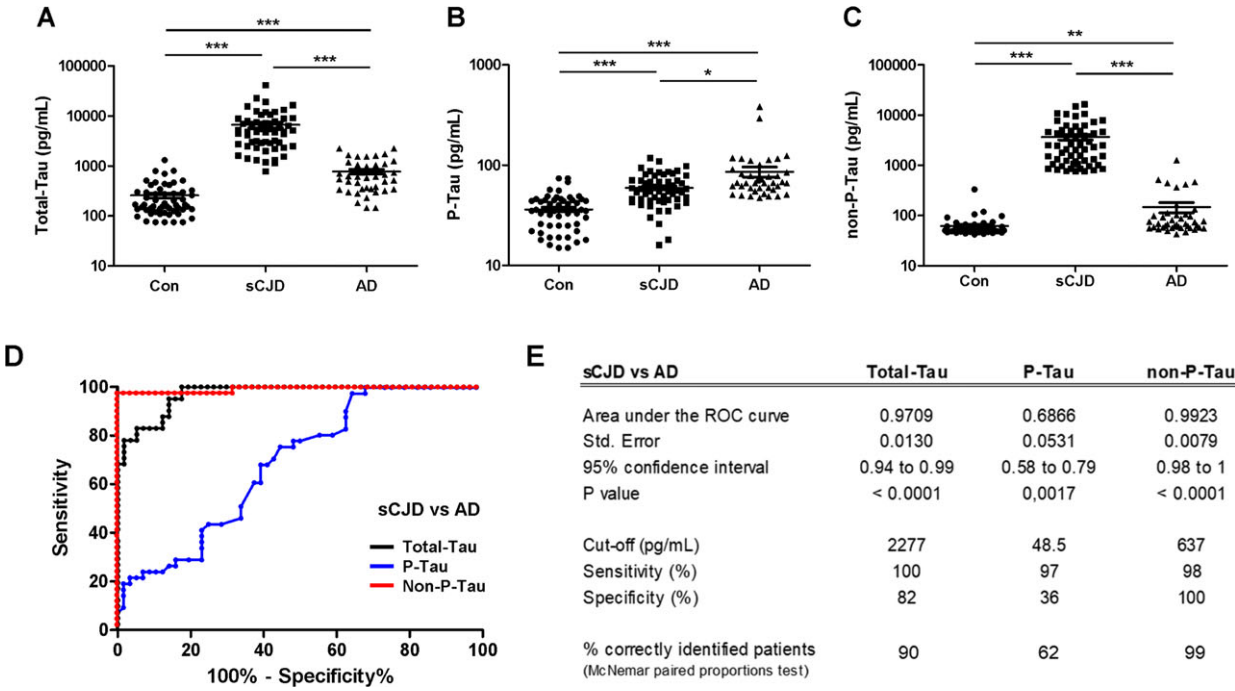
Discussion

The overlap of CSF total-Tau and P-Tau concentrations in CJD and AD patients results in a decreased specificity of both diagnostic tests. Increased concentrations of CSF Tau is assumed to reflect the degree of axonal damage in the brain tissue, thus we hypothesized that Non-P-Tau quantification could improve the diagnostic discrimination between both diseases, as it would exclusively reflect neuroaxonal degeneration in the absence of phosphorylated forms of Tau associated to AD pathology.

While our data validated previous results on total-Tau and P-Tau performance, we evidence that Non-P-Tau greatly improves the discrimination between AD and CJD compared to total-Tau, P-Tau, and 14-3-3, reaching almost full discrimination between both diagnostic groups.

In an original work where the Non-P-Tau assay was established and analytically and clinically characterized, we demonstrated that CSF Non-P-Tau concentrations were increased in AD/Mild Cognitive Impairment patients compared to controls.[6] A very recent report also detected increased Non-P-Tau concentrations in AD compared to controls,[11] however, the authors questioned diagnostic added value of the Non-P-Tau test compared to the core AD biomarkers. Although an increased diagnostic performance of Non-P-Tau for CJD was suggested, total-Tau levels were above detection limit for 89% of these cases, thus, no definite conclusions could be drawn.

Our data reveal that Non-P-Tau quantification could serve as an excellent first discriminatory assay (fast, low expensive and fully standardized) for prion disease diagnosis in nonprion-based clinical dementia centers, for which the results could be later validated with prion-specific approaches such as the real-time quaking induced assay (RT-QuIC).[12]



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sCJD vs AD	Total-Tau	P-Tau	non-P-Tau
Area under the ROC curve	0.9709	0.6866	0.9923
Std. Error	0.0130	0.0531	0.0079
95% confidence interval	0.94 to 0.99	0.58 to 0.79	0.98 to 1
P value	< 0.0001	0,0017	< 0.0001
Cut-off (pg/mL)	2277	48.5	637
Sensitivity (%)	100	97	98
Specificity (%)	82	36	100
% correctly identified patients (McNemar paired proportions test)	90	62	99

Figure 1. Diagnostic accuracy of total-Tau, P-Tau, and Non-P-Tau in the differential diagnostic context of AD and CJD cases. CSF total-Tau (A), P-Tau (B), and Non-P-Tau (C) concentrations in neurological controls (Con), CJD, and AD cases. Asterisks indicates the presence of statistically significant differences between groups (*$P < 0.05$; **$P > 0.01$, ***$P < 0.001$). Kruskal–Wallis test and Dunn's post hoc test was applied. (D) ROC curve for total-Tau, P-Tau, and Non-P-Tau in the comparative analysis between CJD and AD cases. (E) Diagnostic accuracy of total-Tau, P-Tau, and Non-P-Tau quantification in the discrimination of CJD from AD cases. Area under the curve (AUC) derived from receiver operating characteristic (ROC) curves with the 95% confidence intervals and associated p values are indicated. Based on Youden Index, optimal cut-offs (pg/mL), sensitivity, and specificity, as well as percentage (%) of correctly identified patients according to NcNemar paired proportion tests are displayed.

Although validation studies in large independent patient cohorts are needed, our findings soundly support the clinical value of Non-P-Tau as a diagnostic and prognostic tool for prion diseases, by improving the performance achieved by total-Tau quantification.

Finally, our data also suggest that Non-P-Tau assay may be an interesting additional tool for the study of the dissociation between neuronal damage and Tau pathology in the brain and biological fluids of neurodegenerative disorders.

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Authors' Contributions

PL (Piotr Lewczuk) and FL designed the study. NE, PLa, MS, and FL performed the experiments. NE, PL, JK, IZ, and FL analyzed and interpreted data. TK, SG, and IZ contributed to clinical data acquisition and sampling. NE, PL, and FL wrote the manuscript draft. All authors critically revised the manuscript and approved its contents before submission.

Conflicts of Interest

PL received consultation and lectures honoraria from Fujirebio Europe, IBL International, AJ Roboscreen, and Roche. PL and JK are coinventors of the patent application WO2016041553 A3 (Monoclonal antibody against human Tau protein). Other authors report no conflict of interests.

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