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Cerebrospinal fluid tau levels are a marker for molecular
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1Cerebrospinal fluid tau levels are a marker for molecular subtype in sporadic

2Creutzfeldt-Jakob disease

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31Abstract:

32The molecular subtype of sporadic Creutzfeldt-Jakob disease (sCJD) is an important prognostic
33marker for patient survival. However, subtype determination is not possible during life-time. Since the
34rate of disease progression is associated with the molecular subtype, this study aimed at investigating
35if total tau, a marker of neuronal death, allows pre-mortem diagnosis of molecular subtype when codon
36129 genotype is known.

37296 sCJD patients were tested for their cerebrospinal fluid total tau level at time of diagnosis and were
38investigated for their sCJD subtype post-mortem.

39There was a significant association between tau levels and the prion protein type in patients with
40codon 129 MM ($p<0.001$), MV ($p=0.004$) and VV ($p=0.001$) genotype. ROC analyses showed AUC
41values of 0.76 to 0.80 for the different genotypes indicating a good diagnostic validity of the test.

42Total tau can be used as a diagnostic test for the assessment of prion protein type when codon 129
43genotype is known. It provides valuable information for physicians and nexts of kin about the further
44course of disease.

45Highlights:

- 46 • To date, subtype determination in Creutzfeldt-Jakob disease is only possible post-mortem
- 47 • We investigate if total tau can be used as a subtype-specific marker for Creutzfeldt-Jakob
- 48 disease
- 49 • In our study we show that total tau has a good diagnostic validity when codon 129 genotype is
- 50 known
- 51 • Thus, we propose the combination of total tau and codon 129 genotype as a new test for
- 52 molecular subtype
- 53 • This approach provides valuable information for caretakers about the further course of disease
- 54

55Keywords:

56Creutzfeldt-Jakob disease, tau, biomarker, subtype, neurodegeneration

57Introduction

58Creutzfeldt-Jakob disease (CJD) is the most common human prion disease affecting about 1 in
591,000,000 individuals per year (Heinemann, et al., 2007). There are genetic and iatrogenic causes of
60CJD; however, the majority of patients suffers from a sporadic form of Creutzfeldt-Jakob disease
61(sCJD) (Kovacs, et al., 2005,Puoti, et al., 2012). Although sporadic CJD is in any case fatal, there are
62considerable differences in survival between patients. These differences have major implications for
63the patient as rapid action needs to be taken by both the treating physicians and the families in order
64to ensure an appropriate medical and nursing care. One prognostic marker for disease progression
65and patient survival is the patient's molecular subtype (MM1, MM2, MV1, MV2, VV1, VV2) which is
66compound of two different kinds of information: 1) codon 129 genotype (MM, MV or VV) and 2) prion
67protein type (PrP type 1 or 2) (Parchi, et al., 1996,Puoti, et al., 2012). While codon 129 genotype is
68easily available by genetic testing during life-time, information on PrP type can only be obtained by
69neuropathological evaluation which is mostly limited to post-mortems.

70The prognostic ability of the molecular subtype is attributed to the different rates of disease
71progression associated with the subtypes, which might, amongst others, reflect different intensities of
72neuronal degeneration. Cerebrospinal fluid (CSF) levels of one classic marker of neurodegeneration,
73protein 14-3-3, have been shown to differ between molecular subtypes when using a 14-3-3 ELISA
74(Gmitterova, et al., 2009). However, as 14-3-3 is routinely assessed in a binary way by Western Blot
75(resulting in high positivity rates for all subtypes and no detection of quantitative differences), 14-3-3
76has not been adopted in clinical practice in order to differentiate between subtypes during life-time.
77Total tau is another marker of neuronal degeneration that is widely available and is routinely measured
78on a continuous scale by ELISA. It was the aim of the present study to evaluate if CSF levels of total
79tau can be used as a diagnostic test for the differentiation of molecular sCJD subtypes during life-time.

80

81Methods

82This diagnostic study is based on data from an ongoing surveillance study of the German national
83reference centre for Transmissible Spongiform Encephalopathies (Heinemann, et al., 2007).

84Patients diagnosed with definite sCJD between 2001 and 2012 who were investigated for their
85molecular subtype within the routine surveillance process were included in this study. Moreover, a

86control group of patients diagnosed with Alzheimer's disease (AD) in the same centre (using Dubois'
87revised research criteria (Dubois, et al., 2007)) and enrolled in a longitudinal observational study
88(Schmidt, et al., 2013) were tested for their codon 129 genotype and added to the study population.
89Lumbar punctures were performed at time of diagnosis; CSF tau protein levels were quantitatively
90analysed using a commercially available ELISA according to the manufacturer's instructions
91(Innogenetics, Ghent, Belgium) (Otto, et al., 2002).

92Tau values were logarithmized to the base of 10 in order to fulfil the normal distribution assumption
93and were used in their logarithmized form in all analyses. In a first step, total tau was compared
94between sCJD PrP Type 1, sCJD PrP Type 2 and AD patients within each codon 129 genotype using
95ANOVAs (and Tukey Post-hoc tests for pairwise comparisons). In a second step, ROC analyses were
96performed for the comparison of sCJD PrP Type 1 and 2 patients within each codon 129 genotype and
97standard measures of diagnostic validity were calculated for the best cut-off value (sensitivity,
98specificity, predictive values) identified by the Youden index. Neuropathology results obtained post-
99mortem were used as the gold standard against which the proposed diagnostic procedure was tested.
100Associations between tau values and markers of disease progression (time to first tau test, total
101duration of disease) were investigated within and across subtypes using linear regression models.
102Differences of markers of disease progressions between subgroups were assessed using Wilcoxon
103ranksum tests. All data analyses were performed using Stata 12 (StataCorp, USA).

104Informed consent was given by all study participants or their legal next of kin. Ethics approval was
105obtained from the local Ethics Committee of the University of Göttingen (Study 11/11/93).

106Results

107In total, 296 sCJD patients (median age 69, interquartile range 63-73; 57 % female) were enrolled in
108this study. The majority of these patients carried a MM genotype at codon 129 (n=178), whereas MV
109(n=55) and VV (n=63) genotypes were less common (Figure 1E). Most patients with MM genotype
110carried PrP type 1 (91%, n=162), whereas 62% (n=34) of those with MV genotype and 83% of VV
111patients (n=52) showed a PrP type 2 in their post-mortems. Of the 143 AD patients included in this
112study, 60 carried a MM, 61 a MV and 22 a VV genotype. In all genotypes, both sCJD subtypes showed
113tau levels significantly higher than control patients suffering from Alzheimer's disease ($p < 0.001$ for all
114comparisons, Tukey Post-hoc tests after ANOVA, Figure 1B-E). CJD patients with PrP type 1 showed

115significantly higher tau levels in MM ($p<0.001$) and MV genotypes than patients with PrP type 2
116($p=0.008$), but lower levels in patients with VV genotype ($p=0.004$; all Tukey Post-hoc tests after
117ANOVA, Figure 1A). There was no confounding effect of sex or age at diagnosis on the association of
118subtypes and tau levels. Areas under the curve (AUCs) confirmed a good discriminatory ability of total
119tau in sCJD patients when codon 129 genotype was known (AUC [MM]: 0.76 (95%CI: 0.62-0.91), AUC
120[MV]: 0.79 (95%CI: 0.67-0.92), AUC [VV]: 0.80 (95%CI: 0.66-0.93), Figure 2). While 3,000 pg/ml could
121be applied as the optimal cut-off for being classified as PrP type 1 for patients with MM and MV
122genotype (with higher tau levels being predictive for MM1 and MV1 subtype), 6,000 pg/ml was
123identified as the best cut-off for patients carrying a VV genotype (with higher tau levels being predictive
124for VV2 subtype). Using these cut-off points (which were based on the Youden index) we obtained
125sensitivities of 81% (MM), 76% (MV) and 65% (VV) with corresponding specificities of 69% (MM), 72%
126(MV) and 91% (VV).

127In our study, tau levels were inversely associated with duration of disease (-74 (95%CI: -111 - -37)
128pg/ml per 10 days, $p<0.001$) and time to first tau test (-132 (95%CI: -196 - -69) pg/ml per 10 days,
129 $p<0.001$, linear regression). This effect was constant over all subtypes (p for interaction=0.987
130(duration of disease); 0.865 (time to first tau test)). Molecular subtypes associated with higher tau
131values at diagnosis showed also a more rapid disease progression (as measured by disease duration
132and time to first tau test, Table 1).

133

134Discussion

135In this diagnostic study we showed that CSF tau levels can be used as a marker for molecular subtype
136in sporadic Creutzfeldt-Jakob disease when codon 129 genotype is known; we thus provide for the
137first time a widely available and easy to perform pre-mortem test for subgroup differentiation in
138sporadic Creutzfeldt-Jakob disease.

139Tau was able to show a good discriminatory ability only if codon 129 genotype was known. This can be
140attributed to the fact that tau levels were considerably higher in PrP type 1 patients with MM and MV
141genotype, but lower in those with VV genotype. It can thus not be recommended to use tau as a test
142for subtype if no information on codon 129 genotype is available. However, genotypes can be
143assessed easily pre-mortem using routine genetic testing. The combination of genotyping and CSF tau

144testing provides thereby a feasible and valid approach for subtype differentiation in high-income as
145well as in low-income settings.

146Observed differences in tau levels between subtypes can be attributed to the different rates of disease
147progression which are present at point of diagnosis. Within our study we were able to show that
148disease progression rates (measured by time to first tau test and total disease duration) are inversely
149associated with tau levels at point of diagnosis; moreover they differ significantly between subtypes
150with a faster disease progression in those subtypes with higher tau levels at point of diagnosis (Table
1511). As tau is a marker of neurodegeneration, it seems reasonable to consider the speed of neuronal
152loss as a molecular equivalent of disease progression rates; however, other neuropathological
153processes like astrogliosis, PrP deposition or plaque formation might also play a role. Subtype-
154specific findings attributable to different rates of disease progression have been described in a similar
155way for proteins 14-3-3 and MRI profiles (Gmitterova, et al., 2009, Meissner, et al., 2009); however,
156these findings have not been transferred to clinical practice, because 14-3-3 ELISA results are not
157commonly available and MRI profile differentiation needs expert radiological rating. Total tau, on the
158other hand, is a widely available test which can also be applied in resource-poor settings. Another
159advantage of total tau is, that subtype differentiation can be based on information at the point of
160diagnosis and does not need to rely on additional retrospective information about e.g. the course of
161disease before diagnosis which is difficult to obtain and less reliable. In addition, the suggested
162approach can also be helpful for research studies as the distribution of subtypes within a study
163population might play an important role as an additional explanatory variable or as a confounder.
164Again, subtypes are available in a small subset of CJD patients only, which makes comprehensive
165studies in this rare disease more challenging. Knowledge about codon 129 genotype and CSF tau
166levels might help in overcoming the limitations of future studies in the field.

167A potential limitation of total tau might be that the observed sensitivities and specificities are
168considered as acceptable but not high enough for proper diagnostic testing. Therefore, future studies
169should focus on combining information on tau levels with other potential predictive markers available in
170a cross-sectional setting in order to develop a composite diagnostic test procedure which is associated
171with a higher diagnostic validity as CSF tau levels alone. As there is no other test available at the
172moment we are confident that the use of CSF total tau alone will improve patient care although its
173observed diagnostic validity allows correct classification in 70-80% of patients only.

174A major strength of this study is that it represents one of the largest reported samples of sCJD patients
175with a definite subtype. This is not restricted by the fact that it reflects a monocentre experience. Initial
176clinical evaluation and decision on lumbar puncture in this study population has been made by
177numerous hospitals in Germany so that major parts of the study can be considered a multicentre
178study. However, potential biases have been removed by this study design as all tau tests were
179performed in the same laboratory and as all patients received the same standardised clinical work-up
180from the time of diagnosis onwards.

181One limitation of this study is that it is entirely based on patients who were assessed for their
182molecular subtype post-mortem within a routine diagnostic setting. However, a post-mortem was
183offered to all patients and the decision for post-mortem subtype determination was entirely due to the
184wishes of the patients' family. This makes it unlikely that bias has been introduced by the restriction of
185the study population. Moreover, we did not find systematic differences in age, sex or duration of
186disease between sCJD patients with a definite molecular subtype and those without (probable and
187possible sCJD cases according to the updated CJD criteria (Zerr, et al., 2009)), when reviewing all
188patients referred to the German National Reference Center between 2001 and 2012. Another limitation
189of our study is, that we were not able to take into account sCJD patients with mixed PrP type 1/2
190subtypes which have been described in recent years (Puoti, et al., 1999, Uro-Coste, et al., 2008) as
191there were only few cases available in our surveillance study. The presence of mixed subtypes adds
192more complexity to subtype differentiation and might decrease the diagnostic validity of tau as a
193subtype-specific marker. Although the proportion of patients with detected mixed subtypes is low, this
194issue needs to be addressed in future studies.

195Moreover, we only looked at the first tau test during a diagnostic workup even if there were follow-up
196tests available. Follow-up tests showed in general significantly higher tau levels than tests at point of
197diagnosis (difference between first and second tau test (n=24): 3135 pg/ml (95%CI: 1314-4956),
198 $p < 0.001$)); however, it has to be kept in mind, that patients with serial CSF testing underlie a severe
199selection bias as a second CSF test is usually only performed when there is uncertainty in the
200diagnostic process. This happens typically if 14-3-3 or tau are negative in the first test. Data obtained
201within a routine setting might therefore not be generalizable for the longitudinal course of CSF tau
202levels of other sCJD patients. We therefore cannot predict how deviations in the timing of the tau test
203might affect the diagnostic validity of tau. As our study is based on surveillance data from multiple

204hospitals, we think that these deviations in the timing of tau tests are already incorporated in our study
205results and that the reported diagnostic validity of tau is realistic for routine settings with some
206variance in the timing of tau tests

207In summary, CSF total tau levels might serve as a diagnostic test for molecular subtype in sCJD when
208codon 129 genotype is known. Future studies should focus on establishing composite diagnostic tests
209(including clinical markers, MRI and CSF tests) in order to allow an improved subtype differentiation in
210sCJD patients pre-mortem as this is crucial for both appropriate patient care and clinical research.

211

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216

217**Conflict of interests**

218The authors declare that they have no conflict of interest.

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272 **Figure legends:**

273 **Figure 1:** Boxplots showing A) Tau levels of sCJD patients by molecular subtype; B) Tau levels of
274 sCJD patients and AD patients with MM genotype by subtype; C) Tau levels of sCJD patients and AD
275 patients with MV genotype by subtype; D) Tau levels of sCJD patients and AD patients with VV
276 genotype by subtype. Tau levels are displayed on a logarithmic scale (y axis). Boxes represent the
277 interquartile range (upper line: 75% quantile, lower line: 25% quantile, central line: median). Whiskers
278 represent the 90% and 10% quantile, respectively. Outliers are displayed as dots. Means and standard
279 deviations for all subtypes are displayed in E). P values for the comparisons of two groups are
280 indicated in the figure by brackets and numbers (using Tukey Post-hoc tests on the logarithmized tau
281 values after an initial ANOVA testing for global differences).

282

283 **Figure 2:** Diagnostic validity of total tau for molecular subtype differentiation. Displayed are receiver
284 operating characteristics curves of total tau for the comparison of A) MM1 and MM2 subtypes, B) MV1
285 and MV2 subtypes and C) VV1 and VV2 subtypes of sporadic Creutzfeldt-Jakob disease. Measures of
286 diagnostic validity are summarized in D) together with the respective cut-off values identified in this
287 study. *Positive predictive value **Negative predictive value

288

289Table 1: Markers for disease progression by sCJD subtype

290

Subtype (n=296)	MM1 (n=162)	MM2 (n=16)	MV1 (n=21)	MV2 (n=34)	VV1 (n=11)	VV2 (n=52)
Disease duration (in days)*	133 (94-267)	421 (127-632)	155 (91-233)	426 (322-599)	513 (173-599)	180 (137-280)
p value**	0.015		<0.001		0.009	
Time to first tau test (in days)*	83 (59-144)	227 (105-374)	112 (55-186)	290 (180-390)	157 (63-319)	120 (85-182)
p value**	0.001		<0.001		0.397	

291*displayed are medians and interquartile ranges (in brackets)

292**using Wilcoxon ranksum tests

293