Diagnostic performance of cerebrospinal fluid biomarkers in Creutzfeldt-Jakob disease
Results from the Swedish mortality registry

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Cover page art courtesy of Dr. Andreas Horn of the Horn Max-Planck-Institute for Human Development, Berlin. The image was a contestant in the 2012 Brain-Art Competition held by the Neuro Bureau, and depicts the structural connectome, averaged from 20 healthy subjects. Reconstructions obtained using the Gibbs’ Tracking approach on HARDI diffusion weighted data.
Abstract

**Background:** Distinguishing the invariably lethal prion disease Creutzfeldt-Jakob disease (CJD) from non-prion rapidly progressive dementias is important and sometimes difficult, and reliable tools for diagnosis are thus in great demand. Cerebrospinal fluid (CSF) levels of total tau (T-tau) and phosphorylated tau (P-tau) have been used to identify patients with CJD in small studies. Here, we wanted to validate the diagnostic performance of CSF T-tau, P-tau and the T-tau/P-tau-ratio by analyzing results from a large database of clinical routine samples, in combination with diagnosis information from the Swedish mortality registry.

**Methods:** We cross-referenced the Swedish mortality registry with a dataset of CSF measurements of T-tau and P-tau performed in clinical routine at the Clinical Neurochemistry Laboratory of the Sahlgrenska University Hospital, serving most of Sweden. The dataset consisted of 9765 deceased subjects with CSF measures, including 93 with CJD, whereof 56% autopsy verified.

**Results:** Patients who later died of CJD had elevated CSF T-tau and T-tau/P-tau ratio, but not CSF P-tau, compared to all other dementia patients and compared to patients who died of Alzheimer’s disease (AD). The previously defined biomarker profile had a specificity of 99.0%, a sensitivity of 78.5% and a positive likelihood ratio (LR+) of 79.9. When tested against common differential diagnoses, the sensitivity, specificity and LR+ of this profile was 78.5%, 99.6% and 196.6 in relation to AD, and 78.5%, 99.3% and 109.3 in relation to other dementias. In CJD subjects (n=30) with repeated measurements, but not in subjects with AD (n=242) or other dementias (n=258), T-tau and T-tau/P-tau ratios increased over time.

**Conclusion:** In this clinical routine setting, the combination of increased T-tau levels and increased T-tau/P-tau ratios in CJD patients has a very high specificity against important differential diagnoses to CJD, and may serve as a clinically useful diagnostic test.
Introduction

Creutzfeldt-Jakob disease (CJD) is a rare and untreatable disease, leading to rapid and invariably lethal neurodegeneration. CJD is caused by prions, an indigenous yet infectious agent whose existence was hypothesized in the 1960s and first discovered in 1982 by a research team lead by Stanley B. Prusiner [1], who was later awarded the Nobel Prize of Medicine for his research in this field. Prions are normal proteins that are intracellularly misfolded into a form that is protease resistant, and possess the ability to act as a blueprint for protein folding, inducing properly configured proteins to adapt its form. The newly refolded proteins inherit this combination of properties and trigger a chain reaction that leads to an exponential growth in numbers of the prion proteins that eventually forces the hosting cell to burst, spreading the infectious prion proteins to adjacent cells that in their turn repeat the process [2].

All mammalian prion diseases arise from the same protein, the aptly named prion protein (PrP). The normal physiological function of this protein remains elusive, and PrP knockout mice are largely phenotypically intact, although recent studies suggests PrP might play a role in myelin repair in Schwann cells [3]. There are at present four human diseases known to be caused by prions; CJD, Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI) and Kuru [2]. GSS and FFI are extremely rare, almost exclusively inherited diseases caused by mutations in the prion protein gene, PRNP, while Kuru is an endemic disorder affecting tribal regions of Papua New Guinea. Kuru was researched extensively by Daniel Carleton Gajdusek [4], and found to be spread by ingestion, as the funeral practices in the affected tribes included cannibalistic rites. In 1976 Gajdusek was awarded the Nobel Prize of Medicine for his work in the research of Kuru.
CJD is, although exhibiting a worldwide incidence of only 1 / 1 000 000 per year, the most common prion disease affecting humans. CJD is classified by the means of infection into sporadic (sCJD), variant (vCJD), familial (fCJD) and iatrogenic. The sporadic form is the most common (85%), and the variant and familial forms account for the majority of the remaining cases [2]. vCJD was discovered and 1996 and stirred up a media frenzy as tens of cases of the new disease was diagnosed in the UK, and evidence pointed to risk of infection through consummation of food products contaminated with bovine spongiform encephalitis (BSE), a bovine form of prion disease [5]. Since then, precautions have been taken regarding cattle feeding and food preparation and the incidence of vCJD has dropped to virtually zero [6]. Iatrogenic CJD is extremely rare but can in occurring cases be attributed to contaminated transplanted parts of the CNS such as corneal grafts, dural grafts or human pituitary gonadotropin [2].

Despite its rarity some conditions might elicit suspicion on CJD making it a not so uncommon differential diagnosis in the clinic. Distinguishing CJD from other and potentially treatable rapidly progressive dementias such as autoimmune encephalopathy is important, and since there is a risk of misdiagnosis due to variation in the symptoms of CJD, reliable tools for diagnosis are in great demand [7, 8]. Periodic sharp wave complexes in electroencephalography (EEG) are seen in about 2/3 of CJD patients, especially in the later stages of the disease, and high signal intensities in the basal ganglia on magnetic resonance imaging (MRI) has been suggested to discriminate CJD against other dementia disorders, but neither EEG or MRI have diagnostic accuracy high enough for a reliable diagnosis on their own [9-11]. Furthermore, the nature of MRI makes definition of objective criteria difficult, resulting in diverging recommendations for optimal criteria from different studies and expert groups [11, 12]. The severe and progressive neuronal loss that is the hallmark sign of this disease
leads to protein leakage into the cerebrospinal fluid (CSF), and measuring its biomarker quantities is an important and effective aid in the diagnostic process. CSF is collected by lumbar puncture, which is a routine procedure with minimal risk of complications and low cost, carried out by trained medical personnel. The proteins 14-3-3 and total tau (T-tau) have been of particular interest in this setting, but the initial appraisal of the presence of 14-3-3 protein in CSF as a sensitive and specific method of identifying CJD has been disputed and shown to be less accurate than previously thought, especially regarding diagnostic specificity [13-15].

Tau is a protein that is predominantly expressed in CNS neurons, modulating and providing stability in axonal microtubules. CSF levels of tau, or T-tau (the total amount of tau), increase in conditions characterized by grey matter injury and degeneration, due to loss of unmyelinated axons rich in tau-enforced microtubule [16]. Head trauma, stroke and CJD are
all conditions that correlate with high levels of CSF T-tau [17-19]. Further, there is a class of neurological diseases called Tauopathies, with the common denominator of all being associated with pathological aggregation of tau protein in the brain. Alzheimer’s disease (AD), frontotemporal dementia (FTD), progressive supranuclear palsy and dementia pugilistica are all members of this family of diseases [20]. Tau protein phosphorylated at specific amino acid residues (P-tau) is a form of tau that is thought to represent neurofibrillary tangles, a hallmark sign of AD. P-tau is increased in AD, but generally not in other progressive neurological diseases [20].

Predictive values of T-tau and the T-tau/P-tau-ratio suggest that they are of value especially in conjunction with other diagnostic methods in CJD [21-23]. However, most previous CSF studies that include analysis of P-tau and the T-tau/P-tau ratio in CJD are limited by small sample numbers. Here we wanted to assess the diagnostic performance of T-tau, P-tau and the T-tau/P-tau ratio by analyzing results from measurements of biomarkers in CSF taken from a large database of clinical routine samples in combination with diagnosis information from the Swedish mortality registry. We hypothesized that patients who died of CJD would show elevated CSF T-tau and T-tau/P-tau-ratio prior to death, and that a previously defined algorithm using these biomarkers [21] would have high sensitivity and specificity for CJD versus other dementias.

**Materials and methods**

CSF T-tau and P-tau data generated in clinical routine at the Clinical Neurochemistry Laboratory of the Sahlgrenska University Hospital, Sweden from January 1, 2002 to June 1, 2012, were extracted from the laboratory software database. The laboratory performs CSF
biomarker measurements for all of Sweden, and carries out about 15,000 – 20,000 such analyses yearly.

This dataset was cross-referenced against the Swedish mortality registry using the Swedish ten digit unique national identification number (“personal identity number”). The Swedish mortality registry is a national registry maintained by the Swedish National Board of Health and Welfare, which keeps complete records on all deaths in Sweden, including causes of death as established by the medical doctor issuing the death certificate using the International Statistical Classification of Diseases and Related Health Problems as the standard diagnostic tool. This database was queried for dates and causes of death for the subjects in our dataset. The inclusion criteria were that test results of at least one of the analyses T-tau and P-tau were registered for the patient. However, cases where only T-tau measurement was performed were stored in a separate dataset used for longitudinal analysis. Only the first measurement of biomarkers was used for statistical analysis when multiple test results were registered for the same patient, except for longitudinal analysis. The resulting dataset contained CSF tests for 9765 individual subjects. Ninety-three of these patients were reported to have CJD as a main or underlying cause of death, and n=52 (56%) of those were autopsy verified. Thirty of the CJD cases had more than one registered sampling of T-tau and/or P-tau in our dataset.

A previously suggested neurochemical algorithm was applied to determine a biomarker profile suggestive of CJD [21]. This profile consists of a CSF T-tau concentration > 1400 ng/L together with a T-tau/P-tau ratio > 25, and was calculated by finding the optimal Youden index for the combination of these two biomarkers in a cohort of nine autopsy confirmed CJD subjects and a contrast group of 27 patients with clinically suspected CJD who eventually were confirmed CJD negative. In [21] this algorithm had 78% sensitivity and 93% specificity.
In this study we tested the sensitivity and specificity of the biomarker test in the subjects identified in the Swedish mortality registry.

**Biochemical measurements**

CSF T-tau and P-tau were measured using enzyme-linked immunosorbent assays (ELISAs) (hTau Ag and Phospho-tau [181P]; Innogenetics) as previously described [24, 25]. The between-assay coefficients of variation (CV) for the T-tau and P-tau tests were 10.35% and 10.19% respectively (as determined by internal control samples during the entire study period).

All analyses were performed in clinical routine by board-certified laboratory technicians using procedures accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC). Longitudinal stability in the measurements over years was ascertained using an elaborate system of internal quality control samples and testing of incoming reagents (appendix A).

**Data collection procedures/Variable analyses/Statistical methods**

The associations between biomarker levels and possible confounding demographic factors (age and sex) were tested by non-parametric statistics (Spearman correlation and Mann-Whitney U test). Group comparisons of age, biomarker levels and survival were done by Mann-Whitney U and T-test. The associations between diagnosis (dichotomous) and biomarker levels were tested by logistic regression, adjusted for age and sex. The associations between biomarker levels and age, sex and survival were tested by multiple regressions. Biomarker accuracies were calculated as sensitivity and specificity. The sensitivity was the
proportion that tested positive among all true positives (according to the Swedish mortality registry), the specificity was the proportion that tested negative among all true negatives and the LR+ was calculated by sensitivity / (1 − specificity). In addition to using previously defined cut-offs, we determined novel biomarker cut-offs using the Youden index. The Youden index for a cut-off is defined by its sensitivity + specificity -1. Statistical significance was determined at p < 0.05. All statistics, charts and tables were produced in SPSS version 20 (IBM, New York).

Ethics

We did not receive written informed patient consent to perform the study since this was a retrospective study on archived laboratory data, where all laboratory analyses had been performed previously in clinical routine, and where we did not include any clinical information on the participants. The study was approved by the regional ethical committee.

Results

Dataset description

Demographics are summarized in table 1. Sex distribution was slightly skewed against more women in the CJD group, although the difference was not significant in a chi-square test (p=0.245). CJD patients were significantly younger than other patients, and had shorter survival. There were no significant differences in sex, age, time of survival or biomarker levels of T-tau, P-tau or P-tau/T-tau ratio between autopsy verified and non-autopsy-verified CJD patients when tested in a Mann Whitney U test. In the whole patient population, age correlated weakly with T-tau (R = 0.18, p < 0.001) and P-tau (R = 0.23, p < 0.001). Females
had higher levels of T-tau (mean=736 [sd=2661] versus mean=631 [sd=1768], u=1.3E7, p<.001) and P-tau (mean=75 [sd=40] versus mean=70 [sd=39], u=1.3E7, p<.001) than males.

**Table 1.** Demographics. Hypothesis testing conducted using t-test for equality of means.

<table>
<thead>
<tr>
<th></th>
<th>CJD</th>
<th>Non-CJD</th>
<th>Total</th>
<th>CJD vs Non-CJD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>50</td>
<td>4615</td>
<td>4665</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>43</td>
<td>5057</td>
<td>5100</td>
<td></td>
</tr>
<tr>
<td><strong>Age at sampling, years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>67</td>
<td>74</td>
<td>74</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>66</td>
<td>76</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>61-74</td>
<td>70-81</td>
<td>69-81</td>
<td></td>
</tr>
<tr>
<td><strong>Age of death, years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>67</td>
<td>78</td>
<td>78</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>66</td>
<td>79</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>61-74</td>
<td>73-84</td>
<td>73-84</td>
<td></td>
</tr>
<tr>
<td><strong>Time to death at sampling, days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>78</td>
<td>1265</td>
<td>1254</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Standard Deviation</td>
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<td>854</td>
<td>858</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>40</td>
<td>1154</td>
<td>1143</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>22-102</td>
<td>555-1864</td>
<td>541-1857</td>
<td></td>
</tr>
<tr>
<td><strong>T-tau [ng/L]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9794</td>
<td>594</td>
<td>681</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>19994</td>
<td>676</td>
<td>2240</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4300</td>
<td>490</td>
<td>490</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2200-9960</td>
<td>310-730</td>
<td>320-740</td>
<td></td>
</tr>
<tr>
<td><strong>P-tau [ng/L]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>61</td>
<td>73</td>
<td>73</td>
<td>p = 0.006</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>31</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>59</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>38-73</td>
<td>45-91</td>
<td>45-91</td>
<td></td>
</tr>
<tr>
<td><strong>T-tau/P-tau ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>155</td>
<td>8.4</td>
<td>9.8</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>280</td>
<td>7.8</td>
<td>31.6</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>101</td>
<td>7.3</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>34-170</td>
<td>6.2-8.6</td>
<td>6.2-8.7</td>
<td></td>
</tr>
</tbody>
</table>

**Biomarker levels**

We first compared levels of T-tau, P-tau and T-tau/P-tau ratio across patients listed with CJD as a cause of death, and patients listed with other non-CJD causes of death, using continuous biomarker levels. CSF levels of T-tau, but not P-tau, were higher in the CJD group, as were the T-tau/P-tau ratios when compared with non-CJD group (Table 1). In contrast, P-tau was
lower in CJD. Age and sex were possible confounders for this, but in logistic regression analysis \( \log(\text{T-tau/P-tau ratio}) \) was still a significant predictor of CJD group with age and sex as covariates (p < 0.001, B = 6.06, exp(B) = 429.39 [95% confidence interval 226-816]), as was \( \log(\text{T-tau}) \) (p < 0.001, B= 6.27, exp(B) = 527.67 [95% confidence interval 252-1102]), but not \( \log(\text{P-tau}) \) (p=0.17).

*Sensitivity and specificity*

Using the previously defined biomarker profile (T-tau > 1400 ng/L, T-tau/P-tau > 25), 73 of the 93 subjects with CJD as a cause of death had a positive biomarker profile at their first assessment, resulting in a sensitivity of 78.5%. 9577 of the 9672 subjects without CJD as a cause of death had a negative CJD biomarker profile, resulting in a specificity of 99.0%. The LR+ of this profile was 79.9. Figure 1 shows ROC plots of T-tau, P-tau and T-tau/P-tau-ratio as diagnostic predictors in our material.

![ROC plots](image)

**Figure 1.** ROCs plots of the discriminatory power of T-tau, P-tau and the T-tau/P-tau-ratio in CJD against all non-CJD subjects (A), AD (B) and other dementias (C). The T-tau/P-tau-ratio has better performance than T-tau and P-tau individually. Areas under the curve for T-tau, P-tau and T-tau/P-tau ratio were 0.960, 0.588 and 0.989 respectively in CJD vs. non-CJD, 0.939, 0.765 and 0.994 vs. AD, and 0.962, 0.595 and 0.992 vs. other dementias.
Next, we examined the group of biomarker-negative subjects in our dataset. A Mann-Whitney U test showed that their distribution of age at sampling and time of survival from sampling were significantly different (p < 0.001) from the biomarker-positive group. The group of biomarker-negative subjects were older (median = 76, interquartile range [IQR] = 11) and had longer survival time in days (median = 1156, IQR = 1307), when compared to the biomarker-positive group (age: median = 70, IQR = 15, survival: median = 93, IQR = 538).

**Differences in age and survival between biomarker positive and negative patients**

Further we compared the CJD group to the non-CJD group amongst the biomarker-positives, i.e. the true positives to the false positives. A Mann-Whitney U test showed these groups to be significantly (p < 0.001) different in terms of distributions of age at sampling and time of survival. Subjects in the CJD group were younger (median = 66, IQR = 12), than the non-CJD group (median = 74, IQR = 14), and they had a shorter survival time in days (median = 31, IQR = 57) compared to the non-CJD group (median = 439, IQR = 1060).

Next, in the CJD group, the biomarker-positive (true positives) and -negative (false negatives) subsets were compared. When tested by Mann-Whitney U test the two groups differed significantly (p < 0.01) in distribution of days of survival, but did not differ significantly in age-distribution (p = 0.255). The true positives had a shorter survival in days (median = 31, IQR = 57) than the false negatives (median = 118, IQR = 121). Figure 2 shows levels of T-tau, P-tau and T-tau/P-tau-ratio in relation to survival days left at the time of sampling for the CJD group, and their biomarker profile.
Relationship between CSF biomarkers and survival time

In this cross-sectional analysis, levels of both T-tau and T-tau/P-tau ratio increased with disease progress and were at their highest levels just before fatal outcome, while P-tau decreased and was at its lowest levels at that time (figure 2). Separate multiple regression analyses were conducted to predict log(T-tau), log(P-tau) and log(T-tau/P-tau) from sex, age, and days of survival. These variables significantly predicted log(T-tau) (F(3, 89) = 10.3, p < 0.001, R² = .257), but only days of survival (p < 0.001) added significantly to the prediction. They also significantly predicted log(T-tau/P-tau) (F(3, 89) = 13.38, p < 0.001, R² = .311), but only days of survival (p < 0.001) added significantly to the prediction. The prediction of log(P-tau) (F(3, 89) = 1.76, p = 0.16, R²=0.056), was only significantly added to by age at sampling (p = 0.025).
Figure 3. Causes of death among non-CJD subjects in the biomarker-positive group, i.e. the false positives. Cardiac events and infections were the most common causes of death reported. More than half (52%) of the subjects had also received a classification code indicating that some kind of progressive encephalopathy, e.g. unspecified dementia, named other dementia, neurodegeneration or encephalopathy (including epilepsy and psychiatric disturbances), had contributed to their death.

**Biomarker profile validity and optimization**

The validity of the chosen Blennow et al. biomarker profile was tested by comparing it to a new profile computed by applying the Youden index strategy to our dataset. The optimal individual cut-off for T-tau was calculated to 1350 ng/L (Youden index 0.81), and for the T-tau/P-tau ratio 17 (Youden index 0.90). Applying this new biomarker profile increased the sensitivity from the previous 78.5% to 85.0%, while the specificity only decreased from 99.0% to 98.6% and the LR+ decreased from 79.9 to 61.8. An algorithmic approach was also tested, using the T-tau/P-tau ratio as a first diagnostic gate, and only assessing the subject T-
tau value if their T-tau/P-tau ratio surpassed the optimal cut-off at 17. A new Youden index calculation was made using the subjects in our data that passed this first gate, and a T-tau value of 2100 ng/L (Youden index 0.56) was determined as the optimal cut-off. This proved to be the best profile in terms of having the least absolute number of misdiagnosed subjects (false positives + false negatives = 96), and had a sensitivity of 77.4%, a specificity of 99.2% and a LR+ of 99.8. The results of the performance testing of all biomarker profiles are detailed in table 2.

<table>
<thead>
<tr>
<th>Non-CJD (n)</th>
<th>T-tau&gt;1400, T-tau/P-tau ratio&gt;25</th>
<th>T-tau &gt; 1350, T-tau/P-tau ratio&gt;18</th>
<th>T-tau/P-tau ratio&gt;18, T-tau&gt;2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>9577</td>
<td>95</td>
<td>9539</td>
<td>133</td>
</tr>
<tr>
<td>CJD (n)</td>
<td>20</td>
<td>73</td>
<td>14</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>78.5%</td>
<td>85.0%</td>
<td>93.6%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.0%</td>
<td>98.6%</td>
<td>97.1%</td>
</tr>
<tr>
<td>Correctly diagnosed subjects</td>
<td>9650</td>
<td>9618</td>
<td>9479</td>
</tr>
<tr>
<td>Misdiagnosed subjects</td>
<td>115</td>
<td>147</td>
<td>286</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>79.9</td>
<td>61.8</td>
<td>32.3</td>
</tr>
</tbody>
</table>

**Biomarker diagnostic performance and differential diagnoses**

To better validate the accuracy of the tested biomarkers for CJD we also tested them against contrast groups consisting of patients where cause of death were AD (n=2004) or other dementias (n=2645) calculating sensitivity and specificity of the original CJD biomarker profile (defined by a T-tau cut-off at 1400 ng/L in combination with a T-tau/P-tau ratio > 25). When compared to the AD group sensitivity, specificity and LR+ for CJD were 78.5%, 99.6% and 196.6, and when compared to other dementias 78.5%, 99.3% and 109.3 respectively. The ROC plots in panel B and C in figure 1 demonstrate the performance of the biomarker profile in these settings.

**Longitudinal data**

To explore how the levels of biomarkers develop with disease progression, days of survival
left at the time of sampling in relation to biomarker levels were analyzed in the subset of CJD patients with more than one CSF sample registered (n=30). Their individual changes in T-tau, P-tau and T-tau/P-tau-ratio are plotted in figure 4. Higher values of both T-tau and T-tau/P-tau-ratio were seen at later stages in the disease. Close to all repeated measurements, 85% of T-tau and 93% of T-tau/P-tau-ratio, complied with this pattern. The greatest increases in both T-tau and T-tau/P-tau-ratio values were seen closest to death. P-tau had a variable longitudinal pattern, with 54% of subjects having increasing and 46% decreasing values. The same clear trend of rising T-tau, and T-tau/P-tau ratio was not seen in the groups with AD or other dementias listed as cause of death, as AD subjects with multiple measurements (n=242) showed increasing T-tau levels in 46%, and increasing T-tau/P-tau ratios in 58% of the cases closer do death, and subjects with other dementias (n=258) showed increasing T-tau levels in 48%, and increasing T-tau/P-tau ratios in 56% of the cases closer do death.

When evaluating the diagnostic performance of T-tau and T-tau/P-tau ratio using the test results at the time point closest to death, another 7 CJD patients were classified as biomarker-positive resulting in a sensitivity of 86.0% and an LR+ of 87.6, as compared to the original

Figure 4. Spaghetti plots of how biomarker levels develop over time in the CJD cases with more than one recorded biomarker assessment. Most subjects show increasing levels of T-tau and T-tau/P-tau ratio as survival days diminish.
sensitivity of 78.5% and LR+ of 79.9 obtained when the first test results were used. The specificity remained unchanged.

Discussion

In this study, we explored a large dataset of dates and causes of death from the Swedish mortality registry, cross-referenced with a dataset of CSF measurements of T-tau and P-tau performed in clinical routine. Our hypothesis that patients who died of CJD would show elevated CSF T-tau and T-tau/P-tau-ratio prior to death was confirmed, as the CJD-indicative biomarker profile (T-tau > 1400 ng/L and T-tau/P-tau-ratio > 25) had a specificity of 99.0%, a sensitivity of 78.5% and an LR+ of 79.9 in this dataset of 9675 deceased subjects, whereof 93 with CJD. In groups of common differential diagnoses to CJD, the sensitivity, specificity and LR+ was 78.5%, 99.6% and 196.6 in relation to AD, and 78.5%, 99.3% and 109.3 in relation to other dementias. Furthermore, the false negatives (CJD diagnosis but negative biomarker profile) had a longer survival time than the true positives, possibly indicating that the test was taken at an earlier stage of the disease and therefore displaying lower levels and a negative profile due to not as far progressed pathology. The subset of CJD subjects in our dataset with longitudinal data showed a clear trend of rising T-tau and T-Tau/P-tau ratios, further corroborating this notion. The sensitivity of the biomarker profile increased to 86.0% when the latest biomarker measurement was taken into account for this group of patients, which suggests that in cases where clinical presentation and/or other investigations support a diagnosis of CJD, repeated CSF samplings may be of value.

The false positives in our dataset were categorized according to if they had neurological diseases listed as a cause of or contributor to death. This was true in more than half (52%) of the cases. As previously discussed, CJD can be difficult to correctly diagnose due to its
heterogenic clinical presentation, and there is a possibility that there might be a share of clinically misdiagnosed cases in this lot. However, the false positive group differed compared to the true positives in terms of age distribution and survival, and furthermore we did not have access to detailed clinical records for these patients, precluding firm conclusions on the probability of misdiagnosis. Another possible explanation to why some CJD patients display lower T-tau and T-tau/P-tau-ratio levels could be that they suffer from a variant of the disease with lower intensity and longer survival. That such a subset of CJD cases exists has been described in earlier studies [26], and their divergent biomarker profile can be attributed to atypical CJD variants or biological variables such as long disease duration, young age at onset or genetic differences [19].

Previous studies have also shown rising levels of CSF biomarkers during the course of the disease, which eventually level out and return to normal or just slightly elevated in the terminal stage [27, 28]. We could not find evidence to support this notion in our study. Almost all cases where more than one lumbar puncture had been performed showed higher levels of T-tau, and T-tau/P-tau-ratio in later stages of disease, including measurements made mere days before death.

Optimization of the diagnostic biomarker profile proposed by Blennow et al. using Youden index calculation in our material suggested that a lower cut-off for the T-tau/P-tau ratio was more optimal in the present cohort. For T-tau the cut-offs identified here were well aligned with previous suggested values [19, 29]. The best performing biomarker profile in this study was acquired with an algorithmic approach using a T-tau/P-tau ratio threshold as a first diagnostic gate and then applying a second test of a T-tau threshold only to subjects passing the first gate. This method yielded the highest specificity (99.2%), the lowest number of misdiagnosed patients (96) and highest LR+ (99.8), but a lower sensitivity (77.4%). An LR+
of 99.8 could be regarded as diagnostically very reliable, as values exceeding 10 are generally considered satisfactory [30, 31]. It should be noted that the exact cut-off values of these algorithms and profiles might not be directly transferable for use at other laboratories and clinical centers, due to differences in laboratory equipment, assays, routines, and patient cohorts. Also, as for any diagnostic test, the ultimate clinical usefulness of these biomarkers is influenced by the disease prevalence in the tested cohort.

There are several sets of diagnostic criteria for CJD in clinical settings; the most commonly used being those of WHO [World health 32], UCSF criteria [UCSF Memory and aging 33] and the European MRI-CJD Consortium [34]. These criteria all rely on clinical symptoms and presentation as a basis, and can be backed up by typical EEG findings or CSF analysis of 14-3-3 protein as confirmatory tests, or by MRI characteristics in the European MRI-CJD and UCSF criteria, but none of these sets of criteria include CSF levels of T-tau, or T-tau/P-tau-ratio. The validity and performance of these sets of diagnostic criteria, as well as of their constituent parts, has been examined in literature, and concerns have been raised about several of them. Periodic sharp wave complexes in EEG has a poor sensitivity (40-67%) but high specificity, is mainly positive in certain molecular subtypes and can be mimicked by other conditions [7, 10, 34-37]. CSF 14-3-3 protein detection in CSF was when discovered thought to be both highly specific and sensitive for CJD diagnosis [38, 39], but was later found to be expressed constitutively in both neurons and glial cells and released into the CSF in many other non-prionic conditions characterized by extensive neuronal loss such as stroke, multiple sclerosis and meningoencephalitis [14, 40], lowering its credibility as a disease specific marker. The high value of MRI as a diagnostic tool has relatively newly been demonstrated [11, 34, 41], but is sometimes lacking in sensitivity and might not be appropriate in late stage disease [9, 42], and defining MRI criteria has proved a challenging task [11]. Tau levels in CSF reflect various neurodegenerative processes and are usually very high in CJD [28, 29,
and the T-tau/P-tau ratios discriminates CJD from AD and frontotemporal dementia [23]. Furthermore, a CSF biomarker profile is an objective and easily applicable means of substantiating a clinical suspicion of CJD, as opposed to the more subjective nature of MRI and EEG evaluation. In this study, we showed that a CJD biomarker profile effectively distinguished CJD from many other conditions, specifically AD and other dementias. More studies directly comparing different diagnostic modalities for diagnosing CJD versus other dementias are needed.

The main strength of this study was the large size of the dataset used for analysis, which included complete records of registered causes and dates of death in concert with CSF measurements of T-tau and P-tau for all patients that had undergone a diagnostic LP with measurements of dementia markers in the Clinical Neurochemistry Laboratory of the Sahlgrenska University Hospital, serving most of Sweden, between 2002-2012 and later deceased. The diversity of the contrast group makes inclusion of many of the CJD biomarker profile-mimicking conditions highly probable.

There were several limitations to this study, the most important one being the lack of autopsy-confirmation in 44% of the CJD cases, since this is the only definitive way of correctly diagnosing CJD. However, in Sweden, reporting CJD cases to the Swedish Institute for Infectious Disease Control is mandatory, and the specific regulations of this report demands strong evidence for CJD diagnosis, probably minimizing the risk of false positive diagnoses in this material. Furthermore, since all diagnoses in our data were taken from the Swedish mortality registry, we could be treating CJD-positive cases as negatives if CJD was not listed as a cause of death. Again, this is unlikely since it is mandatory to follow through and report clinically suspected CJD cases in Sweden.
Conclusions and implications

CJD patients have elevated T-tau levels and T-tau/P-tau ratio, as compared to the other patients undergoing laboratory testing for dementia biomarkers, including patients diagnosed with Alzheimer’s disease.
Populärvetenskaplig sammanfattning

Creutzfeldt-Jakobs sjukdom (CJD) är en ovanlig, snabbt progredierande demenssjukdom som bryter ned det centrala nervsystemet och leder till döden inom ett par månader efter insjuknandet. Trots att CJD är obotlig är det mycket viktigt att tillförlitligt kunna ställa korrekt diagnos, då det finns behandlingsbara tillstånd som kan misstas för denna sjukdom. Dessvärre kan detta i många fall vara mycket svårt eftersom symptombilden kan variera kraftigt för olika patienter. I dagsläget är det enda säkra sättet att ställa diagnos att ta ett prov av patientens hjärnvävnad, vilket inte är genomförbart på en levande individ. I denna studie kunde vi visa att nivåerna av ett ämne (T-tau) i ryggvätskan är mycket kraftigt förhöjda hos patienter med CJD, samt att ett ryggvätskeprov med analys av en kombination av två ämnen (T-tau och P-tau) kan urskilja denna sjukdom från andra neurologiska sjukdomar med en mycket hög tillförlitlighet.

Proteinet tau är en komponent i nervcellernas intracellulära transportsystem som därför läcker ut i ryggvätskan vid nervcellsdöd. Förhöjda nivåer av tau kan mätas i tillstånd såsom huvudtrauma och stroke, men även vid t.ex. Alzheimers sjukdom och frontallobsdemens.


Vi använde testresultat från ryggvätskeprover, lagrade i en laboratoriedatabas, tagna i klinisk rutin och som analyserats på Sahlgrenska universitetssjukhuset. Denna information kopplades samman med det svenska mortalitetsregistret, varifrån vi hämtade information om dödsdatum och dödsorsaker för de avlidna patienterna i vårt material. Detta resulterade i en databas som
avändes för statistisk analys, och som innehåller information om ryggvätskeprover, dödsdatum och dödsorsaker för 9765 individer, varav 93 med CJD som dödsorsak.

Vi utvärderade sedan tidigare föreslagna CJD-gränsvärden för ryggvätskenivåer av ämnena T-tau och P-tau, som dock inte används i kliniken, och fann att dessa gränsvärden kunde urskilja CJD-patienterna i vårt material med mycket hög säkerhet. Av de 9765 proverna var det bara 115 som klassificerades felaktigt. Genom statistisk analys kunde vi dessutom förbättra dessa gränsvärden så att endast 96 patienter felklassificerades. Detta prov är enkelt att genomföra och att tolka och skulle kunna hjälpa den kliniskt verksamma läkaren att säkrare ställa diagnos vid misstånt CJD.

Vidare undersökte vi hur nivåer av dessa ämnen utvecklade sig under sjukdomsförloppet hos de patienter i vårt material som provtagits mer än en gång. Vi kunde då se hur nivåer av T-tau, men inte P-tau, ökade för nästan samtliga patienter när sjukdomen förvärrades. De högsta nivåerna sågs precis innan patientens bortgång. Denna information är viktig eftersom den visar att det vid kliniskt stark misstanke kan vara värt att ta om ett CJD-negativt första ryggvätskeprov, då det kan ha tagits i ett för tidigt skede av sjukdomen.
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Appendices

Appendix A.

The coefficients of variation (CVs) of the assays for T-tau and P-tau were determined by analysing records of measurements of internal control samples that are routinely carried out at the lab at Mölndal at least twice a week. Control samples are kept in frozen aliquots and reused until depleted and then exchanged. Standard deviations of the averages of all measurements from each control sample were calculated and an average of these standard deviations was used to represent a CV for every analysis.

For T-tau seven different low, and six different high controls were used. The three low controls measured standard deviations of 8.04%, 8.69%, 10.94%, 8.66%, 9.67%, 9.88% and 9.01%, resulting in a mean CV of 9.27%. The high controls measured standard deviations of 19.41%, 4.57%, 7.56%, 13.95%, 10.73% and 9.62%, resulting in a mean CV of 10.97%. The total average of the T-tau measurements was calculated to 10.35%.

For P-tau seven different low, and seven different high controls were used. The seven low controls measured standard deviations of 9.06%, 8.22%, 9.20%, 11.79%, 8.54%, 11.68% and 11.04%, giving a mean CV of 9.94%. The high controls measured standard deviations of 9.80%, 15.49%, 8.91%, 9.30%, 8.47%, 8.85% and 9.38%, giving a mean CV of 10.03%. The total average of the P-tau measurements was calculated to 10.19%.

No measureable longitudinal drift was registered for any of the analyses.