 Association of Cerebrospinal Fluid Prion Protein Levels and the Distinction Between Alzheimer Disease and Creutzfeldt-Jakob Disease

Aline Dorey, PhD; Yannick Tholance, MSc, PharmD; Alain Vighetto, MD; Armand Perret-Liaudet, PharmD; Ingolf Lachman, PhD; Pierre Krolak-Salmon, MD, PhD; Uta Wagner, PhD; Hanne Struyfs, MSc; Peter P. De Deyn, MD, PhD; Benaissa El-Moualij, PhD; Willy Zorzi, PhD; David Meyronet, MD, PhD; Nathalie Streichenberger, MD; Sebastiaan Engelborghs, MD, PhD; Gabor G. Kovacs, MD, PhD; Isabelle Quadrio, PharmD, PhD

**IMPORTANCE** Although typical forms of Alzheimer disease (AD) and Creutzfeldt-Jakob disease (CJD) are clinically distinguishable, atypical AD phenotypes may pose a diagnostic challenge. The major biological diagnostic biomarker for identifying CJD, 14-3-3 protein in cerebrospinal fluid (CSF), unfortunately lacks specificity when confronting a rapid dementia presentation.

**OBJECTIVE** To assess the relevance of total CSF prion protein (t-PrP) levels in the differential biological diagnosis between atypical AD phenotypes and CJD.

**DESIGN, SETTING, AND PARTICIPANTS** A retrospective study in an autopsy-confirmed cohort of 82 patients was performed to evaluate the relevance of CSF t-PrP to distinguish 30 definite cases of AD from 52 definite cases of CJD. Next, CSF t-PrP concentration was measured in a cohort of 104 patients including 55 patients with probable AD, 26 with probable sporadic CJD, and 23 control patients for whom 14-3-3 protein, total tau, phosphorylated tau 181 (P-tau181), and Aβ1-42 were available. We investigated 46 patients diagnosed as having probable AD who presented atypical phenotypes. A diagnosis strategy was proposed to classify atypical AD phenotypes with suspicion of CJD based on a decision tree combining CSF biomarkers.

**MAIN OUTCOMES AND MEASURES** We determined CSF t-PrP levels for all patients. We calculated the ratio of total tau and P-tau181 and determined the diagnostic accuracy of each biomarker alone or in combination. We calculated the misclassification rate for each biomarker that corresponded to the percentage of patients within the group of atypical AD phenotypes wrongly classified as CJD.

**RESULTS** In patients with CJD, CSF t-PrP concentrations were decreased compared with control participants and patients with AD. When considering the differential diagnosis of CJD compared with atypical AD phenotypes, CSF t-PrP determination reached 82.1% sensitivity and 91.3% specificity. The misclassification rate of atypical AD phenotypes decreased from 43.5%, obtained when using the CSF 14-3-3 protein determination alone, to only 4.3% when calculating the ratio total tau/(P-tau181 × t-PrP). The proposed classification tree permitted correct classification of 98.4% of the patients.

**CONCLUSIONS AND RELEVANCE** For unusual phenotypes of AD, especially cases presenting with a biological ambiguity suggesting CJD, determination of CSF t-PrP levels increased diagnostic accuracy. The use of CSF t-PrP levels may be beneficial in clinical practice in addition to the current classic biomarkers.

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Alzheimer disease (AD) is the most common cause of dementia. In typical AD characterized by progressive memory loss, the diagnosis can be performed with good reliability because of its clinical and neuropathological profile as well as neuroimaging. In atypical AD, including either presentation with focal symptoms unrelated to mesial temporal lobe dysfunction, coexistence of motor signs, or rapid evolution of cognitive decline, the diagnosis is made using AD cerebrospinal fluid (CSF) biomarkers. However, these atypical AD phenotypes can present with high levels of total tau (T-tau) protein and/or positive 14-3-3 protein (p14-3-3) in the CSF, reflecting intense neuronal degeneration similar to what is found in Creutzfeldt-Jakob disease (CJD). Therefore, CSF T-tau and p14-3-3 may not be suitable to provide an in vivo differential diagnosis when the clinical phenotype is atypical. Diagnosing these atypical cases remains challenging and raises suspicion of CJD. Ideally, an additional biomarker more closely related to the pathological process would be helpful in these cases. This study addresses relevance of determining the total prion protein (T-PrP) level in CSF using a commercially available enzyme-linked immunosorbert assay to discriminate in vivo AD from CJD, especially when AD presents atypically.

Methods

Study Population

We included the following 232 patients: 82 patients with dementia with autopsy-confirmed pathological diagnoses of AD (30 cases) and CJD (52 cases), 101 patients with probable AD and 26 patients with probable CJD, and 23 patients without dementia as control participants.

For definite diagnosis of AD, the neuropathological criteria of Braak and Braak and Jellinger were applied as previously reported. Definite diagnosis of CJD was based on a brain examination according to World Health Organization criteria except in 7 cases in whom definite genetic CJD was diagnosed owing to the presence of one of the pathogenic genetic mutations in symptomatic patients without postmortem confirmation. The Western blot analysis of brain pathological prion protein was determined using a standard Western blot technique. Genetic analysis of the PRNP gene was performed as previously published once we received written informed consent from the patient or a relative.

Probable AD diagnosis was based on medical history, caregiver interview, neurological examination, neuropsychological battery, structural brain imaging, and a biochemical CSF AD signature. Patients with AD had dementia according to DSM-IV-TR criteria and were diagnosed as having probable AD dementia with evidence of the AD pathophysiological process. In addition, no other diagnosis emerged in these cases in a follow-up period of at least 2 years after CSF analysis.

Among these 101 patients, 55 had a typical clinical presentation and evolution, mainly with predominant memory impairment (typical probable AD) while 46 showed atypical features or evolution (atypical probable AD; atypical AD). Atypical AD was defined as patients with AD with rapid cognitive decline or patients with additional motor signs, such as myoclonus or parkinsonism, independent of cognitive impairment at onset (eTable in the Supplement). At the time of the lumbar puncture, these atypical presentations led clinicians to raise suspicion of CJD and prescribe a CSF p14-3-3 analysis. Probable CJD diagnosis was established according to the updated criteria of the World Health Organization by the European CJD consortium and included presence of rapidly progressive dementia and at least 2 of the following: myoclonus, visual, cerebellar signs, pyramidal or extrapyramidal signs, or a kinetic mutism; at least 1 of the following: periodic sharp-wave complexes on electroencephalography, positive p14-3-3 on CSF, hyperintensities in diffusion-weighted imaging, or fluid-attenuated inversion recovery magnetic resonance images at the level of the caudate and putamen and/or at least 2 cortical regions (temporal, parietal, or occipital).

The age-matched control group included 23 patients without dementia with presenting psychiatric disorders and cognitive complaints, which were unrelated to either AD or to another neurodegenerative brain disease based on the absence of clinical and imaging evidence for an organic brain disease, the absence of progression during the 2-year follow-up, and a normal CSF AD biomarker profile.

Approval was obtained from the local ethics committees at CPP-B Lyon and CME Middelheim. Written informed consent to participate in this study was obtained from all the patients and/or their relatives.

CSF Analysis

All individuals underwent a lumbar puncture to collect CSF using a standard procedure. Cerebrospinal fluid was stored at −80°C until analysis. Cerebrospinal fluid biomarker analyses were performed blind to the clinical diagnosis in the Memory Clinic at the Reference Center for Biological Markers of Dementia at the University of Antwerp concerning samples from patients with autopsy-confirmed AD and in the Neurochemistry Unit, Lyon 1 University, Hospices Civils de Lyon for all other samples. Both laboratories participated in the Alzheimer Association Quality Control program and the national quality control assessment of French laboratories organized by the French National Network of CJD Surveillance.

Western blot immunoassay for p14-3-3 was routinely performed as previously reported. Positive and negative CSF control samples and CSF control samples with trace test results were used in all experiments as internal quality controls.

Cerebrospinal fluid levels of T-tau, phosphorylated tau 181 (P-tau181), and β-amyloid 1-42 (Aβ1-42) were determined using commercially available enzyme-linked immunosorbtent assay kits (INNOTEST htau-Ag, INNOTEST phosphorylated-Tau181, and INNOTEST Aβ42; Innogenetics/Fujirebio Europe) according to the manufacturer’s instructions.

Cerebrospinal fluid T-PrP levels were determined in the Neurochemistry Unit, Lyon 1 University, Hospices Civils de Lyon using commercial BetaPrion Human Enzyme-Linked Immunoassay Test kits (AJ Roboscreen; more details in the eAppendix in the Supplement). The antibodies used in the kits did not distinguish the pathological from the normal form of PrP. Because we did not introduce proteinase k treatment, we measured the levels of total PrP.
Study Design
First, to establish the relevance of CSF t-PrP determination, we compared findings from both the autopsy-confirmed AD and CJD groups. Similarly, we investigated a cohort of patients diagnosed as having probable AD and presenting a typical phenotype and compared them with patients with probable CJD and with control participants. Because values were found to be similar in definite and typical probable AD as well as in definite and probable CJD, we pooled data to form 2 groups, a typical AD group and a CJD group. This allowed us to determine cutoff values that distinguished AD from CJD. Then, CSF biomarker ratios were generated from the different biomarkers analyzed and cutoff values defined in the last step were applied to the atypical probable AD group, for whom clinicians had raised suspicion of CJD at the time of the lumbar puncture. In addition, we proposed a diagnosis strategy to classify atypical clinical AD presentation with suspicion of CJD based on a decision tree that combined the available CSF biomarkers.

Statistical Analyses
Regarding the statistical analyses, we used MedCalc version 11.3.1.0 (http://www.medcalc.be). Receiver operating characteristic analysis was used to assess the ability of the individual biochemical variables to discriminate the CJD group from other diagnostic groups. Differences were considered statistically significant at P < .05.

A CHAID (χ² Automatic Interaction Detector) model was used to build the decision tree with SPSS Statistics (version 17.0; SPSS Inc). This classification method, which works for any type of predictors, identified optimal splits using χ² analysis. The targeted outcome was CJD diagnosis to identify and rank the predictor variables.

Results
Demographic, Clinical, and CSF Data Analysis
Diagnostic groups’ corresponding CSF data are summarized in Table 1. Some differences on sex, Mini-Mental State Examination, and codon 129 polymorphism were observed between the AD and CJD groups. Patients with definite AD were significantly older than control participants and patients with definite CJD. Patients presenting with atypical AD were younger than those presenting with typical AD (including typical probable AD and definite AD), but were comparable in age to patients with CJD. All patients showing typical or atypical AD were still alive after 2 years except 1 patient who died 9 months after being diagnosed as having AD. The time between the first complaint and CSF analysis was shorter in the CJD population than in the AD and control populations.

CSF Biomarker Values in Definite and Probable AD and CJD Groups
As displayed in Figure 1, t-PrP levels were significantly higher in definite AD than in definite CJD. The same result was found in typical probable AD when compared with probable CJD. No difference was found between typical probable AD and definite AD (P = .11). We found a significant difference between probable and definite CJD because t-PrP values were lower in definite than in probable CJD. However, because both groups showed significantly lower values than in both AD groups, we pooled the data to constitute a larger group of CJD. The comparison of the typical AD groups (including typical probable AD and definite AD) with CJD groups yielded a significant difference in t-PrP levels. The populations of CJD and typical AD were then used to determine t-PrP cutoff values. The determination of T-PrP was used to discriminate CJD from typical AD with 82.1% sensitivity and 82.4% specificity, applying a cutoff value of 263 µg/L. The control population showed significantly lower t-PrP levels than the typical AD group and significantly higher values than CJD groups.

No p14-3-3 result was available for patients with definite AD. In the control population, p14-3-3 was negative in 87% of cases and traces were detected in 13%. Sensitivity and specificity for p14-3-3 to discriminate CJD from typical AD were 96.2% and 76.4%, respectively, when traces were considered as positive results, and specificity culminated at 100% when traces were considered as negative results.

As shown in receiver operating characteristic curve comparison analyses (Figure 2), T-tau and P-tau181 levels distinguished typical AD from CJD groups. The optimal cutoff value of T-tau was 1128 ng/L, with 91.3% sensitivity and 92.9% specificity. A value of P-tau181 lower or equal to 63 ng/L distinguished CJD from typical AD, with 69.0% sensitivity and 82.4% specificity. A T-tau:P-tau181 value higher than 13.2 yielded 94.2% sensitivity and 98.8% specificity.

CSF Biomarkers in Atypical Probable AD Compared With CJD and Typical AD Groups
As shown in Figure 1, t-PrP was significantly higher in atypical AD when compared with CJD and typical AD. The cutoff value was 263 µg/L for t-PrP–distinguished CJD and atypical AD, with 82.1% sensitivity and 91.3% specificity. In atypical AD, p14-3-3 test results were positive in 15.2% of cases and negative in 56.5%, and there were traces in 28.3% of cases. To differentiate the atypical AD population from the CJD population, the sensitivity and specificity performance of p14-3-3 varied, respectively, from 95.2% sensitivity and 56.5% specificity to 87.2% sensitivity and 84.8% specificity, depending on whether traces were considered positive or negative. The level of T-tau and the ratio of T-tau to P-tau181 in atypical AD were significantly higher than in typical AD but significantly lower than in CJD. Levels of P-tau181 were significantly higher in atypical AD than in patients with typical AD and in patients with CJD. For levels of Aβ1-42 and the ratio of Aβ1-42 to P-tau181, significant differences were also observed, with lower values in atypical AD than in typical AD and CJD. The T-tau to t-PrP ratio was significantly higher in atypical AD than in typical AD and significantly lower than in CJD. A CJ factor was defined as T-tau/(P-tau181× t-PrP). This ratio was higher in CJD than in AD and a value higher than 0.054 permitted differentiation of typical AD from CJD, with 95.7% sensitivity and 97.7% specificity. The CJ factor was significantly lower in atypical AD than in CJD. The CJ factor cutoff value at 0.054 differentiated CJD and atypical AD, with 100% sensitivity and 95.7% specificity.
Table 1. Demographic and Pathological Parameters of Study Populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AD (n = 111)</th>
<th>Typical AD (n = 85)</th>
<th>Definite AD (n = 30)</th>
<th>Atypical AD (n = 55)</th>
<th>All (n = 78)</th>
<th>Definite CJD (n = 52)</th>
<th>Probable CJD (n = 26)</th>
<th>Control Participants (n = 23)</th>
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<td>78 (100)</td>
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<td>26 (100)</td>
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<td>42 (76)</td>
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<td>768 (389-1114)b</td>
<td>525 (352-1218)b</td>
<td>72 (58-152)b</td>
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<td>75 (59-152)</td>
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<td>No. (%)</td>
<td>85 (100)</td>
<td>30 (100)</td>
<td>55 (100)</td>
<td>46 (100)</td>
<td>69 (88)</td>
<td>43 (83)</td>
<td>26 (100)</td>
<td>22 (96)</td>
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<td>515 (342-713)c</td>
<td>574 (470-802)c,b,c</td>
<td>1304 (1078-1667)c</td>
<td>4192 (2762-6607)c</td>
<td>3743 (2767-6228)c</td>
<td>5110 (2762-10150)c</td>
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<td>No. (%)</td>
<td>85 (100)</td>
<td>30 (100)</td>
<td>55 (100)</td>
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<td>69 (88)</td>
<td>43 (83)</td>
<td>26 (100)</td>
<td>22 (96)</td>
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<tr>
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<td>319 (216-576)c</td>
<td>463 (343-603)c,b,c</td>
<td>501 (337-629)c,b,e,f</td>
<td>154 (60-255)c</td>
<td>98 (52-196)c</td>
<td>249 (153-287)c</td>
<td>303 (214-401)</td>
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<td>T-tau/T-PrP</td>
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<tr>
<td>No. (%)</td>
<td>85 (100)</td>
<td>30 (100)</td>
<td>55 (100)</td>
<td>46 (100)</td>
<td>69 (88)</td>
<td>43 (83)</td>
<td>26 (100)</td>
<td>22 (96)</td>
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<tr>
<td>Median (IQR), (0.98-1.84)c,b,c</td>
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<td>1.36 (1.00-2.44)c</td>
<td>1.43 (0.98-1.81)c,b,c</td>
<td>2.59 (1.86-3.45)c,b,e,f</td>
<td>33 (15.05-81.24)c</td>
<td>37.41 (15.05-44.21)c</td>
<td>29.39 (15.05-44.21)c</td>
<td>0.52 (0.39-0.62)</td>
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<td>P-tau181</td>
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<tr>
<td>No. (%)</td>
<td>85 (100)</td>
<td>30 (100)</td>
<td>55 (100)</td>
<td>46 (100)</td>
<td>71 (91)</td>
<td>45 (87)</td>
<td>26 (100)</td>
<td>23 (100)</td>
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<tr>
<td>Median (IQR), (67-109)c,b,c</td>
<td>86 (67-109)c,b,c</td>
<td>65 (56-104)c</td>
<td>93 (73-110)c,b,c</td>
<td>145 (105-183)c,b,e,f</td>
<td>52 (39-69)c</td>
<td>52 (37-68)c</td>
<td>59 (42-72)c</td>
<td>35 (25-43)</td>
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<tr>
<td>T-tauP-tau181</td>
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<tr>
<td>No. (%)</td>
<td>85 (100)</td>
<td>30 (100)</td>
<td>55 (100)</td>
<td>46 (100)</td>
<td>69 (88)</td>
<td>43 (83)</td>
<td>26 (100)</td>
<td>22 (96)</td>
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<tr>
<td>Median (IQR), (5.8-8.0)c,b,c</td>
<td>6.8 (5.6-5.8)c</td>
<td>6.8 (5.6-5.8)c</td>
<td>6.9 (5.8-7.9)c,b,c</td>
<td>8.3 (7.3-10.7)c,b,e,f</td>
<td>85.7 (47.8-143.4)c</td>
<td>81.0 (37.9-138.9)</td>
<td>96.9 (53.8-176.5)c</td>
<td>4.6 (4.3-6.1)</td>
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<td>Creutzfeldt-Jakob factor</td>
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<td>55 (100)</td>
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<td>69 (88)</td>
<td>43 (83)</td>
<td>26 (100)</td>
<td>22 (96)</td>
</tr>
<tr>
<td>Median (IQR), (0.012-0.024)c,b,c</td>
<td>0.016 (0.014-0.017)c,b,c</td>
<td>0.021 (0.014-0.017)c,b,c</td>
<td>0.014 (0.011-0.021)c,b,c</td>
<td>0.018 (0.012-0.029)c,b,c,</td>
<td>0.780 (0.304-1.305)c,b,e,f</td>
<td>0.855 (0.321-0.541)c,b,e,f</td>
<td>0.590 (0.228-1.053)c,b,e,f</td>
<td>0.016 (0.011-0.025)</td>
</tr>
</tbody>
</table>
Table 2 presents the misclassification rates of patients with atypical AD when using each relevant biomarker for CJD diagnosis. Patients with atypical AD were misclassified in 43.5% of cases when considering p14-3-3 results. They were misclassified in 65.2% of cases when taking into account T-tau. Misclassification rate was only 8.7% for t-PrP, 6.5% for T-tau:t-PrP ratio, and 4.3% for CJ factor.

CHAID Classification Tree

A CHAID classification tree was constructed with p14-3-3 as the required first variable to mimic actual clinical investigation in our population with possible CJD (Figure 3). Sex, age, Mini-Mental State Examination score, the period between the first complaint and lumbar puncture, proteinorachia, Aβ1-42, t-PrP, and the Aβ1-42:P-tau181 ratio were not retained, suggest-
Regulation of neuronal PrPC expression has been reported in 0.964 (T-tau), 0.886 (t-PrP), and 0.803 (P-tau 181). The association of increased CSF t-PrP levels in the AD condition.  

In CJD-AD32 as well as in inflammation or hypoxic/ischemic circumstances. Thus, a rapid evolution of AD is more likely associated with active neuronal stress and with upregulated neuronal PrP\(^{\circ}\) also in CSF, which may explain our observation of increased CSF t-PrP levels in the AD condition. In CJD-infected brains, PrP\(^{\circ}\) is converted into scrapie PrP, generating both soluble oligomers of scrapie PrP and deposition of insoluble scrapie PrP. The CSF t-PrP level decrease could be explained by this conversion. We assumed that the decrease of t-PrP in CSF could be linked to its trapping in aggregates similarly to how the decrease of A\(\beta\)1-42 in CSF of patients with AD was explained by its trapping into amyloid plaques.  

Our results contrasted with those reported in a prior study related to this topic published by Meyne et al.,\(^{35}\) who reported a decrease in t-PrP levels in neurodegenerative diseases without differentiating between the neurodegenerative conditions tested (ie, CJD, AD, dementia with Lewy bodies, and Parkinson disease as compared with control participants). We suggest this discrepancy might lie in the difference of specificity between our enzyme-linked immunosorbent assay and the one used by these authors developed for bovine PrP. Antibodies have been developed and produced against recombinant human PrP and showed a better reactivity with human than with bovine PrP levels.  

Regarding the AD CSF biomarkers, A\(\beta\)1-42 alone had a limited distinguishing capability in the context of CJD distinction. This could be partially explained by the significant association of amyloid deposition with t-PrP deposits as reported in brain examination in sporadic as well as genetic CJD.\(^{36}\) T-tau has been proposed as a candidate marker for neuronal degeneration occurring in CJD and AD.\(^{16,37-41}\) Our data are consistent with the literature because we found a cutoff value for T-tau at 1128 ng/L with less sensitivity than p14-3-3 but higher specificity for diagnostic classification.\(^{37-41}\) In CJD, T-tau levels were much higher than in AD owing to the kinetics of neuronal degeneration; levels of P-tau\(_{181}\) reflecting the formation of neurofibrillary tangles were lower than in AD. This explains the high interest of considering the ratio between T-tau and P-tau\(_{181}\) to differentiate CJD from other dementias, particularly AD.\(^{42,43}\) In our study, findings were consistent with the literature because significantly higher T-tau:P-tau\(_{181}\) ratios were observed in CJD than in AD.  

As a complementary diagnostic biomarker, determining the levels of CSF t-PrP were very helpful in classifying both types of patients at risk of misdiagnosis as shown in the decision tree (Figure 3). The p14-3-3 test results were first retained in the tree,
either the T-tau or T-tau:P-tau181 ratio very significantly en-
ration. Conversely, combining the CSF t-PrP determination with
in our study, these 3 patients presented with a long disease du-
severe neuronal damage in the atypical presentations of AD,
ber of cases presenting positive or uncertain test results for the
ing negative p14-3-3 test results, which were later confirmed
value for CJD diagnosis in the 3 atypical cases of CJD regard-
ing the disease. An underlying process inducing particularly
cific of CJD but reflects the neuronal damage that occurs dur-
ing the disease. An underlying process inducing particularly
severe neuronal damage in the atypical presentations of AD, especially in rapid processing, may explain the significant
numbers of cases presenting positive or uncertain test results for the
CSF detection of p14-3-3 (15% and 28% of cases in our series, respectively), a finding consistent with prior studies.9,10,13,37
In our study, the determination of CSF t-PrP levels decreased the misclassification rate of patients with atypical AD without decreasing the rate of accurate classification of CJD (Table 2). The best use of biomarkers to classify patients with atypical AD was achieved using the CJ factor, with a misclassication rate of only 4.3%. A larger study combining CSF t-PrP determination with other diagnostic criteria, such as magnetic resonance imaging and electroencephalography, should be conducted in the future for several neurological disorders mimicking CJD, particularly for the ones that are treatable. Pending the development of a disease-specific routine test for CJD diagnosis,48-50 this easy enzyme-linked immunoassay test determining total PrP concentration implementable in routine diagnosis laboratories appears to be a relevant diagnostic tool.

Conclusions

In this study, we provided evidence to introduce titration of t-PrP in CSF as a new biomarker to help clinicians exclude CJD in the setting of AD when suspicion of CJD increases owing to unusual presentation, evolution, and/or biological profile ambiguity. We addressed the case of atypical AD because AD is a frequent condition and its diagnosis can now reliably be made in vivo using CSF AD biomarkers. However, in clinical practice, clinicians may face degenerative dementias with atypical features or rapid cognitive decline, for which CJD remains in the differential diagnosis. In these cases, we infer from our study that t-PrP determination in the CSF would be helpful to exclude CJD as a misdiagnosis, especially when currently available nonspecific biomarkers, such as p14-3-3 and T-tau, do not contribute much.
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Bron Cxe, France. (Perret-Liaudet, Quadrio); AJ Roboscreen GmbH, Leipzig, Germany (Lachman, Wagner); Institute of Biological Markers of Demencia, Laboratory for Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium (Struyfs, Engelborghs); Biobank and Laboratory of Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium (De Deyn); Laboratory of Human Histology-CHARP, Institute of Pharmacy-ChU, Liège, Belgium (El-Moualij, Zorzi); Hopsices Civis de Lyon, Groupement Hospitalier Est, Department of Biochemistry, Neurochemistry Unit, Lyon, France; Maison Ménard-Ruiz, MSc, and Bernard Renaud, PhD, for their technical support. We are grateful to Jean-Jacques Martin, PhD, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium, for the neuropathology of the Antwerp patients with AD included in this study. None of the individuals received compensation for their contributions with the exception of Marion Menard-Ruiz for her translation service.

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