



# Cerebrospinal fluid non-phosphorylated tau in the differential diagnosis of Creutzfeldt–Jakob disease: a comparative prospective study with 14-3-3

Franç Llorens<sup>1,2,3</sup> · Anna Villar-Piqué<sup>1,2</sup> · Peter Hermann<sup>2</sup> · Matthias Schmitz<sup>2,4</sup> · Stefan Goebel<sup>2</sup> · Katharina Waniek<sup>5</sup> · Ingolf Lachmann<sup>5</sup> · Inga Zerr<sup>2,4</sup>

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## Abstract

Cerebrospinal fluid (CSF) non-phosphorylated tau (non-p-tau) is increased in sporadic Creutzfeldt–Jakob disease (CJD), but its accuracy in the differential diagnosis has not been previously established. Here, we first used a retrospective cohort of non-CJD ( $n = 135$ ) and CJD ( $n = 137$ ) cases to determine the optimal cutoff point for the discrimination of CJD cases. Next, we prospectively quantified non-p-tau and 14-3-3 protein in a cohort of 1427 cases received for CSF testing at the German National Reference Center for transmissible spongiform encephalopathies. Among them, 36 were subsequently diagnosed as CJD. The diagnostic accuracy of both proteins discriminating CJD cases was evaluated. Using a cutoff of 650 pg/mL, non-p-tau displayed 94.39% accuracy in discriminating CJD cases, while 92.92% accuracy was achieved by 14-3-3 using a cutoff of 20,000 AU/mL. Diagnostic test evaluation for both proteins showed a slightly better performance of non-p-tau compared to 14-3-3. The two biomarkers' concentrations showed a significant positive correlation, both in the total population and in CJD cases ( $p < 0.001$ ). Finally, the analysis of CSF non-p-tau concentrations when undergoing pre-analytical factors showed high stability in front of temperature storage and freeze/thaw cycles. Therefore, we conclude that when used in the appropriate clinical context of a prion disease surveillance center, non-p-tau is a highly sensitive and specific diagnostic marker for CJD.

**Keywords** Cerebrospinal fluid · Creutzfeldt–Jakob disease · Tau · Non-phosphorylated tau · 14-3-3 · RT-QuIC · Biomarker

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Franç Llorens and Anna Villar-Piqué equally contributed to this paper.

✉ Franc Llorens  
franc.llorens@gmail.com

✉ Anna Villar-Piqué  
avillar@gwdg.de

<sup>1</sup> Network Center for Biomedical Research in Neurodegenerative Diseases, (CIBERNED), Institute Carlos III, L'Hospitalet de Llobregat, Barcelona, Spain

<sup>2</sup> Department of Neurology, Clinical Dementia Center and National Reference Center for CJD Surveillance, University Medical School, Göttingen, Germany

<sup>3</sup> Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Spain

<sup>4</sup> German Center for Neurodegenerative Diseases (DZNE), Göttingen, Germany

<sup>5</sup> AJ Roboscreen GmbH, Leipzig, Germany

## Introduction

Sporadic Creutzfeldt–Jakob disease (CJD) is a rapidly progressive, invariably fatal neurodegenerative disorder caused by the abnormal isoform of the prion protein (PrP). CJD occurs worldwide with an estimated annual incidence of 1–2 cases per million person-years with typical onset of symptoms occurring at about the age of 65 [1]. CJD diagnosis is based on clinical symptoms, neuroimaging, electroencephalogram and cerebrospinal fluid (CSF) tests [2, 3]. However, definite diagnosis can only be achieved by post-mortem examination. Among CSF tests, detection of 14-3-3 protein [4, 5], total-tau quantification [5] and the real-time quaking-induced conversion (RT-QuIC) assay [3], are considered as the gold standards in the diagnosis of CJD.

Several new CSF biomarkers for prion diseases have been proposed in the recent years presenting high accuracies in distinguishing CJD cases [6, 7], especially in the context of the differential diagnosis of neurodegenerative dementias [2, 8–10]. However, their precise performance has not been

scrutinized in the setting of a CJD surveillance center using a large prospective cohort. In this regard, the differential diagnosis includes not only neurodegenerative dementias such as Alzheimer disease (AD) and Dementia with Lewy bodies (DLB) but a variety of alternative conditions such as vascular, autoimmune, paraneoplastic, infectious, demyelinating and toxic-metabolic disorders [11–13].

In a previous study, we detected highly increased non-phosphorylated tau (non-p-tau) CSF concentrations in CJD compared to AD and neurological controls (non-demented). We observed that at the cutoff of 637 pg/mL, non-p-tau reached sensitivity of 98% and specificity of 100% discriminating CJD from AD cases, significantly improving the proportion of correctly classified patients compared to that achieved by CSF total-tau, phospho-tau and 14-3-3 proteins [10]. However, we did not investigate the diagnostic accuracy of non-p-tau in the context of a CJD surveillance system.

In the present study, we evaluated the performance of CSF non-p-tau in the diagnosis of CJD cases in comparison to 14-3-3. To achieve this goal, we quantified CSF non-p-tau and 14-3-3 using two well-established ELISA assays in a large cohort of cases received for CSF testing at the German National Reference Center for CJD Surveillance.

## Material and methods

### Patients and CSF sampling

The study included 1699 CSF samples from patients with neuropsychiatric disorders. The majority of the cases had clinical suspicion of CJD and CSF samples were received for testing at the German National Reference Center for CJD Surveillance, University Medical School, Göttingen, Germany. Among the total amount of cases, 272 were retrospectively collected and used to define the cutoff for CSF non-p-tau in the discrimination of CJD cases and 1427 were prospectively collected for the assessment of non-p-tau accuracy in the diagnosis of CJD. Among the 137 CJD cases of the retrospective cohort, 54 were definite (neuropathologically confirmed) and 83 probable CJD according to established diagnostic criteria [14]. Among the 36 cases from the prospective cohort diagnosed as CJD, 5 were definite and 31 probable CJD.

### CSF analyses

Non-p-tau (capturing molecules unphosphorylated at Thr-175 and Thr-181 based on antibody 1G2 directed to KTPP motif [15]) (non-pTAU ELISA—AJ Roboscreen, Leipzig, Germany), and 14-3-3 (CircuLex 14-3-3γ ELISA Kit—MBL International) concentrations were analyzed using

commercially available ELISA kits according to the manufacturer's instructions and as described before [4, 15]. For the non-p-tau assay, intra-coefficient of variation (CV) was 7% and inter CV was 10%. For the 14-3-3 assay, intra-CV was 6% and inter-CV was 10%. RT-QuIC was performed as described before [16].

To evaluate the stability of non-p-tau in CSF under pre-analytical conditions, CSF samples from 5 randomly selected cases were stored in polypropylene tubes at room temperature (RT) and 4 °C for 1, 2, 3, 5 and 10 days. In addition, CSF samples were subjected up to five repeated freeze/thaw cycles and up to five tube transfers. Non-p-tau concentrations were quantified as described above and data were normalized at each time point, cycle or transfer as percentage of control (time point zero), which was defined as 100%.

### Statistical analysis

According to distributional features, Mann–Whitney *U* test was used to compare non-p-tau concentrations between non-CJD and CJD cases. To assess the diagnostic accuracy of non-p-tau and 14-3-3 in the discrimination of non-CJD from CJD groups, receiver operating characteristic (ROC) curve analyses were carried out and areas under the curve (AUC) with 95% confidence intervals (95% CI) were calculated using GraphPad-Prism 6.01. The best cutoff values were estimated based on the Youden index. Spearman rank correlation coefficients were used to assess associations between continuous biomarker levels. Comparison between AUC was performed using the DeLong's test [17], available in the R package pROC [18]. McNemar tests were used to compare sensitivities and specificities and generalised score statistics were used to compare predictive values; both analyses were conducted with the R package DTComPair [19]. For the evaluation of non-p-tau stability on different pre-analytical conditions, data were assessed for normality and repeated-measures ANOVA followed by Bonferroni post-hoc analysis was applied.

## Results

### Determination of CSF non-p-tau cutoff in the discrimination of CJD

In the present work, we quantified non-p-tau concentrations in 137 retrospectively collected CSF cases diagnosed with probable or definite CJD and in 135 cases diagnosed with neurological diseases suspicious of dementia/CJD where later on CJD diagnosis was excluded (non-CJD).

Non-p-tau was associated neither with age ( $p=0.40$ ) nor with sex of the patients ( $p=0.83$ ) in the whole population. Mean non-p-tau concentrations in CJD cases (4145 pg/

mL, 95% CI 3323–4947 pg/mL) were significantly higher than in non-CJD cases (110 pg/mL, 95% CI 84–136 pg/mL,  $p < 0.001$ ) (Fig. 1a). AUC was 0.9946 (95% CI 0.98–1) indicating a high value of non-p-tau distinguishing between both diagnostic groups (Fig. 1b). Based on Youden index, at the cutoff of 650 pg/mL, non-p-tau reached sensitivity of 95% and specificity of 99% discriminating CJD from non-CJD cases (Fig. 1b). When only definite CJD cases were used, mean non-p-tau value (3966 pg/mL) was not significantly different than that achieved when definite and probable cases were analysed together (4145 pg/mL,  $p = 0.82$ ), so the discriminatory value was not altered.

### Comparative analysis of non-p-tau and 14-3-3 in suspected dementia cases

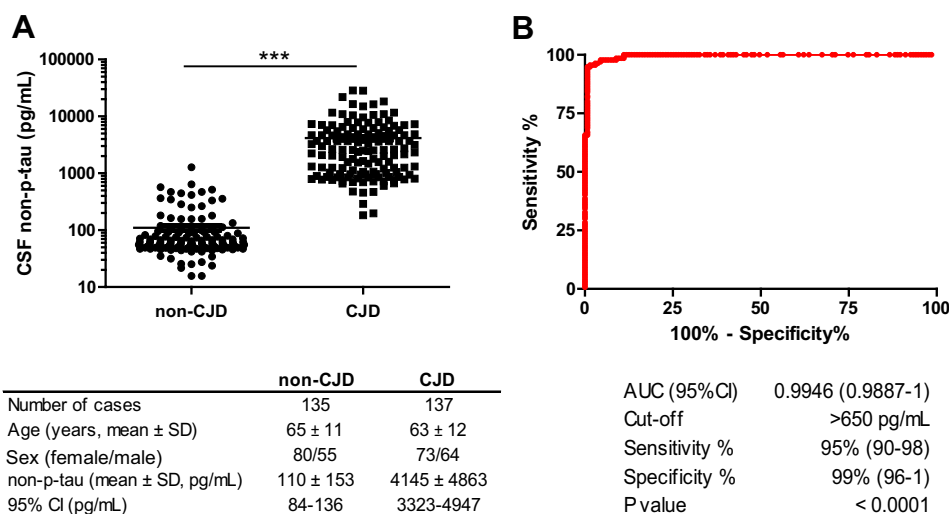
Next we quantified non-p-tau and 14-3-3 protein in 1427 prospectively collected CSF samples. For non-p-tau, 112 samples were over the CJD cutoff (>650 pg/mL), representing 7.8% of the total cases (Fig. 2a). Using a previously established CJD cutoff of >20,000 AU/mL [4], 14-3-3 concentrations were over the CJD cutoff in 129 cases, representing 9.0% of the total cases (Fig. 2b). 78 cases were over non-p-tau and 14-3-3 cutoffs for CJD, representing the 47.9% of the total positive cases, while 34 cases (20.9%) were positive exclusively for non-p-tau and 51 cases (31.3%) were positive only for 14-3-3 (Fig. 2c).

A strong positive correlation between non-p-tau and 14-3-3 concentrations was observed in the total study population ( $r = 0.64$ ,  $p < 0.001$ ) (Fig. 2d).

### Accuracy of non-p-tau and 14-3-3 in the discrimination of CJD cases

Among the 1427 prospectively quantified cases, 36 were classified as probable or definite CJD (2.5%) according to established diagnostic criteria [14].

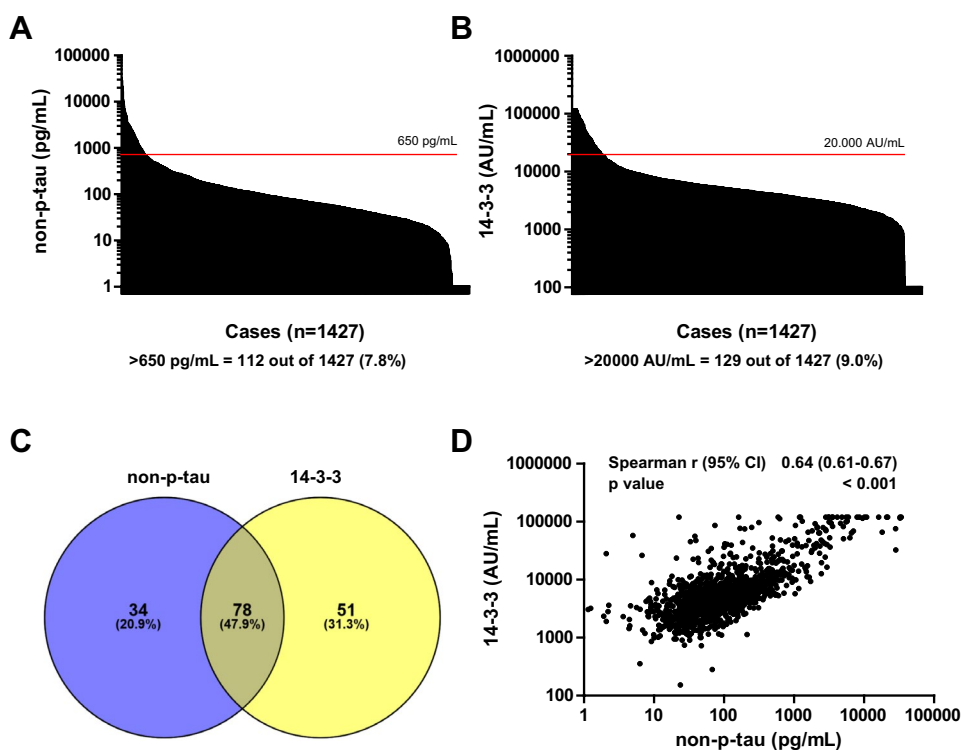
Non-p-tau and 14-3-3 discriminated with high accuracy CJD from non-CJD cases, with AUCs of 0.9674 (95% CI 0.9502–0.9847) and 0.9665 (95% CI 0.9545–0.9784), respectively. AUC values were not significantly different between both biomarkers ( $p = 0.9017$ ). Using previously established cutoffs, evaluation metrics were calculated for diagnostic performance of both tests (Tables 1, 2). Overall accuracy was higher for non-p-tau (94.39%) than for 14-3-3 (92.92%). Non-p-tau correctly identified 34 out of 36 CJD cases and 14-3-3 identified 32 out of 36 CJD cases, rendering sensitivities of 94.44% and 88.89%, respectively (Table 2, Fig. 3a–c). The number of false positive results was 78 with non-p-tau and 97 with 14-3-3, rendering specificities of 94.39% and 93.03%, respectively (Table 2, Fig. 3a, b). McNemar tests were applied in the subgroups of CJD and non-CJD cases to compare sensitivities and specificities of both biomarkers, respectively. Significant difference was found in specificities ( $p = 0.0348$ ) but not in sensitivities ( $p = 0.3173$ ). Given the class imbalance intrinsic to the detection of CJD-positive samples, positive predictive value (PPV) and negative predictive value (NPV) were also calculated. Although NPV was similar for both tests (NPV > 99%,  $p = 0.3085$ ), for non-p-tau, PPV was significantly raised from 24.81% for 14-3-3 to 30.36% ( $p = 0.0230$ ). Finally, we also



**Fig. 1** Diagnostic accuracy of CSF non-p-tau as CJD biomarker. **a** CSF non-p-tau concentrations in non-CJD and CJD cases. Statistically significant differences were detected between both groups. Number of cases, age, sex, non-p-tau concentrations and 95% CI are indicated. Mann–Whitney  $U$  test was used to assess significant dif-

ferences. \*\*\* $p < 0.001$ .  $SD$  standard deviation,  $CI$  confidence interval. **b** ROC curve for non-p-tau in the comparative analysis between non-CJD cases and CJD cases. AUC with 95% CI, cutoff based on Youden index, sensitivity, specificity and  $p$  value are indicated. AUC area under the curve

**Fig. 2** Non-p-tau and 14-3-3 concentrations in suspected dementia. **a** Non-p-tau and **b** 14-3-3 concentrations in 1427 prospectively collected cases received for CSF testing in the German National Reference Center for CJD Surveillance. Cutoffs (red lines), number and percentage of cases above CJD cutoffs (>650 pg/mL for non-p-tau and > 20,000 AU/mL for 14-3-3) are indicated. **c** Venn diagram displaying the number of common and uncommon cases above CJD cutoffs for non-p-tau (>650 pg/mL) and 14-3-3 (20,000 AU/mL). **d** Correlation analysis for CSF non-p-tau (pg/mL) and 14-3-3 (AU/mL) in the prospective cohort. Spearman rank test was applied. Upper limit of detection for 14-3-3 was 120,000 AU/mL



**Table 1** Confusion matrices for 14-3-3 and non-p-tau

	CJD	non-CJD
Non-p-tau		
> 650 pg/mL	34	78
< 650 pg/mL	2	1313
14-3-3		
> 20,000 AU/mL	32	97
< 20,000 AU/mL	4	1294

**Table 2** Evaluation of diagnostic performance of 14-3-3 and non-p-tau using established optimal cutoff points

	Non-p-tau	14-3-3
Accuracy	94.39	92.92
Sensitivity	94.44	88.89
Specificity	94.39	93.03
Positive predictive value	30.36	24.81
Negative predictive value	99.85	99.69
F1-score	0.4595	0.3879

computed F1 score, which was higher for non-p-tau than for 14-3-3 (Table 2).

From the non-CJD cases over biomarkers' cutoffs (false positives), 47 were commonly identified by non-p-tau and 14-3-3 (36.7%). From the 36 CJD cases (true positives), 31

were correctly classified by both biomarkers (88.6%) and 35 correctly classified by at least one of the biomarkers (Fig. 3c).

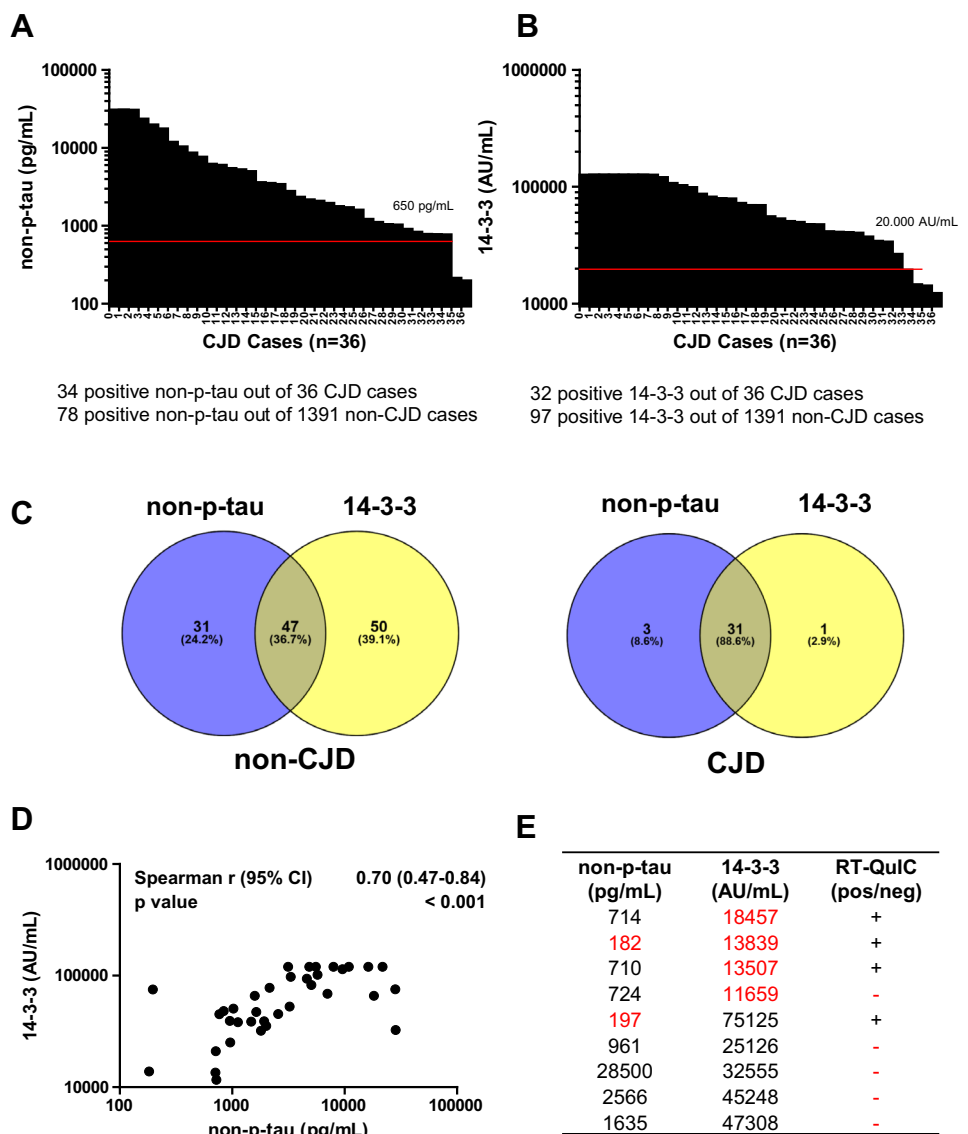
A strong positive correlation between non-p-tau and 14-3-3 concentrations was observed among CJD cases ( $r=0.70, p<0.001$ ) (Fig. 3d).

Regarding the cases not correctly identified by one of the two biomarkers; among the four CJD cases not correctly classified by 14-3-3, one was not correctly classified by non-p-tau and the other three were on borderline non-p-tau values (710 pg/mL, 714 pg/mL and 724 pg/mL). Among the two CJD cases not correctly classified by non-p-tau, one was not correctly classified by 14-3-3 (13,839 AU/mL) and the second one displayed unambiguous positive 14-3-3 value (75,125 AU/mL) (Fig. 3e).

**RT-QuIC analysis in CJD cases**

Next we compared non-p-tau and 14-3-3 performance in detecting CJD cases with the one achieved by the RT-QuIC assay, which is characterized by its high specificity, but sensitivities in range with those achieved by surrogate disease markers [16, 20, 21]. In the subset of 36 CJD cases, RT-QuIC tested positive in 31 cases and negative in 5. This leads to an overall sensitivity of 86%, which is lower than non-p-tau (94.44%) and 14-3-3 (88.89%) sensitivities. The CJD case with negative non-p-tau and 14-3-3 values was positive for RT-QuIC, and importantly, four out of

**Fig. 3** Non-p-tau and 14-3-3 in the discrimination of CJD. **a** Non-p-tau and **b** 14-3-3 concentrations in 36 cases diagnosed as CJD from the prospective cohort. Cutoffs (red lines), and number of true positive cases and false positive cases are indicated. **c** Venn diagrams displaying the number and percentages of non-CJD cases over the non-p-tau and 14-3-3 cutoffs (left), and the number and percentages of CJD cases over the non-p-tau and 14-3-3 cutoffs (right). **d** Correlation analysis for CSF non-p-tau (pg/mL) and 14-3-3 (AU/mL) in 36 CJD cases. Spearman rank test was applied. Upper limit of detection for 14-3-3 was 120,000 AU/mL. **e** non-p-tau and 14-3-3 concentrations and RT-QuIC outcome in CJD cases not correctly identified by at least one of the two biomarkers and in RT-QuIC-negative cases



the five RT-QuIC negative cases displayed unambiguous positive values for non-p-tau and 14-3-3 (Fig. 3e).

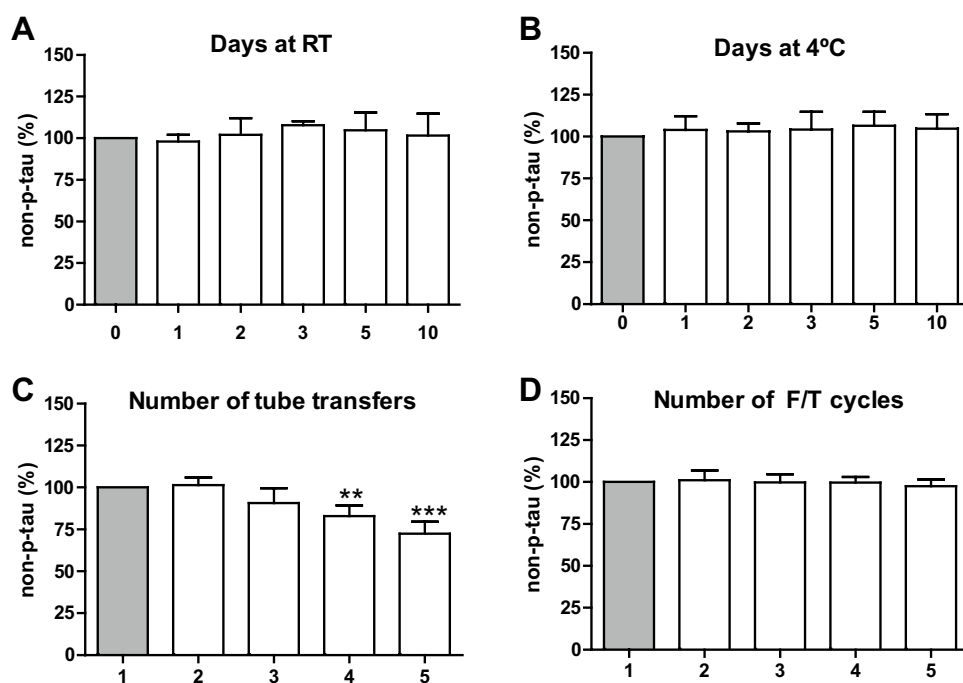
**Impact of pre-analytical variables in CSF non-p-tau concentrations:**

To determine the stability of CSF non-p-tau, samples were treated under different pre-analytical conditions. Non-p-tau was stable up to 10 days storage at RT (Fig. 4a) and at 4 °C (Fig. 4b). In contrast, significantly lower non-p-tau concentrations were observed after four ( $p < 0.01$ ) and five ( $p < 0.001$ ) tube transfers (Fig. 4c) compared to non-transferred samples. Non-p-tau was stable up to five freeze/thaw cycles (Fig. 4d).

**Discussion**

In the present study, we validate previous observations on the potential role of CSF non-p-tau as a highly accurate biomarker for CJD diagnosis [10, 22]. While in our preceding work we retrospectively analysed CJD, AD and neurological controls, here we investigated non-p-tau performance in the context of a CJD surveillance centre and compared its diagnostic accuracy with 14-3-3 protein, a gold standard biomarker in CJD diagnosis. Compared to total-tau, we already observed that non-p-tau provides better discrimination capacity between CJD and AD [10]. Although CJD entails severe neuronal damage, it is not characterized by pathological tau hyper-phosphorylation,

**Fig. 4** Effect of different storage conditions on non-p-tau concentrations. The stability of non-p-tau in CSF under pre-analytical conditions was tested in 5 randomly selected cases: **a** storage at room temperature (RT), **b** storage at 4 °C, **c** tube transfers and **d** freeze/thaw cycles (F/T). Non-p-tau concentrations were determined as indicated in “Material and methods” and are shown relative to the reference sample (time point 0), which was set as 100%. Error bars represent standard deviations (SD). Repeated-measures ANOVA followed by Bonferroni post-hoc analysis was applied.  $**p < 0.01$  and  $***p < 0.001$



in contrast to AD, putting non-p-tau in an excellent position as a discriminatory tool between both diseases. It should also be noted that observations from prion disease surveillance centres indicate that AD is one of the most common causes of rapid progressive dementia after CJD [12, 23, 24]. Thus, a correct differential diagnosis between CJD and AD, especially for AD cases displaying fast progressive course, is relevant in the context of prion disease surveillance centres.

In the prospective cohort, both biomarkers displayed similar percentage of positive cases and significant correlation on their concentrations. However, the number of common positive samples detected by both biomarkers was rather low (47.9% of the cases were over both cutoffs). This is in contrast with the high degree of agreement in the detection of CJD cases, since 88.6% of the total CJD cases were correctly identified by both biomarkers. Additionally, the cases not identified as CJD based on biomarkers cutoffs displayed borderline concentrations towards a correct diagnosis. Interestingly, the concentrations of both biomarkers in CJD cases were highly correlated, although a limitation of the study is the absence of real 14-3-3 concentrations for cases above 120,000 AU/mL. Thus, it is not possible to conclude whether correlations between biomarkers are higher in the total prospective cohort or in the CJD subgroup. Since increased CSF non-p-tau and 14-3-3 in CJD are associated with decreased tau and 14-3-3 levels in the brain of CJD cases [25], it is tempting to speculate that both proteins in the CSF reflect the degree of neuroaxonal damage occurring in the brain tissue of CJD cases.

Regarding the diagnostic accuracy in the discrimination of CJD from non-CJD cases, we could not find differences in the AUC values between biomarkers. However, upon the establishment of an optimal cutoff, non-p-tau demonstrated a slightly superior performance compared to 14-3-3 in all computed evaluation metrics. Although statistical significant differences were present between test specificities (94.39% for non-p-tau vs. 93.03% for 14-3-3;  $p = 0.0348$ ) but not sensitivities (94.44% for non-p-tau vs 88.89% for 14-3-3;  $p = 0.3173$ ), it should be noted that these p-values are strongly determined by different sample size (36 CJD cases vs. 1391 non-CJD cases). Given the low event rate for disease condition, a larger study cohort should be necessary to confirm the trend of non-p-tau higher sensitivity. Although NPV was similar for both biomarkers, a significantly higher PPV was obtained for non-p-tau (30.36%) compared to 14-3-3 (24.81%) ( $p = 0.0330$ ), indicating the increased capacity to predict CJD cases upon overcoming the cutoff value in the quantification test. Even though predictive values depend on the disease prevalence, it makes sense to consider them in the context of a specific CJD surveillance centre.

It is equally relevant that in the tested cohort, non-p-tau also displayed higher sensitivity (94%) compared to RT-QuIC (86%). While RT-QuIC is an extremely specific technique (values close to full specificity), its sensitivity ranges from 80 to 95% [20, 21, 26]. Therefore, the higher sensitivity of non-p-tau in contrast to 14-3-3 and RT-QuIC unveils the power of non-p-tau to reduce the number of false negative CJD cases, which converts this potentially new biomarker

to an excellent front-line tool for the detection of CJD cases that can later be validated by alternative approaches such as RT-QuIC. In this regard, non-p-tau may also serve as a supporting biomarker in the case of negative RT-QuIC outcome, since positive non-p-tau concentrations were detected in four out of five RT-QuIC negative cases.

In this study, we did not compare the diagnostic accuracy of non-p-tau with total-tau. On one side, our previous work [10] already suggested the improved performance of non-p-tau compared to total-tau in the distinction between CJD and AD. On the other side, data on total-tau levels were only available in 17 out of 36 CJD cases. This is a limitation of this work, because such a small number of cases with total-tau information is insufficient to provide robust statistical estimates when assessing its discriminatory capacity. Nonetheless, previous studies did compare ELISA 14-3-3 with total-tau in the diagnosis of CJD, and although sensitivity was the same for both CSF biomarkers, 14-3-3 displayed higher specificity (94%) compared to total-tau (90%) [27], supporting our study design that compares non-p-tau with 14-3-3.

An important consideration regarding the experimental design of the study is that 14-3-3 is one of the criteria used to define probable CJD cases according to established diagnostic criteria [14]. In spite of potential bias intrinsic to this set up, we found more false negative cases with 14-3-3 ( $n=4$ ) than with non-p-tau ( $n=2$ ), arguing in favour of non-p-tau as a more sensitive biomarker for CJD.

The accuracy of the 14-3-3 protein discriminating CJD cases in the present study was highly resembling to the one achieved in a previous retrospective study including 231 CJD and 2035 control patients with sensitivity and specificity values of 88% and 96%, respectively [4]. Previously, using the semi-quantitative detection of 14-3-3 by western-blot, it was reported that test specificity varied with respect to differential diagnosis, with high 14-3-3 specificity in differentiation to other neurodegenerative diseases (95–97%) and non-neurological conditions (91–97%), but lower specificity in the differential diagnoses of acute neurological diseases (82–87%) [28]. In this regard, the diagnostic accuracy of non-p-tau discriminating CJD cases in the clinical context of our CJD surveillance center (AUC=0.97) is slightly lower than the one previously achieved discriminating AD from CJD cases (AUC=0.99) [10].

Despite the fact that comparison of sensitivity accuracies is limited by the reduced amount of CJD cases in the prospective cohort, it is important to stress that the sensitivities for the three biomarkers in detecting CJD cases are in range with those detected in other studies [4, 10, 20, 21].

The experimental design of the present study underscores the importance of investigating biomarkers' performance in the appropriate clinical context. Indeed, accuracies in retrospective studies may be biased by sample selection, which may

not faithfully represent the prevalence and type of diseases relevant in the differential diagnostic context of CJD. Thus, a relevant strength of this study is the establishment of the diagnostic parameters of both biomarkers in real clinical settings. By contrast, one of the limitations is that, as a referral centre, the CJD surveillance group was not involved in further clinical investigation of cases when initial CJD suspicion had been dispelled due to improvement of symptoms or obvious clinical evidence for alternative diagnoses. Thus, no information is available on the diagnosis of the non-CJD cases included in the study.

Finally, CSF non-p-tau displays good stability regarding pre-analytical conditions, which is superior to the one achieved by 14-3-3 regarding temperature storage. In this regard, 14-3-3 is significantly decreased after 2 days at RT and after 4 days at 4 °C [4] in contrast to non-p-tau, which is not affected by temperature storage up to 10 days. This is a pre-analytical aspect to take into account when samples need to be shipped to centralized prion disease surveillance centres. In contrast, non-p-tau concentrations were significantly lower after four tube transfers, in line with previous observations on the effect of tube transfer in total-tau [29]. Adhesiveness to the micro-tube walls was suggested as the cause of reduced total-tau concentration, which could also happen in the case of non-p-tau. However, further studies are needed to corroborate this hypothesis.

Overall, our data indicate that CSF non-p-tau is a robust, stable and accurate surrogate marker of neuronal damage for the detection of CJD cases in the context of a prion disease surveillance centre with slightly higher diagnostic accuracy than 14-3-3, a gold standard biomarker in CJD diagnosis. Therefore, we suggest that non-p-tau may be useful as an optional marker in cases with suspected CJD and inconclusive 14-3-3 or RT-QuIC results. It may also improve the diagnostic accuracy of clinical diagnostic criteria, which still has to be validated in large independent cohorts.

**Author contributions** FL, AV-P, and IZ designed the study. AV-P and FL performed experiments. FL, AV-P, PH and MS analysed data and interpreted the results. PH, SG and IZ contributed to sample collection and characterization. KW and IL contributed to technical expertise. FL, AV-P and PH wrote the manuscript draft. All authors critically revised the manuscript and approved its content before submission.

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## Compliance with ethical standards

**Conflicts of interest** Dr. Lachmann and Dr. Waniek report they are employers of AJ Roboscreen GmbH, Leipzig, Germany. No other conflict of interest is reported.

**Ethical approval** This study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines and was approved by local Ethics committee (Reference number 11/11/93), Universitätsmedizin Göttingen, Germany).

## References

- Puoti G, Bizzi A, Forloni G et al (2012) Sporadic human prion diseases: molecular insights and diagnosis. *Lancet Neurol* 11:618–628. [https://doi.org/10.1016/S1474-4422\(12\)70063-7](https://doi.org/10.1016/S1474-4422(12)70063-7)
- Zerr I, Schmitz M, Karch A et al (2018) Cerebrospinal fluid neurofilament light levels in neurodegenerative dementia: evaluation of diagnostic accuracy in the differential diagnosis of prion diseases. *Alzheimers Dement*. <https://doi.org/10.1016/j.jalz.2017.12.008>
- Hermann P, Laux M, Glatzel M et al (2018) Validation and utilization of amended diagnostic criteria in Creutzfeldt–Jakob disease surveillance. *Neurology*. <https://doi.org/10.1212/WNL.00000000000005860>
- Schmitz M, Ebert E, Stoeck K et al (2015) Validation of 14-3-3 protein as a marker in sporadic Creutzfeldt–Jakob disease diagnostic. *Mol Neurobiol*. <https://doi.org/10.1007/s12035-015-9167-5>
- Sanchez-Juan P, Green A, Ladogana A et al (2006) CSF tests in the differential diagnosis of Creutzfeldt–Jakob disease. *Neurology* 67:637–643. <https://doi.org/10.1212/01.wnl.0000230159.67128.00>
- Llorens F, Schmitz M, Zerr I (2017) Progress in CSF biomarker discovery in sCJD. *Oncotarget* 8:5666–5667. <https://doi.org/10.18632/oncotarget.13998>
- Thompson AGB, Mead SH (2019) Review: Fluid biomarkers in the human prion diseases. *Mol Cell Neurosci* 97:81–92
- Llorens F, Kruse N, Schmitz M et al (2016) Evaluation of  $\alpha$ -synuclein as a novel cerebrospinal fluid biomarker in different forms of prion diseases. *Alzheimers Dement*. <https://doi.org/10.1016/j.jalz.2016.09.013>
- Villar-Piqué A, Schmitz M, Lachmann I et al (2018) Cerebrospinal fluid total prion protein in the spectrum of prion diseases. *Mol Neurobiol*. <https://doi.org/10.1007/s12035-018-1251-1>
- Ermann N, Lewczuk P, Schmitz M et al (2018) CSF nonphosphorylated Tau as a biomarker for the discrimination of AD from CJD. *Ann Clin Transl Neurol* 5:883–887. <https://doi.org/10.1002/acn3.584>
- Paterson RW, Torres-Chae CC, Kuo AL et al (2012) Differential diagnosis of Jakob–Creutzfeldt disease. *Arch Neurol* 69:1578–1582. <https://doi.org/10.1001/2013.jamaneurol.79>
- Zerr I, Hermann P (2018) Diagnostic challenges in rapidly progressive dementia. *Expert Rev Neurother* 18:761–772
- Geschwind MD, Murray K (2018) Differential diagnosis with other rapid progressive dementias in human prion diseases. In: *Handbook of clinical neurology*. pp 371–397
- Zerr I, Kallenberg K, Summers DM et al (2009) Updated clinical diagnostic criteria for sporadic Creutzfeldt–Jakob disease. *Brain* 132:2659–2668. <https://doi.org/10.1093/brain/awp191>
- Lewczuk P, Lelental N, Lachmann I et al (2016) Non-phosphorylated tau as a potential biomarker of Alzheimer’s disease: analytical and diagnostic characterization. *J Alzheimers Dis* 55:159–170. <https://doi.org/10.3233/JAD-160448>
- Schmitz M, Cramm M, Llorens F et al (2016) The real-time quaking-induced conversion assay for detection of human prion disease and study of other protein misfolding diseases. *Nat Protoc* 11:2233–2242. <https://doi.org/10.1038/nprot.2016.120>
- DeLong ER, DeLong DM, Clarke-Pearson DL (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 44:837. <https://doi.org/10.2307/2531595>
- Robin X, Turck N, Hainard A et al (2011) pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinform*. <https://doi.org/10.1186/1471-2105-12-77>
- Stock C, Hielscher T (2014) DTComPair: comparison of binary diagnostic tests in a paired study design. R package version 1.0.3. <https://CRAN.R-project.org/package=DTComPair>. Accessed 2014
- Cramm M, Schmitz M, Karch A et al (2016) Stability and reproducibility underscore utility of RT-QuIC for diagnosis of Creutzfeldt–Jakob disease. *Mol Neurobiol* 53:1896–1904. <https://doi.org/10.1007/s12035-015-9133-2>
- McGuire LI, Peden AH, Orru CD et al (2012) Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt–Jakob disease. *Ann Neurol* 72:278–285. <https://doi.org/10.1002/ana.23589>
- Goossens J, Bjerke M, Struyfs H et al (2017) No added diagnostic value of non-phosphorylated tau fraction (p-tau<sub>re</sub>) in CSF as a biomarker for differential dementia diagnosis. *Alzheimers Res Ther* 9:49. <https://doi.org/10.1186/s13195-017-0275-5>
- Poser S, Mollenhauer B, Krauß A et al (1999) How to improve the clinical diagnosis of Creutzfeldt–Jakob disease. *Brain* 122:2345–2351. <https://doi.org/10.1093/brain/122.12.2345>
- Geschwind M et al (2008) Rapidly progressive dementia. *Ann Neurol* 64:97–108. <https://doi.org/10.1038/jid.2014.371>
- Llorens F, Zafar S, Ansoleaga B et al (2015) Subtype and regional regulation of prion biomarkers in sporadic Creutzfeldt–Jakob disease. *Neuropathol Appl Neurobiol* 41:631–645. <https://doi.org/10.1111/nan.12175>
- Atarashi R, Satoh K, Sano K et al (2011) Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* 17:175–178. <https://doi.org/10.1038/nm.2294>
- Leitão MJ, Baldeiras I, Almeida MR et al (2016) Sporadic Creutzfeldt–Jakob disease diagnostic accuracy is improved by a new CSF ELISA 14-3-3 $\gamma$  assay. *Neuroscience* 322:398–407. <https://doi.org/10.1016/j.neuroscience.2016.02.057>
- Stoeck K, Sanchez-Juan P, Gawinecka J et al (2012) Cerebrospinal fluid biomarker supported diagnosis of Creutzfeldt–Jakob disease and rapid dementias: a longitudinal multicentre study over 10 years. *Brain* 135:3051–3061. <https://doi.org/10.1093/brain/aws238>
- Toombs J, Paterson RW, Schott JM, Zetterberg H (2014) Amyloid-beta 42 adsorption following serial tube transfer. *Alzheimer’s Res Ther*. <https://doi.org/10.1186/alzrt236>