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RESEARCH ARTICLE

Diagnostic and prognostic performance of CSF α-synuclein in prion disease in the context of rapidly progressive dementia

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Abstract

Introduction: Surrogate cerebrospinal fluid (CSF) biomarkers of neurodegeneration still have a central role in the first-line screening of patients with suspected Creutzfeldt-Jakob disease (CJD). Recently, CSF α-synuclein, a marker of synaptic damage, showed a close to optimal performance in distinguishing between CJD and other neurodegenerative dementias.

Methods: We evaluated the diagnostic value of CSF α-synuclein in patients with prion disease, non-prion rapidly progressive dementias, and non-neurodegenerative controls. Additionally, we studied its distribution across the different prion disease subtypes and evaluated its association with survival.

Results: CSF α-synuclein levels were significantly higher in patients with prion disease than in the other groups but showed a lower diagnostic value than CSF total tau or 14-3-3. Moreover, CSF α-synuclein was significantly associated with survival in the whole prion cohort and the most frequent clinicopathological subtypes.

Discussion: In the clinical setting, CSF α-synuclein does not exceed the diagnostic performance of currently used surrogate markers, but it might constitute a robust prognostic indicator.

KEYWORDS
alpha-synuclein, cerebrospinal fluid, Creutzfeldt-Jakob disease, diagnosis, fatal familial insomnia, prognosis, rapidly progressive dementias

1 | INTRODUCTION

Prion disease comprises a heterogeneous group of neurodegenerative disorders caused by tissue deposition of a misfolded form (PrP^{Sc}) of the cellular prion protein (PrP^{C}). Major clinicopathological phenotypes include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI), and variably protease-sensitive prionopathy (VPSPr).[1] The sporadic form of CJD (sCJD) is by far the most common entity in this group. It includes six major clinicopathological subtypes, which are classified according to the genotype (methionine, M or valine, V) at the polymorphic codon 129 in the prion protein gene (*PRNP*) and the PrP^{Sc} type (1 or 2) that is detected in the brain (e.g., MM1, MM2, VV1, etc.).[2,3]

The early discrimination of prion disease from other forms of rapidly progressive dementia (RPD) is crucial due to the possibility of starting a medical therapy when an underlying treatable etiology is identified. Unfortunately, the goal is often hindered in clinical practice by the lack of specificity of the most frequent presenting symptoms and signs.[4]

During the last two decades, the in vivo diagnosis of prion disease has progressively improved, thanks to the introduction of CSF assays for surrogate protein biomarkers of neurodegeneration, such as 14-3-3 and total tau (t-tau);[5–12] the availability of magnetic resonance imaging (MRI) techniques with increased sensitivity for spongiform change;[13–16] and, more recently, the development of the prion real-time quaking-induced conversion assay (RT-QuIC).[17–22] The latter provided virtually complete specificity and optimal sensitivity by allowing for the first-time PrP^{Sc} detection in biofluids and accessible tissues. Unfortunately, the need for a non–commercially available recombinant substrate and the lack of adequately standardized procedures currently limit its systematic employment as a screening test. Therefore, surrogate markers of neurodegeneration still play a central role in the diagnostic work-up of patients with RPD.[12]

Recently, patients with prion disease showed a marked increase in cerebrospinal fluid (CSF) α-synuclein levels compared to those with other neurodegenerative disorders, yielding an almost maximal diagnostic accuracy for this marker of synaptic degeneration.[23,24] However, the diagnostic performance of CSF α-synuclein has never been investigated in a group of subjects with RPD, namely in a cohort reflecting the clinical routine of a reference center for the diagnosis of prion disease.

In this study, we measured CSF α-synuclein levels in a large cohort of patients with RPD, including both prion and non-prion patients, and compared its diagnostic value to the one obtained by other CSF surrogate biomarkers. Moreover, we studied, for the first time, the distribution of CSF α-synuclein concentration according to prion disease subtypes. Finally, we assessed the association between levels of CSF α-synuclein and survival in prion disease.

RESEARCH IN CONTEXT

1. **Systematic review**: We reviewed the current research literature on the use of cerebrospinal fluid (CSF) α-synuclein for the diagnosis of prion disease using online databases. Llorens et al. (2017) reported a high diagnostic accuracy (94% sensitivity and 96% specificity) for this marker of synaptic degeneration.
2. **Interpretation**: In a patient cohort representative of the clinical routine setting, we showed that the diagnostic value of CSF α-synuclein does not reach that of protein total tau (t-tau) and does not exceed that of protein 14-3-3. The parallel distribution of CSF α-synuclein and t-tau values across the prion disease subtypes seems to reject, at least in rapidly progressive dementias, the concept of a distinct diagnostic value between "synaptic" (i.e., α-synuclein) and "neuroaxonal" (i.e., t-tau) biomarkers.
3. **Future directions**: Future studies involving patients with rapidly progressive dementias are encouraged to confirm our findings and identify the most suitable first-line biomarker to diagnose prion disease.

2 | METHODS

2.1 | Ethical approval

The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines and approved by the ethics committee "Area Vasta Emilia Centro" (approval number AVEC:18025, 113/2018/OSS/AUSLBO) and Istituto Superiore di Sanità (CE-ISS 09/266; May 29, 2009). Written informed consent was given by study participants or the next of kin.

2.2 | Inclusion criteria

We retrospectively analyzed CSF samples submitted to the Neuropathology Laboratory (NP-Lab) of the Institute of Neurological Sciences of Bologna (ISNB) or to the National CJD Surveillance Unit at the Istituto Superiore di Sanità in Rome between January 2005 and September 2020. Both are major referral centers for patients with suspected prion disease in Italy. Neuropathological studies were performed at ISNB (NP-Lab). All CSF samples were from patients suffering a RPD that raised the suspicion of prion disease. We included patients with a definite (i.e., with neuropathology) or a probable clinical diagnosis, and a sufficient CSF volume to perform all biomarker assays. A total of 486 patients were included, comprising 292 individuals affected by prion disease and 130 individuals with a non-prion rapidly progressive dementia (np-RPD). We also examined the CSF in 64 subjects lacking clinical or neuroradiological evidence of central nervous system disease, as a control group. Among the prion cases, 222 had a definite diagnosis (171 sCJD, 3 FFI, 1 VPSPr, and 47 genetic CJD [gCJD]), while 70 received a diagnosis of probable sCJD based on clinical findings, MRI, and prion RT-QuIC results, according to current diagnostic criteria.[22] Each patient with a definite diagnosis of sCJD was given a subtype classification according to Parchi et al.[2] (100 MM[V]1, 32 VV2, 24 MV2K, 9 MM[V]2C, 3 MM2T, 2 VV1, 1 not classifiable). Patients with a mixed subtype were classified based on the dominant histotype (e.g., MM1+2C classified as MM1 when the MM2C histotype was only seen focally), and then merged into the corresponding pure subtype. For the analysis of the diagnostic role of α-synuclein across the different CJD subtypes, patients with a probable diagnosis and a high level of certainty for a given subtype were merged with the corresponding group of patients with a definite diagnosis. However, to exclude a bias related to possible misdiagnosis, separate analyses including only the definite cases were also performed.

Regarding the analysis of the prognostic value of CSF α-synuclein in prion disease, survival was calculated as the time (in months) from the lumbar puncture (LP) to death or the time from LP to akinetic mutism when the available medical records indicated the adoption of life-extending treatments (e.g., enteral/parental nutrition, tracheostomy). Eight prion patients were excluded from the survival analysis due to lack of information on disease duration. Further details regarding the selection criteria and the clinical-pathological entities included in the np-RPD and control groups are shown in Tables S1 and S2 in supporting information.

2.3 | CSF biomarker analyses

CSF samples were obtained by LP at the L3/L4 or L4/L5 level following a standard procedure, centrifuged in case of blood contamination, divided into aliquots, and stored in polypropylene tubes at −80°C until analysis. We measured CSF t-tau, neurofilament light (NfL), and 14-3-3 gamma isoform using commercially available enzyme-linked immunosorbent assay (ELISA) kits as described.[12] CSF α-synuclein concentration was determined with the single molecule array (Simoa) technology. The assay was run on a SIMOA SR-X instrument (Quanterix) using the commercially available α-Synuclein Discovery kit (Quanterix). All CSF samples from patients without autopsy examination classified as probable CJD or np-RPD were tested by second generation prion CSF RT-QuIC.[20]

2.4 | Statistical analyses

Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software) and Stata 14.2 SE (StataCorp). Data were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) basing on the distribution of values. For continuous variables, depending on the data distribution and number of groups, the Mann-Whitney *U* test, *t* test, Kruskal-Wallis test (followed by Dunn-Bonferroni post hoc test), or the one-way analysis of variance (followed by Tukey's post hoc test) were performed. All reported *P*-values were adjusted for multiple comparisons, and differences were considered statistically significant at $P < .05$. The Chi-square test was used for categorial variables. Receiver operating characteristic (ROC) analyses were performed to calculate the sensitivity, the specificity, and the diagnostic accuracy of each biomarker with relative 95% confidence interval (95% CI). The optimal cut-off value for each biomarker was defined using the maximized Youden's index. For the analysis of survival, the concentration of CSF α-synuclein was natural log-transformed to fulfil the normal distribution. The Kaplan-Meier estimate was adopted to calculate the cumulative time-dependent probability of death. Univariate and multivariate Cox regression analyses were then performed to assess the association between survival, continuous values or tertiles of CSF α-synuclein, and other variables known as prognostic factors in prion disease (age at LP, time from symptoms onset to LP, codon 129 genotype, and clinicopathological subtype).[25,26] Both analyses were first run in the whole prion cohort. Secondly, to establish the prognostic value of the biomarker for each clinicopathological subtype, univariate and multivariate analyses were repeated in the following four groups: (1) sCJD MM(V)1 plus gCJD E200K-129 M, V210I-129 M or 4-OPRI-129 M; (2) sCJD VV2; (3) sCJD MV2K; or (4) rare, slowly progressive prion subtypes, including sCJD MM(V)2C, MM2T, VPSPr, FFI, or gCJD D178N-129V. The results are presented as hazard ratios (HRs) and 95% CIs. The assumption of proportional hazard was assessed by Schoenfeld residuals.

3 | RESULTS

3.1 | Demographic variables and distribution of CSF α-synuclein values in the diagnostic groups

Demographic features and CSF biomarker results in the diagnostic groups are shown in Table 1.

Patients with np-RPD were significantly older than those with prion disease ($P < .001$), while neurologic controls were younger than patients with prion disease ($P < .001$). There was no difference in sex distribution between diagnostic groups (Table 1). Data on t-tau, NfL, and 14-3-3 were previously reported.[12] CSF α-synuclein levels were higher in patients with prion disease than in those with np-RPD ($P < .001$), and neurologic controls ($P < .001$; Table 1, Figure 1A).

In the CJD group, α-synuclein CSF concentrations did not differ significantly between sCJD and gCJD cases. Similarly, we found no statistically significant differences between patients with a neurodegenerative disease and individuals with a probable alternative etiology ($P = .38$). Nevertheless, in the neurodegenerative group, Alzheimer's disease (AD) patients showed higher CSF α-synuclein levels than those with dementia with Lewy bodies (DLB; $P = .04$). In contrast, there were no significant differences between patients with AD and frontotemporal dementia (FTD) or between DLB and FTD (Table 2).

3.2 | Diagnostic performance of CSF α-synuclein

When evaluating the ROC curves, CSF α-synuclein yielded an accuracy of 85% in discriminating between prion and np-RPD patients (area under the curve [AUC] 0.853 ± 0.018). The diagnostic value of CSF α-synuclein exceeded that of CSF NfL (AUC 0.624 ± 0.035) but was slightly inferior to that of CSF 14-3-3 (AUC 0.878 ± 0.019) and CSF tau (AUC 0.904 ± 0.017), which exhibited the best diagnostic performance (Table 3, Figure 1C). When we limited the analysis to the most frequent and rapidly progressing sCJD subtypes (i.e., MM[V]1 and VV2), the diagnostic accuracy of CSF α-synuclein increased (AUC 0.946 ± 0.011); nevertheless, the performance did not reach that of CSF t-tau (AUC 0.958 ± 0.012). Finally, CSF α-synuclein showed a higher diagnostic value in the distinction between prion disease and np-RPD when the comparison was limited to the np-RPD subgroup with a neurodegenerative etiology (AUC 0.878 ± 0.020). Further details on the diagnostic performance of the different CSF biomarkers across the diagnostic groups are reported in Table 3.

TABLE 1 Demographic variables and CSF biomarkers in the major diagnostic groups

	Prion (n = 292)	np-RPD (n = 130)	Controls (n = 64)	*P*
Age at LP* (years)	66.1 ± 9.6	69.9 ± 12.0	60.8 ± 10.9	< .001
Female (%)	49.6	51.5	51.6	.11
CSF t-tau† (pg/mL)	4706 (2086–10073)	587.5 (331.8–1231)	–	< .001
CSF NfL† (pg/mL)	7187 (4112–11863)	3270 (1305–14088)	–	< .001
CSF 14-3-3† (pg/mL)	65650 (30525–139750)	10650 (6007–21600)	–	< .001
CSF α-syn† (pg/mL)	6123 (2502–12513)	1446 (836.8–2282)	598 (457–908)	< .001

Abbreviations: α-syn, alpha-synuclein; CSF, cerebrospinal fluid; LP, lumbar puncture; NfL, neurofilament light chain; np-RPD, non-prion rapidly progressive dementia; t-tau, total tau.
*Mean ± SD.
†Median (interquartile range).

TABLE 2 Distribution of CSF α-synuclein in all subgroups of the prion and np-RPD cohorts

Diagnostic group	N	CSF α-syn† (pg/mL)
Prion	**292**	**6123 (2502–12513)**
sCJD	241	6132 (2539–13362)
sCJD MM(V)1*	113	9945 (4416–20141)
sCJD VV2*	54	9954 (5829–15574)
sCJD MV2K*	51	1998 (1445-3169)
sCJD MM(V)2C*	15	2111 (1011–6124)
sCJD MM2T*	3	818; 922; 4738
sCJD VV1*	2	6669; 6877
gCJD	47	6257 (2512–10406)
gCJD E200K-129 M	19	2601 (1599–6941)
gCJD V210I-129 M	25	9556 (5897–16179)
gCJD D178N-129V	1	2965
gCJD 4-OPRI-129 M	1	8113
gCJD E219K-129 M	1	7468
VPSPr	1	3906
FFI (D178N-129 M)	3	318; 418; 498
np-RPD	**130**	**1446 (836.8–2282)**
Non neurodegenerative group	81	1464 (840.0–2556)
Neurodegenerative	49	1396 (841.5–1997)
AD	31	1504 (1078–2073)
DLB	12	684 (522–2413)
FTD	4	1267 (1191–1388)

Abbreviations: α-syn, alpha-synuclein; 4-OPRI, 4-octapeptide repeat insertion; AD, Alzheimer's disease; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FFI, fatal familial insomnia; FTD, frontotemporal dementia; gCJD, genetic Creutzfeldt-Jakob disease; np-RPD, non-prion rapidly progressive dementia; sCJD, sporadic Creutzfeldt-Jakob disease; VPSPr, variably protease-sensitive prionopathy.
*Both patients with a definite diagnosis of a specific subtype and patients with a probable diagnosis and a high level of certainty for a given subtype are included.
†Median (interquartile range).

3.3 | Distribution of CSF α-synuclein according to prion disease subtypes

After stratification according to the sCJD subtype, CSF α-synuclein showed significantly higher levels in MM(V)1 and VV2 patients than in those with MV2K and MM(V)2C (MM[V]1 vs. MV2K, $P < .001$; MM[V]1 vs. MM[V]2C, $P < .001$; VV2 vs. MV2K, $P < .001$; VV2 vs. MM[V]2C, $P < .001$). All differences remained statistically significant after the exclusion of probable cases. CSF α-synuclein levels did not differ significantly between MM(V)1 and VV2 patients. In the genetic form, FFI patients showed the lowest CSF α-synuclein levels of all prion groups, with a statistically significant difference against the MM(V)1 and VV2 patients (MM[V]1 vs. FFI, $P = .022$; VV2 vs. FFI, $P = .022$). Genetic CJD patients with the V210I mutation showed CSF α-synuclein levels in the range of the MM(V)1 and VV2 subgroups, with a significant difference with the MV2K patients (V210I vs. MV2K, $P < .001$) and those with MM(V)2C (V210I vs. MM[V]2C, $P = .014$). Finally, patients carrying the E200K mutation showed CSF α-synuclein values comparable to those of the MV2K patients and significantly lower than those of the MM(V)1 and VV2 subtypes (E200K vs. MM(V)1, $P = .003$; E200K vs. VV2, $P = .007$). All findings regarding gCJD patients remained significant after excluding the probable sCJD cases, except for the comparison between V210I and MM(V)2C. Details on the CSF α-synuclein levels in the different subgroups of sCJD patients and the gCJD cases linked to different mutations are reported in Table 2 and Figure 1B. The profiles of the remaining CSF biomarkers stratified by prion disease subtypes are shown in Table S3 in supporting information.

3.4 | Prognostic value of CSF α-synuclein in prion disease

Considering the whole prion cohort, CSF α-synuclein levels were significantly associated with survival even after accounting for covariates known to affect disease progression, including codon 129 genotype and the clinicopathological subtype (Table 4, Figure 2).

TABLE 3 Diagnostic performance of CSF α-synuclein and other surrogate biomarkers

CSF biomarker	AUC (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)	Cut-off
Prion vs. np-RPD				
CSF α-syn	0.853 ± 0.018 (0.817–0.889)	73.3% (67.9–78.0)	82.3% (74.8–87.9)	2701 pg/mL
CSF t-tau	0.904 ± 0.017 (0.871–0.938)	84.9% (80.4–88.6)	83.8% (76.6–89.2)	1511 pg/mL
CSF 14-3-3	0.878 ± 0.019 (0.840–0.916)	87.3% (83.0–90.7)	76.1% (68.1–82.7)	21750 pg/mL
CSF NfL	0.624 ± 0.035 (0.554–0.693)	91.8% (88.1–94.4)	44.62% (36.3–53.2)	2467 pg/mL
Typical prion (MM(V)1 + VV2) vs np-RPD				
CSF α-syn	0.946 ± 0.011 (0.923–0.968)	90.4% (85.0–94.9)	82.3% (74.8–87.9)	2701 pg/mL
CSF t-tau	0.958 ± 0.012 (0.935–0.981)	96.4% (92.4–98.3)	87.7% (80.9–92.3)	1767 pg/mL
CSF 14-3-3	0.938 ± 0.015 (0.908–0.967)	95.2% (90.8–97.5)	81.5% (74.0–87.3)	26550 pg/mL
CSF NfL	0.653 ± 0.036 (0.583–0.723)	94.6% (90.1–97.1)	45.4% (37.1–53.9)	2494 pg/mL
Prion vs. neurodegenerative np-RPD				
CSF α-syn	0.878 ± 0.020 (0.838–0.918)	73.3% (67.9–78.0)	91.8% (80.8–96.8)	2701 pg/mL
CSF t-tau	0.944 ± 0.020 (0.904–0.985)	92.5% (88.9–95.0)	89.8% (78.2–95.6)	1111 pg/mL
CSF 14-3-3	0.953 ± 0.015 (0.923–0.982)	92.5% (88.9–95.0)	89.8% (78.2–95.6)	16200 pg/mL
CSF NfL	0.776 ± 0.048 (0.681–0.870)	92.5% (88.9–95.0)	65.3% (51.3–77.1)	2245 pg/mL

Abbreviations: α-syn, alpha-synuclein; AUC, area under the curve; CI, confidence interval; CSF, cerebrospinal fluid; NfL, neurofilament light chain; np-RPD, non-prion rapidly progressive dementia; t-tau, total tau.

In the specific subanalysis of disease subtypes, CSF α-synuclein concentrations inversely correlated with disease duration in patients with sCJD MM(V)1, gCJD E200K-129 M, V210I-129 M or 4-OPRI-129 M, and sCJD VV2. The result remained statistically significant in the multivariate analysis after adjusting for age, time from onset to LP, and codon 129 subtype. In contrast, we found no significant associations between CSF α-synuclein and total disease duration in the other clinicopathological subgroups (i.e., sCJD MV2K and slowly progressive/atypical prion subtypes; Table 4).

4 | DISCUSSION

The combined use of CSF surrogate markers of neurodegeneration and the prion RT-QuIC seeding assays currently ensures an accurate in vivo identification of most patients affected by CJD.[21,22,25,26] In most centers, protein 14-3-3 or t-tau are used to select samples for RT-QuIC analysis due to the current unavailability of RT-QuIC as a large-scale screening test. Given the suboptimal accuracy of 14-3-3 and t-tau proteins,[9,12,22] the search for an alternative marker with a higher diagnostic value to use as the first discriminatory test remains relevant. Among the potential novel candidates, CSF α-synuclein recently showed a close to optimal accuracy in discriminating CJD patients,[23,24] prompting further investigations. To assess the diagnostic value of CSF α-synuclein in the clinical context, we analyzed a large group of patients with a rapidly progressive neurological syndrome that raised the suspicion of prion disease. By doing so, we aimed to build a cohort of individuals representative of the referrals to a reference center for the diagnosis of prion disease. Our analyses confirmed the marked increase of CSF α-synuclein levels in the prion patients compared to np-RPD cases and controls, reflecting the more severe neuronal damage in the former group. However, comparing the performance of CSF α-synuclein in discriminating patients with prion disease from cases with other forms of RPD, we obtained lower accuracy values compared to other established surrogate biomarkers such as CSF t-tau and protein 14-3-3. Our results differ from those previously reported on the diagnostic accuracy of CSF α-synuclein, which showed diagnostic accuracy values close to those of the second-generation prion RT-QuIC assay. The main reason for this significant discrepancy almost certainly depends on the selected control cohorts used to calculate the diagnostic performance, which in previous studies mainly comprised patients with typical ND (i.e., non-rapidly progressive). However, the relatively high number of patients with MV2K and other slowly progressive prion subtypes included in our cohort might also have contributed to the lower overall sensitivity and specificity of CSF α-synuclein given that CSF α-synuclein, as all surrogate biomarkers of neurodegeneration, performs better against the two most frequent and typical clinicopathological sCJD subtypes (i.e., MM[V]1 and VV2). Furthermore, our results show that limiting the analysis to the specific context of neurodegenerative disorders also increases CSF α-synuclein diagnostic value, even when the comparison is limited to atypical, rapidly progressive forms. Given that other laboratory assays can reasonably exclude the most frequent non-neurodegenerative forms of RPD, the use of CSF α-synuclein in the differential diagnosis of rp-NDs might have a clinical value in the context of neurodegenerative dementia. In this regard, it has been suggested that α-synuclein has a higher value than t-tau because it is probably less affected by the secondary tauopathy occurring in AD.[23] However, when we specifically compared the value of CSF t-tau and α-synuclein in the discrimination between prion disease and AD we found that the former still exhibited a better diagnostic performance (data not shown).

TABLE 4 Associations of CSF α-synuclein with survival time in the whole prion cohort and after stratification according to the disease subtype

		Survival time (months)	Univariate Cox regression		Multivariate Cox regression Codon 129-genotype adjusted*		Multivariate Cox regression Clinicopathological subgroup-adjusted*	
Diagnostic group		mean ± SD	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Whole prion cohort (n = 284)	Continuous value		1.63 (1.44–1.85)	<.001	1.48 (1.29–1.71)	<.001	1.24 (1.07–1.44)	.004
	Low tertile	6.3 ± 8.4	Ref	Ref	Ref	Ref	Ref	Ref
	Mid tertile	4.0 ± 6.7	1.39 (1.04–1.85)	.027	1.00 (0.72–1.39)	.987	0.91 (0.65–1.27)	.575
	High tertile	1.4 ± 1.8	2.93 (2.16–3.97)	<.001	2.22 (1.56–3.15)	<.001	1.31 (0.93–1.85)	.118
sCJD MM(V)1 + gCJD E200K-129 M, V210-129 M and 4-OPRI-129 M (n = 152)	Continuous value		1.25 (1.05–1.48)	.010	1.21 (1.01–1.45)	.035	–	–
	Low tertile	2.1 ± 3.5	Ref	Ref	Ref	Ref	–	–
	Mid tertile	1.3 ± 0.9	1.21 (0.77–1.90)	.402	0.95 (0.60–1.51)	.832	–	–
	High tertile	1.4 ± 2.0	1.31 (0.86–2.00)	.204	1.14 (0.73–1.75)	.567	–	–
sCJD VV2 (n = 52)	Continuous value		2.04 (1.20–3,46)	.008	1.95 (1.14–3.31)	.014	–	–
	Low tertile	1.7 ± 1.0	Ref	Ref	Ref	Ref	–	–
	Mid tertile	3.0 ± 1.5	0.53 (0.20–1.45)	.216	0.58 (0.20–1.67)	.311	–	–
	High tertile	1.5 ± 1.3	1.21 (0.46–3.17)	.700	1.31 (0.50–3.49)	.583	–	–
sCJD MV2K (n = 51)	Continuous value		1.03 (0.65–1.63)	.891	1.04 (0.64–1.70)	.886	–	–
	Low tertile	7.0 ± 5.5	Ref	Ref	Ref	Ref	–	–
	Mid tertile	8.0 ± 6.2	0.77 (0.39–1.53)	.011	0.90 (0.45–1.81)	.770	–	–
	High tertile	2	4.92 (0.62–38,9)	.132	6.22 (0.76–50.7)	.088	–	–
Slowly progressive prion disease (n = 25)	Continuous value		0.86 (0.58–1.29)	.468	0.52 (0.29–0.95)	.033	–	–
	Low tertile	14.8 ± 14.7	Ref	Ref	Ref	Ref	–	–
	Mid tertile	16.4 ± 14.7	0.88 (0.38–2.02)	.757	0.42 (0.13–1.31)	.134	–	–
	High tertile	–	–	–	–	–	–	–

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; gCJD, genetic Creutzfeldt-Jakob disease; HR, hazard ratio; Ref, reference; sCJD, sporadic Creutzfeldt-Jakob disease; SD, standard deviation.

*Both multivariate Cox regression analyses included age and time from symptoms onset to LP as covariates.

Note: Bold values indicate statistically significant hazard ratios.

Comparison of CSF α-synuclein values across prion disease subtypes demonstrated a striking similarity with the distribution of CSF t-tau (and 14-3-3) levels.[9] Patients belonging to the VV2 and MM(V)1 subtypes showed the highest protein levels. In contrast, both MV2K and MM2C subtypes showed much lower CSF α-synuclein values, with significant overlap with those detected in other neurodegenerative dementias.[27] Similarly, FFI patients showed the lowest CSF α-synuclein concentrations within the prion disease group, as already reported for CSF t-tau and 14-3-3. Therefore, the degree of neuronal injury and its progression rate mostly determine CSF α-synuclein levels, as is the case for CSF t-tau. These results also have implications for the definition, classification, and pathophysiology of currently available surrogate markers of neurodegeneration. Based on difference in the intraneuronal compartment distribution, these markers are increasingly distinguished in "synaptic", "generic neuroaxonal" and "myelinated axonal." Following this view, and the notion that α-synuclein is enriched in synapses,[28] whereas t-tau mainly colocalizes with the neuronal cytoskeleton, and NfL is mainly found in myelinated axons,[29] these three markers well represent the three categories outlined above. Increasing evidence seems to indicate that each of the three categories of biomarkers may provide different information reflecting the prevalent regional and subcellular pathology of a given disorder. In this regard, the results we obtained in this study argue against a significant discriminatory diagnostic role between "synaptic" (i.e., α-synuclein) and "neuroaxonal" (t-tau) markers of neurodegeneration, at least in prion disease. In contrast, the preferential distribution of NfL in myelinated axons makes it much more sensitive than the other markers for sCJD subtypes with diffuse subcortical damage and a relatively slow disease progression (e.g., the MV2K subtype).[11]

We also looked for an association between CSF α-synuclein levels and disease duration in prion disease. We found a significant correlation between CSF α-synuclein levels and survival even after account-

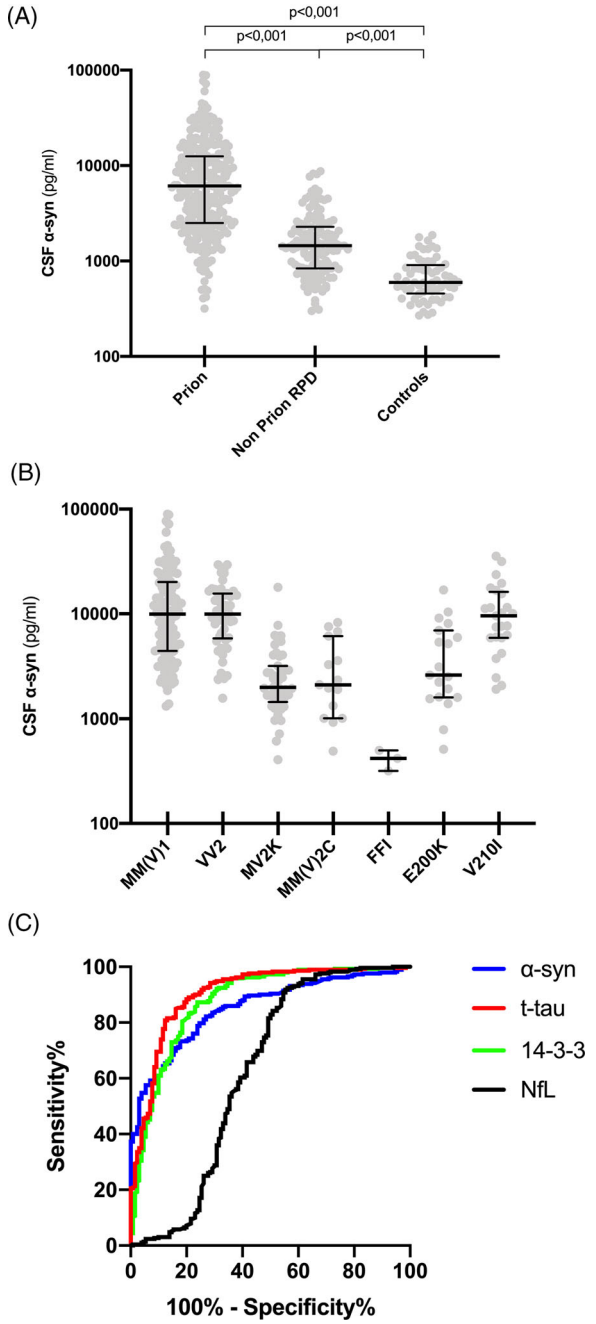

FIGURE 1 CSF α-synuclein levels in the diagnostic groups and ROC curves for CSF α-synuclein and other CSF surrogate biomarkers. A, CSF α-synuclein values in prion disease, non-prion rapidly progressive dementia (non-prion RPD), and controls. B, CSF α-synuclein levels in sporadic Creutzfeldt-Jakob disease (sCJD) subtypes MM(V)1, VV2, MV2K, and MM(V)2C, FFI and genetic Creutzfeldt-Jakob disease (gCJD) with E200K and V210I mutations. Thick lines represent medians and interquartile range. CSF α-synuclein values are expressed in logarithmic scale. See the main text (section 3.3) for all the *P*-values (Kruskal-Wallis followed by Dunn-Bonferroni post hoc test). C, ROC curves for CSF α-synuclein (blue), CSF total tau (red), CSF 14-3-3 (green), and CSF NfL (black) in the comparison between patients with prion disease and those with non-prion RPD. α-syn, alpha-synuclein; CSF, cerebrospinal fluid; FFI, fatal familial insomnia; NfL, neurofilament light chain; ROC, receiver operating characteristic; t-tau, total tau

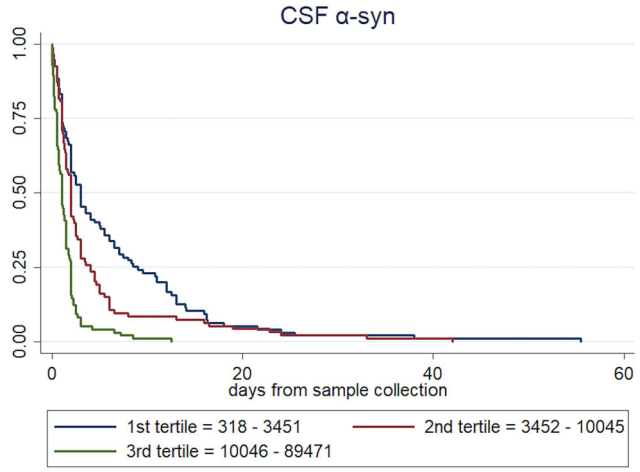

FIGURE 2 Prognostic value of CSF α-syn. Survival curve in patients of the whole prion cohort according to the values of CSF α-synuclein. α-syn, alpha-synuclein; CSF, cerebrospinal fluid

ing for covariates known to have a prognostic role, and that are available *ante mortem*, such as age, time from symptoms onset to LP, and codon 129 genotype. Notably, CSF α-synuclein still predicted survival in the whole group after accounting for the disease subtype, the most potent prognostic factor in prion disease, which currently can only be defined by neuropathological examination. However, when we limited the analysis to each disease subtype, our results demonstrated a positive association between CSF α-synuclein levels and disease duration only in the MM(V)1 and VV2 groups. Comparing the strength of the predictive value of CSF α-synuclein to those of other CSF and plasma biomarkers reported in previous studies,[25,26] CSF t-tau and 14-3-3 proteins showed a stronger association with survival than CSF α-synuclein. However, the three markers showed a comparable performance after taking into account the clinicopathological subtype.[26]

We believe that selecting a cohort of patients with a non-prion rapidly progressive neurological syndrome to compare to the prion group best represents the clinical scenario and constitutes a strength of our study, together with the high percentage of neuropathological confirmation in the prion group. In contrast, the relatively low number of *post mortem* definite diagnosis in the np-RPD cohort represents a limitation of the study. Nonetheless, we can reasonably exclude that many misdiagnoses occurred in the latter group based on the negative result at the prion RT-QuIC assay, the clinical follow-up, and the neuroradiological/biochemical evidence of an alternative disease.

In conclusion, our study demonstrates that, when evaluated in subjects with RPD raising the suspicion of CJD, the diagnostic accuracy of CSF α-synuclein in discriminating the prion cases is lower compared to other CSF surrogate biomarkers such as t-tau and 14-3-3 proteins. The biomarker showed a higher diagnostic value in the context of neurodegenerative RPD, but still did not exceed the performance of t-tau. Finally, our data indicate that CSF α-synuclein could serve as a predictor of survival in prion disease, with a comparable strength to CSF t-tau and 14-3-3 proteins when the clinicopathological subtype is taken into account.

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AUTHOR CONTRIBUTIONS

AMas, S.B., and P.P. designed the study. AMam and B.P. performed biomarker experiments and genetic analyses. P.P. and S.B. performed neuropathological examinations. AMas, S.B., S.C., C.Z., and P.P. analyzed data and interpreted the results. A.L. and A.P. provided samples and clinical data. AMas and P.P. wrote the manuscript draft. All authors critically revised the manuscript and approved its content before submission.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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