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RESEARCH ARTICLE

Clinical value of novel blood-based tau biomarkers in Creutzfeldt–Jakob disease

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Abstract

BACKGROUND: The diagnostic and prognostic performance of the novel fluid biomarkers brain-derived tau (BD-tau) and phospho-tau217 (p-tau217) in Creutzfeldt–Jakob disease (CJD) is not defined.

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METHODS: We measured cerebrospinal fluid (CSF) and plasma BD-tau, p-tau217, p-tau181, total tau (t-tau), neurofilament light (NfL), and 14-3-3 in 100 CJD patients, 100 with non-prion rapidly progressive dementia (np-RPD), 92 with mild cognitive impairment due to Alzheimer's disease (AD-MCI), and 55 healthy controls (HC).

RESULTS: Plasma BD-tau performed comparably to plasma t-tau but had lower performance than CSF t-tau ($p < 0.001$) and 14-3-3 ($p = 0.014$) in CJD versus np-RPD differential diagnosis. Plasma BD-tau diagnostic accuracy increased when ratioed to plasma p-tau217, matching CSF 14-3-3. Plasma BD-tau levels were associated with survival ($p < 0.001$), outperforming t-tau and NfL.

DISCUSSION: Plasma BD-tau is a valuable marker for CJD prognostication. In the clinical setting, the plasma BD-tau/p-tau217 ratio provides an accurate, fast marker supporting the clinical diagnosis of CJD.

KEYWORDS
Alzheimer's disease, biomarker, brain-derived tau, cerebrospinal fluid, Creutzfeldt–Jakob disease, phospho-tau, plasma, prion, p-tau181, p-tau217, rapidly progressive dementia

Highlights

- The increase of plasma BD-tau levels parallels that of CSF t-tau in CJD.
- CSF p-tau217 levels are significantly increased in CJD, reflecting a prion-specific secondary tauopathy.
- Plasma p-tau217 shows a distinct profile than CSF p-tau217 in CJD.
- Plasma BD-tau/p-tau217 ratio is as accurate as CSF 14-3-3 in distinguishing CJD from np-RPDs, including AD.
- BD-tau represents a valuable blood-based biomarker for CJD prognostication.

1 | BACKGROUND

Creutzfeldt–Jakob disease (CJD) is a rare neurodegenerative disorder related to prion protein (PrP) misfolding with heterogeneous etiology (sporadic, genetic, or acquired). Sporadic CJD (sCJD), the most common form (85% of cases), encompasses six major clinicopathological subtypes that are determined by the combination of the polymorphic codon 129 genotype (encoding methionine, M, or valine, V) of the PrP gene (*PRNP*) and the type (1 or 2) of misfolded PrP (PrP^{Sc}) accumulating in the brain (e.g., MM1, MV1, MM2, etc.).[1] Besides sCJD, genetic CJD (gCJD), caused by pathogenic *PRNP* mutations, comprises 10%–15% of the cases. Similar to sCJD, the type of PrP^{Sc} (1, 2, or intermediate size) and the codon 129 genotype of *PRNP* mutated allele are the main determinants of gCJD clinicopathological subtypes (e.g., M1, M2C, M2T, etc.).[2] Each sCJD and gCJD subtype is associated with distinct clinical presentations, neuropathological profiles, and disease progression rates.[1,2]

Differentiating CJD from other rapidly progressive dementias (RPDs) presents significant clinical challenges due to overlapping symptoms, especially in early phases. Moreover, the variability in disease course among CJD subtypes complicates or often prevents adequate disease monitoring and prognostication. Last, given the phenotypic heterogeneity of CJD, it would be useful to have an accessible marker to prompt consideration of prion disease in less specialized settings.

To date, the in vivo diagnosis of patients with prion disease has reached virtually full accuracy using the second-generation prion real-time quaking-induced conversion (RT-QuIC) amplification assay on cerebrospinal fluid (CSF), olfactory mucosa brushes or skin punches, and magnetic resonance imaging (MRI).[3–6] Besides, the combination of markedly increased total tau (t-tau) levels in CSF and a marked increase in the t-tau to phosphorylated tau (p-tau) ratio (t-tau/p-tau ratio) showed close to 100% diagnostic accuracy for typical CJD (MM1, MV1 subtypes).[7,8] However, both RT-QuIC and the t-tau/p-tau ratio require CSF collection by lumbar puncture (LP), which will reduce their suitability for screening purposes, and RT-QuIC has limited availability and is not performed outside specialized laboratories. Similarly, an MRI-based diagnosis requires expertise that may not be available in all hospitals.[9] Regarding blood-based diagnostic markers, t-tau, neurofilament light (NfL), S100B, and β-synuclein levels (variably assayed through enzyme-based immunoassays or mass spectrometry) have been proven to rise significantly in blood derivatives from CJD patients and to accurately discriminate them from both neurodegenerative dementias and non-neurodegenerative controls.[10–13] However, when

investigated in the clinical setting (i.e., in rapidly progressive dementia cohorts), blood β-synuclein was the only one to yield high diagnostic performance in the range of CSF t-tau and 14-3-3.[9,14]

Regarding prognosis, some CSF biomarkers (e.g., t-tau and 14-3-3) have shown significant prognostic power.[14] However, accurate blood biomarkers allowing for noninvasive disease monitoring would be very useful in the clinic. Despite promising pilot data for plasma t-tau and NfL,[15,16] their prognostic value remains lower than that of established CSF markers.[14,17]

It has recently been reported that CSF levels of novel tau biomarkers phospho-tau217 (p-tau217) and phospho-tau181 (p-tau181), usually thought to reflect Alzheimer's disease (AD) pathology, as well as brain-derived tau (BD-tau) reflecting the intensity of neurodegeneration or neuronal injury, may show increased levels also in CJD.[4,18,19] In this study, we aimed to investigate the diagnostic and prognostic value of CSF and plasma BD-tau, p-tau217, and p-tau181 in an RPD cohort (comprising both CJD and non-prion RPD [np-RPD]) and compare it to that of traditional CSF and blood surrogate neurodegeneration biomarkers (i.e., t-tau, 14-3-3, and NfL). Moreover, we studied the biomarkers distribution across CJD subtypes and in a wide cohort comprising healthy controls (HC) and AD patients.

RESEARCH IN CONTEXT

1. **Systematic review**: We searched PubMed for publications regarding cerebrospinal fluid (CSF) and plasma brain-derived tau (BD-tau) and phospho-tau variants (p-tau217 and p-tau181) in Creutzfeldt–Jakob disease (CJD). CSF levels of these biomarkers have been shown to increase in CJD, independently from AD co-pathology. However, data regarding their plasma levels and clinical (diagnostic and prognostic) value are lacking.
2. **Interpretation**: Our findings indicate that CSF and plasma BD-tau and p-tau variant levels vary in CJD without evidence of AD co-pathology, depending on the clinicopathological CJD subtype and the matrix analyzed. In CJD differential diagnosis, plasma BD-tau, p-tau181, or p-tau217 alone do not provide added diagnostic value compared to established CSF and plasma surrogate biomarkers. However, their diagnostic value increases when evaluated in a ratio. Moreover, BD-tau represents the most accurate blood-based biomarker for CJD prognostication.
3. **Future directions**: Replicating the results in additional cohorts will be important for clinically validating our findings.

2 | METHODS

2.1 | Participant selection

In this multicentric study, we retrospectively analyzed the CSF and plasma samples in two independent cohorts. Cohort 1 included patients with RPD whose samples were submitted to the neuropathology laboratory (NP-Lab) at the Institute of Neurological Sciences of Bologna for diagnostic purposes between 2004 and 2023. Patients with a definite neuropathological or probable clinical diagnosis according to current criteria were included. Specifically, the cohort comprised 100 randomly selected patients with np-RPD and 100 with CJD. The CJD group included 88 participants with sCJD (of which 30 had a neuropathological diagnosis, and 58 had a clinical diagnosis of probable sCJD according to the current criteria,[1,3] and were all positive by prion RT-QuIC[4]), and 12 patients with a diagnosis of gCJD.[2] The sCJD cases with a definite (i.e., neuropathological) diagnosis were also classified into subtypes according to Parchi et al. (i.e., MM(V)1, VV2, MV2K, etc.).[1] Among these, 20 patients showing a mixed subtype, mainly belonging to the MM1+2C group, were classified based on the dominant histotype according to published criteria.[20] For the biomarker analysis according to the molecular subtype, we merged the patients with definite sCJD with those with a probable diagnosis and a high level of certainty for a given subtype, as described.[14] Overall, 12 M1, 31 MM(V)1, 24 VV2, 25 MV2K, and 8 MM2C were included. The np-RPD group comprised patients presenting with RPD who tested negative by prion RT-QuIC. For diagnostic accuracy analyses, the np-RPD cohort was split into two main subgroups, that is, rpAD, including patients showing biological evidence of amyloid β (Aβ) deposition (defined by pathological CSF Aβ_{42}/Aβ_{40} ratio), and nonAD-RPD, a heterogeneous group well-representative of the most common RPD alternative etiologies, for example, inflammatory, toxic-metabolic, and so forth (see Supplementary Material and Table S1 for details).

Cohort 2 included participants from the Dementia Disease Initiation (DDI), a Norwegian national multicentric study.[21] The cohort comprises non-demented individuals between 40 and 80 years of age, primarily recruited from memory clinics and advertisements in local news media. The present study included randomly selected participants with pathological levels of CSF Aβ_{42}/Aβ_{40} ratio and p-tau181 with mild cognitive impairment (AD-MCI) (n = 92), according to NIA-AA criteria,[22] as well as HC with normal levels of CSF Aβ_{42}/Aβ_{40} ratio and p-tau181 (n = 55). The HC reported no subjective experience of cognitive impairment or decline and performed within the normal range on neuropsychological test scores. Detailed inclusion criteria for both cohorts are reported in the Supplementary Material.

2.2 | CSF and plasma biomarker analyses

We measured BD-tau and p-tau217 in CSF and plasma, Aβ_{40} and Aβ_{42} in CSF, and p-tau181 and NfL in plasma in both cohorts, as reported.[23–25] Moreover, we quantified CSF and plasma t-tau, CSF NfL, p-tau181, and 14-3-3 levels and performed prion and α-synuclein RT-QuIC seeding amplification assays in NP-Lab participants, as reported.[4,26] All analyses were performed by personnel blinded to

clinical diagnosis. Sample collection protocol and quantification of fluid biomarkers are presented in the Supplementary Material.

2.3 | Survival analyses in the CJD cohort

Regarding the biomarkers' prognostic performance assessment in CJD, we defined survival as the time (in months) from sample (CSF or plasma) collection to death or akinetic mutism. The latter was used in place of time to death exclusively when the revision of medical charts indicated the adoption of life-extending treatments (e.g., enteral/parenteral nutrition, tracheostomy), as previously reported.[14,27] Four patients were excluded from the survival analyses due to the lack of information on disease duration. Moreover, each CJD patient was assigned a disease stage by computing the ratio between disease onset to sampling and the disease duration.[27] Survival analyses were performed in the whole CJD cohort and three subgroups, stratifying for clinicopathological subtype as follows: (1) sCJD MM(V)1 + gCJD M1 (i.e., all cases related to the *PRNP* codon 129-M genotype and PrP^{Sc} type 1 combination); (2) sCJD VV2; (3) "atypical" (i.e., slowly progressive) CJD, including all the other subtypes (i.e., sCJD MV2K and MM2C).

2.4 | Statistical analyses

Statistical analyses were performed using Stata 18 SE (StataCorp), R (version 4.3.2), and GraphPad Prism 9 (Graph-Pad Software). Between-group age comparisons were assessed with linear regression, while sex distributions were evaluated using Fisher´s exact test. To examine group differences in plasma and CSF biomarkers, multiple linear regression models were fitted with the diagnostic group as the independent variable and the respective biomarker as the dependent variable, including age and sex as covariates. Due to departures from normality in the regression residuals, all biomarkers were log-transformed for the final models. The final models demonstrated adequate distributions of the regression residuals concerning normality and heteroscedasticity. In all models, HC was compared to the diagnostic groups. Only CJD cases with normal CSF $A\beta_{42}/A\beta_{40}$ ratio (Aβ-/CJD) were included in this analysis. No adjustments for multiple comparisons were made. Plots were generated using the "ggplot2" R package. For the diagnostic section, receiver operating characteristic (ROC) analyses were performed to calculate each biomarker's sensitivity, specificity, and diagnostic accuracy with a relative 95% confidence interval (95% CI). Maximized Youden's index was used to define the optimal cutoff value for each biomarker. The areas under the curve (AUC) between the ROC curves were compared using the DeLong test. For survival analyses, biomarker concentration was naturally log-transformed to fulfill the normal distribution. The Kaplan–Meier estimate was used to calculate the cumulative time-dependent probability of death. Next, we assessed the biomarkers' prognostic value by fitting all fluid biomarkers (continuous values and tertiles) in separate Cox proportional hazard models, alone (univariate analysis) or by including other known prognostic factors in prion disease (i.e., codon 129 genotype, age at sampling, and time from onset to sample collection) as covariates (multivariate analysis), as reported.[17] Survival analysis results are presented as hazard ratios (HRs) and 95% CI. The assumption of proportional hazard was assessed by Schoenfeld residuals. Further details on survival analyses are provided in the Supplementary Material. Spearman's correlation coefficients were calculated to test the possible association between biomarkers' concentrations and disease stage.

3 | RESULTS

3.1 | Demographic variables

The study included a total of 347 participants (mean [SD] age, 68.06 [10.2] years; 186 females [53.5%]). All three main subgroups, AD-MCI (mean [SD] age: 67.7 [8.2] years old), CJD (mean [SD] age: 67.1 [9.5] years old), and np-RPD (mean [SD] age: 73.1 [10.5] years old), were significantly older than HC (mean [SD] age: 61.3 [9.8] years old) (AD-MCI, CJD, and np-RPD, all $p < 0.001$). There were no significant differences in sex distribution between groups. Details on the participants' demographic and biomarker data for the main cohorts are reported in Table 1 and Table S2.

3.2 | The differential effect of AD pathology and CJD clinicopathological subtype on CSF and plasma biomarker values

In the first part of the study, we sought to assess the differential effect of CJD and AD pathological changes on CSF and plasma biomarker levels. For this purpose, we studied the biomarker value distribution in a wide cohort of typical AD-MCI patients and various CJD clinicopathological subtypes (i.e., MM(V)1, VV2, MV2K, MM2C, and M1) compared to HC (Figure 1 and Figure S1). For this analysis, we only considered Aβ-/CJD patients, that is, CJD participants with no evidence of Aβ deposition (defined by a normal $A\beta_{42}/A\beta_{40}$ ratio) to avoid the confounding effect of AD co-pathology.

In CSF, BD-tau levels were significantly elevated in all groups compared to HC (AD-MCI, MM(V)1, VV2, MV2K, MM2C, and M1, all $p < 0.001$), with a higher increase in CJD subtypes than in the AD group. Furthermore, CSF BD-tau was most prominently elevated in the M1, MM(V)1, and VV2 subtypes, compared with the MV2K and MM2C groups. CSF p-tau217 levels were also significantly elevated in the AD-MCI group ($p < 0.001$) as well as all CJD groups compared to HC (MM(V)1, VV2, MV2K, MM2C, and M1, all $p < 0.001$). Notably, VV2, MV2K, and MM2C showed higher values than MM(V)1 and M1 subtypes and AD-MCI participants. The CSF BD-tau/p-tau217 ratio was significantly increased in M1, MM(V)1, and VV2 groups compared to HC (M1, MM(V)1, and VV2, all $p < 0.001$). Conversely, it was significantly reduced compared to HC in AD-MCI ($p < 0.001$) and MV2K ($p = 0.001$) groups. CSF p-tau181 levels were excluded from

TABLE 1 Demographic characteristics and biomarker levels in the main subgroups.

Parameter	HC ($n = 55$)	AD-MCI ($n = 92$)	CJD ($n = 100$)	np-RPD ($n = 100$)
Age at onset	61.3 (9.8)	67.7 (8.2)	67.1 (9.5)	73.1 (10.5)
Female *N* (%)	34 (62)	46 (50)	54/100 (54)	52/100 (52)
CSF				
t-tau[a]	N/A	N/A	4796 (2103–10006)	621 (372–1244)
BD-tau[a]	199 (144–237)	487 (389–714)	3268 (1086–5264)	279 (154–628)
p-tau217[a]	36 (32–53)	232 (187–294)	314 (233–495)	195 (84–405)
p-tau181[a]	N/A	N/A	60 (41–82)	57 (32–87)
NfL[a]	N/A	N/A	6208 (3545–11350)	3078 (1162–12959)
14-3-3[b]	N/A	N/A	56,300 (24,100–126,000)	11,200 (6772–25,200)
$A\beta_{42}/A\beta_{40}$	1.01 (0.95–1.08)	0.48 (0.43–0.55)	0.84 (0.72–0.95)	0.62 (0.47–0.86)
Plasma				
t-tau[a]	N/A	N/A	10.0 (4.1–28.8)	2.8 (1.7–5.1)
BD-tau[a]	4.1 (3.1–5.6)	5.6 (4.1–14.6)	30.0 (12.7–65.7)	9.3 (7.0–17.9)
p-tau217[a]	1.5 (1.2–1.9)	3.6 (2.5–4.8)	2.6 (1.9–3.7)	3.6 (1.8–6.9)
p-tau181[a]	8.4 (7.2–10.0)	15.1 (11.7–21.0)	25.3 (17.7–33.6)	29.9 (17.2–45.0)
NfL[a]	21.6 (14.7–27.7)	28.1 (22.8–43.0)	115.3 (64.7–208.1)	78.8 (35.6–204.9)

Note: Age at onset is expressed as mean (standard deviation). Biomarker values are expressed as median (interquartile range).
Abbreviations: Aβ, amyloid β; AD-MCI, Alzheimer's disease-mild cognitive impairment; BD-tau, brain-derived tau; CJD, Creutzfeldt–Jakob disease; CSF, cerebrospinal fluid; HC, healthy controls; N/A, not assayed; NfL, neurofilament light chain; np-RPD, non-prion rapidly progressive dementia; p-tau181, phospho-tau181; p-tau217, phospho-tau217; t-tau, total tau.
[a]pg/mL.
[b]AU/mL.

this analysis as they were measured with different assays between the two cohorts (see Supplementary Material).

Plasma BD-tau concentrations were higher than HC in all groups (AD-MCI, MM(V)1, VV2, MV2K, MM2C, and M1, all $p < 0.001$), with the most prominent elevations in the M1 and MM(V)1 CJD groups, and to a lesser extent in the MM2C and VV2 groups. Compared to HC, plasma p-tau217 levels were significantly increased in all groups (AD-MCI, MV2K, MM2C, and M1, $p < 0.001$; MM(V)1, $p = 0.047$), apart from VV2, with the most prominent mean differences observed in MM2C, followed by gCJD M1, AD-MCI, and MV2K. In contrast, plasma p-tau181 concentrations were elevated in all groups compared to HC (AD-MCI, MM(V)1, VV2, MV2K, MM2C, and M1, $p < 0.001$). Among sCJD subtypes, MM2C and MV2K, and to a lesser extent the VV2, showed the highest mean values. Plasma BD-tau/p-tau217 ratio was increased in M1, MM(V)1, VV2, and MV2K compared to HC (M1, MM(V)1, and VV2, $p < 0.001$; MV2K, $p = 0.012$). Similarly, plasma BD-tau/p-tau181 ratio compared to HC was increased in M1, MM(V)1, and VV2 (M1 and MM(V)1, $p < 0.001$; VV2, $p = 0.001$), but not MV2K. For both ratios, AD-MCI and MM2C groups showed values in the range of HC.

3.3 | Biomarker diagnostic performance

Next, we calculated ROC curves for all biomarkers and the plasma tau ratios to assess their diagnostic performance in the differential diagnosis between CJD and np-RPD, representing the main question in the clinical setting (Table S3).

In CSF (Figure 2), BD-tau (AUC, 0.89; 95% CI: 0.84–0.93) yielded a diagnostic performance in the range of t-tau (AUC, 0.89; 95% CI: 0.84–0.93), overpowering 14-3-3 (AUC, 0.83; 95% CI: 0.78–0.89), NfL (AUC, 0.63; 95% CI: 0.55–0.71), p-tau181 (AUC, 0.52; 95% CI 0.44–0.60), and p-tau217 (AUC, 0.65; 95% CI: 0.57–0.73) (all $p < 0.001$). Both p-tau181 and p-tau217 were outperformed by all the other biomarkers (all $p < 0.001$), except for NfL ($p = 0.051$ and $p = 0.731$, respectively). Of note, p-tau217 overpowered p-tau181 ($p < 0.001$).

In plasma (Figure 2), BD-tau (AUC, 0.76; 95% CI: 0.70–0.83) diagnostic accuracy was in the range of that of plasma t-tau (AUC, 0.79; 95% CI: 0.73–0.85), and better than that of NfL (AUC, 0.58; 95% CI: 0.50–0.66) ($p < 0.001$), p-tau181 (AUC, 0.57; 95% CI: 0.48–0.65) ($p < 0.001$), and p-tau217 (AUC, 0.61; 95% CI: 0.53–0.69) ($p = 0.006$); however, lower than that of traditional biomarkers measured in CSF, that is, t-tau ($p < 0.001$) and 14-3-3 ($p = 0.014$). Both p-tau181 and p-tau217 yielded a similar diagnostic performance to NfL and were outpowered by t-tau (both $p < 0.001$). The diagnostic accuracy of plasma BD-tau increased when ratioed to p-tau217 (AUC, 0.81; 95% CI: 0.75–0.87), matching that of CSF 14-3-3 (AUC 0.83), but not CSF t-tau (AUC 0.89, $p = 0.002$) or BD-tau (AUC 0.89, $p < 0.001$) (Table S3). This was especially evident in the differential diagnosis with rpAD (AUC, 0.92; 95% CI: 0.88–0.96). Conversely, the plasma BD-tau/p-tau181 ratio did not provide any diagnostic advantage over CSF t-tau ($p < 0.001$) and 14-

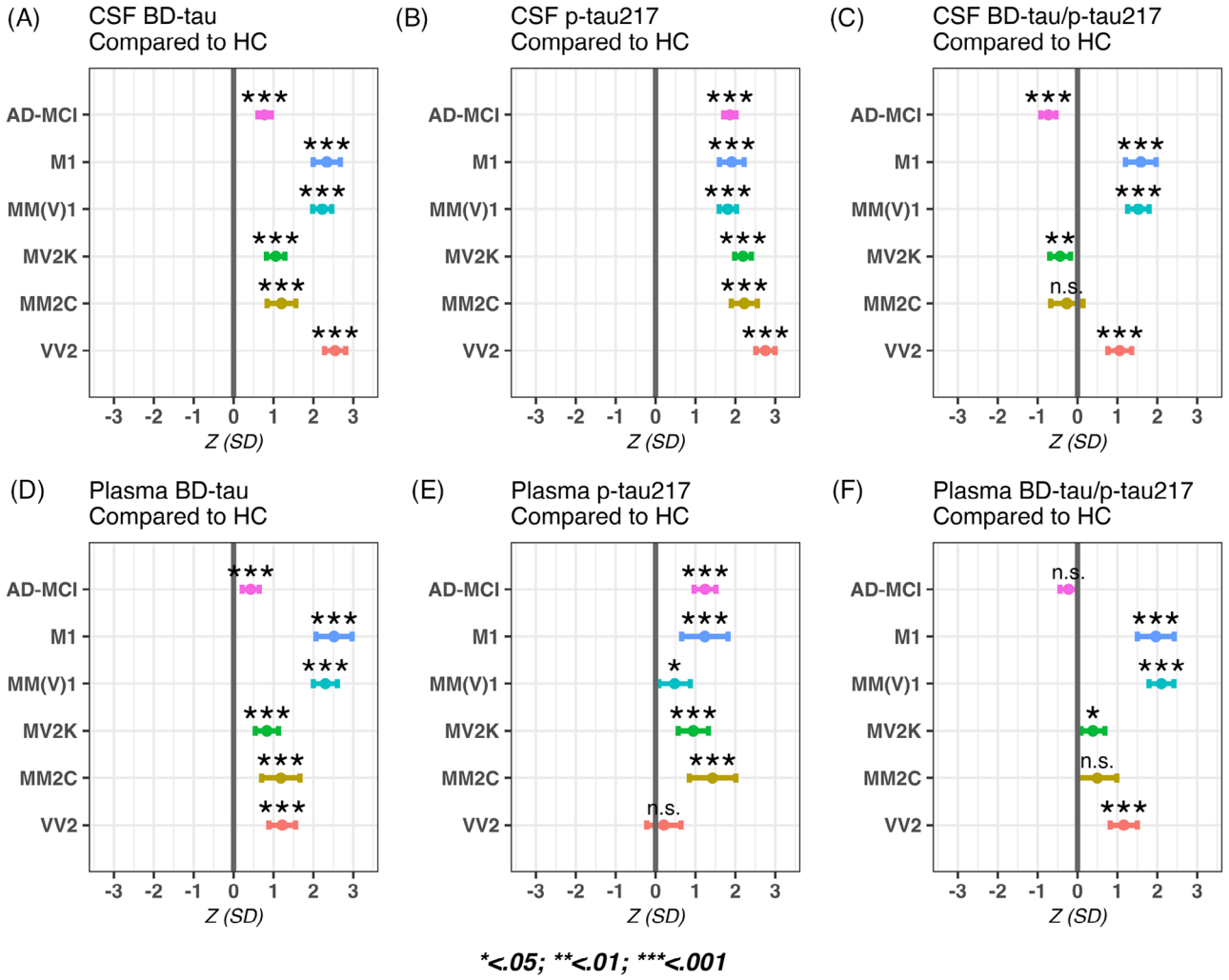

FIGURE 1 CSF and plasma BD-tau, p-tau217, and related ratios mean differences in AD-MCI and Aβ-/CJD subtypes compared to HC. Forest plots show the mean elevation of biomarker levels in CSF and plasma compared to HC across the diagnostic groups. Point estimates are log-transformed and standardized (Z-log), expressed as standard deviations relative to HC participants (represented by a gray vertical bar, normalized to mean 0 as the reference). Error bars denote 95% CIs. All statistical tests were two-sided and unadjusted for multiple comparisons. Aβ, amyloid β; AD-MCI, Alzheimer's disease-mild cognitive impairment; BD-tau, brain-derived tau; CIs, confidence intervals; CJD, Creutzfeldt–Jakob disease; CSF, cerebrospinal fluid; HC, healthy controls; ns, non-significant; p-tau217, phospho-tau217.

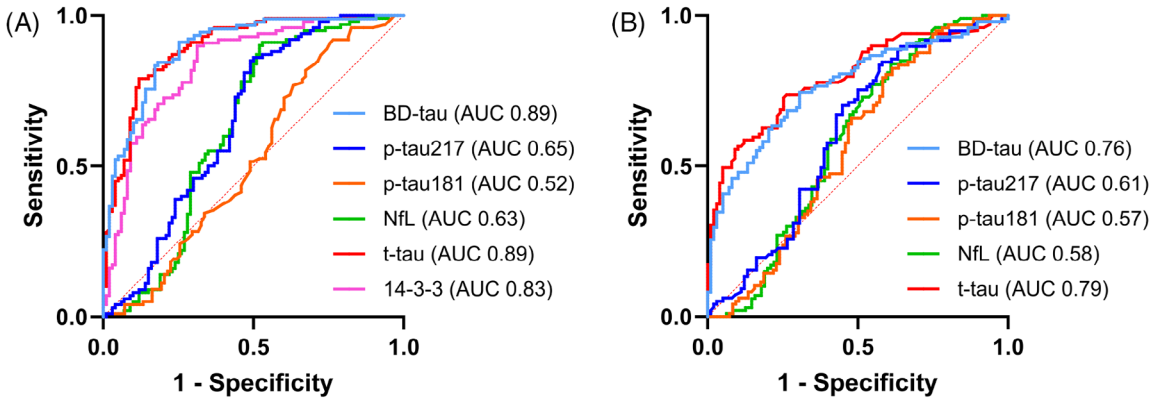

FIGURE 2 Biomarker diagnostic accuracy in discriminating CJD versus np-RPD. ROC curve analyses were performed to assess the accuracy of CSF (A) and plasma (B) biomarkers to discriminate between outcome groups. BD-tau, brain-derived tau; CJD, Creutzfeldt–Jakob disease; CSF, cerebrospinal fluid; NfL, neurofilament light chain; np-RPD, non-prion rapidly progressive dementia; p-tau181, phospho-tau181; p-tau217, phospho-tau217; ROC, receiver operating characteristic; t-tau, total tau.

3-3 ($p = 0.045$). Further details regarding the biomarkers' diagnostic accuracy in the different clinical scenarios are reported in Table S3.

3.4 | Prognostic value of novel tau biomarkers

When considering the whole CJD cohort, CSF and plasma BD-tau levels were significantly associated with survival in both univariate (CSF: HR, 1.51; 95% CI: 1.25–1.82; $p < 0.001$; plasma: HR, 1.63; 95% CI: 1.35–1.97; $p < 0.001$) and multivariate (CSF: HR, 1.48; 95% CI: 1.19–1.84; $p < 0.001$; plasma: HR, 1.68; 95% CI: 1.32–2.14; $p < 0.001$) Cox regression analyses (Table 2). When stratifying for clinicopathological subtype (Tables S4–S6), plasma BD-tau could not predict survival in any subgroup, whereas CSF BD-tau levels negatively correlated with survival only in atypical CJD in the multivariate analysis (HR, 1.54; 95% CI: 1.09–2.19; $p = 0.014$). Conversely, both CSF and plasma p-tau217 levels were not associated with survival either in the whole CJD cohort or in each subtype, except for CSF p-tau217, which significantly correlated with survival in atypical CJD in the multivariate analysis (HR, 3.05; 95% CI: 1.14–8.16; $p = 0.026$). Similarly, CSF and plasma p-tau181 concentrations did not predict survival in the whole CJD group, MM(V)1+M1, and atypical CJD. Conversely, p-tau181 was significantly associated with survival in both plasma (univariate: HR, 4.49; 95% CI: 1.34–15.08; $p = 0.015$; multivariate: HR, 7.37; 95% CI: 1.71–31.5; $p = 0.007$) and CSF (HR, 3.62; 95% CI: 1.04–12.57; $p = 0.043$) (albeit only in the high tertile of multivariate analysis) of VV2 patients. Survival curves for CSF and plasma BD-tau, p-tau217, and p-tau181 are shown in Figure 3 and Figure S2. Survival analysis results in the distinct subgroups are reported in Tables S4–S6.

Regarding the comparison among plasma biomarkers in the whole CJD cohort, in terms of prognostic power BD-tau outperformed both t-tau (univariate: HR, 1.42; 95% CI: 1.21–1.66; $p < 0.001$; multivariate: HR, 1.49; 95% CI: 1.24–1.78; $p < 0.001$) and NfL (univariate: HR, 1.43; 95% CI: 1.11–1.83; $p = 0.005$; not significant in the multivariate analysis) (Table S7 and Figure S2).

3.5 | Association between novel tau biomarkers and disease stage

We also investigated the possible correlation between CSF biomarker levels and the disease stage (Table S8). CSF BD-tau levels were associated with the disease stage in the whole CJD cohort (rho = 0.242, $p = 0.024$), VV2 (rho = −0.485, $p = 0.042$) and atypical CJD (rho = 0.305, $p = 0.001$). Higher plasma BD-tau levels correlated with later disease stages in the whole CJD cohort, but not after stratifying for the clinicopathological subtype. CSF and plasma p-tau217 concentrations were not associated with the disease stage either in the whole CJD cohort or across CJD subtypes, apart from plasma p-tau217, which positively correlated with the disease stage (rho = 0.492, $p = 0.004$) in atypical CJD. Of note, CSF and plasma p-tau181 showed similar behavior, that is, no significant association with disease stage except for plasma levels in VV2 (rho = 0.478, $p = 0.029$).

4 | DISCUSSION

CSF and plasma p-tau217 and p-tau181 are increasingly used in the clinical assessment of neurodegenerative disorders, as they are commonly thought to reflect AD amyloid-associated changes in tau phosphorylation status or secretion.[18,25,28] Moreover, BD-tau is emerging as an accurate blood-based marker of neurodegeneration intensity in the central nervous system.[29,30] Investigating the behavior of these novel markers in CJD, a proteinopathy associated with severe neurodegeneration and extensive phenotypic heterogeneity, is of interest not only to study their value in CJD differential diagnosis and prognostication but also to investigate factors affecting their biofluid levels independently from AD pathology.

In line with a previous report,[18] we demonstrated a marked increase in CSF and plasma BD-tau in AD, and especially in Aβ-/CJD patients compared to HC. CSF BD-tau was most clearly increased in sCJD MM(V)1 and VV2 and gCJD M1 subtypes, reflecting (like t-tau) the massive neurodegeneration and rapid progression. Consistently, plasma BD-tau levels were also significantly increased, although to a lesser extent in VV2 than in MM(V)1 and M1 groups. Besides BD-tau, we demonstrated a significant increase in CSF and plasma p-tau217 and plasma p-tau-181 concentrations in CJD, likely reflecting the secondary tauopathy occurring in this disorder, which was shown to mainly affect the CJD subtypes related to the V2-CJD strain (i.e., VV2 and MV2K).[4,31] Consistently, CSF p-tau217 showed marked increases in VV2 and MV2K individuals. An analogous trend, but less significant, has been previously reported for CSF p-tau181.[4] The present findings underline the conceptual limitation of considering p-tau181 and p-tau 217 and related "occupancy ratios" such as the %p-tau217 as markers indicating "β-amyloidosis,"[32–34] given that they can also increase secondary to other proteinopathies. A definition of early (pre-tangle in case of AD) markers of molecular/biochemical tauopathy would be more correct.

Interestingly, when assayed in plasma, the increase in p-tau181 and p-tau217 levels showed a relative reduction in sCJD VV2 and MV2K, compared to the MM(V)1 subtype, resulting in similar biomarker levels among the three sCJD groups. This effect, already observed with other plasma biomarkers (e.g., tau, NfL, β-synuclein, glial fibrillary acidic protein [GFAP]),[9,14,35] could be related to the different regional lesion profiles between sCJD subtypes. Specifically, the early and prominent cortical involvement in MM2C and MM(V)1, which is not seen in MV2K and VV2 subjects, may lead to a higher spillover of these molecules in the blood than that from subcortical regions.

In the present study, we measured CSF and plasma biomarker concentrations in a large RPD cohort comprising both CJD and np-RPD to compare their diagnostic performance in the main clinical scenarios exemplifying CJD differential diagnosis in clinical practice: CJD versus rpAD and CJD versus nonAD-RPDs (i.e., primarily inflammatory-related conditions and subacute dementias). Overall, CSF t-tau and BD-tau performed tendentially better than all the other CSF and plasma biomarkers. Specifically, neither plasma BD-tau nor p-tau alone added diagnostic value compared to established CSF and plasma surrogate biomarkers (i.e., t-tau and 14-3-3). However, plasma t-tau and

TABLE 2 Biomarker association with survival in the whole CJD cohort.

	Survival time	Univariate Cox regression		Multivariate Cox regression	
Diagnostic group and biomarker	**Median ± IQR (months)**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Whole CJD cohort ($n = 96$)					
CSF BD-tau					
Continuous value	2.0 (1.0–6.0)	**1.51 (1.25–1.82)**	**< 0.001**	**1.48 (1.19–1.84)**	**< 0.001**
Low tertile	7.0 (2.5–11.0)	Ref	Ref	Ref	Ref
Mid tertile	1.7 (1.0–3.0)	**2.80 (1.60–4.88)**	**< 0.001**	**2.85 (1.58–5.14)**	**< 0.001**
High tertile	1.7 (0.9–3.0)	**2.56 (1.47–4.43)**	**0.001**	**1.95 (1.04–3.64)**	**0.035**
Plasma BD-tau					
Continuous value	2.0 (1.0–6.0)	**1.63 (1.35–1.97)**	**< 0.001**	**1.68 (1.32–2.14)**	**< 0.001**
Low tertile	6.2 (2.3–11.0)	Ref	Ref	Ref	Ref
Mid tertile	2.0 (0.8–3.5)	**2.33 (1.39–3.92)**	**0.001**	**2.37 (1.38–4.06)**	**0.002**
High tertile	1.0 (0.7–1.7)	**3.92 (2.29–6.71)**	**< 0.001**	**3.91 (1.93–7.92)**	**< 0.001**
CSF p-tau217					
Continuous value	2.0 (1.0–6.0)	1.19 (0.79–1.79)	0.401	1.38 (0.86–2.19)	0.171
Low tertile	1.6 (1.0–3.7)	Ref	Ref	Ref	Ref
Mid tertile	2.5 (0.9–7.5)	0.84 (0.51–1.38)	0.492	1.12 (0.66–1.88)	0.668
High tertile	2.0 (1.0–3.0)	1.14 (0.69–1.89)	0.601	1.61 (0.88–2.94)	0.120
Plasma p-tau217					
Continuous value	2.0 (1.0–6.0)	1.08 (0.75–1.55)	0.653	1.23 (0.82–1.84)	0.304
Low tertile	1.9 (0.9–5.0)	Ref	Ref	Ref	Ref
Mid tertile	2.0 (1.0–6.0)	1.00 (0.60–1.66)	0.988	0.91 (0.54–1.52)	0.731
High tertile	1.7 (1.0–6.0)	1.11 (0.67–1.84)	0.675	1.23 (0.68–2.21)	0.483
CSF p-tau181					
Continuous value	2.0 (1.0–6.0)	1.16 (0.72–1.85)	0.529	1.22 (0.76–1.95)	0.396
Low tertile	1.8 (1.0–3.5)	Ref	Ref	Ref	Ref
Mid tertile	2.8 (0.9–6.5)	0.96 (0.58–1.58)	0.878	1.22 (0.73–2.03)	0.445
High tertile	1.8 (1.0–4.5)	1.16 (0.70–1.92)	0.543	1.36 (0.81–2.29)	0.236
Plasma p-tau181					
Continuous value	2.0 (1.0–6.0)	0.98 (0.61–1.59)	0.963	1.08 (0.64–1.83)	0.763
Low tertile	1.5 (1.0–6.0)	Ref	Ref	Ref	Ref
Mid tertile	2.0 (1.0–6.0)	0.88 (0.53–1.47)	0.647	0.94 (0.55–1.58)	0.824
High tertile	2.0 (0.8–8.0)	0.91 (0.55–1.51)	0.735	0.97 (0.54–1.73)	0.922

Note: Bold values indicate statistically significant HRs. All multivariate Cox regression analyses included codon 129 genotype, age at sampling, and time from onset to sample collection as covariates.

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; gCJD, genetic Creutzfeldt–Jakob disease; HR, hazard ratio; IQR, interquartile range; Ref, reference; sCJD, sporadic Creutzfeldt–Jakob disease.

BD-tau over p-tau217 (the BD-tau/p-tau217 ratio) matched the diagnostic accuracy of CSF 14-3-3, thus providing an accurate blood-based biomarker for a rapid RPDs diagnostic assessment, especially when the differential diagnosis with rpAD is an issue.[8,36] Having highly accurate blood tests in the clinic that would eliminate the need to perform an LP would be a great improvement, leading to much higher accessibility, for example, in primary care.

In this study, we employed previously validated prognostic models for CJD[17] to study the association between biomarker levels and survival. In contrast to p-tau181 and p-tau217, CSF and plasma BD-tau levels could predict survival in the CJD cohort. This most likely depends on the biological processes these biomarkers reflect, with BD-tau mirroring the massive CNS neurodegeneration[18] and p-tau the prion-related secondary tauopathy.[4] Regarding the strength of the association between biomarker levels and survival, BD-tau was the best-performing biomarker in plasma (overpowering both t-tau and NfL), and it even outperformed some CSF biomarkers included in our cohort (NfL and BD-tau) or previously investigated (α-synuclein, β-synuclein, neurogranin, and GFAP).[14,17,27,35,37] Specifically, the higher prognostic power over t-tau depends on BD-tau correlating better

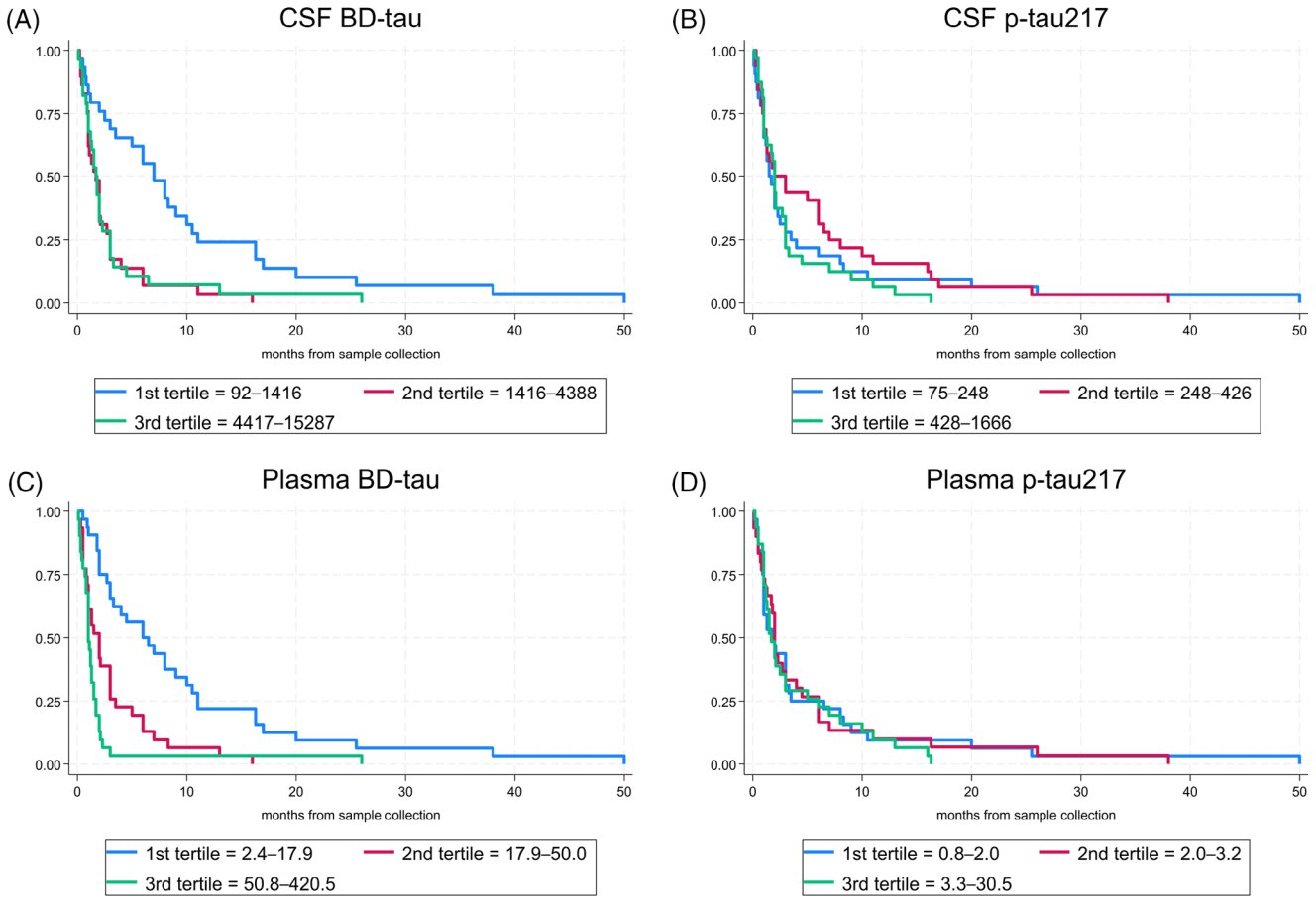

FIGURE 3 Biomarker prognostic performance. Kaplan–Meier survival curves displaying time from sampling to death in the whole CJD cohort as a function of biomarker levels. Biomarker concentrations were binned in tertiles. All patients were deceased at the time of the analysis. BD-tau, brain-derived tau; CSF, cerebrospinal fluid; CJD, Creutzfeldt–Jakob disease; p-tau217, phospho-tau217.

than plasma t-tau with CSF t-tau levels, considered the gold standard peripheral biomarker for CJD prognosis.[17,18]

Of note, plasma BD-tau prognostic performance remained lower than that of traditional CSF biomarkers, specifically t-tau and 14-3-3, as well as some novel (i.e., SNAP-25[27]) surrogate CSF biomarkers, most likely due to the lower capacity of mirroring the neurodegeneration occurring in the CNS (especially in VV2) compared to these CSF biomarkers. Nonetheless, our results support its use as the first-line blood-based (i.e., noninvasive) test for disease monitoring and prognostication in CJD.

When stratifying for clinicopathological subtypes, we found no significant association between CSF and plasma BD-tau levels and survival, except for CSF BD-tau in atypical CJD. In line with this result, within the limitations of having used cross-sectional rather than longitudinal data in predicting the biomarkers biofluid dynamics along the disease course, we reported that CSF (but not plasma) BD-tau levels surge early and remain stable as the disease progresses in the most common subtypes (i.e., MM(V)1 and VV2) but not in atypical CJD, in which BD-tau concentrations increase gradually over the disease course, likely due to the slower progressing neurodegenerative process. This effect, which has also been observed for CSF t-tau, 14-3-3, NfL, SNAP-25,[14,27,38] implies that in atypical CJD, its CSF levels accurately mirror the course (rather than simply the presence) of the disease and therefore have high prognostic value. Regarding plasma BD-tau, we found no association between blood concentrations and disease stage across CJD subtypes, similarly to other plasma biomarkers, thus likely justifying their poor prognostic performance.

Regarding p-tau181 and p-tau217 prognostic value across CJD subtypes, we only found a significant association between survival and CSF p-tau217 in atypical CJD (which mainly includes MV2K cases), and both plasma and CSF p-tau181 (albeit the latter with statistical significance only in the third tertile) in VV2 patients. This might suggest that CJD secondary tauopathy acts synergistically and independently with prion pathology in worsening prognosis in such cases. Alternatively, a more likely explanation is that secondary tauopathy-related biomarkers simply indicate a more advanced disease stage (and therefore reduced survival). This hypothesis is supported by the significant association between CSF p-tau217 and plasma p-tau181 levels with the disease stage in atypical CJD and VV2, respectively.

As a limitation of our study, we recognize that CJD diagnosis and subtype classification could have been mistaken due to the lack of a neuropathological assessment in some patients. However, we have effectively minimized the risks of patient misdiagnosis and misclassification by carefully analyzing medical records, codon 129 genotyping,

and the performance of second-generation prion RT-QuIC. Regarding survival analyses, we are aware that the employment of akinetic mutism in place of time to death when life-extending treatments were adopted might have introduced a bias in the calculation of survival (as we did not use the same variable for all patients). However, we believe that eliminating the significant effect of life-extending treatments would be more accurate than ignoring this variable, which would also introduce a bias in the calculation of disease duration.

In conclusion, our results show that CSF and plasma BD-tau do not provide added diagnostic value compared to the established CSF (14-3-3 and t-tau) and plasma (t-tau) surrogate biomarkers. However, especially when the differential diagnosis with rpAD is an issue, plasma BD-tau (or t-tau) ratios with p-tau variants provide a diagnostic accuracy in the range of CSF 14-3-3. Moreover, we found that plasma BD-tau is a highly accurate blood-based marker for CJD prognostication, envisioning its future use for noninvasive disease monitoring. Finally, our study provides new evidence indicating that increased plasma p-tau181 and p-tau217 levels in CJD strongly depend on the clinicopathological subtype, reflecting the extent of secondary tauopathy. Future studies in independent cohorts should validate our findings.

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CONFLICT OF INTEREST STATEMENT

G.M.B., F.G.O., S.B., A.M. (Andrea Mastrangelo), A.M. (Angela Mammana), S.C., and P.P. declare that they have no competing interests. H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, Roche, and WebMD, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). K.B. has served as a consultant and at advisory boards for Abbvie, AC Immune, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Neurimmune, Novartis, Ono Pharma, Prothena, Roche Diagnostics, Sanofi, and Siemens Healthineers; has served at data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials, and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. B.E.K. has served as a consultant for Biogen and on an advisory board for Eisai. T.F. has served as a consultant and on advisory boards for Biogen, Eisai, Novo Nordisk, Eli Lilly, and Roche. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines and approved by the local ethics committee (approval number AVEC:18025, 113/2018/OSS/AUSLBO). Written informed consent was given by study participants or the next of kin. The DDI study was approved by the Regional Committees for Medical and Health Research Ethics in Norway (REK: 2013/150). All participants provided written informed consent prior to participating in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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