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RESEARCH ARTICLE

Alzheimer's disease biomarker utilization at first referral enhances differential diagnostic precision with simultaneous exclusion of Creutzfeldt-Jakob disease

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Abstract

Most suspected Creutzfeldt-Jakob disease (CJD) cases are eventually diagnosed with other disorders. We assessed the utility of investigating Alzheimer's disease (AD) biomarkers and neurofilament light (NfL) in patients when CJD is suspected. The study cohort consisted of cerebrospinal fluid (CSF) samples referred for CJD biomarker screening wherein amyloid beta 1-42 (Aβ1-42), phosphorylated tau 181 (p-tau181), and total tau (t-tau) could be assessed via Elecsys immunoassays (n = 419) and NfL via enzyme-linked immunosorbent assay (ELISA; n = 161). In the non-CJD sub cohort (n = 371), 59% (219/371) had A+T− (abnormal Aβ1-42 only) and 21% (79/371) returned A+T+ (abnormal Aβ1-42 and p-tau181). In the 48 CJD subjects, a similar AD biomarker profile distribution was observed. To partially address the prevalence of likely pre-symptomatic AD, NfL was utilized to assess for neuronal damage. NfL was abnormal in 76% (25/33) of A+T− subjects 40 to 69 years of age, 80% (20/25) of whom had normal t-tau. This study reinforces AD as an important differential diagnosis of suspected CJD, highlighting that incorporating AD biomarkers and NfL at initial testing is worthwhile.

KEYWORDS
Alzheimer's disease, amyloid beta 1-42, cerebrospinal fluid biomarker, Creutzfeldt-Jakob disease, differential diagnosis, Elecsys, neurofilament light, phosphorylated tau181, rapidly progressive dementia, total tau/phosphorylated tau181 ratio

1 | BACKGROUND

Dementia, characterized by progressive cognitive decline and accompanying behavioral changes, usually advances slowly across many years, but some forms can be rapidly progressive, moving from initial symptoms to dementia and even death within months.[1] Creutzfeldt-Jakob disease (CJD) is prototypical of a rapidly progressive dementia and a most concerning differential diagnosis due to inexorable decline, lack of disease-modifying treatment, and potential transmissibility.[2] A clinical diagnosis of CJD is assisted by laboratory investigations such

as estimation of cerebrospinal fluid (CSF) 14-3-3 and total tau (t-tau) protein levels, as well as the real-time quaking-induced conversion (RT-QuIC) assay to detect misfolded prion protein seeding activity.[3–6] The 14-3-3 and t-tau proteins are non-specific markers of neuronal degeneration, and although the specificity of the RT-QuIC assay is excellent, sensitivity is reduced in some sporadic and genetic CJD subtypes, often those with more atypical clinical presentations.[7] In addition, the differential diagnosis of CJD can be challenging due to non-specific presenting symptoms, which may overlap other neurodegenerative disorders, as well as primarily non-neurodegenerative disorders such as encephalitis, vascular dementia, and psychiatric disorders.[1,8–10]

Long-term observational studies at national prion disease surveillance centers have reported that in a large proportion of cases when sporadic CJD was initially suspected, the diagnosis was eventually excluded and a variety of alternative diagnoses, including potentially treatable disorders, were eventually confirmed.[1,11,12] Alzheimer's disease (AD) is generally the most common alternative diagnosis in rapidly progressive dementias wherein CJD is initially suspected,[13,14] highlighting AD as an important consideration in the differential diagnosis of rapidly progressive dementia despite AD historically being associated with a slower progression. One center reported that of the confirmed non-CJD referrals, 51% were diagnosed with AD whereas another center reported 27% of their confirmed non-CJD referrals being diagnosed with AD.[1] Indeed, ≈25%–30% of AD may be associated with more rapid clinical progression,[15] Hence, to maximize the diagnostic yield from referred CSF samples at first contact, targeted investigation of CSF biomarkers for the most common differential diagnoses of CJD together with CJD CSF biomarkers may offer improved diagnostic utility.

As described, CSF biomarker screening remains a common method for assisting the clinical diagnosis of suspected CJD, underscored by the development of sensitive and highly specific tests such as the RT-QuIC assay. Despite CJD being a rare disease, surveillance centers, such as the Australian National Creutzfeldt-Jakob Disease Registry (ANCJDR), are receiving increasingly more CSF referrals for investigation of suspected CJD.[16] AD CSF biomarkers, including amyloid beta 1-42 (Aβ1-42), a component of amyloid plaques, and phosphorylated tau181 (p-tau), the main component of neurofibrillary tangles, have been validated against amyloid positron emission tomography (PET), the current "gold standard" for the premortem diagnosis of AD, with over 90% concordance.[17–21] The combination of these two CSF biomarkers more effectively discriminates AD from other causes of dementia,[22,23] with reduced CSF Aβ1-42 alone offering reduced specificity such that this change may be observed in vascular dementia and even 38% of CJD patients.[24–27] Technical factors may also contribute to the apparent reduction in CSF Aβ1-42 levels, especially prior to the establishment of pre-analytical handling protocols.[28,29] Strict adherence to pre-analytical CSF handling requirements is necessary for AD biomarker results to be the most reliable and interpretable. As a stand-alone biomarker, CSF p-tau may provide higher specificity than CSF Aβ1-42 in differentiating AD from other dementias, suggesting closer alignment to AD pathogenesis,[23,30] although concerns regarding the sensitivity of diagnostic cut-points have been raised.[31]

RESEARCH IN CONTEXT

Systematic Review: We reviewed the literature using PubMed and Google Scholar for reports from national prion disease surveillance centers and for studies assessing the utility of cerebrospinal fluid (CSF) biomarkers in rapid dementias, especially in Alzheimer's disease (AD) and Creutzfeldt-Jakob disease (CJD).

Interpretation: This study examined the AD biomarker profiles and neurofilament light (NfL) levels in patients with suspected CJD referred to the Australian National Creutzfeldt-Jakob Disease Registry (ANCJDR). Our findings reinforce AD as a major differential diagnosis in suspected CJD, as reported by other national prion disease surveillance centers, and they emphasize that incorporating AD biomarker and the neuronal damage marker NfL at initial CSF screening is worthwhile.

Future Directions: Future studies should capitalize on the increasingly standardized pre-analytical handling requirements of CSF specimens for the routine assessment of AD CSF biomarker assessment and look to reinforce the clinical utility of blood-based biomarkers for CJD and other neurodegenerative diseases.

CSF p-tau may also have greater practical utility due to relative resistance to pre-analytical factors that frequently adversely impact CSF Aβ1-42 levels.[28] Neurofilament light (NfL) is a protein that contributes to the stability of the neuronal cytoskeleton and thereby can act as a non-specific marker of neuro-axonal injury,[32] with neuronal damage inducing increased release of NfL into the CSF. CSF NfL has been reported to differentiate CJD from neurological and non-neurological disorders, such as primary psychiatric illnesses.[14,33,34]

Analogous to the experience of other, longstanding, national CJD surveillance centers, most cases referred to the ANCJDR for biomarker testing for suspected sporadic CJD are eventually diagnosed with other neurological or psychiatric disorders, with prima facie review often determining a low likelihood of CJD. Acknowledging that AD is the most common confirmed alternative diagnosis in patients with suspected CJD, the present study assessed the utility of including AD biomarkers and NfL assessment at initial CSF specimen referral.

2 | METHODS

2.1 | Study cohort and ascertainment of diagnostic outcomes

The ANCJDR receives CSF samples nationally for routine CJD biomarker testing (14-3-3, t-tau, and the RT-QuIC assays); between May 1, 2021, and June 30, 2022, a total of 749 CSF specimens were

received. Routine ANCJDR surveillance activities were undertaken for all referred CSF specimens, allowing classification of CJD according to internationally recognized criteria.[35] The study cohort consisted of all referred CSF samples from this period with sufficient remnant volume after the completion of CJD CSF biomarker analysis to allow additional AD CSF biomarker testing ($n = 419$) and when possible NfL assessment ($n = 161$) at the National Dementia Diagnostics Laboratory (NDDL). The cohort was then divided into CJD ($n = 48$) and non-CJD ($n = 371$) sub-cohorts. For those subjects wherein CJD had been confidently excluded, follow-up letters were systematically sent to the referring clinicians to obtain an updated working clinical diagnosis.

2.2 | CSF specimens

The ANCJDR provides recommendations to referring clinicians regarding CSF specimen collection, temporary storage, and transport, designed for CJD CSF biomarker testing. Prior to March 2022, the ANCJDR recommended a minimum of 2 mL of CSF collected into polypropylene tubes and shipped frozen to the ANCJDR laboratory. After March 2022, the ANCJDR provided an updated protocol, recommending that 2.5 mL of CSF be collected directly into Sarstedt low-binding polypropylene tubes (catalog number 63.614.625) and shipped non-frozen; however, the ANCJDR continued to accept and perform CJD biomarker screening on CSF specimens not conforming to these parameters, so long as the additional 14-3-3 testing requirements for the CSF specimens were met (i.e., red blood cell counts $<500 \times 10^6$/L and white blood cell counts $<10 \times 10^6$/L). All specimens with sufficient volume after the CJD CSF biomarker assays underwent AD CSF biomarker analysis, and, when possible, NfL assessment. Acknowledging the known potential pre-analytical specimen handling effects on CSF Aβ1-42 concentrations, samples were considered compliant for AD CSF biomarker analysis if they were received in polypropylene tubes before March 2022 and after March 2022, if received non-frozen in the Sarstedt low-binding, polypropylene tubes following the update of the Elecsys assay. Specimens received outside of these conditions were considered as non-compliant.

2.3 | Elecsys Aβ1-42, p-tau, and t-tau immunoassays

CSF Aβ1-42, p-tau, and t-tau concentrations were measured in all 419 CSF specimens via electrochemiluminescence immunoassays (Elecsys) on a Roche Cobas e 601 analyzer at the NDDL according to the manufacturer's instructions (Roche Diagnostics Australia, North Ryde, NSW). CSF Aβ1-42 concentrations in specimens referred to the ANCJDR prior to March 2022 were measured via the Elecsys Aβ1-42 Generation I (GI) assay ($n = 312$), whereas concentrations in specimens referred after March 2022 were measured via the Elecsys Aβ1-42 Generation II (GII) assay ($n = 107$) following the update of the assay. The detection ranges and cut-points supporting AD were, respectively: Aβ1-42 GI (200–1700 pg/mL; ≤1000 pg/mL); Aβ1-42 GII (150–2500 pg/mL; ≤1030 pg/mL); p-tau (8–120 pg/mL; >27 pg/mL), and t-tau (80–1300 pg/mL; >300 pg/mL). Concentrations outside the detection range were recorded as the maximum or minimum detectable values for CSF Aβ1-42 and p-tau concentrations, as appropriate, and were used to classify AD biomarker profiles. AD biomarker profiles were compiled based on the AT(N) (A, amyloid; T, tau; N, neurodegeneration) system using the core CSF Aβ1-42 and p-tau concentrations dichotomized through the previously determined cut-points.[36] Subjects with low CSF Aβ1-42 and high p-tau concentrations were considered positive for AD (A+T+) and were assigned profiles "Consistent with AD." Subjects with positive Aβ1-42 and normal p-tau concentrations (A+T–) were assigned profiles "May be consistent with AD pathological change," and subjects with normal Aβ1-42 concentrations (A–) were assigned profiles "Inconsistent with AD" regardless of CSF P-tau concentrations. If Aβ1-42 level was abnormal and the p-tau level was within 5% of the cut-point, as assessed by the coefficient of variation of the assay from internal quality controls, the p-tau/Aβ1-42 ratio was utilized and AD supported, if the ratio was abnormal (GI >0.023).

2.4 | NfL ELISA

CSF NfL was measured in 161 specimens via enzyme-linked immunosorbent assay (ELISA; UmanDiagnostics, Sweden, distributed by Abacus dx, QLD) following the manufacturer's instructions (detection range: 50–5000 pg/mL). Specimens with CSF NfL concentrations above the detection ranges were diluted until detectable concentrations within the calibration curve were measurable, as described previously.[14] Normal reference ranges were determined previously for subjects between 40 and 59 and between 60 and 69 years of age (normal cognition and negative for AD biomarkers) but had not been established for subjects outside of these age ranges.[14,37]

2.5 | Statistical analysis

Age was compared between groups using independent-samples *t*-tests, and sex using the chi-square test. CSF Aβ1-42 concentrations were compared between compliant and non-complaint specimens using the independent-samples *t*-test. CSF Aβ1-42, p-tau, t-tau, and NfL concentrations and the t-tau/p-tau ratio were compared between the A+T+, A+T–, A–, and the CJD groups using the independent-samples *t*-test or the non-parametric Mann–Whitney *U* test where appropriate. Bonferroni correction was applied to account for multiple comparisons for group-wise assessment of CSF concentrations (six tests, $0.05/6 = \alpha < 0.01$). Where *p*-values were greater than the Bonferroni corrected alpha yet less than 0.05, results were classed as nominally significant. Any *p*-values less than the Bonferroni corrected alpha were classed as statistically significant. Comparisons of NfL between CJD and non-CJD groups were performed with and without adjusting for age via a generalized linear modeling. Receiver-operating characteristic (ROC) curves were used to produce area under the curve (AUC),

sensitivity, and specificity of t-tau and t-tau/p-tau in distinguishing CJD from the non-CJD and the A+T+ groups. Statistical analyses were performed using GraphPad Prism (version 9.4.1 software, California USA) and the R statistical environment (Version 4.2.2 R Core Team (2022). R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria: https://www.R-project.org/).

3 | RESULTS

3.1 | Cohort demographic and biomarker profiles

Within the study period, 749 CSF specimens were received by ANCJDR for CJD biomarker assessment, of which 419 specimens with sufficient remnant volume were selected for this study. An overview of the study and the key sub-cohorts, with their demographic and biomarker profiles, are shown in Figures 1, 2 and Table 1. There were no statistically significant differences in sex or age distributions across groups ($p > 0.05$). CSF Aβ1-42 concentrations were compared between compliant ($n = 177$) and non-compliant ($n = 242$) specimens, and no statistically significant difference was found ($p = 0.602$) (Figure SA.1): therefore, all specimens were included for further analysis.

CJD was diagnosed in 11% (48/419) of the study cohort, with half confirmed by neuropathological examination (definite CJD) and the other half deemed "probable sporadic CJD" after detailed review of clinical and biomarker information according to internationally recommended diagnostic criteria for sporadic CJD,[16] leaving a non-CJD cohort of 371 CSF specimens. Among the CJD sub-cohort, 56% (27/48) had biomarker profiles that may be consistent with AD neuropathological change (A+T-) and a further 19% (9/48) had biomarker profiles consistent with AD (A+T+). Within the non-CJD sub-cohort, similar rates of biomarker outcomes were observed, with 59% (219/371) displaying A+T− biomarker profiles and a further 21% (79/371) having A+T+ biomarker profiles (two of whom had normal p-tau levels but abnormal Aβ1-42 and p-tau/Aβ1-42 ratio). In the non-CJD sub-cohort, CSF Aβ1-42 concentrations were similar between the A+T− and A+T+ profiles ($p = 0.2246$).

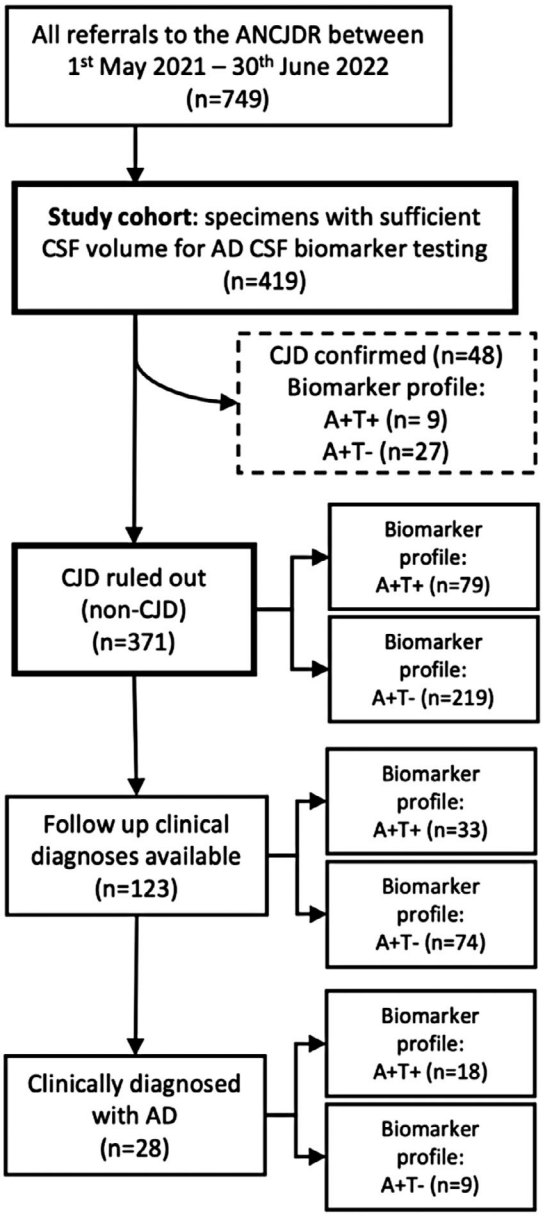

FIGURE 1 Overview of the study cohort and sub-cohorts selected from all referrals to the ANCJDR for CSF biomarker testing between May 1, 2021, and June 30, 2022 ($n = 749$). Note: A+/− = abnormal/normal CSF Aβ1-42 concentration; P+/− = abnormal/normal CSF p-tau concentration.

3.2 | Non-CJD sub-cohort clinical diagnoses and correlation with AD CSF biomarker profile

For the non-CJD sub-cohort, there were 123 responses (33%) from the mailout request to provide follow-up clinical diagnoses. AD was the most common follow-up clinical diagnosis in the non-CJD sub-cohort, determined in 28 subjects (23%), whereas a variety of other neurological and non-neurological diagnoses were established in the remaining 95 subjects. Of those 28 individuals clinically diagnosed with AD, 32% (9/28) had A+T− biomarker profiles and a further 64% (18/28) had A+T+ biomarker profiles, leaving one case of clinical AD with a biomarker profile inconsistent with AD (A−). Of the 95 individuals given non-AD clinical diagnoses, 68% (65/95) had A+T− biomarker profiles, and a further 16% (15/95) had A+T+ biomarker profiles. In total, 60% (74/123) of the non-CJD sub-cohort with available follow-up clinical diagnoses had been assigned A+T− biomarker profiles with a further 33 of 123 (27%) harboring A+T+ biomarker profiles. A detailed breakdown of the clinical diagnoses and the AD biomarker profiles in each group is shown in Table SA.1.

3.3 | CSF NfL, t-tau, and t-tau/p-tau

Sufficient remnant CSF volume after AD biomarker screening (which included t-tau concentrations) allowed NfL to be measured in 161 subjects, including 41 subjects classified with CJD (19 definite CJD and 22

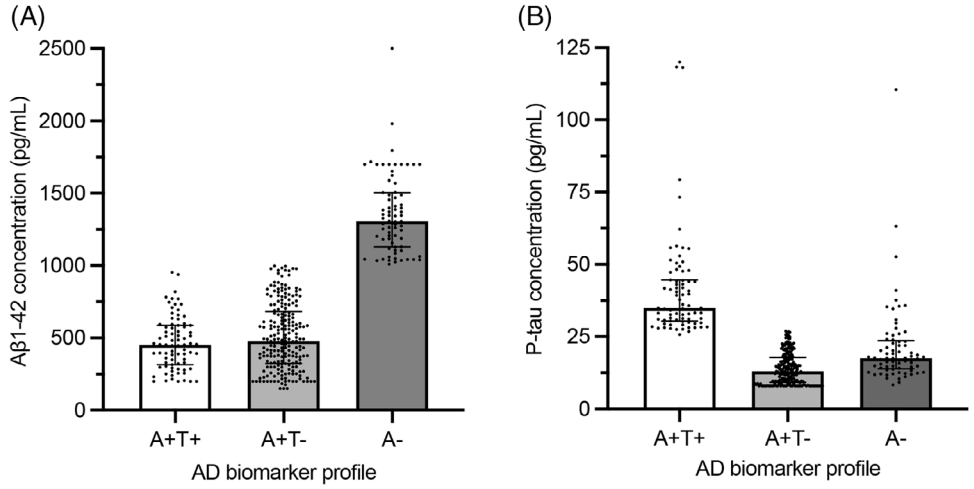

FIGURE 2 Scatter plots of CSF Aβ1-42 (2A) and p-tau (2B) concentrations in the non-CJD sub-cohorts. A+T+, consistent with AD; A+T−, consistent with AD pathologic change; and A−, not consistent with AD. Lines and error bars represent medians and interquartile ranges.

TABLE 1 Demographic and AD biomarker profiles of the non-CJD sub-cohort.

	A+T+ (consistent with AD)	A+T− (consistent with AD pathologic change)	A− (not consistent with AD)		Total
Aβ1-42	P	P	N	N	
p-tau	P	N	P	N	
Total (%)	79 (21%)	219 (59%)	12 (3%)	61 (17%)	371
Female (%)	39 (49%)	90 (41%)	6 (50%)	32 (52%)	167 (45%)
Age (years)	73 (63, 79)	71 (62, 77)	67 (63, 74)	67 (60, 74)	70 (62, 77)
Median Aβ1-42 pg/mL (IQR)	453 (319, 587)	479 (323, 678)	1602 (1395, 1700)	1275 (1117, 1454)	
Median p-tau pg/mL (IQR)	35 (31, 45)	13 (9, 18)	36 (34, 44)	17 (13, 19)	

Abbreviations: Aβ1-42, amyloid β 1-42; IQR, interquartile range; N, concentration is negative for AD; P, concentration is positive for AD; p-tau, phosphorylated tau181.

probable CJD) and 120 non-CJD subjects. Demographic, CSF NfL and t-tau concentrations, the t-tau/p-tau ratio, as well as AD biomarker profiles for the 161 subjects in this group are shown in Table 2 and Figure 3. In non-CJD subjects, 61% (73/120) were assigned A+T− biomarker profiles and a further 27% (32/120) with A+T+. CSF t-tau concentrations and the t-tau/p-tau ratio were significantly higher in those classified as CJD compared to each of the three non-CJD groups (all $p < 0.001$), and both were also significantly higher in subjects with A+T+ compared to those with A+T− ($p < 0.0001$), and those with A− nominally significant ($p < 0.05$).

Subjects with CSF NfL results were age-stratified into the following groups: <40 years ($n = 5$), 40–59 years ($n = 34$), 60–69 years ($n = 44$), and >70 years ($n = 78$) according to predetermined cut-points (≥689 pg/mL for 40–59 years and ≥891 pg/mL for 60–69 years).[14] As observed for the t-tau results, CSF NfL concentrations were significantly higher in subjects classified as CJD compared to each of the three non-CJD groups ($p < 0.001$); however, no statistically significant difference was found between the three non-CJD groups ($p > 0.05$). Adjusting for age did not alter the outcome (unadjusted for age, $p = 9.92^{*}10^{-6}$; adjusted for age, $p = 9.71^{*}10^{-6}$; age correlation with NfL was not significant, $p = 0.328$). CSF NfL abnormality was additionally assessed in non-CJD subjects in each of the A+T−, A+T+, and A− groups and compared to t-tau abnormality in subjects between 40 and 69 years of age ($n = 58$). In subjects with the A+T− profile, 76% (25/33) had abnormal NfL concentrations, surprisingly, 80% (20/25) of whom also had normal t-tau levels. Among subjects with the A+T+ profile, 93% (13/14) had abnormal NfL concentrations, two of whom had normal t-tau levels. In subjects with the A− profile, 45% (5/11) had abnormal NfL concentrations, one of whom had normal t-tau levels.

3.4 | t-tau and t-tau/p-tau ROC curve analysis

ROC curve analysis for definite and probable CJD ($n = 48$) versus non-CJD ($n = 371$) was conducted for t-tau and the t-tau/p-tau ratio (Figure 4A). t-tau achieved an AUC of 0.92 and t-tau/p-tau an AUC of

TABLE 2 Demographic, CSF NfL and t-tau concentrations, AD biomarker profiles and CJD Classification in the NfL sub-cohort.

	CJD Excluded				
	A+T+ (consistent with AD)	A+T− (may be consistent with AD)	A− (inconsistent with AD)	CJD	Total
Total	32	73	15	41	161
Female (%)	17 (53%)	33 (45%)	10 (67%)	20 (49%)	80 (49%)
Age (years)	71 (63, 75)	70 (60, 76)	59 (56, 67)	67 (60, 77)	69 (60, 75)
Median t-tau pg/mL (IQR)	449 (342, 652)	183 (136, 276)‡	238 (188, 526)†	1300 (928, 1300)*	
t-tau/p-tau	10 (9, 12)	14 (11, 22)‡	13 (11, 17)†	41 (30, 65)*	
Median NfL pg/mL (IQR)	1555 (1294, 2912)	1395 (777, 9305)	833 (466, 3700)	7363 (3938, 9780)*	
<40 years (n)	0	3	0	2	5
Median NfL§ pg/mL (IQR)	0	633 (515, 92822)	0	14383 (10873, 17893)	
40–59 years	4	14	8	8	34
Median NfL pg/mL (IQR)	1359 (1135, 2135)	5725 (713, 13386)	551 (406, 1593)	9355 (6631, 15667)	
60–69 years	10	19	3	12	44
Median NfL pg/mL (IQR)	1294 (1160, 1466)	1471 (898, 8021)	972 (750, 2664)	7845 (4648, 11728)	
≥70 years	18	37	4	19	78
Median NfL§ pg/mL (IQR)	2651 (1555, 4092)	1370 (866, 3872)	7521 (4108, 12121)	6520 (1643, 8655)	

Note: Comparison between CJD and non-CJD groups and between A+T+ and A+T− and A− groups were performed using non-parametric Mann–Whitney *U* test with Bonferroni correction.
Abbreviations: IQR, interquartile range.
*$p < 0.001$ between the CJD group and each of the three CJD excluded groups.
†$p < 0.05$ between A+T+ and A+T− groups.
‡$p < 0.0001$ between A+T+ and A− groups.
§NfL concentrations in the <40 years and ≥70 years age groups could not be interpreted due to lack of established cut-points.

0.95. A cut-point of 522.0 pg/mL for t-tau produced a sensitivity of 85.4% and specificity of 89.2%, and a cut-point of 23.4 for t-tau/p-tau produced a sensitivity of 95.8% and specificity of 90.0%. Next, ROC curve analysis for CJD ($n = 48$) versus AD (A+T+) ($n = 79$) was conducted for t-tau and the t-tau/p-tau ratio (Figure 4B). t-tau achieved an AUC of 0.86 and t-tau/p-tau an AUC of 0.98. A cut-point of 659.4 pg/mL for t-tau produced a sensitivity of 83.3% and specificity of 83.5% and a cut-point of 23.4 for t-tau/p-tau produced a sensitivity of 95.8% and specificity of 94.9%. The t-tau/p-tau ratio was superior to t-tau alone both when separating CJD from non-CJD and from AD.

4 | DISCUSSION

CSF biomarker testing is being utilized increasingly to assist in the differential diagnosis of suspected CJD, as reflected by the steadily increasing number of sample referrals to the ANCJDR since 2016.[16] With acknowledging that the majority of CSF samples referred for testing will not support a diagnosis of sporadic CJD (≈90%), this provides an opportunity to utilize these referred CSF specimens to explore alternative diagnoses at this first point of contact. The clinical utility of different biomarker panels in the differential diagnosis and diagnosis of CJD have been explored previously.[38] The present study aimed primarily to assess the utility of routinely incorporating AD CSF biomarkers (Aβ1-42 and p-tau) and NfL testing for rapidly progressive dementias, once sporadic CJD had been excluded, to maximize the diagnostic outcome from CSF specimens and improve diagnostic precision at the time of initial CSF sampling.

A somewhat unexpected finding in the present study was that after ruling out CJD, ≈20% of non-CJD cases had biomarker profiles supportive of AD (A+T+), with a further ≈60% showing findings that may be consistent with the presence of AD neuropathology (A+T−). Abnormal CSF Aβ1-42 concentrations have strong correlation with amyloid PET, and when combined with abnormal CSF p-tau levels, A+T+ subjects are highly likely to have AD neuropathology.[18,19,21] The remaining ≈60% had abnormal CSF Aβ1-42 with normal p-tau concentrations (A+T−), findings that may be consistent with the presence of AD neuropathological changes, although a recent study reported that up to 73% of individuals with abnormal CSF Aβ1-42 and normal p-tau concentrations still evinced AD neuropathology at autopsy.[31] Such observations raise concerns regarding the optimal cut-points for CSF p-tau to support AD diagnosis, which perhaps could be

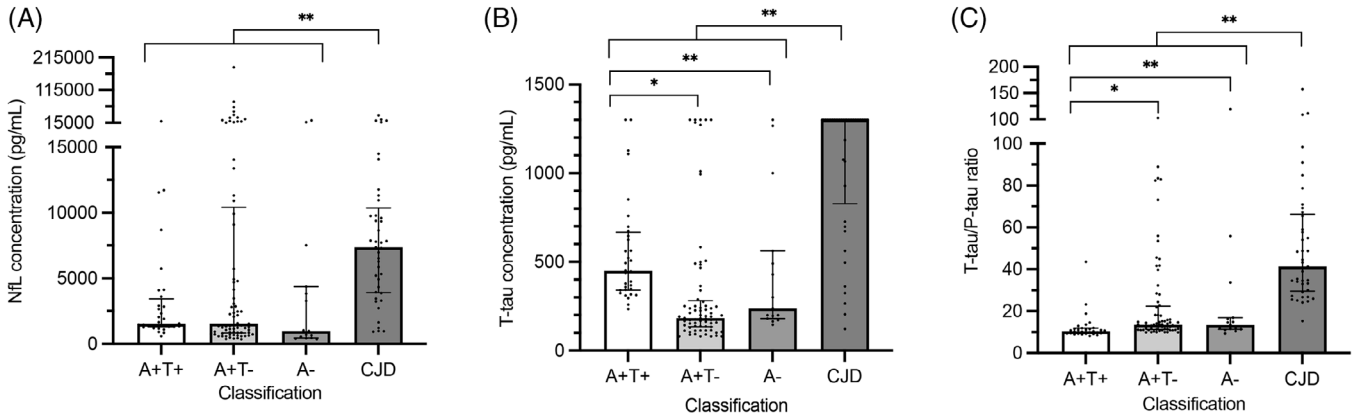

FIGURE 3 Scatter plots of CSF NfL (3A), t-tau (3B) concentrations, and the t-tau/p-tau ratio (3C) in the NfL sub-cohorts. A+T+, consistent with AD; A+T−, consistent with AD pathologic change; and A−, not consistent with AD. Lines and error bars represent medians and interquartile ranges. $^{*}p < 0.01$, $^{**}p < 0.0001$.

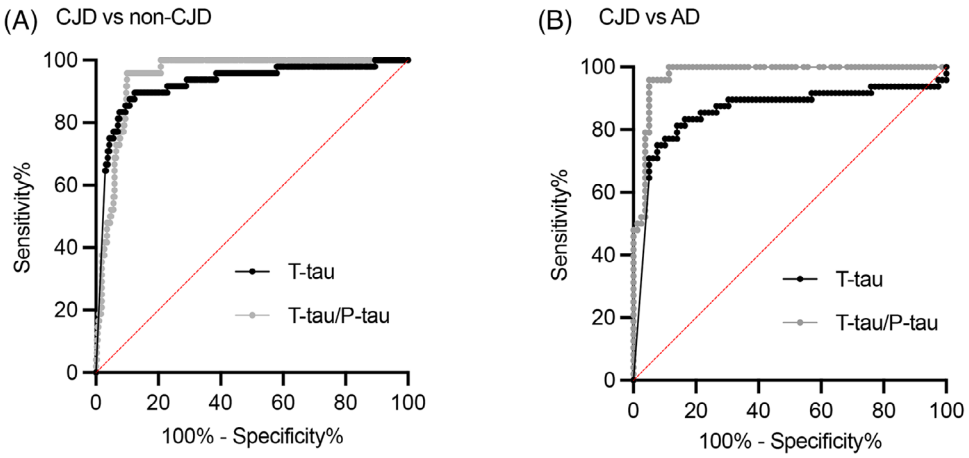

FIGURE 4 ROC curve of t-tau and the t-tau/p-tau ratio for CJD versus non-CJD (4A) and CJD versus AD (4B). AD is classified by being A+T+ (consistent with AD pathologic change. AD, Alzheimer's disease; CJD, Creutzfeldt-Jacob disease; ROC, receiver-operating characteristic.

age-adjusted given that CSF p-tau may exhibit age-related increases similar to CSF t-tau.[39–41] Also noteworthy was that the breakdown of AD CSF biomarker profiles was similar between the CJD and non-CJD sub-cohorts, which may be reflective of the background, especially pre-symptomatic AD neuropathology in the general population of this age group (mean ages 67 and 69 years),[42] although abnormal reduction in CSF Aβ1-42 has been commonly observed (with or without increased p-tau) in definite sporadic CJD with minimal or no AD neuropathology.[24] It is also well established that CSF Aβ1-42 concentrations are extremely sensitive to pre-analytical variables[28,29] and given that some of our samples were received under non-compliant conditions, an uncertain number of CSF Aβ1-42 concentrations across the study cohort could have been artifactually lowered, thereby possibly producing falsely positive A+ profiles. However, given that CSF Aβ1-42 concentrations were compared between compliant ($n = 177$) and non-compliant ($n = 242$) specimens and no statistically significant difference was found ($p = 0.602$), we believe that systematic artifactual bias of this type is unlikely to be a major factor. Also of reassurance, CSF p-tau, t-tau, and NfL are more robust and not affected by the same pre-analytical variables.[28,29]

An additional caveat to the detection of A+T+ and A+T− CSF profiles is their clinical significance. Although the presence of A+T+ is likely to accurately reflect underlying AD neuropathology, this may not explain extant clinical symptoms, as co-morbidities increase in prevalence with age and correct attribution may be difficult. Moreover, akin to what has been reported for CJD in the absence of AD neuropathological changes, studies have reported that patients with Lewy body dementia can exhibit a classic AD biomarker profile (A+T+), with reduced Aβ1-42 being especially common.[43,44] Thus, a biomarker profile being consistent with AD may also be indicative of the presence of Lewy body pathology. Nevertheless, despite these caveats, the presence of an A+T+ CSF profile determines that AD, especially rapidly progressive forms, is a major differential diagnosis of suspected sporadic CJD and is consistent with previous reports by other CJD referral centers identifying AD as a major differential diagnosis of suspected CJD.[1]

In the NfL sub-cohort comprising both CJD and non-CJD subjects, both CSF NfL and t-tau concentrations and the t-tau/p-tau ratio were increased significantly in subjects with CJD compared to the non-CJD sub-cohort, reinforcing the utility of these biomarkers for assisting the diagnosis of CJD, which has been explored previously.[5,14,45,46] For subjects with CJD excluded, CSF t-tau concentrations were increased significantly in those with biomarker profiles consistent with AD (A+T+) compared to other profiles, suggesting those with A+T+ biomarker profiles may harbor more extensive neuronal damage compared to those with A+T- or A− biomarker profiles. On the other hand, CSF NfL did not differentiate between the three non-CJD sub-cohorts, although utility in distinguishing rapidly progressive, including neurodegenerative disorders as a group and from primary psychiatric disorders, has been demonstrated previously.[14] In addition, we investigated the utility of using CSF NfL in combination with Aβ1-42, p-tau, and t-tau to assist differential diagnosis. As expected, CSF NfL concentrations were abnormal in the majority of A+T+ subjects. However, CSF NfL concentrations were also abnormal in 76% of A+T− subjects, a majority of whom also had normal t-tau levels, indicating that NfL can identify abnormality undetected by p-tau and t-tau.

Although CSF NfL may have a limited ability to discriminate between most neurodegenerative and/or neuro-inflammatory disorders, an abnormally elevated NfL concentration in CSF supports an organic basis to neuropsychiatric presentations.[14] CSF NfL, therefore, may provide additional utility when used in combination with AD CSF biomarkers for assisting differential diagnosis after CJD had been excluded.

At only ≈12%, the proportion of confirmed CJD cases among CSF referrals to the ANCJDR is lower than reported for other surveillance centers. For example, an American dementia clinic at the University of California San Francisco (UCSF) reported that 75% of their referred suspected CJD cases were eventually confirmed with CJD, although the total number of referrals at this center was much less than referrals made to the ANCJDR.[1] This suggests that CJD CSF biomarker testing may be employed more sparingly in different milieu with perhaps higher clinical thresholds utilized before entertaining suspicion of CJD or potentially reflecting differences in public health protocols or cost implications in different countries.

The ability of t-tau and the t-tau/p-tau ratio to distinguish CJD from non-CJD, which consists of both neurological and non-neurological conditions (Table SA.1), was assessed. Both t-tau and the t-tau/p-tau ratio achieved an AUC of over 90, with the t-tau/p-tau ratio being marginally superior to t-tau alone, which performed similarly to a previous report.[5] Moreover, the discriminatory power of t-tau and the t-tau/p-tau ratio in differentiating CJD from AD was confirmed. The t-tau/p-tau ratio achieved excellent AUC and was superior to t-tau alone. These results indicate that although RT-QuIC remains the gold standard for the diagnosis of CJD, the t-tau/p-tau ratio can be effectively utilized to differentiate CJD from AD and other differential diagnoses and could be an excellent alternative diagnostic marker for laboratories that lack the feasibility and ability to perform the RT-QuIC assay.

4.1 | Limitations

A sizeable proportion of the CSF specimens in the study cohort were non-compliant with AD Elecsys immunoassay requirements. Notwithstanding CSF Aβ1-42 concentrations were compared between compliant ($n = 177$) and non-complaint ($n = 242$) specimens, and no statistically significant difference was found ($p = 0.602$); consequently, a systematic artifactual bias appears unlikely to be a significant factor. An uncertain number of CSF Aβ1-42 concentrations across the study cohort could have been artifactually lowered, thereby possibly producing false-positive A+ profiles. Fortunately, the many pre-analytical factors known to adversely impact CSF Aβ1-42 concentrations do not appear to significantly influence CSF p-tau, t-tau, and NfL.[28,29] Another limitation is that follow-up clinical diagnoses were available for only a third of the non-CJD sub-cohort, despite follow-up letters being systematically sent to all referring clinicians. Moreover, since these clinical diagnoses were provided non-systematically by different clinicians, the quality of AD and other diagnoses could have varied. Finally, CSF NfL concentration is known to increase and become more variable with increasing age[14]. A significant proportion of the NfL sub-cohort were aged over 70 years for whom a validated cut-point had not been determined and hence we were therefore unable to interpret CSF NfL concentrations in a large proportion of subjects with NfL results.

5 | CONCLUSION

For national diagnostic referral centers attempting to maximize the yield from CSF specimens received, the present study supports attempts to include broader, albeit the most hierarchically relevant, biomarker screening at first specimen testing. Given that the majority of suspected CJD cases referred for testing are eventually diagnosed with other disorders and that AD is a major differential diagnosis, we emphasize the importance of ensuring that pre-analytical CSF handling requirements for AD biomarker testing are adhered to. Furthermore, our study additionally underscores the utility of isolated elevated concentrations of NfL in CSF for discriminating organic from primary psychiatric disorders in persons presenting with neuropsychiatric symptoms. Finally, the development of blood-based biomarkers for CJD and other neurodegenerative diseases is an active area of research[47] and future studies should reinforce findings in clinical cohorts for these novel biomarkers to gain clinical utility.

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CONFLICT OF INTEREST STATEMENT

The authors of no conflicts of interest to report.

CONSENT STATEMENT

Consent was not necessary for suspected CJD diagnostic testing, and ethics approval allowing the use of remnant diagnostic CSF for biomarker studies has been granted by The University of Melbourne Human Research Ethics Committee (Ethics approval ID: 2022-23857-34809-3; Surveillance ID: 20361).

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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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