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Cerebrospinal fluid neurofilament light chain differentiates primary psychiatric disorders from rapidly progressive, Alzheimer and frontotemporal disorders in clinical settings

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PP has received speaker honoraria or consultancy fees to his institution from Chiesi, Eisai, LivaNova, Novartis, Sun Pharma, Supernus, and UCB Pharma. He is an Associate Editor for Epilepsia Open. HZ has served at scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. MW has received research funding, speaker honoraria and has served on advisory boards for Pfizer, Glaxo SmithKline, Eli Lilly, Actelion, Vtesse, and Biomarin pharmaceuticals. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon,

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Statistical Analysis conducted by:

Dr Dhamidhu Eratne

Dr Charles Malpas

Research in context

Systematic review:

We reviewed the literature on PubMed on neurofilament light (NfL). There is extensive data on NfL in neurodegenerative disorders (ND), including recent comprehensive reviews. However, there is limited literature on primary psychiatric disorders (PSY), and especially on NfL assisting on the common clinical diagnostic dilemma of differentiating PSY from ND.

Interpretations:

Our findings significantly extend the literature and provide strong evidence for the diagnostic utility of NfL in real-world clinical settings, to distinguish broad ranges of PSY, from diverse ND, including rapidly progressive ND.

Future directions:

We are building on these findings, investigating diagnostic, clinical, and health economic utility of blood NfL, in a range of specialist and primary care settings, aiming for clinical translation: a simple test to reduce diagnostic delay and misdiagnosis, and dramatically improve the assessment and care for patients with cognitive and psychiatric symptoms, and outcomes for them, their families, clinical trials, and healthcare systems.

ABSTRACT

INTRODUCTION:

Many patients with cognitive and neuropsychiatric symptoms face diagnostic delay and misdiagnosis. We investigated whether cerebrospinal fluid neurofilament light (NfL) and total-tau (T-tau) could assist in the clinical scenario of differentiating neurodegenerative from psychiatric disorders, and rapidly progressive disorders.

METHODS:

Biomarkers were examined in patients from specialist services (neurodegenerative (ND) and psychiatric disorders (PSY)) and a national Creutzfeldt-Jakob registry (CJD and rapidly progressive dementias/atypically rapid variants of common ND, RapidND).

RESULTS:

498 participants were included: 197 ND, 67 PSY, 161 CJD, 48 RapidND, 20 controls. NfL was elevated in ND compared to PSY and controls, with highest levels in CJD and RapidND. NfL distinguished ND from PSY with 95%/78% positive/negative predictive value, 92%/87% sensitivity/specificity, 91% accuracy. NfL outperformed T-tau in most real-life clinical diagnostic dilemma scenarios, except distinguishing CJD from RapidND.

DISCUSSION:

We demonstrated strong generalisable evidence for the diagnostic utility of CSF NfL in differentiating neurodegenerative from psychiatric disorders, with high accuracy.

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INTRODUCTION

There is a great need to improve the timely and accurate diagnosis of neurodegenerative disorders. Despite major advances in clinical neurosciences, many patients with cognitive and neuropsychiatric symptoms still face a significant diagnostic odyssey, of several years of multiple assessments, diagnostic uncertainty and delay, misdiagnoses, imprecise management and prognostication, with significant negative outcomes for patients, families, and healthcare systems.[1,2] These challenges are greater in younger people (onset of symptoms less than 65 years of age), where atypical presentations are more common, and differential diagnoses are broader.[3–5] Clinical diagnoses of behavioural variant frontotemporal dementia (bvFTD) and Alzheimer disease (AD) have an appreciable error rate.[6,7] Multiple factors contribute to the diagnostic odyssey, and even older patients and those with more 'typical' presentations that could present less of a diagnostic challenge, often still face delay and misdiagnosis.[1,8–10] Despite gold-standard, costly and, at times, invasive multimodal and multidisciplinary assessments, many patients continue to face diagnostic uncertainty.[11] In particular, distinguishing neurodegenerative from primary psychiatric disorders is a frequent diagnostic dilemma. Many patients eventually diagnosed with a neurodegenerative dementia, are initially misdiagnosed with psychiatric disorders.[1,12,13] Given numerous challenges that patients face to get timely and accurate diagnosis, a biomarker that could assist in the differentiation between neurodegenerative and psychiatric disorders would have significant implications for the care and assessment, and outcomes of patients.

Neurofilament light chain (NfL), an essential component of the neuronal cytoskeleton, has been shown to be a reliable diagnostic marker of neuronal injury and neurodegeneration in diverse neurological and neurodegenerative disorders.[14–22] In our previous work, we found that CSF NfL distinguished neurodegenerative from primary psychiatric disorders with

high accuracy (AUC 0.94, 87% sensitivity, 90% specificity).[14,15] Elevated CSF total tau (T-tau) concentrations have also been shown to be a non-specific marker of neuronal injury in a range of conditions such as AD, Creutzfeldt-Jakob disease (CJD), and traumatic brain injury.[23–32] Elevations in CSF T-tau appear to be restricted to AD and CJD, with normal levels in other disorders, including most forms of FTD, progressive supranuclear palsy and diseases that have a tau based pathology.[33] NfL and T-tau are markers of neuronal, particularly axonal injury, but are distributed differently in the brain. NfL, although present throughout the neuron is mainly in larger myelinated axons; T-tau is highly prevalent in thin, cortical interneuron non-myelinated axons.[17,32] We thus anticipated that NfL and T-tau levels differed between different broad diagnostic groups and subgroups, and potentially demonstrated differential utility.

This study, part of The Markers in Neuropsychiatric Disorders Study (The MiND Study), aimed to extend our earlier pilot work in a large, diverse cohort of individuals presenting with cognitive and neuropsychiatric symptoms, in whom the differential diagnoses included psychiatric and neurodegenerative disorders. The primary aim was to compare CSF NfL and T-tau levels in patients with neurodegenerative and psychiatric disorders, and control participants. We included patients with neurodegenerative (ND) and primary psychiatric (PSY) disorders seen in a clinical neuropsychiatry service, and patients referred to a national CJD registry who had post-mortem diagnostic confirmation. This second group included patients diagnosed with CJD and patients diagnosed with other rapidly progressive neurodegenerative (RapidND) disorders. Secondary aims included assessing the diagnostic utility of NfL and T-tau in differentiating between ND, PSY and CJD/RapidND groups, and between specific diagnoses within these groups.

METHODS

This study included retrospective and prospective data collected between January 2009 and August 2020, from patients 1) referred for diagnostic assessment of a possible neurodegenerative neurocognitive disorder to a clinical neuropsychiatry service, and neurology/neuropsychiatry clinical trials; 2) with symptoms of a rapidly progressive dementia whose CSF samples were sent to the Australian National Creutzfeldt-Jakob Disease Registry (ANCJDR) for suspected CJD and where there was a post-mortem diagnosis; and 3) control participants.

Neuropsychiatry is a tertiary clinical service in Melbourne, Australia, providing diagnostic input to people with a range of neuropsychiatric presentations, including younger onset dementia.[11] Patients received comprehensive multidisciplinary and multimodal investigations (e.g. MRI and SPECT/PET brain, neuropsychiatry, neurology, neuropsychology, CSF analysis) and received gold standard consensus diagnosis based on established diagnostic criteria, such as NIA-AA criteria for Alzheimer dementia, and international consensus criteria for bvFTD.[34,35] One-hundred-and-twenty-eight participants from our previous study were included (77 ND and 31 PSY from Neuropsychiatry, 20 controls).[14]

ANCJDR is the national CJD surveillance centre, to which patients with a rapidly progressive dementia (including atypically rapid presentations/variants of more common neurodegenerative disorders such as AD, Lewy body dementia), and a suspicion of CJD, are referred for CSF biomarker analysis and diagnosis. ANCJDR operational and surveillance methods, and methods of reviewing clinical criteria have been previously described [36,37]. We included all patients with a post-mortem diagnosis during the study period who had remnant CSF specimens after diagnostic 14-3-3 testing.

Control data was accessed from the Australian Imaging, Biomarker & Lifestyle (AIBL) study of ageing [38]. Inclusion criteria included age less than 70, no neurological or active psychiatric disorder diagnosis at time of CSF, no recent diagnosis (within 18 months of CSF) of neurological disorder, stroke, transient ischaemic attack, traumatic brain injury, or psychiatric disorder, negative amyloid PET, and normal CSF AB42, T-tau and phospho-tau levels.

Group categorisation (according to most recent diagnosis on longitudinal follow up) was made blinded to the NfL levels.

CSF was stored at -80°C, and NfL and T-tau levels measured at the National Dementia Diagnostic Laboratory (NDDL), Melbourne, according to manufacturers' protocols, described previously.[14,15,39] NfL was measured using a commercial ELISA (NF-light; UmanDiagnostics, Sweden). Diluted CSF 1+1 and reconstituted standards were added to the plate in duplicate, incubated, washed. Samples displaying concentrations above the highest standard point were further diluted and re-assayed. T-tau was measured using INNOTEST ELISA (Fujirebio, Belgium). CSF, ready to use calibrators and run validation controls were added to the plate in duplicate, incubated, washed. Two internal controls of pooled CSF were included in each NfL and T-Tau plate. Mean intra-assay coefficient for NfL and T-Tau were 6.2% and 4% respectively, inter-assay coefficient of variation 11.3% and 8.4% respectively.

Consent was not necessary, but where appropriate, participants provided informed consent. This study was approved by Human Research Ethics Committees at Melbourne Health

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Statistical Analysis

Statistical analyses were performed using IBM SPSS 27. General linear models (GLM) were estimated to examine relationships between NfL and clinical variables. Age at CSF was included as a covariate where appropriate, given its strong correlation with NfL.[16,40] Receiver operator characteristic (ROC) curves were computed to determine areas under the curve (AUC), sensitivity, and specificity of NfL and T-tau in distinguishing ND from PSY, and between various subgroups. Optimal cut-off was determined using Youden's (1959) method. Paired-sample area difference under the curve determined differences in biomarker performance. As normality of sampling distribution could not be assumed for all variables, robust statistical methods were used for all analyses. Inference was performed using bias-corrected and accelerated (BCa) confidence intervals, computed for all GLMs via nonparametric bootstrapping (1000 replicates). Statistical significance was defined as any confidence interval not capturing the null-hypothesis value (at the 95% level). These robust statistical methods were selected because they mitigate the effects of distributional violations, including presence of outliers.

RESULTS

Study cohort details (Tables 1 and 2)

A total of 498 participants were included, mean age 63 years, 49% female. Diagnostic groups were neurodegenerative disorders (ND, n=197), primary psychiatric disorders (PSY, n=67), ANCJDR cohort (n=161), mild cognitive impairment (n=5), and controls (n=20).

ND included: older-onset AD (OlderAD, n=52), younger-onset AD (YoungerAD, n=59), behavioural variant FTD (bvFTD, n=33), and other neurodegenerative disorders (OtherND, n=53). PSY were all patients referred to a specialist service for diagnostic assessment of a possible neurodegenerative disorder, and ultimately diagnosed with PSY: schizophrenia (n=17), major depressive disorder (n=16), bipolar disorder (n=12), and other psychiatric disorders (OtherPSY) (n=22). ANCJDR consisted of: CJD (n=161), and other severe/rapidly progressive neurodegenerative disorder (RapidND, n=48). As detailed in Table 2, RapidND included many atypically rapid variants/presentations of more common neurodegenerative disorders (such as AD (n=13), and Lewy body dementia (n=7)), where CJD was suspected clinically due to the aggressive presentation.

ND patients were older than PSY (62 vs 49 years; mean difference, Mdiff=13, 95%CI:[10, 16]) and controls were older than PSY (66 vs 49 years; Mdiff=17, 95%CI:[11, 23]).

CSF NfL and T-tau in neurodegenerative, primary psychiatric, and control groups

As demonstrated in Table 1 and Figure 1, CSF NfL levels were elevated in ND (mean, M=1528pg/mL) compared to PSY (M=435pg/mL), and controls (M=523pg/mL). Adjusting for age, the difference between NfL levels in ND and PSY groups was large (GLM, Mdiff=1089, 95%CI:[640, 1539]), as was the difference between ND and controls (GLM Mdiff=1007, 95%CI:[653, 1359). There was no difference between PSY and controls, after adjusting for age (GLM Mdiff=82, 95%CI:[-174, 338]). The spread of NfL levels in PSY was much narrower than in ND, and no patients in PSY had an NfL level greater than 802pg/mL (Figure 1).

CSF T-tau was significantly elevated in ND (M=514pg/mL), compared to PSY (M=157pg/mL; Mdiff=250, 95%CI:[190, 309]), and controls (M=182pg/mL; Mdiff=370, [233, 507]), Table 1 and Figure 2. There was no difference between PSY and controls (Mdiff=120, 95%CI:[-37, 389])

NfL distinguished ND from PSY with high accuracy (AUC 0.94, 92% sensitivity, 87% specificity at 582pg/mL cut-off), Figure 3 and Table 3. NfL outperformed T-tau (AUC 0.88, 82% sensitivity, 83% specificity at 198pg/mL cut-off), paired-sample AUC difference, AUCdiff=0.06, 95%CI:[0.01, 0.10].

NfL at the 582pg/mL cut-off distinguished ND from PSY with a 95% positive predictive value (PPV), 78% negative predictive value (NPV), 6.8 positive likely ratio (LR+ve), 0.09 negative likely ratio (LR-ve), accurately classifying 91% (239/264) of patients, diagnostic odds ratio of 73 (Table 4). NfL performed better than T-tau, which at a cut-off of 198pg/mL was associated with 94% PPV, 60% NPV, 4.8 LR+ve, 0.22 LR-ve, 82% accuracy, diagnostic odds ratio of 22.

CSF NfL and T-tau in CJD and RapidND

The highest NfL and T-tau levels were seen in CJD (mean NfL=4767pg/mL and mean T-tau=4921pg/mL), and other severe/rapidly progressive disorders (RapidND), mean NfL=4857pg/mL and mean T-tau=1123pg/mL), Table 2 and Figures 1, 2, 4 and 5. T-tau better distinguished CJD from RapidND, than NfL (sensitivity 83% vs 80%, specificity 79% vs 50%, AUC 0.86 vs 0.60, AUCdiff=0.25, 95%CI:[0.16, 0.35]).

As demonstrated in Table 3 and Supplementary Material, NfL performed better at distinguishing RapidND from ND, compared to T-tau. NfL and T-tau were not significantly different in distinguishing RapidND from PSY. NfL and T-tau both distinguished CJD from PSY with extremely high accuracy. Combining all neurodegenerative disorders in our cohort (i.e. CJD, RapidND from ANCJDR, and ND from Neuropsychiatry) in to a combined 'CJD+RapidND+ND' group, improved the diagnostic performance of NfL and T-tau in differentiating from PSY (compared to ND (without ANCJDR) vs PSY).

CSF NfL and T-tau levels in diagnostic subgroups

To reflect real-life clinical diagnostic dilemma scenarios, NfL and T-tau levels and diagnostic performance were compared between a range of combinations of diagnostic subgroups (Table 3 and Supplementary Material). NfL levels in all ND, and each ND subgroup (OlderAD, YoungerAD, bvFTD, OtherND), were higher than levels in all PSY, and each PSY subgroup (schizophrenia, MDD, BPAD and OtherPSY). NfL levels in CJD and RapidND were higher compared to ND, ND subgroups, PSY, PSY subgroups. No differences were found between YoungerAD and OlderAD, between ND subgroups, between PSY subgroups, or between all PSY subgroups and controls.

CSF T-tau was not consistently elevated in ND subgroups, compared to PSY subgroups. T-tau levels were not different between bvFTD and several PSY subgroups (schizophrenia, OtherPSY, MDD and BPAD), and bvFTD and controls (Table 3 and Supplementary Material). Furthermore, T-tau levels were not different between OtherND and MDD, OtherND and BPAD, and OtherND and controls. There were no differences in T-tau between PSY subgroups.

As demonstrated in Table 3 and Supplementary Material, NfL was superior to T-tau in distinguishing: ND from PSY, bvFTD from PSY, bvFTD from schizophrenia, bvFTD from bipolar disorder, bvFTD from depression, and RapidND from ND. T-tau performed better than NfL in distinguishing CJD from RapidND, and CJD from ND.

DISCUSSION

Our findings in a large cohort of patients from real-world clinical settings showed that CSF NfL levels distinguished diverse neurodegenerative disorders (ND) from primary psychiatric disorders (PSY), with high accuracy. CSF NfL levels can assist the clinician in the common, clinical challenge of distinguishing neurodegenerative from primary psychiatric disorders, leading to earlier, accurate diagnosis. These findings confirm and extend our previous work.[14] A significant strength and substantial contribution to the literature, is that data was derived from real-world Australian tertiary and quaternary services and clinical settings, to which patients presented with diverse clinical presentations and diagnostic uncertainty. Additional strengths included longitudinal follow up for most patients, consistent gold-standard multimodal and multidisciplinary assessments, diagnoses based on established diagnostic criteria, and broad types of ND and PSY.

Given the PPV of 95%, an elevated CSF NfL in a patient with an initial neuropsychiatric presentation reduces the likelihood of a primary psychiatric misdiagnosis, and indicates need for further investigation. In a patient with an existing primary psychiatric diagnosis, an elevated NfL level would prompt further investigation for a neurodegenerative diagnosis. Conversely, a low level could reassure that a neurodegenerative disorder is unlikely, reinforce the need for psychiatric treatment, and reduce the need for further investigations

and referrals to specialist services. Caution and close clinical monitoring would still be warranted, given the NPV of 78% and that a low NfL does not entirely rule out a neurodegenerative disorder.

Our study adds important data to the literature on the high accuracy of NfL in assisting in a broad range of frequently faced, specific clinical diagnostic dilemmas (Table 3), especially in younger people. Distinguishing bvFTD from PSY can be particularly challenging; bvFTD is associated with greater diagnostic delay and rates of psychiatric misdiagnoses.[6] Recent expert consensus recommendations highlighted the potential role of biomarkers such as NfL.[6] Our study included patients with bvFTD and PSY referred explicitly with a question of whether the clinical picture was due to a primary psychiatric or neurodegenerative condition, unlike previous studies which included patients from general psychiatric services where there was no clear suspected or differential diagnosis of bvFTD, or excluded patients with comorbidities (e.g. substance abuse, head injuries). Despite relatively small numbers of patients with bvFTD, our findings, demonstrating high accuracy in this challenging clinical scenario, provides evidence for the real-world diagnostic utility of NfL in distinguishing bvFTD from PSY. Given the often non-diagnostic neuroimaging findings, and lack of diagnostic biofluid markers, a strong case can be made for NfL as a first-tier test.[6] As a single biofluid biomarker for the distinguishing AD from PSY, NfL has superior AUC, sensitivity and specificity, compared to MRI to distinguish AD from controls.[41] Further clinical utility and health economic analysis studies are needed, to determine whether NfL (especially plasma NfL) has a place as a first-tier routine test for all patients and differential diagnoses, or best reserved for specific situations.

A recent study of 162 patients referred for evaluation of cognitive disorders investigated the diagnostic utility of CSF NfL and T-tau.[42] For differentiating ND from PSY, this study found

AUCs of NfL / T-tau of 0.877 / 0.857 respectively, sensitivity 76.6% / 93.8%, specificity 86.4% / 67%. This contrasts with our larger, younger cohort, where NfL differentiated ND from PSY with superior AUC, sensitivity, specificity, PPV, NPV. We found NfL outperformed T-tau in distinguishing ND from all PSY, and even more so for distinguishing ND+CJD+RapidND from all PSY. Also, we found no benefit for a combination of NfL and T-tau, or sequential use (except for differentiating CJD from RapidND).

Patients were referred to a tertiary neuropsychiatry service, most commonly by neurologists and psychiatrists, for diagnostic assessment and clarification. The referral reason in most cases was to assist with distinguishing neurodegenerative from psychiatric differential diagnoses. CSF analysis was performed where clinically indicated, and not routinely if other assessments were clearly diagnostic. This study thus focusses on a clinical population which is more 'atypical' and complex than may be seen in routine practice. However, not every such patient gets referred, nor do most places in the world have access to such tertiary services. Patients in other clinical settings and with less complexity, also face diagnostic dilemmas. Therefore, even though our study builds definitive evidence for diagnostic utility in tertiary and quaternary services, it is of relevance to secondary specialist (e.g., neurology, psychiatry, geriatrics) and other clinical settings where most patients with cognitive and neuropsychiatric symptoms assessed. Further study to confirm generalisability to less specialised and lower prevalence settings are needed, and are underway.

Extremely high CSF NfL levels were seen in CJD, consistent with other studies.[43] Similarly elevated levels were seen in RapidND (such as encephalitis, rapidly progressive presentations of AD and DLB), suggesting NfL is a marker not only of neurodegeneration, but also of the severity and rate of progression in these presentations. This contrasts with similar studies that found lower NfL in comparable RapidND groups (which also included

disorders such as AD, DLB).[43,44] This could possibly reflect more severe cases in our cohort. Our findings suggest that T-tau may help differentiate CJD, from RapidND with a suspicion of CJD. These findings extend the literature on CJD biomarkers.[26,31,43–48]. As our RapidND group consisted mostly of atypically rapid/aggressive variants of more common neurodegenerative disorders, further study is needed on NfL and T-tau in a broader range of rapidly progressive and rarer dementias. Further study is also needed on NfL, T-tau and specific tests such as RT-QuIC in CJD and RapidND, and to determine whether NfL could act as a screening test in the earliest stages of CJD, in particular in less common instances of psychiatric presentations and differentials, and prior to the onset of frank neurological signs.

Limitations of our study include its retrospective nature, reliance on clinical diagnosis, lack of pathological or genetic confirmation in the ND group (all AD cases were sporadic). A significant limitation is the small number of controls, and the older, narrow age range in this group. Younger ND still had greater NfL levels than controls, demonstrating much greater increases in NfL levels due to neurodegeneration, relative to well-known age-related increases.[16] The older controls may have reduced our ability to find a difference from younger PSY. Patients recruited later in the study period had 8-12 months of follow up information, and potentially less certainty based on time and serial assessments, compared to earlier patients. Although people with MCI seemed to have intermediate NfL levels (between ND and PSY), the very small number in this group limited any comparisons and interpretations. It is important to note that T-tau levels were incorporated into the clinical diagnoses (as compared to NfL levels, which were blinded to clinical diagnoses and diagnostic categorisation), therefore it is likely that T-tau performance was overestimated. This is particularly likely to be the case in AD diagnoses where CSF AB42, T-tau and phosphorylated tau were incorporated into diagnostic decisions as per diagnostic criteria for AD. The overall study cohort when viewed as a whole, can appear unbalanced, given the

large CJD group relative to other groups. We focused on distinguishing between ND and PSY groups (comparable or larger than similar studies in clinical settings), and between specific NDs and PSYs, considering these as the most generally clinically relevant distinctions. Data on one of the largest post-mortem confirmed cohorts of CJD and RapidND where CJD was suspected clinically, demonstrated important biomarker performance findings in and comparisons between, an even more extended and diverse set of presentations.

This study demonstrated the diagnostic utility of CSF NfL to distinguish a broad range of neurodegenerative disorders from primary psychiatric disorders and controls, in real-world, generalisable clinical settings, with high accuracy. NfL performed well in a range of commonly faced clinical diagnostic dilemmas, such as differentiating bvFTD from a range of primary psychiatric disorders, where this was an explicit clinical question. A patient with a significantly elevated NfL level could quickly dismiss primary psychiatric disorder differentials, and lead to appropriate, tailored investigations and referrals. An extremely high NfL level (with or without an elevated T-tau) would prompt urgent investigations and assessments, and even hospitalisation. Conversely, a low NfL could reduce the chance of a misdiagnosis of a neurodegenerative disorder, support investigations and a treatment of a primary psychiatric disorder, and reduce unnecessary investigations and referrals. Stability of levels on serial testing, could offer further reassurance. An added potential of NfL as a 'first tier' test for neurodegeneration, could be to help facilitate research and precision use of more specific tests, (e.g., phosphorylated tau for AD).

Given CSF and plasma NfL levels correlate strongly,[16,22] the availability of a blood test could dramatically alter the care, assessment, diagnosis and treatment of patients. A plasma NfL level could form a part of a 'dementia blood screen' ordered by a general practitioner.

The level could inform and guide initial clinical management, referrals, and triaging. We are building on our findings, investigating the diagnostic and health economic utility of plasma NfL in specialist and primary care settings, with the aim of developing an inexpensive blood test which can act as a “CRP” for the brain, that could significantly reduce diagnostic delay and misdiagnosis, and improve outcomes for patients, families, and healthcare systems.

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FIGURES (in separate files)

Figure 1. Cerebrospinal fluid neurofilament light chain levels in rapidly progressive disorders, neurodegenerative disorders, primary psychiatric disorders, and controls. Light red dotted line=optimal cut-off of ND vs PSY from ROC curve analyses ***: p<0.001; ANCJDR PM: Australian National CJD registry rapidly progressive post-mortem patients with CJD and other rapidly progressive disorders; CSF: cerebrospinal fluid; ND: neurodegenerative disorders; NfL: neurofilament light chain; PSY: primary psychiatric disorders

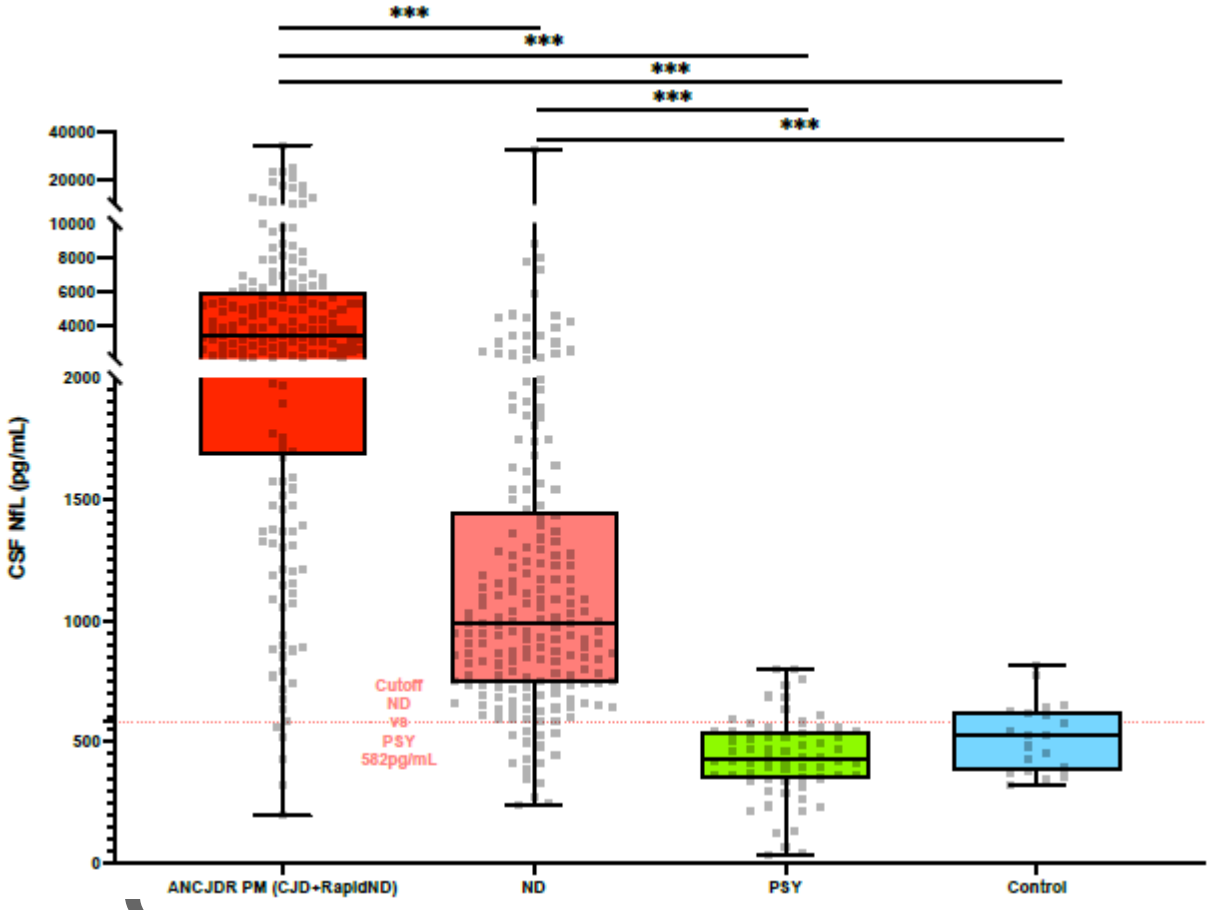


Figure 2. Cerebrospinal fluid total tau levels in rapidly progressive disorders, neurodegenerative disorders, primary psychiatric disorders, and controls. Light red dotted line = optimal cut-off of ND vs PSY from ROC curve analyses.***: p<0.001; ANCJDR PM: Australian National CJD registry rapidly progressive post-mortem patients; CSF: cerebrospinal fluid; ND: neurodegenerative disorders; PSY: primary psychiatric disorders; T-tau: total tau

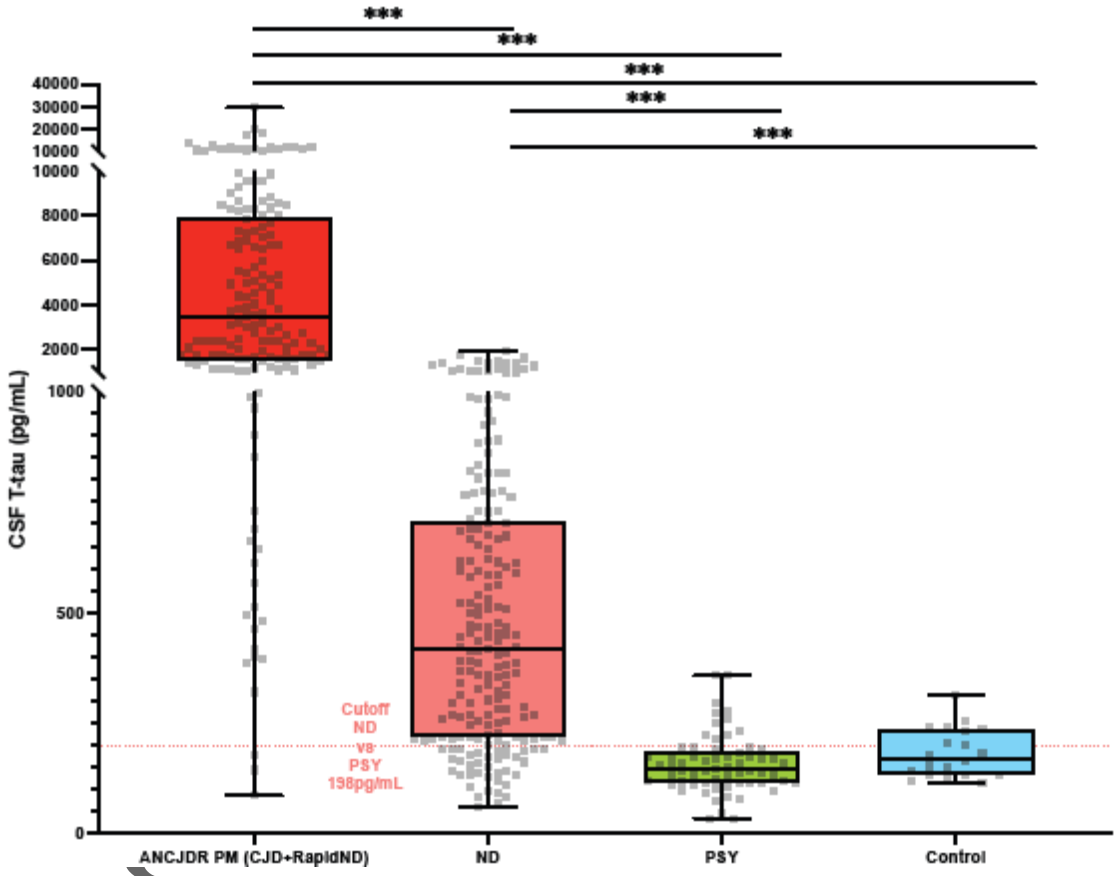


Figure 3. ROC curve for neurodegenerative versus primary psychiatric disorders

NfL: neurofilament light chain protein; T-tau: total tau

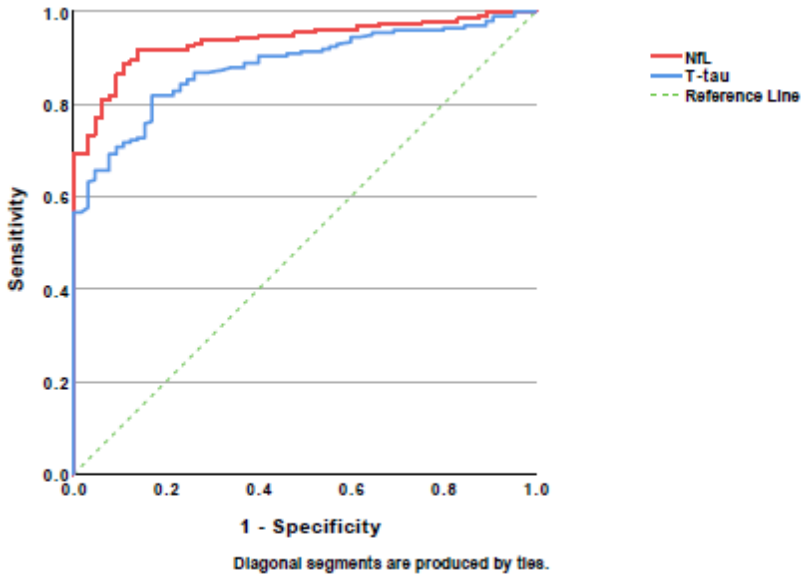


Figure 4. Cerebrospinal fluid neurofilament light chain levels in rapidly progressive disorders from ANCJDR (dark red), neurodegenerative disorders (ND, light red), psychiatric disorders (green) and controls. Light red dotted line = optimal cut-off of ND vs PSY from ROC curve analyses.

ANCJDR: Australian National Creutzfeldt-Jakob Disease Registry severe rapidly progressive post-mortem confirm patients; AD: Alzheimer disease; BPAD: bipolar affective disorder; bvFTD: behavioural variant frontotemporal dementia; OlderAD: older-onset Alzheimer disease; MCI: mild cognitive impairment; MDD: major depressive disorder; ND: neurodegenerative disorder; RapidND: other severe rapidly progressive neurodegenerative disorder; PM: post mortem; PSY: primary psychiatric disorder; YoungerAD: younger-onset Alzheimer disease

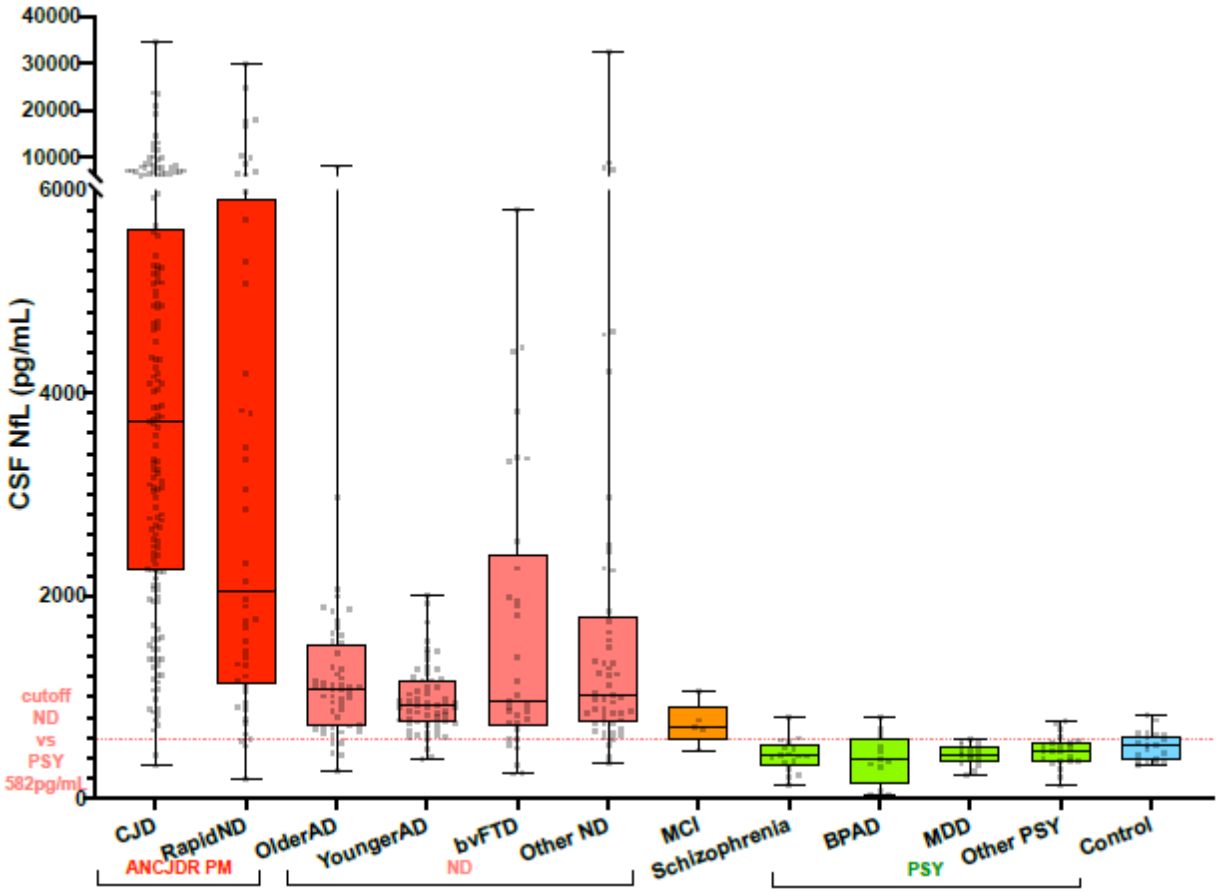
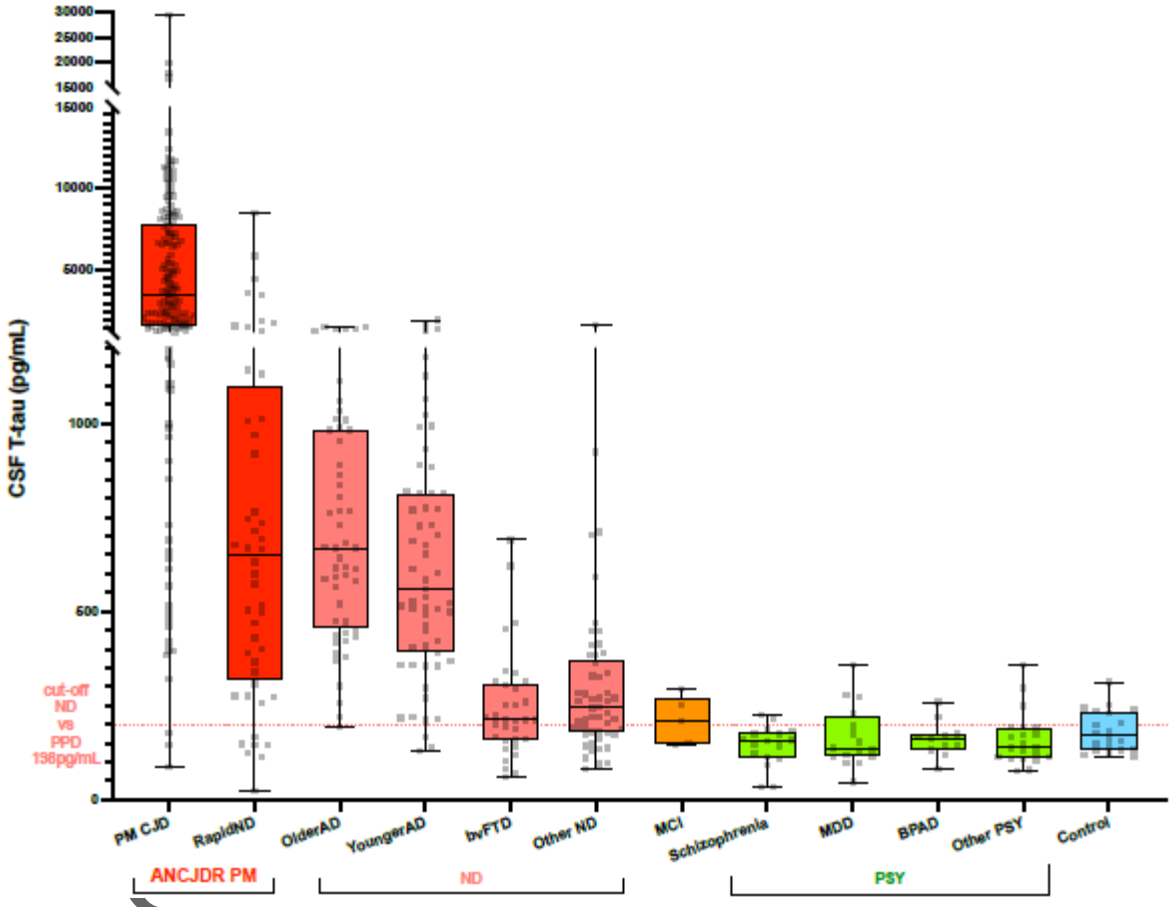


Figure 5. CSF T-tau levels in rapidly progressive disorders from ANCJDR (dark red), neurodegenerative disorders (ND, light red), psychiatric disorders (green) and controls. Light red dotted line = optimal cut-off of ND vs PSY from ROC curve analyses.

ANCJDR: Australian National Creutzfeldt-Jakob Disease Registry severe rapidly progressive post-mortem confirm patients; AD: Alzheimer disease; BPAD: bipolar affective disorder; bvFTD: behavioural variant frontotemporal dementia; OlderAD: older-onset Alzheimer disease; MCI: mild cognitive impairment; MDD: major depressive disorder; ND: neurodegenerative disorder; RapidND: other severe rapidly progressive neurodegenerative disorder; PM: post mortem; PSY: primary psychiatric disorder; YoungerAD: younger-onset Alzheimer disease



TABLES AND FIGURES

	ANCJDR (CJD + RapidND)	Neurodegenerative disorders (ND)	Primary psychiatric disorders (PSY)	Control	Difference*
N	**209**	**197**	**67**	**20**	
Age at CSF, y	**68 [67, 69]**	**62** [60, 63]	**49** [46, 52]	**66** [65, 67]	PM>ND, PM>PSY, ND>PSY, C>ND, C>PSY
Female	**107 (51%)**	91 (46%)	32 (48%)	15 (75%)	C>ND, C>PSY, C>PM
NfL, pg/mL	**4792 [4109, 5476]**	**1528** [1168, 1888]	**435** [394, 476]	**523** [457, 590]	PM>ND, PM>PSY, PM>C, ND>PSY, ND>C
T-tau, pg/mL	**4049 [3465, 4632]**	**514 [463, 565]**	**157 [141, 173]**	**182 [156, 208]**	PM>ND, PM>PSY, PM>C, ND>PSY, ND>C

Table 1. Demographics of Australian National Creutzfeldt-Jakob Disease Registry rapidly progressive disorders, neurodegenerative disorders, primary psychiatric disorders, and controls

Data is Mean, [95% CI] or n, (%)

* details only differences in groups where confidence intervals do not capture the null hypothesis value

ANCJDR: Australian National Creutzfeldt-Jakob Disease Registry; CJD: Creutzfeldt-Jakob disease; ND: neurodegenerative disorder; PSY: primary psychiatric disorder; RapidND: other severe/rapidly progressive disorders referred for suspected CJD

	ANCJDRPM		ND				PSY				MCI	Control
Subgroup	**CJD**	**RapidND[a]**	**OlderAD[z]**	**YoungerAD[z]**	**bvFTD**	**OtherND[b]**	**Schizophrenia**	**MDD**	**BPAD**	**OtherPSY[c]**		
N	**161**	**48**	**52**	**59**	**33**	**53**	**17**	**16**	**12**	**22**	**5**	**20**
Age at CSF, y	**67** [66, 69]	**71** [68, 74]	**73** [72, 75]	**58** [57, 60]	**56** [53, 59]	**58** [55, 61]	**42 [34, 49]**	**54 [48, 60]**	**46 [36, 56]**	52 [47, 57]	**60 [52, 67]**	**66** [65, 67]
Female	87 (54%)	20 (42%)	28 (54%)	33 (56%)	9 (27%)	21 (40%)	10 (59%)	7 (44%)	5 (45%)	10 (45%)	1 (20%)	15 (75%)
NfL, pg/mL	**4767** [4063, 5472]	**4875** [3005, 6745]	**1263** [963, 1562]	**975** [887, 1063]	**1694** [1186, 2202]	**2301** [1035, 3567]	**426 [342, 509]**	**433 [379, 487]**	**389 [228, 549]**	469 [299, 538]	**739 [478, 1001]**	**523** [457, 590]
T-tau, pg/mL	**4921 [4229, 5613]**	**1123 [662, 1584]**	**737 [643, 833]**	**651 [554, 748]**	**246 [195, 297]**	**308 [240, 377]**	**143 [115, 171]**	**165 [122, 209]**	**161 [128, 193]**	**161 [129, 193]**	**209 [129, 289]**	**182 [156, 208]**

Table 2. Breakdown of study population demographics and results

Data is Mean, [95% CI] or n, (%)

a: RapidND group included AD (n=13), dementia not-otherwise-specified (n=10), Lewy body dementia (n=7), leukoencephalopathy (n=4), encephalitis/meningitis (n=3), lymphoma (n=2), mixed AD/DLB (n=2), mixed AD/vascular/cerebral amyloid angiopathy (n=2), and n=1 each of astrocytoma, glioblastoma multiforme, gliomatosis cerebri, hemiplegic migraine, vascular dementia.

b: Other ND included vascular dementia (n=5), corticobasal syndrome (n=4), dementia with Lewy bodies (n=4), multiple system atrophy (n=2), Niemann-Pick Type C (n=3), Parkinson disease dementia (n=2), primary progressive aphasia (n=3), dementia not-otherwise-

specified (n=23), and n=1 each of CJD, CNS vasculitis, Huntington disease, mixed AD/vascular, multiple sclerosis, posterior cortical atrophy, progressive supranuclear palsy.

c: Other PSY included functional cognitive/neurological disorders (n=11), anxiety disorders (n=3), adjustment disorder (n=2), delusional disorder (n=2), obsessive compulsive disorder (n=2), post-traumatic stress disorder (n=1) and tic disorder (n=1).

z: Demographics and results for combined/all AD are available in Supplementary Table 3

ANCJDR: Australian National Creutzfeldt-Jakob Disease Registry severe rapidly progressive post-mortem confirm patients; AD: Alzheimer disease; BPAD: bipolar affective disorder; bvFTD: behavioural variant frontotemporal dementia; OlderAD: older-onset Alzheimer disease; MCI: mild cognitive impairment; MDD: major depressive disorder; ND: neurodegenerative disorder; RapidND: other severe rapidly progressive neurodegenerative disorder; PM: post mortem; PSY: primary psychiatric disorder; YoungerAD: younger-onset Alzheimer disease

Diagnostic group comparison		Area under the curve	95% CI	Optimal cut-off	Sensitivity	Specificity
ND vs PSY	NfL*	**0.94**	0.91, 0.97	582	92	87
	T-Tau*	0.88	0.84, 0.92	198	82	83
CJD+RapidND+ND vs PSY	NfL*	**0.96**	0.95, 0.98	634	92	91
	T-Tau*	0.93	0.90, 0.95	300	79	97
CJD+RapidND vs PSY	NfL*	0.99	0.97, 1.00	809	94	100
	T-tau*	0.97	0.95, 0.99	361	92	100
AD[z] vs PSY	NfL*	0.95	0.92, 0.98	610	91	90
	T-Tau*[a]	0.97	0.95, 0.99	300	89	97
AD[z] vs BPAD	NfL*	0.94	0.88, 1.00	686	82	92
	T-Tau*[a]	0.97	0.94, 1.00	264	91	100
AD[z] vs MDD	NfL*	0.97	0.94, 0.99	586	93	100
	T-Tau*[a]	0.97	0.94, 1.00	363	85	100
bvFTD vs PSY	NfL*	**0.89**	0.81, 0.98	583	85	87
	T-Tau[NS]	0.72	0.61, 0.84	198	62	83
bvFTD vs	NfL*	**0.90**	0.81,	579	85	88

schizophrenia			0.99			
	T-Tau^{NS}	0.76	0.63, 0.89	191	65	88
bvFTD vs BPAD	NfL*	**0.90**	0.80, 0.99	683	79	92
	T-Tau^{NS}	0.71	0.56, 0.87	182	68	82
bvFTD vs MDD	NfL*	**0.91**	0.82, 1.00	582	85	100
	T-Tau^{NS}	0.70	0.54, 0.86	155	79	63
CJD vs RapidND	NfL*	0.60	0.50, 0.71	1970	80	50
	T-Tau*	**0.86**	0.80, 0.91	1150	83	79
CJD vs ND	NfL*	0.87	0.84, 0.91	2057	79	88
	T-Tau*	**0.94**	0.91, 0.96	1076	86	93
CJD vs PSY	NfL*	0.99	0.98, 1.00	809	97	100
	T-Tau*	0.99	0.98, 1.00	371	98	100
RapidND vs ND	NfL*	**0.74**	0.65, 0.83	1307	71	71
	T-Tau*	0.63	0.53, 0.72	626	52	70
RapidND vs PSY	NfL*	0.96	0.92, 1.00	840	85	100
	T-Tau*	0.91	0.84, 0.97	271	83	94
CJD+RapidND+ND vs control	NfL*	0.94	0.92, 0.97	814	82	100
	T-Tau*	0.91	0.87,	313	78	100

			0.94			
ND vs control	NfL*	0.91	0.86, 0.95	654	84	90
	T-Tau*	0.84	0.78, 0.90	255	70	95
CJD vs control	NfL*	0.99	0.97, 1.00	814	97	100
	T-tau*	0.99	0.97, 1.00	316	98	100
RapidND vs control	NfL*	0.95	0.89, 1.00	846	85	100
	T-tau*	0.88	0.80, 0.97	256	85	95

Table 3. ROC curve analyses for NfL and T-tau

***: statistically different levels between diagnostic groups (p<0.05)**

NS: no statistical difference in levels between diagnostic groups

Bolded and underlined area under the curve: statistically greater than the other biomarker area under the curve (p<0.05)

a: Values for T-tau are likely to be overestimations, as T-tau was often used to inform the clinical diagnosis, particularly in AD vs non-AD

z: Combined/all AD (demographics and results available in Supplementary Table 3)

ANCJDR: Australian National Creutzfeldt-Jakob Disease Registry severe rapidly progressive post-mortem confirm patients; AD: Alzheimer disease; BPAD: bipolar affective disorder; bvFTD: behavioural variant frontotemporal dementia; OlderAD: older-onset Alzheimer disease; MCI: mild cognitive impairment; MDD: major depressive disorder; ND: neurodegenerative disorder; RapidND: other severe rapidly progressive neurodegenerative disorder; PM: post mortem; PSY: primary psychiatric disorder; YoungerAD: younger-onset Alzheimer disease

NfL cut-off 582pg/mL			T-tau cut-off 198pg/mL		
	ND	PSY		ND	PSY
Above cut-off 'test positive'	181	9	Above cut-off 'test positive'	162	11
Below cut-off 'test negative	16	58	Below cut-off 'test negative	36	54

Table 4. Crosstabs for neurofilament light chain and T-tau in distinguishing neurodegenerative from primary psychiatric disorders

ND: neurodegenerative disorder; NfL: neurofilament light chain protein; PSY: primary psychiatric disorder; T-tau: total tau

APPENDIX 1: Collaborators

Collaborators associated with The MiND Study Group:

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