Plasma neurofilament light in mood and psychotic disorders, behavioural variant frontotemporal dementia, and an interactive web application based on a large reference control cohort

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ABSTRACT

Objective

There has been an exponential increase in the understanding of blood biomarkers of neuronal injury such as neurofilament light (NfL) in neurodegenerative disorders, but a relative lack of research in primary psychiatric disorders (PPD) such as mood and psychotic disorders. Improved understanding of NfL in a diverse range of PPDs, particularly those of clinical relevance with overlapping symptoms and neurodegenerative differential diagnoses, the role and performance of large normative/reference data sets, and the influence of covariates, will be critical for future clinical translation. This study aimed to investigate plasma NfL in a range of PPDs, the diagnostic utility in differentiating PPD from behavioural variant frontotemporal dementia (bvFTD, a neurodegenerative disorder commonly misdiagnosed initially as PPD), and develop an interactive tool based on a large reference cohort.

Methods

Plasma NfL was analysed using Single molecule array (Simoa) technology in major depressive disorder (MDD, n=42), bipolar affective disorder (BPAD, n=121), treatment-resistant schizophrenia (TRS, n=82), and bvFTD (n=22). Comparisons were made between the four clinical cohort groups, and age-matched controls (Control Group 1, n=96), and the reference cohort (Control Group 2, n=1926). Different models were used to explore impact of weight and different control groups.

Results

Plasma NfL was elevated in BPAD compared to Control Group 1 ($p=0.020$), Control Group 2 ($p<0.001$), and TRS ($p=0.003$). Levels were similar in MDD, TRS, and controls. Large differences were seen between bvFTD (mean NfL 34.9pg/mL) and all PPDs and controls (all <11pg/mL). Plasma NfL distinguished bvFTD from PPD with high accuracy; a cut-off of 13.3pg/mL resulted in 86% sensitivity, 88% specificity. Models using the large Control Group 2 without weight were similar to models that included weight as a covariate, an internet-based application was developed to provide individualised z-scores and percentiles from this reference cohort.

Conclusions

The finding of higher plasma NfL levels in the largest cohort of BPAD to date should prompt further investigation. This study adds further evidence on the strong diagnostic utility of NfL to distinguish bvFTD from clinically relevant PPDs. Studies investigating clinical and diagnostic utility of plasma NfL and the serviceability of the internet-based application for diverse neurodegenerative and primary psychiatric conditions in real-world primary care and specialist clinical settings are underway.

INTRODUCTION

There has been a great deal of research into blood-based biomarkers for neurological and neurodegenerative conditions, with great potential implications for clinical trials and clinical translation to improve early diagnosis (even pre-clinical diagnosis), care and treatment. In particular, neurofilament light chain (NfL) was demonstrated to be a highly sensitive biomarker of neuronal injury in a diverse range of conditions (Bridel et al., 2019; Eratne, Loi, Walia, et al., 2020; Eratne, Loi, et al., 2022; Gaetani et al., 2019, 2021; Khalil et al., 2018).

NfL is of particular interest as a potential diagnostic biomarker, as it may help distinguish disorders with significant neuronal degeneration from those without, for example distinguishing neurodegenerative dementias from primary psychiatric disorders (PPD). This is a frequent clinical diagnostic dilemma and one associated with uncertainty, misdiagnosis, and negative impacts for patients and healthcare systems (Loi et al., 2020; Tsoukra et al., 2021; Woolley et al., 2011). One of the most challenging clinical distinctions associated with the most diagnostic uncertainty/instability and misdiagnoses is distinguishing primary psychiatric disorders (PPD) from behavioural variant frontotemporal dementia (bvFTD), a neurodegenerative condition associated with variable personality, behavioural and psychiatric changes (Ducharme et al., 2020). While NfL has been extensively investigated in neurological and neurodegenerative disorders, there remain significant gaps in our understanding of NfL levels in severe PPDs that can often present or 'mimic' conditions like bvFTD, and/or are associated themselves with cognitive and neuroimaging abnormalities. Improved understanding of the ranges and performance of NfL in a diverse range of PPDs, particularly those of clinical relevance with overlapping symptoms and neurodegenerative differential diagnoses, the role and performance of large normative/reference data sets, and the influence of covariates, will be critical for future clinical translation. Finally, improved understanding of how well models that include and exclude covariates that influence plasma NfL levels, in particular weight, compare, and the performance of models based on large reference cohorts that facilitate precision interpretation of individual levels and move beyond coarse age-binned cut-offs, will have significant implications for future research and clinical translation.

Most studies examining NfL in PPDs have primarily focussed on comparing NfL levels to primary neurological disorders, rather than specifically focussing on large, well-characterised cohorts of PPD themselves. For example, studies have investigated blood and cerebrospinal fluid (CSF) NfL in bvFTD compared to PPD (Al Shweiki et al., 2019; Ashton et al., 2021; Katisko et al., 2020; Vijverberg et al., 2017) and a range of other neurodegenerative conditions (Eratne, Loi, et al., 2022; Fourier et al., 2020), finding significantly elevated levels in neurodegenerative disorders compared to PPDs. Fewer studies have specifically studied blood NfL in PPD, with mixed findings. Higher blood NfL levels were seen in MDD compared to controls in one study (Bavato et al., 2021), but not in another study (Ashton et al., 2021). Mixed findings have also been seen in psychotic disorders: while some studies reported no differences between controls and schizophrenia (Al Shweiki et al., 2019; Bavato et al., 2021), between controls and treatment-resistant clozapine-treated schizophrenia (Eratne et al., 2021), one study reported slightly higher NfL levels in both schizophrenia and clozapine-treated patients compared to controls (Rodrigues-Amorim et al., 2020). Most studies did not include weight or BMI as a covariate, and none used very large control groups.

Given the relative lack of research on blood NfL in PPD, and in particular the lack of studies in bipolar affective disorder (BPAD) with larger sample sizes, we assessed plasma NfL in a range of psychiatric disorders to investigate differences between them, and between control groups. We also explored aspects important for future research and potential clinical utility and translation: to investigate differences between clinically relevant PPD and bvFTD, and to assess the sensitivity of different control groups and body weight as a covariate with a view to develop a tool for future research.

Aim 1 of this study was to investigate plasma NfL levels in a range of PPDs, compared to each other, and to age-matched healthy controls, using models that did not include weight (Model 1), and included weight (Model 2).

Aim 2 was to compare plasma NfL levels in bvFTD to primary psychiatric conditions that can often appear like or 'mimic' bvFTD, such as BPAD, MDD, and schizophrenia, which are common initial misdiagnoses or prodromes of bvFTD.

Aim 3 of this study was to use a comprehensive and sophisticated model of percentiles of NfL across the lifespan from a large reference range cohort of healthy controls, to interpret NfL levels in primary psychiatric disorders and bvFTD, to determine how sensitive comparisons/analyses are to using different control groups, and to including or excluding weight as a covariate. These will be used to develop an interactive web-based application to allow visualisation of an individual's NfL level compared to this large reference cohort, with individualised centiles and z-scores, to facilitate future research of diagnostic and clinical utility, and the potential of a simple tool to assist clinicians interpret an individual patient's level in routine clinical practice.

METHODS

Participant recruitment and data

Participant samples and data were included from four patient cohorts and two control groups, detailed below. More detailed information on recruitment and eligibility criteria have been previously published (Berk et al., 2019; Bousman et al., 2019; Dean et al., 2014, 2017; Eratne et al., 2021; Mostaid et al., 2017; Ooi et al., 2022; Simrén et al., 2022).

Cohort 1, bipolar affective disorder (BPAD)

Baseline (pre-intervention) samples and data were collected during a 16-week, three-arm, double-blind, randomised control trial (RCT) of adjunctive mitochondrial agents and *N*-acetylcysteine for bipolar depression [ACTRN12612000830897] (Berk et al., 2019). Participants were at least 18 years old, met DSM-IV-TR diagnostic criteria for bipolar disorder (assessed via structured clinical interview), and experiencing a bipolar depressive episode of at least moderate severity. Participants who were under any form of therapy needed to remain on stable therapy for at least 1 month prior entering the study. Trial sites included: Barwon Health and The Geelong Clinic, Geelong, Australia;

The Melbourne Clinic, Melbourne, Australia; and The CADE Clinic at Royal North Shore Hospital, Sydney, Australia. Recruitment occurred between 2013 and 2015.

Cohort 2, major depressive disorder (MDD)

Baseline (pre-intervention) samples and data were collected during a 12-week, two-arm, double-blind RCT of adjunctive minocycline for unipolar depression [ACTRN12612000283875] (Dean et al., 2014, 2017). Participants were at least 18 years old, met DSM-IV diagnostic criteria for unipolar depression (assessed via structured clinical interview), and experiencing a current depressive episode of at least moderate severity. Participants currently under any therapy needed to remain on stable treatment for at least 2 weeks prior entering the study. Recruitment sites included: Barwon Health and The Geelong Clinic, Geelong, Australia; The Melbourne Clinic, Melbourne, Australia; and Chulalongkorn University, Bangkok, Thailand. Recruitment occurred between 2013 and 2015.

Cohort 3, treatment-resistant schizophrenia (TRS)

Participants were from the Cooperative Research Centre (CRC) Psychosis Study, a cross-sectional study that recruited people aged 18-65 years from inpatient and outpatient services in Melbourne, Australia, between 2012-2017, who were on clozapine and had a diagnosis of treatment-resistant schizophrenia (TRS), defined as failure to respond to adequate trials of two or more antipsychotics, as previously described (Bousman et al., 2019; Eratne et al., 2021; Mostaid et al., 2017)

Cohort 4, behavioural variant frontotemporal dementia (bvFTD)

Patients were recruited from the Eastern Cognitive Disorders Clinic, Eastern Health, Melbourne, Australia, a specialist cognitive neurology service with expertise in diagnosis and management of bvFTD. Included in this study were patients who met diagnostic criteria for probable or definite bvFTD based on expert multidisciplinary and multimodal investigations, as previously described (Ooi et al., 2022).

Control Group 1, local control group

In order to maximise the size and age range of controls, samples and data were pooled from healthy people included in the CRC Psychosis Study (healthy controls age-matched to TRS, and healthy parents and siblings of participants with TRS) (Eratne et al., 2021).

Control Group 2, large reference normative control group

Control data from 1926 people aged 5-90 with no history or clinical symptoms or signs of neurological disorder, as described in detail previously (Simrén et al., 2022), was used as a large reference population for modelling of NfL levels across most of the lifespan.

For Cohorts 3 and 4, Control Group 1, Control Group 2, there were no: significant renal impairment, severe/uncontrolled diabetes or other general medical conditions, and no known stroke or head injury, within at least 12 months of recruitment. This data was not available for Cohorts 1 and 2.

All the previously mentioned studies that contributed cohort data and samples to this study, had ethical approval at the relevant Human Research Ethics Committees, and all participants provided written informed consent prior to participation. This study, which is part of The Markers in Neuropsychiatric Disorders Study (The MiND Study, https://themindstudy.org), was approved by the Melbourne Health Human Research Ethics Committee (MH HREC 2020.142).

Sample analysis

Plasma aliquots from all samples were stored at -80°C. Patient cohorts and Control Group 1 samples were randomised before analysis, and analyses were blinded to diagnosis. All plasma NfL levels were measured on the Quanterix HD-X and HD-1 analyzers using Simoa NF-Light kits, according to the manufacturer's recommendations (Quanterix Corporation, Billerica, MA USA).

Statistical analysis

All statistical analyses were performed using R version 4.2.2 (2022-10-31). General linear models (GLMs) were used to examine relationships between NfL levels, diagnostic group, and relevant clinicodemographic variables. For these models, Log_{10}-transformed NfL was entered as the dependent variable. Diagnostic group (using Control Group 1 as the reference class), age, sex, and weight (where available) were included as independent variables, given their previously demonstrated relationships with plasma NfL levels (Eratne et al., 2021; Simrén et al., 2022). Analyses were performed with and without weight to investigate contribution of this covariate to overall results. 95% confidence intervals were computed for all GLMs via nonparametric bootstrapping (1000 replicates), with statistical significance defined as any confidence interval not including the null (at the 95% level). Receiver operator characteristic (ROC) curves were computed to determine area under the curve (AUC), sensitivity, and specificity of NfL in distinguishing between groups. Optimal cut-off was determined using Youden's method. For Aim 3, all patient cohorts and Control Group 1 were compared to the large reference cohort, Control Group 2. Z-scores were calculated from age-adjusted percentiles from Control Group 2, which were derived using generalised additive models for location, scale, and shape (GAMLSS), followed by single-sample t-tests to test the hypothesis that the mean z-score was 0 (i.e., no difference / equal to the mean of Control Group 2). Welch Two Sample two-tests were used to compare z-scores between groups. The GAMLSS model was used to develop the web-based application. Model residuals were inspected for normality and homoscedasticity.

RESULTS

The final cohort included 258 participants with psychiatric disorders: 42 MDD, 121 BPAD, 82 TRS, ranging from 20 to 79 years of age (Table 1), 22 participants with bvFTD (mean age 66 years, range 43-80), 96 participants in Control Group 1 (mean age 45 years, range 18-77), and 1926 participants in Control Group 2 (mean age 54 years, range 5-90). MDD patients were older (mean 55 years) than

BPAD and TRS (44 and 40 years, respectively). Weight data was not available for two groups: bvFTD, and Control Group 2. The bvFTD group was the oldest group (mean 66 years). Participants with TRS were heavier (mean weight 95.8kg) compared to the other psychiatric groups (BPAD 84.3kg, MDD 84.6kg) and Control Group 1 (77.3kg).

Plasma NfL in primary psychiatric disorders

Plasma NfL levels are detailed in Table 1 and Figure 1. Before adjustments for covariates, raw NfL in psychiatric disorders were highest in MDD (mean M=10.9pg/mL 95%CI [9.1, 13.2]), with lowest levels in TRS (M=6.6pg/mL [5.8, 7.6]), which were also the oldest and youngest groups, respectively.

Model 1

A GLM adjusting for age and sex (without weight) was used to compare mean log NfL differences between psychiatric disorders and Control Group 1. Levels in Control Group 1 were not different to BPAD (standardised β=0.13 95%CI: [-0.08, 0.33], p=0.244) and MDD (β=0.02 [-0.22, 0.28], p=0.826), but TRS had lower levels (β=-0.31 [-0.59, -0.04], p=0.030). Comparing psychiatric groups to each other, levels were not different between MDD and BPAD (β=0.10 [-0.32, 0.15], p=0.408), but were lower in TRS compared to BPAD (β=-0.43 [-0.69, -0.17], p=0.004) and MDD (β=-0.33 [-0.66, -0.01], p=0.042).

Model 2

Weight was added for this model, given association between increased weight and lower plasma NfL concentrations, and given the greater weights in TRS compared to the other groups. Adding weight to the GLM resulted in no significant differences between TRS and MDD (β=-0.20 [-0.51, 0.12], p=0.198) and TRS and Control Group 1 (β=-0.10 [-0.39, 0.16], p=0.446). However, levels remained statistically significantly lower in TRS compared to BPAD (β=-0.32 [-0.59, -0.07], p=0.020). Including weight also resulted in statistically higher levels in BPAD compared to Control Group 1 (β=0.22 [0.02, 0.42], p=0.028). Age had the highest coefficients in both Models 1 and 2 (β=0.57 [0.51, 0.65], p<0.001, and β=0.61 [0.54, 0.68], p<0.001, respectively).

Plasma NfL in behavioural variant frontotemporal dementia compared to primary psychiatric disorders

As demonstrated in Table 1 and Figure 1, unadjusted plasma NfL levels were significantly elevated in bvFTD (M=34.9pg/mL), approximately three times higher compared to all other groups: (mean levels all below 11pg/mL).

Performing a GLM adjusting for age and sex, demonstrated statistically significant and large differences between bvFTD and all other groups: BPAD (β=1.66 [1.03, 2.28], p<0.001), MDD (β=1.80 [1.09, 2.39], p<0.001), TRS (β=1.98 [1.36, 2.62], p<0.001), and Control Group 1 (β=1.78 [1.10, 2.38], p<0.001) and Control Group 2 (β=1.94 [1.35, 2.51], p<0.001).

ROC curve analyses were performed to assess the ability of plasma NfL distinguish bvFTD from other groups. Plasma NfL distinguished between bvFTD and all psychiatric disorders, with high accuracy (area under the curve (AUC)=0.95 (95%CI [0.91, 0.99]), optimal cut-off of 13.3pg/mL, 86% sensitivity, 88% specificity). Diagnostic performance remained high even when restricting psychiatric disorders to the age range of the bvFTD group (43-80 years): AUC 0.91 [0.85, 0.98], 13.3pg/mL cut-off, 86% sensitivity, 78% specificity. Further details are available in the Supplementary Material.

Regarding differences seen in Models 1 and 2, ROC curve analyses demonstrated that while statistical significances were found in Model 1 and Model 2, plasma NfL did not demonstrate strong diagnostic utility to distinguish between TRS and BPAD (AUC 0.66 [0.58, 0.74], 5.6pg/mL cut-off, 72% sensitivity, 59% specificity), TRS and MDD (AUC 0.74 [0.65, 0.83], 6.2pg/mL cut-off, 79% sensitivity, 63% specificity), or BPAD and Control Group 2 (AUC 0.58 [0.53, 0.63], 6.9pg/mL cut-off, 67% sensitivity, 50% specificity). Plasma NfL did not accurately distinguish BPAD from Control Group 1 (AUC 0.46 [0.38, 0.53]).

Plasma NfL in all groups compared to large reference control cohort, Model 3

To explore the utility of a large normative control dataset, age-adjusted percentiles for plasma NfL were derived from the reference cohort Control Group 2, using generalised additive models for location, scale, and shape (GAMLSS). The centile plot of this model is demonstrated in Figure 2.

To investigate any differences, z-scores for individuals in every cohort were computed from this reference cohort model. As demonstrated in Figure 3: highest z-scores were in bvFTD, with slightly higher levels in BPAD and MDD, and slightly lower levels in TRS, similar to patterns seen in Figure 1.

Simple t-tests were used to test the null hypothesis (i.e., that the mean z-scores in each diagnostic group was zero). The results were similar to the model comparing to the local Control Group 1 where weight was included as a covariate (Model 2). The mean z-score for BPAD was greater than Control Group 2 (2.02 vs 0), suggesting a small effect (difference=0.44 [0.24, 0.64], p <0.001; Cohen's d = 0.39 [0.20, 0.57]). BPAD was also greater than TRS (mean z-score 0.44 vs -0.10, difference=0.54 [0.19, 0.90], p = 0.003; small effect Cohen's d = 0.44 [0.15, 0.73]). Z-scores in the other groups - MDD, TRS, and Control Group 1 - were not different to Control Group 2. bvFTD was significantly greater than Control Group 2, and the effect was large (difference = 2.02 [1.41, 2.62], p < .001; Cohen's d = 1.47 [0.85, 2.07]). bvFTD was also greater than BPAD, MDD, TRS, Control Group 1 (differences=1.58, 1.75, 2.02, 1.84, respectively), and all with large effect sizes (Cohen’s d=1.25, 1.48, 1.58, 1.43, respectively).

Of note, although Model 3, like Model 1, did not include weight, unlike Model 1, Model 3 did not demonstrate the differences between TRS and controls and TRS and MDD (that were lost when weight was included in Model 2). This suggests that in a large reference cohort, GAMLSS modelling and use of z-scores, outperforms other models that do not include weight, and there was no evidence for a difference in performance and findings when compared to models that included weight adjusted comparisons.

Based on Model 3, an interactive web-based application was developed, available via https://themindstudy.org/apps. As demonstrated in Figure 4, the application allows input of an individual's age and plasma NfL level, providing estimated centiles and z-scores and allowing visualisation on the centiles reference chart, compared to the large reference cohort, Control Group 2.

DISCUSSION

This study investigated plasma NfL levels in a range of primary psychiatric conditions, finding higher levels in BPAD compared to controls, and compared to TRS. In addition, there were significant and large differences between bvFTD and all other groups, and high diagnostic accuracy of plasma NfL to distinguish bvFTD from PPD. This provides important replication of plasma NfL differences between PPDs and bvFTD, and the strong diagnostic performance and potential for NfL to assist in this common, often very challenging, clinical distinction (Al Shweiki et al., 2019; Ducharme et al., 2020; Eratne, Keem, et al., 2022; Katisko et al., 2020; Ooi et al., 2022).

Our finding of elevated plasma NfL levels in 121 people with BPAD compared to controls, is to our knowledge in the largest group of BPAD described to date. A previous study found elevated serum NfL levels in 45 people with bipolar depression (Aggio et al., 2022), although the control group was not age-matched and the difference between raw/unadjusted mean levels between BPAD and controls was much larger in that study (9.13pg/mL vs 4.28pg/mL), compared to our study (8.4pg/mL in BPAD, vs 9.4 and 9.9pg/mL in Control Groups 1 and 2, respectively). Elevated CSF NfL levels in BPAD have previously been described (Jakobsson et al., 2014; Rolstad et al., 2015). Our finding of elevated BPAD levels compared to controls may suggest some degree of mild and/or slow rate of neuronal injury in BPAD, greater than in controls/healthy ageing, but less than what is seen in clearly neurodegenerative disorders such as bvFTD and Alzheimer disease (Ashton et al., 2021). While there was statistical significance at group levels between BPAD and controls and between BPAD and TRS, the differences and effects were small, and there was significant overlap between groups, as demonstrated in Figures 1 and 3, and corresponding poor performance on ROC curve analyses. Thus, the clinical relevance of using plasma NfL to distinguish BPAD from other primary psychiatric conditions in individual patients is low, while the clinical utility to differentiate BPAD and other psychiatric conditions from neurodegenerative disorders such as bvFTD, remains high.

We did not find differences between MDD, psychotic disorders, and healthy controls. This is similar to other studies (Al Shweiki et al., 2019; Ashton et al., 2021), and our previous findings in CSF (Eratne, Loi, et al., 2022; Eratne, Loi, Li, et al., 2020; Eratne, Loi, Walia, et al., 2020). A study found elevated serum NfL levels in MDD compared to controls, but no differences between schizophrenia and controls (Bavato et al., 2021), whereas another found elevated serum NfL in schizophrenia compared to controls (Rodrigues-Amorim et al., 2020). Of note, only Bavato et al. included weight (BMI) in analyses, and control groups used in these studies (including reference cohorts), were smaller. Our contrary findings are not obviously explained by age (our cohorts were older or similar), or clinical or other study population factors. Studies with larger samples sizes, serial NfL levels,

associations with additional biomarkers (e.g., of inflammation/neuroinflammation) and neuroimaging, medication use, different stages/phases of illness (e.g., bipolar mania), and longitudinal comprehensive follow up, will be valuable to further extend these findings.

Our BPAD group was exclusively comprised of participants with bipolar depression. Further study is required to investigate NfL changes during manic episodes, as well as during acute episodes of other psychiatric illnesses (e.g., in patients admitted to acute inpatient psychiatric wards). A limitation is the lack of serial plasma NfL levels and longer term follow up clinical information in the groups. There were several participants with quite elevated NfL levels in MDD, BPAD, including one extremely high level in Control Group 1. These were unexpected as they did not appear to be explained by any pre-analytical or analysis factors, nor any obvious clinical characteristic. It is possible that the utility of plasma NfL in psychiatric symptoms (or indeed people without any symptoms), is underestimated without this follow-up data. Not having MRI data on most psychiatric groups is an additional limitation, as it is possible that subclinical cerebrovascular disease - both acute and more chronic small vessel ischaemic, especially in older patients with mood disorders - could have explained some of the higher levels seen. We pooled data from several well-characterised cohorts and analysed all samples in the same lab, which while relative strengths, also limit generalisability to real-world clinical settings. Nonetheless this study provides important replication and similar findings to other studies that have compared separate cohorts in distinguishing bvFTD from PPDs (Al Shweiki et al., 2019; Katisko et al., 2020).

We explored the sensitivity of using different control groups, and used the limitation of the lack of weight data for bvFTD and Control Group to explore the influence of weight as a covariate. We found that Model 3, using a large control reference data set (Simrén et al., 2022) without weight, had the same results as analyses from specifically recruited local controls and when weight was included as a covariate (Model 2). In particular, it avoided some of the spurious findings of other models that similarly did not include weight (i.e., lower levels in TRS compared to controls and MDD in Model 1). The finding, that weight may not be required when using a large control data set and using modelling such as in Model 3, requires further investigation, but has potential important implications. For example, significant efficiencies and cost reductions may be possible for future studies by potentially not requiring local control group recruitment, and instead focusing on facilitation of data pooling. In addition, while there is growing evidence of weight and other factors that influence plasma NfL levels, the overall impact of these are relatively small (Akamine et al., 2020; Fitzgerald et al., 2022). Considering clinical translation, a clinician only having to consider and input only three simple and easily/immediately obtainable variables for many patients – age, sex, and plasma NfL level – would be more feasible for busy clinicians in primary care and via telehealth assessments, and even has implications for laboratories reporting on plasma NfL levels, while reducing the potential for variability introduced by additional measurements in clinical and research settings. To extend our findings, future studies should incorporate as many of these variables into analyses and modelling, and in particular to specifically investigate the clinical utility of including these variables, compared to a minimum set (e.g., age, sex, plasma NfL).

We developed an online interactive web application based on Model 3, available via https://themindstudy.org/apps. This builds on other similar applications that have been developed

recently (Benkert et al., 2022; Vermunt et al., 2022), but to our knowledge is the first app to use GAMLSS modelling and providing both individualised percentiles and z-scores. This application could be used for academic and research interests, and will be used in studies underway to investigate the clinical and diagnostic utility and validity of such tools, feasibility and utility for clinicians, all with a view to possible implementation in routine clinical care in the future, where a clinician may use such an application, similar to using growth charts, to help quickly facilitate a precision interpretation of an individual's NfL level.

This study found no significant differences between MDD, TRS, and controls, and small elevations in BPAD compared to controls, adding to our understanding of these disorders and evidence suggesting a lack of significant neuronal injury and degeneration (axonal in particular) in primary PPDs. In addition, this study demonstrated the strong diagnostic utility of plasma NfL in distinguishing bvFTD from clinically relevant PPDs, building the accumulating evidence base for a relatively simple test to assist with this common yet challenging diagnostic dilemma. The app developed demonstrates how visualisation of an individual's NfL level and minimal additional data (age), using a large reference cohort and sophisticated modelling, moves beyond coarser age-based binary cut-offs and starts to facilitate the individualised and precise medicine interpretation required for best translation into real-world clinical care. Studies are underway to investigate the clinical and diagnostic utility of plasma NfL in diverse neurodegenerative and primary psychiatric conditions in real-world primary care and specialist clinical settings.

REFERENCES

Aggio V, Fabbella L, Finardi A, et al. (2022) Neurofilaments light: Possible biomarker of brain modifications in bipolar disorder. *Journal of Affective Disorders* 300: 243–248. DOI: 10.1016/j.jad.2021.12.122.

Akamine S, Marutani N, Kanayama D, et al. (2020) Renal function is associated with blood neurofilament light chain level in older adults. *Scientific Reports* 10(1): 20350. DOI: 10.1038/s41598-020-76990-7.

Al Shweiki MR, Steinacker P, Oeckl P, et al. (2019) Neurofilament light chain as a blood biomarker to differentiate psychiatric disorders from behavioural variant frontotemporal dementia. *Journal of Psychiatric Research* 113(February). Elsevier: 137–140. DOI: 10.1016/j.jpsychires.2019.03.019.

Ashton NJ, Janelidze S, Al Khleifat A, et al. (2021) A multicentre validation study of the diagnostic value of plasma neurofilament light. *Nature Communications* 12(1): 3400. DOI: 10.1038/s41467-021-23620-z.

Bavato F, Cathomas F, Klaus F, et al. (2021) Altered neuroaxonal integrity in schizophrenia and major depressive disorder assessed with neurofilament light chain in serum. *Journal of Psychiatric Research* 140: 141–148. DOI: 10.1016/j.jpsychires.2021.05.072.

Benkert P, Meier S, Schaedelin S, et al. (2022) Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *The Lancet Neurology* 21(3): 246–257. DOI: 10.1016/S1474-4422(22)00009-6.

Berk M, Turner A, Malhi GS, et al. (2019) A randomised controlled trial of a mitochondrial therapeutic target for bipolar depression: mitochondrial agents, N-acetylcysteine, and placebo. *BMC medicine* 17(1): 18. DOI: 10.1186/s12916-019-1257-1.

Bousman CA, Luza S, Mancuso SG, et al. (2019) Elevated ubiquitinated proteins in brain and blood of individuals with schizophrenia. *Scientific Reports* 9(1): 2307. DOI: 10.1038/s41598-019-38490-1.

Bridel C, Van Wieringen WN, Zetterberg H, et al. (2019) Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. *JAMA Neurology* 76(9): 1035–1048. DOI: 10.1001/jamaneurol.2019.1534.

Dean OM, Maes M, Ashton M, et al. (2014) Protocol and rationale-the efficacy of minocycline as an adjunctive treatment for major depressive disorder: a double blind, randomised, placebo controlled trial. *Clinical Psychopharmacology and Neuroscience: The Official Scientific Journal of the Korean College of Neuropsychopharmacology* 12(3): 180–188. DOI: 10.9758/cpn.2014.12.3.180.

Dean OM, Kanchanatawan B, Ashton M, et al. (2017) Adjunctive minocycline treatment for major depressive disorder: A proof of concept trial. *Australian & New Zealand Journal of Psychiatry* 51(8): 829–840. DOI: 10.1177/0004867417709357.

Ducharme S, Dols A, Laforce R, et al. (2020) Recommendations to distinguish behavioural variant frontotemporal dementia from psychiatric disorders. *Brain* 143(6): 1632–1650. DOI: 10.1093/brain/awaa018.

Eratne D, Loi SM, Walia N, et al. (2020) A pilot study of the utility of cerebrospinal fluid neurofilament light chain in differentiating neurodegenerative from psychiatric disorders: A 'C-reactive protein' for psychiatrists and neurologists? *Australian and New Zealand Journal of Psychiatry* 54(1): 57–67. DOI: 10.1177/0004867419857811.

Eratne D, Loi SM, Li QX, et al. (2020) Cerebrospinal fluid neurofilament light chain is elevated in Niemann–Pick type C compared to psychiatric disorders and healthy controls and may be a marker of treatment response. *Australian and New Zealand Journal of Psychiatry* 54(6): 648–649. DOI: 10.1177/0004867419893431.

Eratne D, Janelidze S, Malpas CB, et al. (2021) Plasma neurofilament light chain protein is not increased in treatment-resistant schizophrenia and first-degree relatives. *Australian & New Zealand Journal of Psychiatry*: 000486742110586. DOI: 10.1177/00048674211058684.

Eratne D, Keem M, Lewis C, et al. (2022) Cerebrospinal fluid neurofilament light chain differentiates behavioural variant frontotemporal dementia progressors from non-progressors. *Journal of the Neurological Sciences* 442: 120439. DOI: 10.1016/j.jns.2022.120439.

Eratne D, Loi SM, Li Q-X, et al. (2022) Cerebrospinal fluid neurofilament light chain differentiates primary psychiatric disorders from rapidly progressive, Alzheimer's disease and frontotemporal disorders in clinical settings. *Alzheimer's & dementia : the journal of the Alzheimer's Association*. United States. DOI: 10.1002/alz.12549.

Fitzgerald KC, Sotirchos ES, Smith MD, et al. (2022) Contributors to Serum NfL Levels in People without Neurologic Disease. *Annals of Neurology* 92(4): 688–698. DOI: 10.1002/ana.26446.

Fourier A, Formaglio M, Kaczorowski F, et al. (2020) A combination of total tau and neurofilaments discriminates between neurodegenerative and primary psychiatric disorders. *European Journal of Neurology* 27(7): 1164–1169. DOI: 10.1111/ene.14247.

Gaetani L, Blennow K, Calabresi P, et al. (2019) Neurofilament light chain as a biomarker in neurological disorders. *Journal of Neurology, Neurosurgery & Psychiatry* 90(8): 870–881. DOI: 10.1136/jnnp-2018-320106.

Gaetani L, Parnetti L, Calabresi P, et al. (2021) Tracing Neurological Diseases in the Presymptomatic Phase: Insights From Neurofilament Light Chain. *Frontiers in Neuroscience* 15: 672954. DOI: 10.3389/fnins.2021.672954.

Jakobsson J, Bjerke M, Ekman CJ, et al. (2014) Elevated concentrations of neurofilament light chain in the cerebrospinal fluid of bipolar disorder patients. *Neuropsychopharmacology* 39(10): 2349–2356. DOI: 10.1038/npp.2014.81.

Katisko K, Cajanus A, Jääskeläinen O, et al. (2020) Serum neurofilament light chain is a discriminative biomarker between frontotemporal lobar degeneration and primary psychiatric disorders. *Journal of Neurology* 267(1): 162–167. DOI: 10.1007/s00415-019-09567-8.

Khalil M, Teunissen CE, Otto M, et al. (2018) Neurofilaments as biomarkers in neurological disorders. *Nature Reviews Neurology* 14(10). Nature Publishing Group: 577–589. DOI: 10.1038/s41582-018-0058-z.

Loi SM, Goh AMY, Mocellin R, et al. (2020) Time to diagnosis in younger-onset dementia and the impact of a specialist diagnostic service. *International Psychogeriatrics*: 1–9. DOI: 10.1017/S1041610220001489.

Mostaid MS, Lee TT, Chana G, et al. (2017) Elevated peripheral expression of neuregulin-1 (NRG1) mRNA isoforms in clozapine-treated schizophrenia patients. *Translational Psychiatry* 7(12): 1280. DOI: 10.1038/s41398-017-0041-2.

Ooi S, Patel SK, Eratne D, et al. (2022) Plasma Neurofilament Light Chain and Clinical Diagnosis in Frontotemporal Dementia Syndromes. *Journal of Alzheimer's disease: JAD*. DOI: 10.3233/JAD-220272.

Rodrigues-Amorim D, Rivera-Baltanás T, del Carmen Vallejo-Curto M, et al. (2020) Plasma β-III tubulin, neurofilament light chain and glial fibrillary acidic protein are associated with neurodegeneration and progression in schizophrenia. *Scientific Reports* 10(1): 14271. DOI: 10.1038/s41598-020-71060-4.

Rolstad S, Jakobsson J, Sellgren C, et al. (2015) Cognitive performance and cerebrospinal fluid biomarkers of neurodegeneration: a study of patients with bipolar disorder and healthy controls. *PloS one* 10(5): e0127100–e0127100. DOI: 10.1371/journal.pone.0127100.

Simrén J, Andreasson U, Gobom J, et al. (2022) Establishment of reference values for plasma neurofilament light based on healthy individuals aged 5–90 years. *Brain Communications* 4(4): fcac174. DOI: 10.1093/braincomms/fcac174.

Tsoukra P, Velakoulis D, Wibawa P, et al. (2021) The Diagnostic Challenge of Young-Onset Dementia Syndromes and Primary Psychiatric Diseases: Results From a Retrospective 20-Year Cross-Sectional Study. *The Journal of Neuropsychiatry and Clinical Neurosciences*: appineuropsych20100266. DOI: 10.1176/appi.neuropsych.20100266.

Vermunt L, Otte M, Verberk IMW, et al. (2022) Age- and disease-specific reference values for neurofilament light presented in an online interactive support interface. *Annals of Clinical and Translational Neurology* 9(11): 1832–1837. DOI: 10.1002/acn3.51676.

Vijverberg EGB, Dols A, Krudop WA, et al. (2017) Cerebrospinal fluid biomarker examination as a tool to discriminate behavioral variant frontotemporal dementia from primary psychiatric disorders. *Alzheimer's and Dementia: Diagnosis, Assessment and Disease Monitoring* 7. Elsevier Inc.: 99–106. DOI: 10.1016/j.dadm.2017.01.009.

Woolley JD, Khan BK, Murthy NK, et al. (2011) The diagnostic challenge of psychiatric symptoms in neurodegenerative disease: Rates of and risk factors for prior psychiatric diagnosis in patients with early neurodegenerative disease. *Journal of Clinical Psychiatry* 72(2): 126–133. DOI: 10.4088/JCP.10m06382oli.

TABLES AND FIGURES

	Bipolar affective disorder (BPAD)	Major depressive disorder (MDD)	Treatment-resistant schizophrenia (TRS)	bvFTD	Control Group 1	Control Group 2
N	121	42	82	22	96	1926
Age at sample, y	44.1 [42.0, 46.3]	55.0 [51.1, 58.9]	40.3 [38.4, 42.4]	65.7 [61.6, 69.7]	44.7 [41.8, 47.4]	54.4 [53.8, 55.0]
Age range, y	20-72	26-79	22-61	43-80	20-77	5-90
Sex, n female (%)	75 (62%)	24 (57%)	23 (28%)	4 (18%)	50 (52%)	1218 (63%)
Weight, kg	84.3 [81.0, 87.8]	84.6 [79.3, 89.8]	95.8 [90.2, 102.3] (n=71)	-	77.3 [74.1, 80.7] (n=87)	-
Plasma NfL (pg/mL)	8.4 [7.7, 9.2]	10.9 [9.1, 13.2]	6.6 [5.8, 7.6]	34.9 [25.4, 46.3]	9.4 [7.3, 12.3]	9.9 [9.6, 10.2]
Log10NfL	0.87 [0.83, 0.91]	0.97 [0.90, 1.00]	0.73 [0.67, 0.79]	1.54 [1.38, 1.68]	0.85 [0.80, 0.91]	0.93 [0.91, 0.94]
z-scores	0.44 [0.23, 0 64]	0.26 [-0.04, 0.57]	-0.10 [-0.39, 0.19]	2.02 [1.32, 2.58]	0.18 [-0.06, 0.43]	0 (reference group)

Table 1. Study demographics and plasma neurofilament light levels

bvFTD: behavioural variant frontotemporal dementia.

Data presented are mean [bootstrapped 95% confidence interval] or number (%).

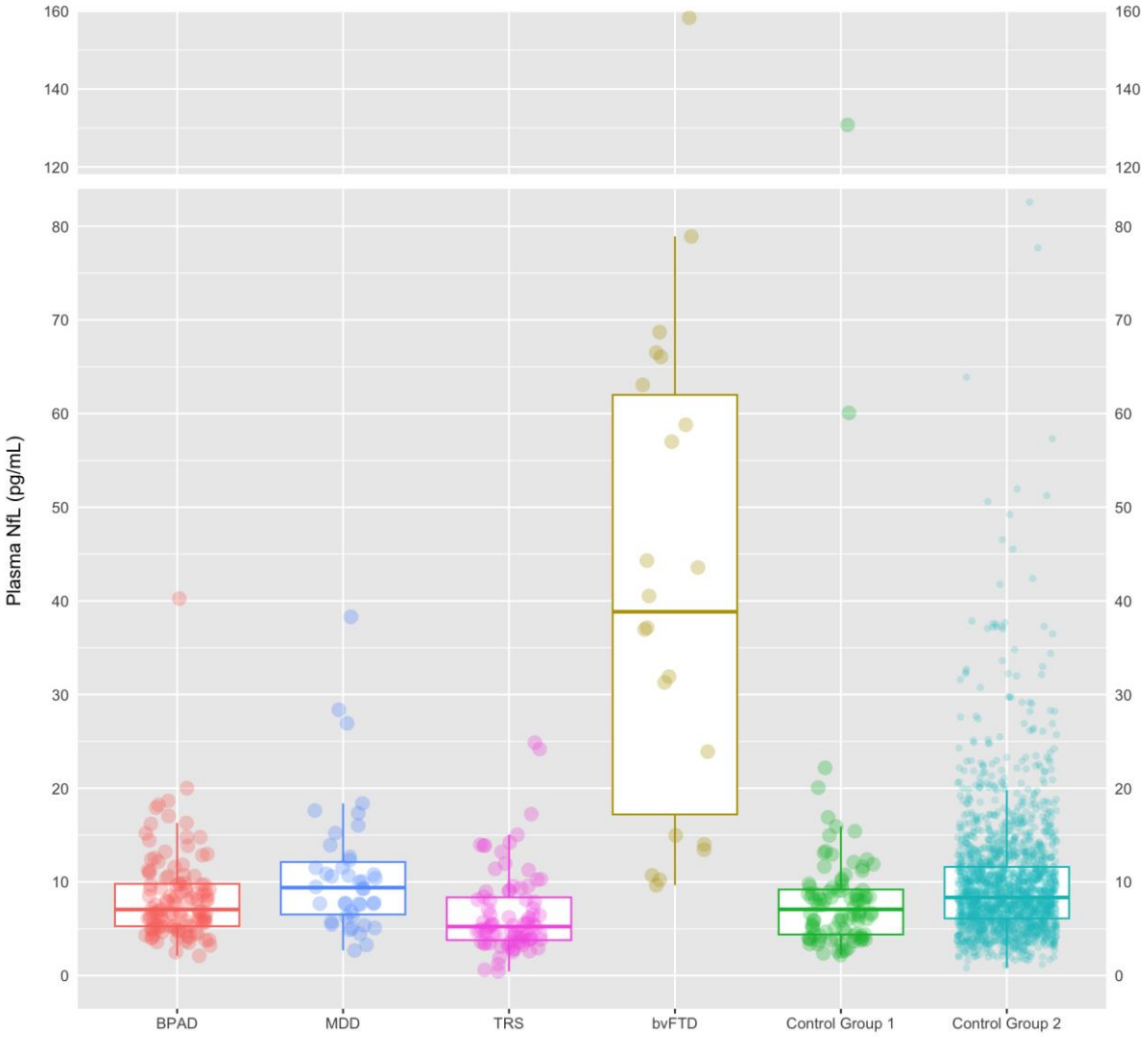

Figure 1. Plasma NfL levels in primary psychiatric disorders, behavioural variant frontotemporal dementia, and controls

BPAD: bipolar disorder; bvFTD: behavioural variant frontotemporal dementia; MDD: major depressive disorder; TRS: treatment-resistant schizophrenia

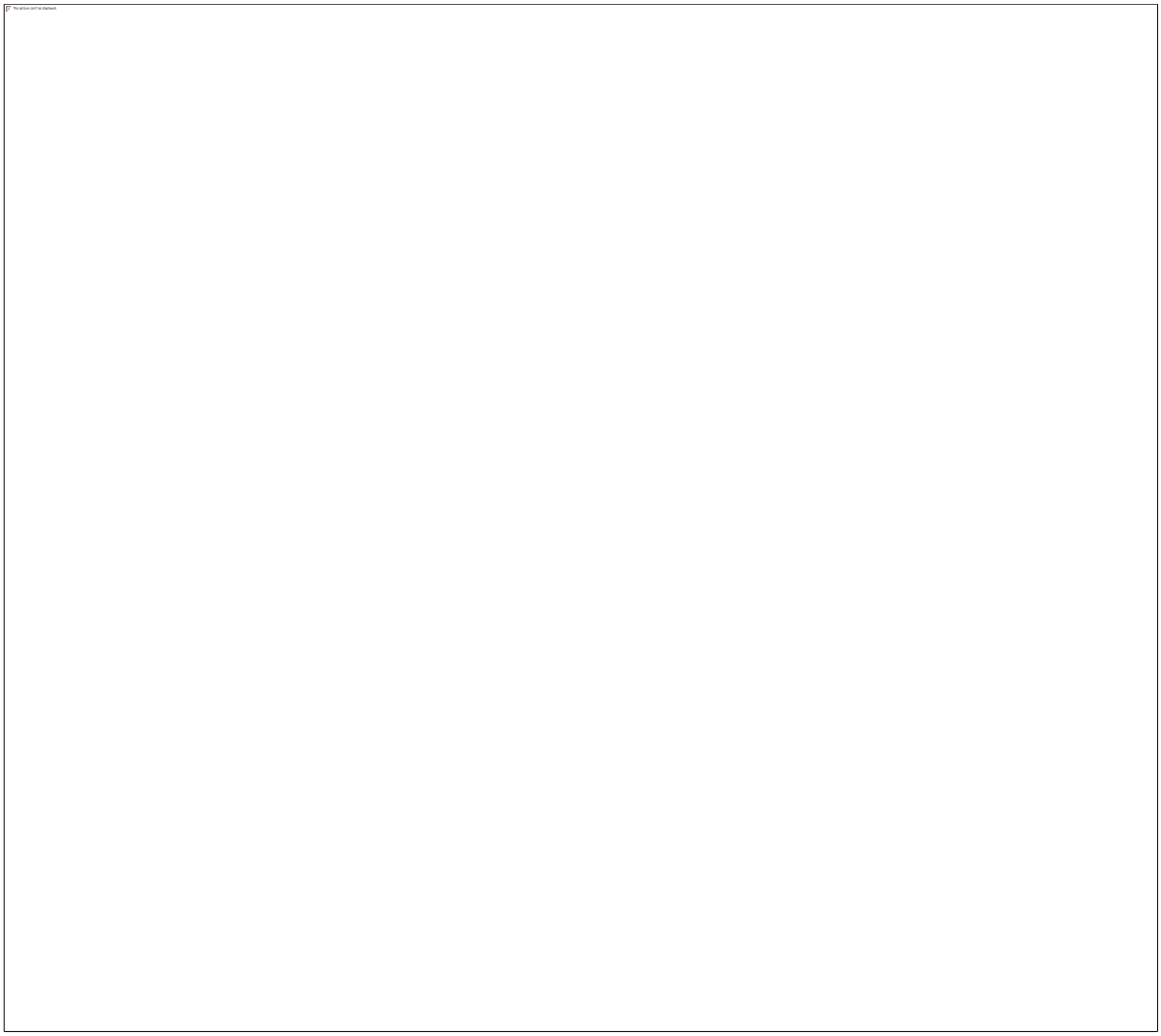

Figure 2. Percentiles derived from generalised additive models for location, scale, and shape, from 1926 healthy controls, Control Group 2 (Simrén et al., 2022)

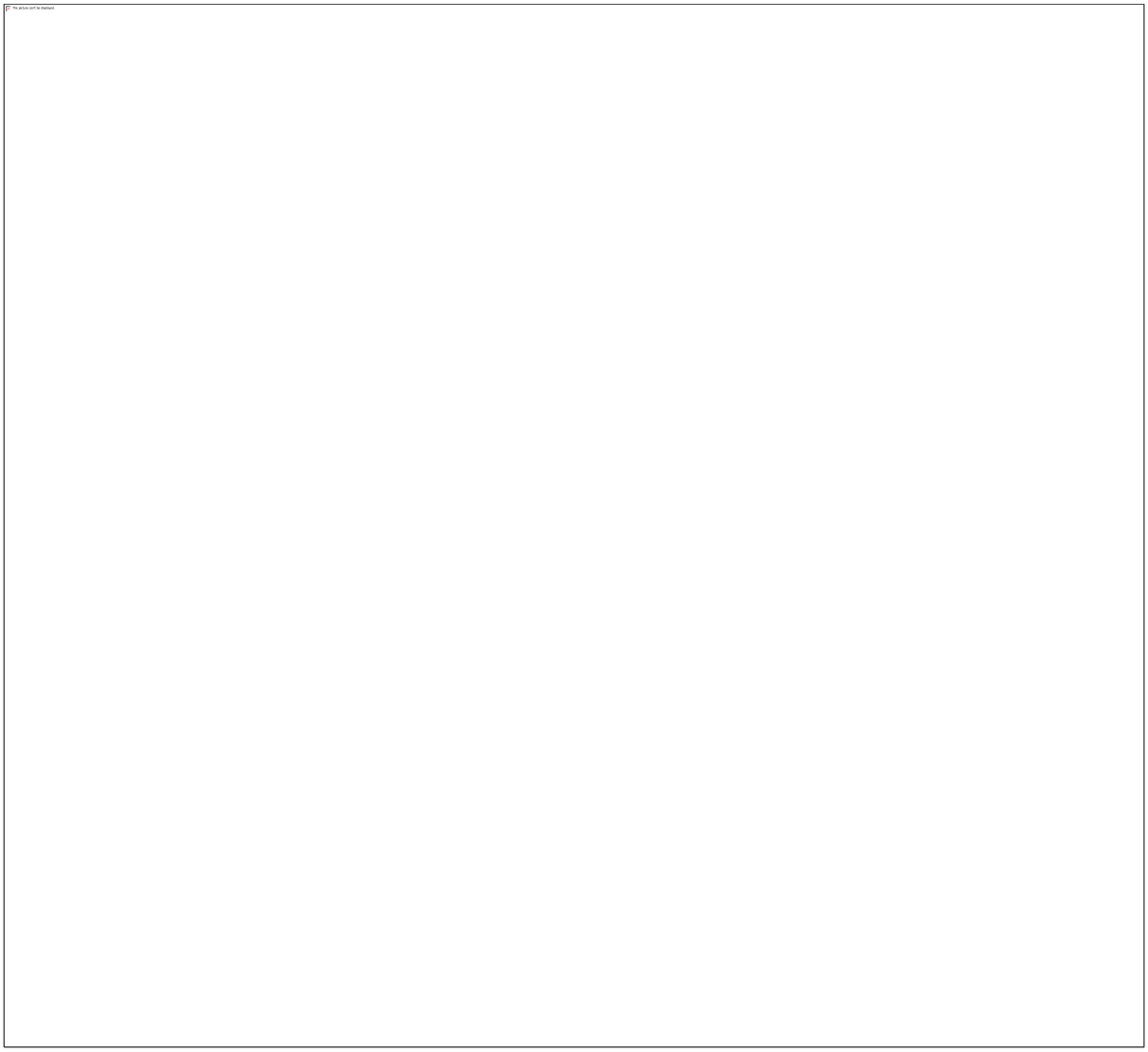

Figure 3. Z-scores (compared to Control Group 2) of plasma NfL levels in primary psychiatric disorders, behavioural variant frontotemporal dementia, and controls

BPAD: bipolar disorder; bvFTD: behavioural variant frontotemporal dementia; MDD: major depressive disorder; TRS: treatment-resistant schizophrenia

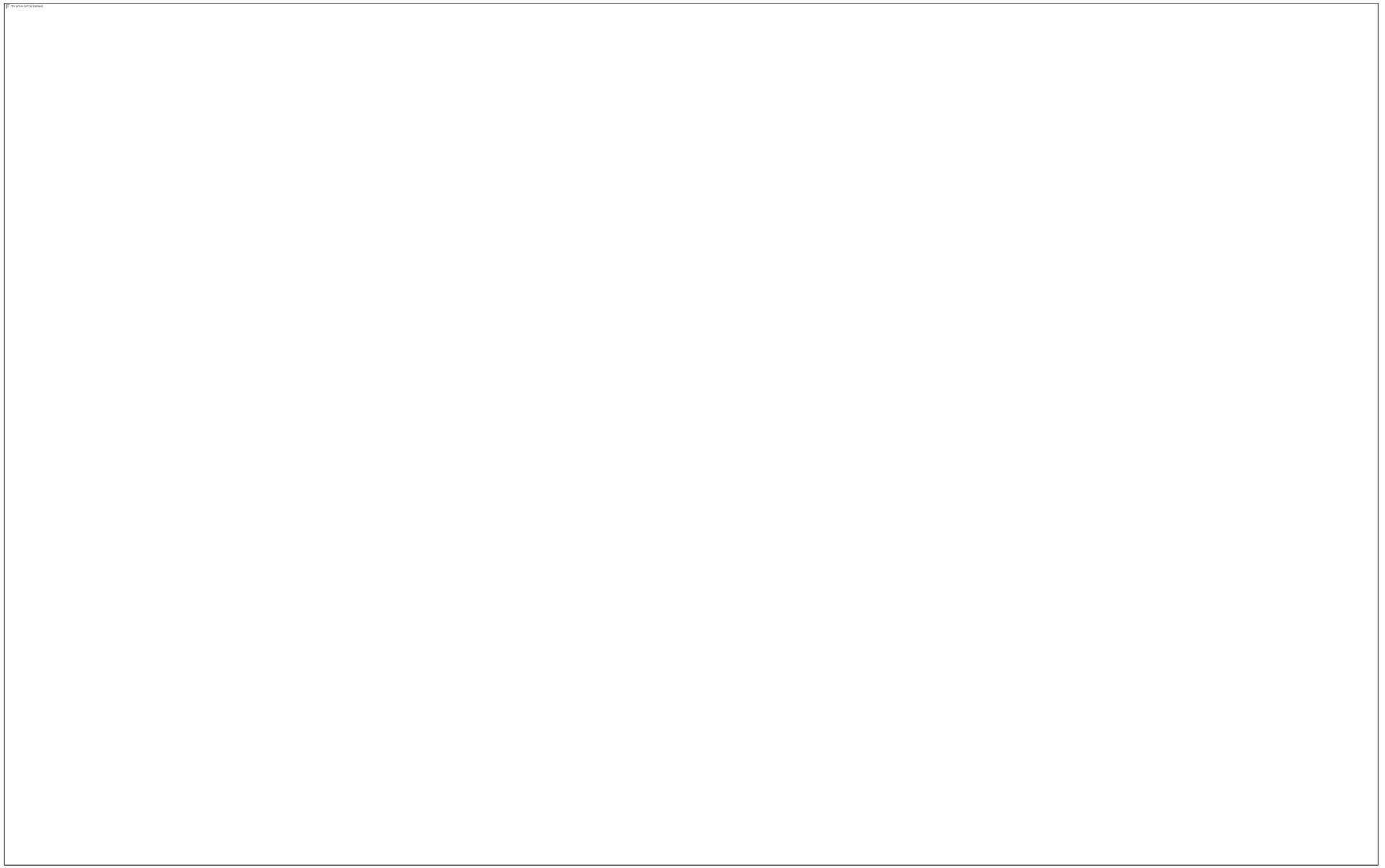
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Figure 4. The interactive web-based application available at https://themindstudy.org/apps.

The user is able to input individual ages and plasma NfL levels, providing estimated centiles and z-scores and allowing visualisation on the centiles reference chart, compared to the large reference cohort, Control Group 2. Two examples have been entered (real patients, not part of the study cohort). The red cross refers to patient 1, a 55-year-old man who was initially diagnosed with late onset psychosis and delirium, but on a third subsequent reassessment he was eventually re-diagnosed with probable behavioural variant frontotemporal dementia (bvFTD), and ultimately definite bvFTD (genetic testing confirmed the *C9orf72* repeat expansion mutation). In hindsight the psychosis diagnosis was very likely a prodrome/misdiagnosis. His plasma NfL level was 55pg/mL, which as the figure conveys visually was significantly elevated (99.96th percentile and z-score of 3.38 for a 55-year-old), was consistent with a neurodegenerative disorder and could have quickly dismissed primary psychiatric and non-neurodegenerative differential diagnoses. Conversely, patient 2's level of 8pg/mL (blue cross, z-score of 0.15) could have potentially dismissed or at the very least added caution to an initial diagnosis of probable bvFTD, which in hindsight was a misdiagnosis and this 56-year-old woman was eventually re-diagnosed to bipolar disorder.

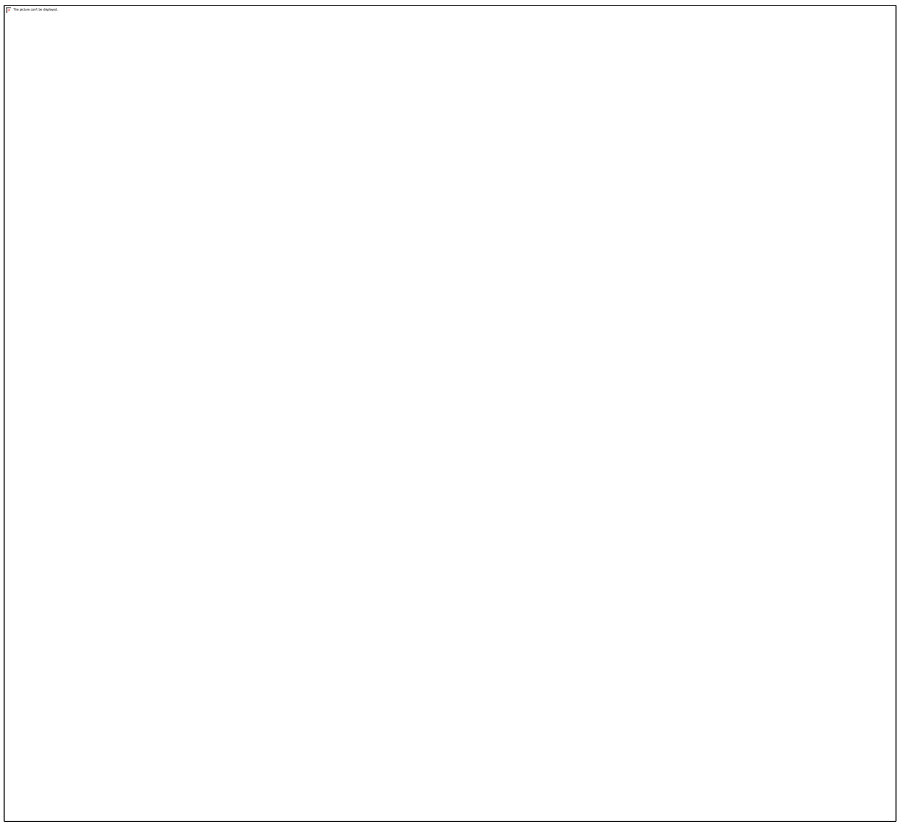
Supplementary figure 1. Plasma NfL levels by age in primary psychiatric disorders, behavioural variant frontotemporal dementia, and controls

Control is Control Group 1. Control Group 2 is detailed in small grey circles, to improve readability.

BPAD: bipolar disorder; bvFTD: behavioural variant frontotemporal dementia; MDD: major depressive disorder; TRS: treatment-resistant schizophrenia

Model 1: GLM lognfl ~ dx + age + sex, standardised coefficients, reference group = Control Group 1, bootstrapped

Parameter	Coefficient	95% CI	p
(Intercept)	-5.13e-03	[-0.15, 0.13]	0.956
dx [BPAD]	0.13	[-0.08, 0.33]	0.244
dx [MDD]	0.02	[-0.22, 0.28]	0.826
dx [TRS]	-0.31	[-0.59, -0.04]	0.030
age	0.57	[0.51, 0.65]	< .001
sex string [Male]	0.04	[-0.14, 0.22]	0.640



Model 1: GLM lognfl ~ dx + age + sex, standardised coefficients, reference group = TRS, bootstrapped

Parameter	Coefficient	95% CI	p

(Intercept) | -0.31 | [-0.55, -0.08] | 0.020

dx [BPAD] | 0.43 | [0.17, 0.69] | 0.004

dx [Control] | 0.31 | [0.04, 0.59] | 0.030

dx [MDD] | 0.33 | [0.01, 0.66] | 0.042

age | 0.57 | [0.51, 0.65] | < .001

sex string [Male] | 0.04 | [-0.14, 0.22] | 0.640

Model 2: GLM lognfl ~ dx + weight + age + sex, standardised coefficients, reference group = Control Group 1, bootstrapped

Parameter | Coefficient | 95% CI | p

(Intercept) | 0.89 | [0.54, 1.23] | < .001

dx [BPAD] | 0.22 | [0.02, 0.42] | 0.028

dx [MDD] | 0.09 | [-0.15, 0.35] | 0.454

dx [TRS] | -0.10 | [-0.39, 0.16] | 0.446

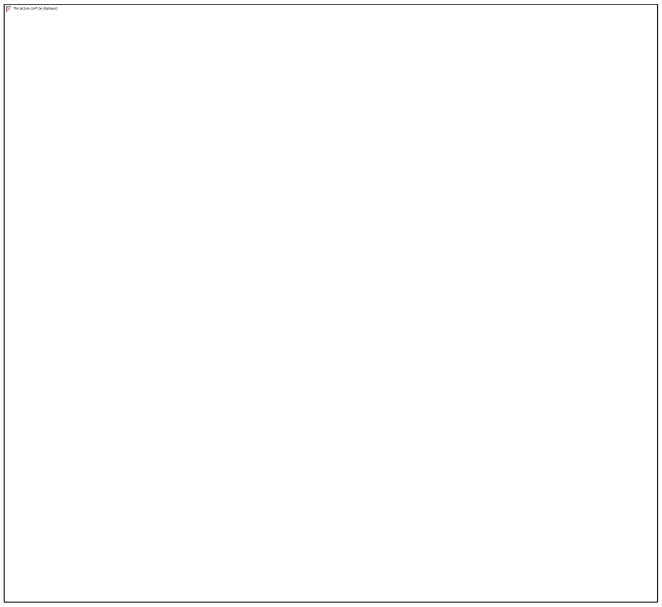
age | 0.61 | [0.54, 0.68] | < .001

sex string [Male] | 0.19 | [0.02, 0.36] | 0.028

weight | -0.01 | [-0.02, -0.01] | < .001

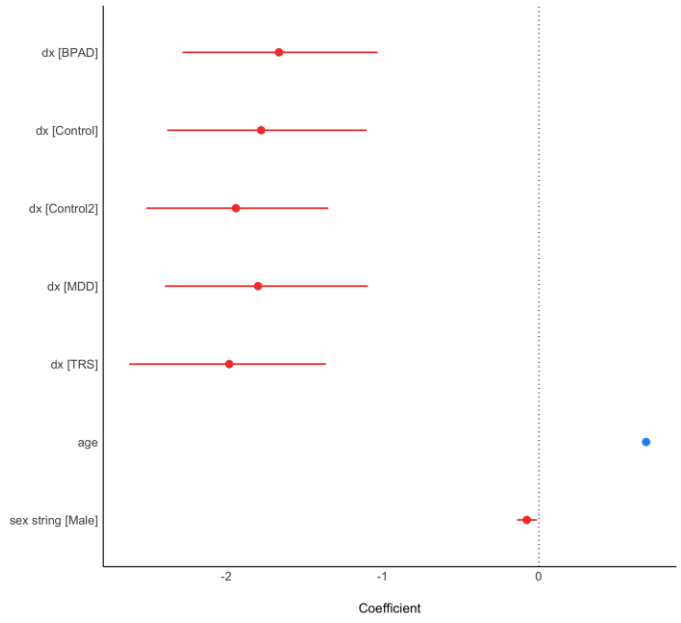
Model 2: GLM lognfl ~ dx + weight + age + sex, standardised coefficients, reference group = TRS,, bootstrapped

Parameter	Coefficient	95% CI	p
(Intercept)	0.79	[0.29, 1.25]	0.004
dx [BPAD]	0.32	[0.07, 0.59]	0.020
dx [Control]	0.10	[-0.16, 0.39]	0.446
dx [MDD]	0.20	[-0.12, 0.51]	0.198
age	0.61	[0.54, 0.68]	< .001
sex string [Male]	0.19	[0.02, 0.36]	0.028
weight	-0.01	[-0.02, -0.01]	< .001



Model 1 for bvFTD: GLM lognfl ~ dx + age + sex, standardised coefficients, reference group = bvFTD, bootstrapped

Parameter	Coefficient	95% CI	p
(Intercept)	1.93	[1.34, 2.50]	< .001
dx [BPAD]	-1.66	[-2.28, -1.03]	< .001
dx [Control]	-1.78	[-2.38, -1.10]	< .001
dx [Control2]	-1.94	[-2.51, -1.35]	< .001
dx [MDD]	-1.80	[-2.39, -1.09]	< .001
dx [TRS]	-1.98	[-2.62, -1.36]	< .001
age	0.69	[0.66, 0.71]	< .001
sex string [Male]	-0.08	[-0.14, -0.01]	0.008



bvFTD versus:	AUC [95%CIs]	Cut-off (pg/mL)	Sensitivity	Specificity
All psychiatric disorders (MDD, BPAD, TRS)	0.95 [0.91, 0.99]	13.3	86	88
All psychiatric disorders (MDD, BPAD, TRS) (age-matched, n=131)*	0.91 [0.85, 0.98]	13.3 (22)^	86 (73)	78 (95)
BPAD	0.95 [0.91, 0.99]	13.3	86	89
MDD	0.91 [0.83, 0.98]	13.1 29.8	86 68	79 98
TRS	0.97 [0.94, 1.00]	9.6 (13.3)^	100 (86)	83 (90)
Control Group 1	0.95 [0.91, 0.99]	9.6 (13.4)^	100 (86)	78 (92)
Control Group 2	0.93 [0.88, 0.98]	13.4	86	83

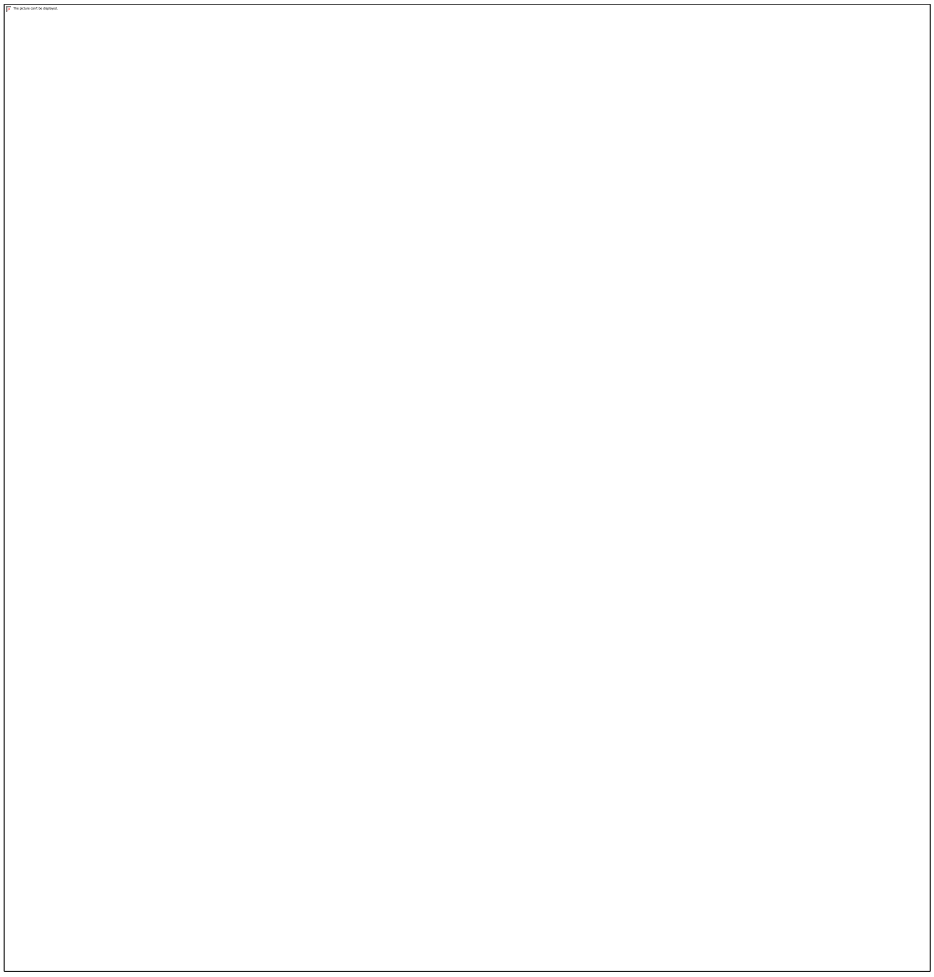
Supplementary Table 1. Receiver operating characteristic (ROC) curve analyses for bvFTD versus other clinically relevant groups.

Levels above the cut-off indicate bvFTD. Results were similar when restricted to age range of bvFTD (43-80 years of age).

*: BPAD n=66, MDD n=35, TRS n=30

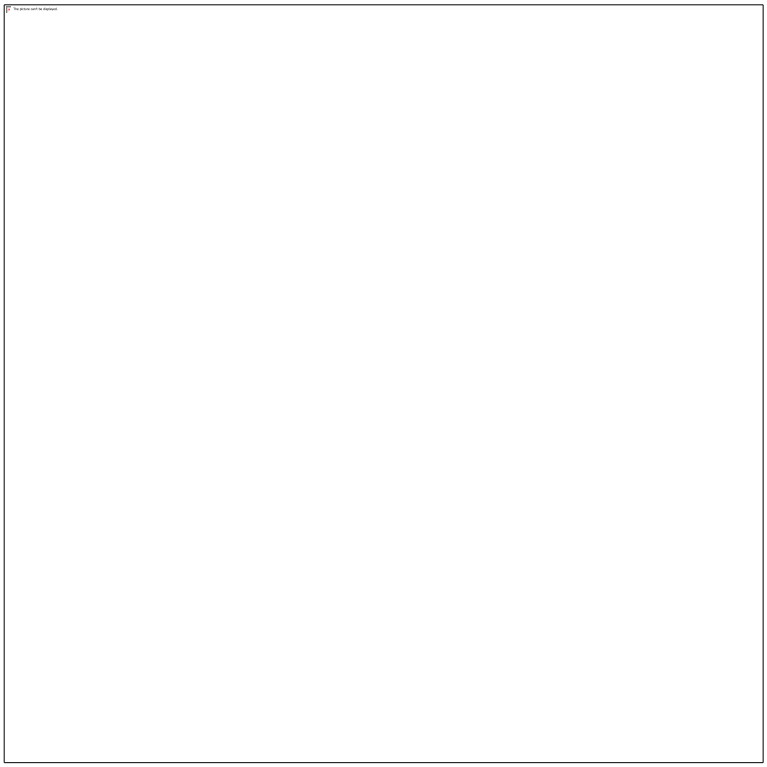
^: indicates alternative cut-off to Youden's method, but optimising for specificity

AUC: area under the curve; BPAD: bipolar disorder; bvFTD: behavioural variant frontotemporal dementia; CIs: confidence intervals; MDD: major depressive disorder; NfL: neurofilament light; TRS: treatment-resistant schizophrenia


The picture can't be displayed.

Extras for Cassie!

Lognfl x age



(GAMLSS Nfl z-score adjusted for age) x age – TRS holds up

<u>Dx * age interactions</u>

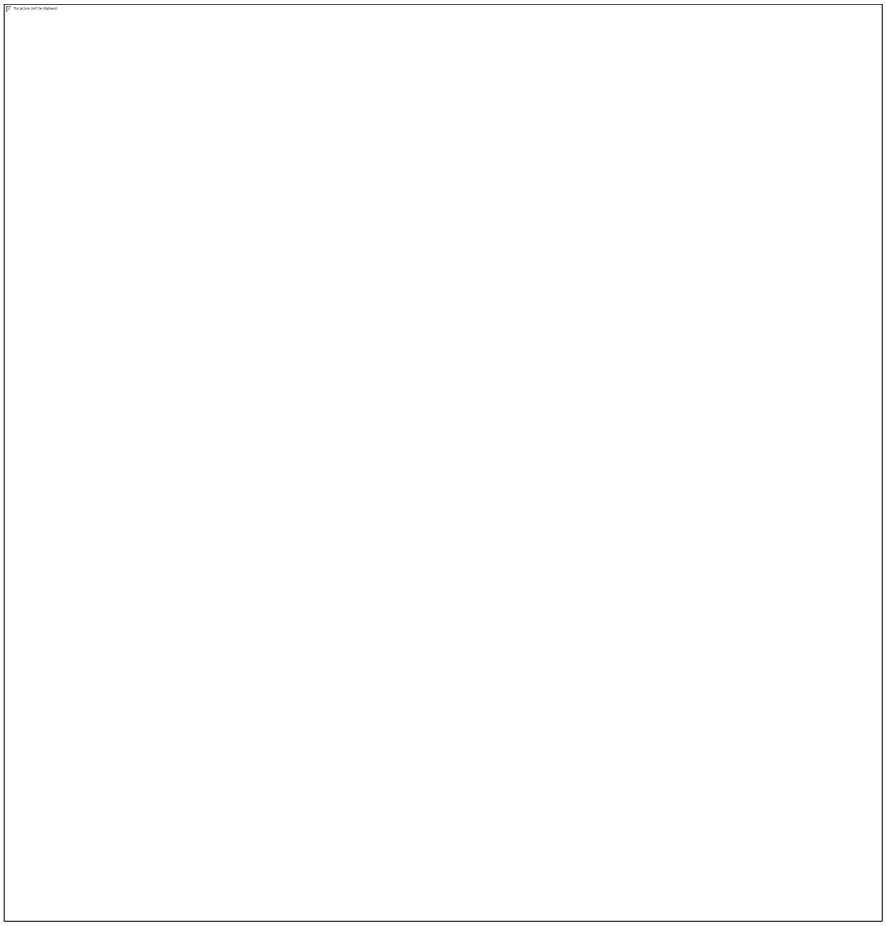
#looking at age * group interaction

model1intdata <- data.raw.full %>% mutate(dx=relevel(dx, ref="Control"))

model1int <- lm(scale(lgnfl) ~ dx + scale(age) + sex_string + dx * scale(age), data=model1intdata) #STANDARDISED

```
model1intparam <- parameters(model1int, bootstrap = TRUE)
# plot(model1int)
summary(model1int)
plot(model1intparam)
```

Parameter	Coefficient	95% CI	p
(Intercept)	0.10	[-0.11, 0.35]	0.368
dx [BPAD]	0.02	[-0.26, 0.30]	0.878
dx [bvFTD]	2.54	[1.27, 3.46]	0.002
dx [Control2]	-0.11	[-0.37, 0.10]	0.320
dx [MDD]	0.03	[-0.29, 0.29]	0.824
dx [TRS]	0.22	[-0.20, 0.59]	0.344
age	0.60	[0.44, 0.80]	< .001
sex string [Male]	-0.07	[-0.13, -0.01]	0.020
dx [BPAD] × age	-0.15	[-0.40, 0.08]	0.192
dx [bvFTD] × age	-0.77	[-1.55, 0.21]	0.108
dx [Control2] × age	0.09	[-0.11, 0.26]	0.372
dx [MDD] × age	0.19	[-0.11, 0.47]	0.182
dx [TRS] × age	0.50	[0.13, 0.91]	0.010



now add weight

model1int <- lm(scale(lgnfl) ~ dx + scale(age) + sex_string + scale(weight) + dx * scale(age), data=model1intdata) #STANDARDISED

model1intparam <- parameters(model1int, bootstrap = TRUE)

plot(model1int)

summary(model1intparam)

plot(model1intparam)

Parameter | Coefficient | 95% CI | p

(Intercept) | -0.18 | [-0.33, -0.02] | 0.020

dx [BPAD] | 0.22 | [0.01, 0.41] | 0.034

dx [MDD] | -9.40e-03 | [-0.29, 0.24] | 0.932

dx [TRS] | 0.04 | [-0.24, 0.33] | 0.770

age | 0.57 | [0.43, 0.70] | < .001

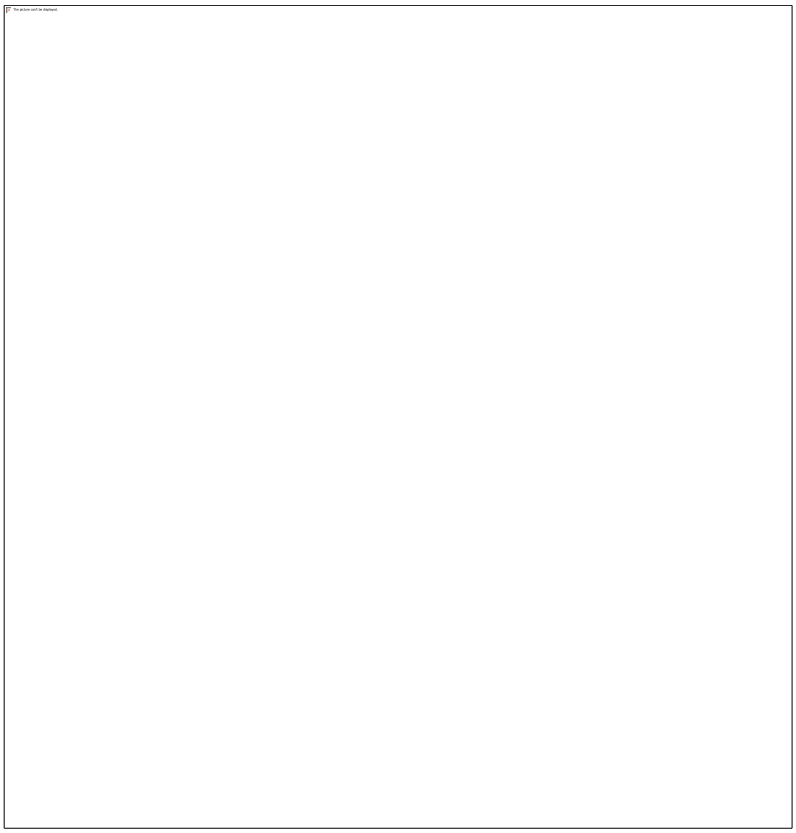
sex string [Male] | 0.21 | [0.04, 0.39] | 0.014

weight | -0.25 | [-0.34, -0.16] | < .001

dx [BPAD] × age | -0.11 | [-0.28, 0.07] | 0.274

dx [MDD] × age | 0.16 | [-0.05, 0.37] | 0.132

dx [TRS] × age | 0.47 | [0.15, 0.81] | 0.002



<u>Now with Control Group 2 = reference</u>

model1intdata <- data.raw.full %>% mutate(dx=relevel(dx, ref="Control2"))

model1int <- lm(scale(lgnfl) ~ dx + scale(age) + sex_string + dx * scale(age), data=model1intdata) #STANDARDISED

model1intparam <- parameters(model1int, bootstrap = TRUE)

plot(model1int)

summary(model1intparam)

plot(model1intparam)

Parameter | Coefficient | 95% CI | p

(Intercept) | -0.01 | [-0.04, 0.01] | 0.398

dx [BPAD] | 0.13 | [-0.03, 0.31] | 0.130

dx [bvFTD] | 2.67 | [1.36, 3.60] | < .001

dx [Control] | 0.11 | [-0.09, 0.33] | 0.322

dx [MDD] | 0.12 | [-0.06, 0.31] | 0.190

dx [TRS] | 0.32 | [-0.02, 0.67] | 0.062

age | 0.70 | [0.66, 0.73] | < .001

sex string [Male] | -0.07 | [-0.13, -0.01] | 0.024

dx [BPAD] × age | -0.23 | [-0.38, -0.08] | 0.004

dx [bvFTD] × age | -0.86 | [-1.64, 0.13] | 0.082

dx [Control] × age | -0.10 | [-0.27, 0.10] | 0.332

dx [MDD] × age | 0.11 | [-0.12, 0.33] | 0.328

dx [TRS] × age | 0.41 | [0.08, 0.78] | 0.014