

RESEARCH ARTICLE

Novel CSF biomarkers to discriminate FTLD and its pathological subtypes

Marta del Campo¹, Daniela Galimberti², Naura Elias¹, Lynn Boonkamp¹, Yolande A. Pijnenburg³, John C. van Swieten^{3,4}, Kelly Watts⁵, Silvia Paciotti⁶, Tommaso Beccari⁶, William Hu⁵ & Charlotte E. Teunissen¹

¹Neurochemistry Laboratory and Biobank, Department of Clinical Chemistry, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

²Department of Neurological Sciences, Pathophysiology and Transplantation, "Dino Ferrari" Center, University of Milan, Fondazione Ca' Granda, IRCCS Ospedale Policlinico, Milan, Italy

³Alzheimer Centre and Department of Neurology, Neuroscience Campus Amsterdam, VU University Medical Centre, Amsterdam, The Netherlands

⁴Department of Neurology, Erasmus Medical Center, Rotterdam, The Netherlands

⁵Department of Neurology, Center for Neurodegenerative Diseases Research, Alzheimer's Disease Research Center, Emory University School of Medicine, Atlanta, Georgia

⁶Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy

Correspondence

Marta del Campo Milan, Department of Clinical Chemistry, Neurochemistry laboratory VU University Medical Center (VUmc), PK1 Br016, De Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands.
E-mail: m.delcampomilan@vumc.nl

Funding Information

We would like to acknowledge the Association for Frontotemporal Degeneration and Alzheimer's Drug Discovery Foundation, and the ZonMw Memorabel program project "PRODIA," as part of the Deltaplanning Dementie, for their grant support of this study.

Received: 29 April 2018; Revised: 19 June 2018; Accepted: 5 July 2018

Annals of Clinical and Translational Neurology 2018; 5(10): 1163–1175

doi: 10.1002/acn3.629

Abstract

Objective: Frontotemporal lobar degeneration (FTLD) is the second most prevalent dementia in young patients and is characterized by the presence of two main protein aggregates in the brain, tau (FTLD-Tau) or TDP43 (FTLD-TDP), which likely require distinct pharmacological therapy. However, specific diagnosis of FTLD and its subtypes remains challenging due to largely overlapping clinical phenotypes. Here, we aimed to assess the clinical performance of novel cerebrospinal fluid (CSF) biomarkers for discrimination of FTLD and its pathological subtypes. **Methods:** YKL40, FABP4, MFG-E8, and the activities of catalase and specific lysosomal enzymes were analyzed in patients with FTLD-TDP ($n = 30$), FTLD-Tau ($n = 20$), AD ($n = 30$), DLB ($n = 29$), and nondemented controls ($n = 29$) obtained from two different centers. Models were validated in an independent CSF cohort ($n = 188$). **Results:** YKL40 and catalase activity were increased in FTLD-TDP cases compared to controls. YKL40 levels were also higher in FTLD-TDP compared to FTLD-Tau. We identified biomarker models able to discriminate FTLD from nondemented controls (MFG-E8, tTau, and $A\beta_{42}$; 78% sensitivity and 83% specificity) and non-FTLD dementia (YKL40, pTau, p/tTau ratio, and age; 90% sensitivity, 78% specificity), which were validated in an independent cohort. In addition, we identified a biomarker model differentiating FTLD-TDP from FTLD-Tau (YKL40, MFG-E8, β HexA together with β HexA/tHex and p/tTau ratios and age) with 80% sensitivity and 82% specificity. **Interpretation:** This study identifies CSF protein signatures distinguishing FTLD and the two main pathological subtypes with optimal accuracy (specificity/sensitivity > 80%). Validation of these models may allow appropriate selection of cases for clinical trials targeting the accumulation of Tau or TDP43, thereby increasing their efficiency and facilitating the development of successful therapies.

Introduction

Frontotemporal lobar degeneration (FTLD) is the second most prevalent dementia in patients below 65 years old^{1,2}

and has the worst life expectancy among non-prion dementia.³ Two main pathological subtypes have been described based on the proteinopathy found in the brain: around half of the cases develop aggregates of the

microtubule-associated protein tau (FTLD-Tau), while the other half are characterized by cytoplasmic inclusions of the transactivator regulatory DNA-binding protein 43 (TDP43, FTLD-TDP).⁴ These two main pathologies likely require distinct pharmacological therapy, and thus, discrimination of both subtypes is strongly needed. However, the clinical presentation of the FTLD pathological subtypes is heterogeneous and overlapping.⁵ So far, there are still no effective early biomarkers available to discriminate FTLD and its two main pathological subtypes, hampering the selection of appropriate patients for clinical trials targeting the specific proteinopathy (i.e., Tau or TDP43).^{6,7}

Most biomarker studies have been performed in pathologically heterogeneous populations.⁸ The few studies analyzing antemortem cerebrospinal fluid (CSF) with known underlying neuropathology have revealed several candidate biomarkers, such as the pTau₁₈₁ to tau ratio, which discriminates FTLD-TDP from FTLD-Tau cases with approximately 80% sensitivity and 60% specificity.^{9–11} Despite these promising results, their specificity is far from optimal, most of the identified markers are awaiting further validation and their diagnostic accuracy remains to be evaluated.

In order to unravel novel specific biomarkers for FTLD subtypes, we previously mapped and validated changes in the proteome of antemortem CSF of well-characterized FTLD patients with confirmed tau or TDP43 pathology and nondemented controls.¹² In this study, we externally validated and assessed the clinical performance of the identified novel CSF biomarkers (chitinase-3-like protein 1 [CHI3L1 or YKL-40], milk fat globule-EGF factor 8 protein [MFG-E8], fatty acid-binding protein 4 [FABP4], catalase activity, and specific lysosomal enzymes' activity), as single biomarkers or combined, in discriminating FTLD and its different pathological subtypes using two independent cohorts biobanked at the Emory University and Milan University Hospital Policlinico.

Methods

Human CSF samples

CSF material was obtained from the Emory University ($n = 100$, USA) and Milan University Hospital Policlinico ($n = 45$, Italy) (discovery cohort, Table 1). FTLD patients with an underlying TDP43 pathology (FTLD-TDP, $n = 30$) were selected based on autopsy ($n = 8$) and C9orf72/GRN mutations ($n = 15$).^{13,14} Diagnostic groups were enriched with CSF from patients with FTLD-Plus syndromes that reflect high correlation with a specific neuropathology. Thus, the FTLD-TDP group was enriched with FTLD patients with amyotrophic lateral

sclerosis (FTLD-ALS, $n = 7$), associated with TDP43 pathology.¹⁵ FTLD cases with tau neuropathology (FTLD-Tau, $n = 20$) were selected based on autopsy ($n = 2$), MAPT mutations ($n = 2$),⁶ and familial history of autopsy confirmed FTLD-Tau ($n = 1$). The FTLD-Tau group was also enriched with CSF from patients with FTLD-Plus syndromes related to tau pathology such as progressive supranuclear palsy (FTLD-PSP, $n = 10$) or corticobasal syndrome (FTLD-CBS, $n = 5$).^{16,17} Noteworthy, six FTLD-Tau and six FTLD-TDP patients had a positive AD CSF biomarker profile (low CSF β -amyloid 1–42 (A β 42) and high p or t-Tau level, applying local laboratory standards), suggesting potential AD copathology in those cases. Non-demented healthy controls (CON, $n = 29$, 4 of them with positive CSF AD biomarker profile) and patients with other types of dementia, such as AD ($n = 30$) and DLB ($n = 29$), were also selected to test the specificity of the biomarker signatures to FTLD. An additional independent CSF cohort was used for validation of the resulting CSF protein biomarker signatures (validation cohort: [subjective cognitive decline (SCD) = 59, FTLD-TDP = 42, FTLD-Tau = 50, AD = 17, and DLB = 20]), consisting of samples recruited at the Erasmus Medical Center (MC) and the VU Medical Center (VUmc; Table 1).

All participants underwent standard neurological and cognitive assessments and diagnosis was assigned according to consensus criteria.^{18–24} The control group of the validation cohort were labeled during a multidisciplinary consensus meeting as SCD when they presented with subjective cognitive complaints, but objective cognitive and laboratory investigations (including AD CSF biomarkers) were normal and thus comparable to controls (CON). Non-demented healthy control or SCD cases did not meet criteria for mild cognitive impairment and had no signs of inflammatory or neurodegenerative disorders, or family history of neurodegenerative diseases. All CSF samples of all cohorts were stored in agreement with the JPND-BIO-MARKAPD guidelines.²⁵ Demographic data, concentration of CSF A β 42, t-Tau, pTau, and type of diagnosis of all cases used in each cohort and the biomarkers measured are summarized in Table 1. The studies were approved by the Institutional Ethical Review Boards of each center. Informed consent was obtained from all subjects or their authorized representatives.

Biomarker analysis

CSF levels of the biomarkers were measured using specific immunoassays that have been previously validated for CSF analysis¹² (Data S1). Intra- and interassays CVs were calculated using two CSF pools as quality controls, resulting in 1.8% and 10% for YKL-40, 3.1% and 9.5% for

Table 1. Descriptive statistics of demographics by diagnostic groups

	CON	FTLD-TDP	FTLD-Tau	AD	DLB
Cohort 1 (Emory + Milan)					
<i>n</i> (M/F)	29 (14/15)	30 (18/12)	20 (13/7)	30 (15/15)	29 (15/14)
Age, years (mean ± SD)	62 (15)	66 (7)	65 (10)	69 (8)	68 (8)
<i>n</i> (Emory/Milan)	20/9	20/10	20/0	20/10	20/9
A β_{1-42} (pg/mL)	408 (307) ^{a,c, d,e}	230 (452) ^b	176 (101) ^b	218 (211) ^b	298 (279) ^b
t-Tau (pg/mL)	46 (36) ^a	78 (114) ^a	43 (35) ^a	171 (156) ^{b,c,d,e}	43 (199) ^a
pTau (pg/mL)	18 (12) ^{a,e}	23 (21) ^{a,e}	18 (14) ^a	72 (46) ^{b,c,d,e}	33 (19) ^{a,b,c}
FTLD subgroups		8 autopsy 15 mutations 7 FTLT-ALS	2 autopsy 2 mutations 1 family history 10 PSP 5 CBS		
Cohort 2 (VUmc + ErasmusMC)					
<i>n</i> (M/F)	59 (32/27)	42 (23/19)	50 (26/24)	17 (9/8)	20 (17/3)
Age, years (mean ± SD)	59 (10)	60(8)	64 (9)	64 (6)	63 (5)
A β_{1-42} (pg/mL)	983 (342) ^{a,c, d,e}	874 (328) ^{a,b}	795 (335) ^{a,b}	461 (137) ^{b,c,d,e}	648 (483) ^{a,b}
t-Tau (pg/mL)	249 (106) ^{a,c}	353 (137) ^{a,b}	290 (182) ^a	622 (237) ^{b,c,d,e}	299 (118) ^a
pTau (pg/mL)	45 (20) ^a	38 (15) ^{a,e}	39 (21) ^a	84 (26) ^{b,c,d,e}	53 (24) ^a
FTLD subgroups		15 autopsy 21 mutations 6 FTLT-ALS	3 autopsy 5 mutations 22 PSP 20 CBS		

CON, nondemented controls; FTLT, frontotemporal lobar degeneration; AD, Alzheimer's disease; DLB, dementia with Lewy body; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; *n*, number of cases; M, Male; F, female.

^a*P* < 0.05 compared to AD.

^b*P* < 0.05 compared to CON.

^c*P* < 0.05 compared to FTLT-TDP.

^d*P* < 0.05 compared to FTLT-Tau.

^e*P* < 0.05 compared to DLB.

FABP4, 10.8% and 24.2% for MFG-E8, and 3.1% and 13.7% for catalase activity. The levels of AD-related biomarkers (total and phosphorylated tau [t-Tau and pTau₁₈₁]) were analyzed in the corresponding sample collection center using the commercially available kits (Emory: INNO-BIA AlzBio3; Milan: Innostest A β (1-42), hTAUAg, phosphor-Tau(181P); Fujiribo, Ghent, Belgium, both using the same antibodies) as previously described.^{9,26} The levels of neurofilament light change (NfL) were measured in a subset of cases within the validation cohort using a validated immunoassay ELISA of UmanDiagnostics (Umeå, Sweden) as previously described.²⁷ All biomarkers were analyzed by a single experienced technician blinded to the clinical groups.

Effects of preanalytical factors

The effects of age, sex, and storage duration on CSF analytes were assessed by statistical evaluation of the results as described below. The effect of freeze-thaw cycles was experimentally determined for YKL40, MFG-E8, and catalase activity by freezing and thawing independent CSF

samples (*n* = 2–3) up to four times, leaving the samples each time at least 2 hours at room temperature, and next store at –80°C for at least 12 h.

Statistical analysis

Statistical analyses were performed using SPSS (Chicago, IL, USA). The influence of different preanalytical variables on biomarker levels was analyzed by linear regression after normalizing skewed data using two-step transformation.^{28,29} Between-group analyses of demographic variables were performed using the Student's *t*-test or Pearson's chi-square test in normally distributed data. Non-Gaussian distributed data were analyzed using the Mann–Whitney test. In the discovery cohort, difference in the biomarker levels between the clinical groups was evaluated by ANCOVA using normalized values and including either center and age or length of storage as covariate followed by Fisher's least significant difference (LSD, equivalent to Mann–Whitney *U* test for adjusted means). Noteworthy, not every differentially expressed marker necessarily has discriminatory power in a classification

exercise.³⁰ Thus, we next used multivariate stepwise backward logistic regression based on likelihood ratio to find the classification signature that gives maximum predictive performance in the demarcation of the specific diagnostic groups including all the CSF markers analyzed and age. The resulting predicting probabilities were used to assess the diagnostic value of biomarker combination using receiver operator characteristic (ROC). Multilayer perceptron analysis was used to validate the models by randomly selecting subset of samples from the whole cohort. Areas under the curve (AUC), sensitivity, and specificity values were calculated. The performance of the models was classified as poor (AUC:0.6–0.7), moderate (AUC = 0.7–0.8), good (AUC = 0.8–0.9), and optimal (AUC = 0.9–1). To further validate the biomarker models, we next analyzed the corresponding biomarker combinations in the independent validation cohort by logistic regression and ROC analysis. For models that could not be further tested in the validation cohort, data were reanalyzed using support vector machine (SVM), which randomly splits the original samples of analysis into training and validation sets (70–30%, respectively). Values with $P < 0.05$ were considered significant.

Results

Demographic and preanalytical effects

No difference in age or sex was observed between FTLD pathological subtypes (Table 1). Samples from the Emory cohort had a shorter storage time and patients had a lower age compared to those obtained from Milan ($P < 0.001$). We observed that the overall levels of all biomarkers with the exception of FABP4 were higher in samples collected at Emory University (Table S1).

Freeze–thaw cycles did not influence the levels of YKL-40, MFG-E8, or catalase activity (Fig. S1), except for

lysosomal activities, which change only after two freeze–thaw cycles.³¹ Longer storage time was associated with decreased levels of MFG-E8 ($P < 0.0001$), as well as the activities of catalase ($P < 0.0001$) and all lysosomal enzymes ($P < 0.01$). Patient age influenced only the levels of YKL-40 ($P < 0.01$) and FABP4 ($P < 0.01$). Sex did not influence any of the biomarkers analyzed (Table S2).

In summary, we observed that age influenced the levels of YKL-40 and FABP4. The center in which samples were collected had a strong influence on all the CSF biomarkers analyzed and the length of storage negatively influenced MFG-E8 concentration and the activities of catalase and lysosomal enzymes. Thus, analysis of the data was performed always correcting for center and either length of storage or age when applicable.

Change in levels of YKL-40, MFG-E8, and catalase activity in CSF across different diagnostic groups

YKL40 was increased in the overall FTLD group compared to nondemented controls ($P < 0.01$) and was higher in FTLD-TDP compared to FTLD-Tau cases ($P < 0.05$). YKL40 was also increased in FTLD-TDP compared to DLB and nondemented controls ($P < 0.0001$), but did not differ from those in AD (Fig. 1A). ROC analysis showed that CSF YKL-40 levels had a moderate performance discriminating nondemented healthy controls (CON) from the overall FTLD patients (AUC: 0.74; 95% CI: 0.62–0.85, $P < 0.0001$) or FTLD-TDP subtype (AUC: 0.78; 95% CI: 0.67–0.90, $P < 0.0001$, Fig. 2D) but did not reach sufficient sensitivity/specificity values (<80%, Table 2).

The levels of MFG-E8 were decreased in the overall FTLD group compared to AD ($P < 0.05$). No significant difference was observed between FTLD-TDP and FTLD-Tau. MFG-E8 levels were especially lower in FTLD-TDP patients compared to AD ($P < 0.05$, Fig. 1B). MFG-E8

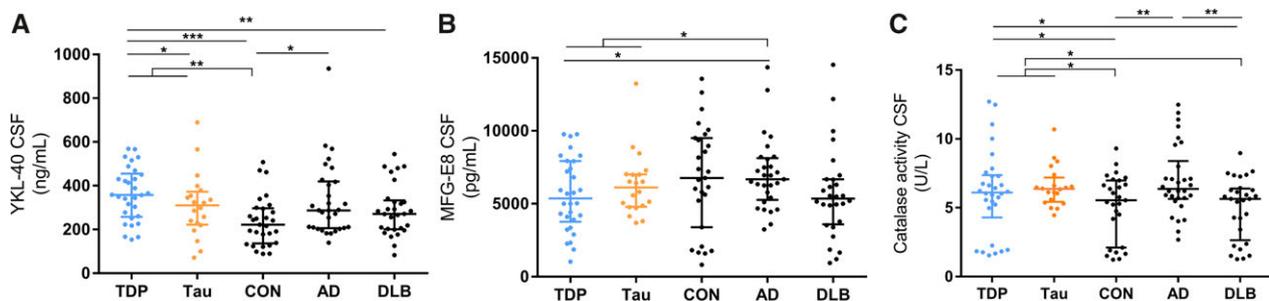


Figure 1. YKL-40, MFG-E8 and catalase activity in CSF were changed across the different diagnostic groups. Dot plot displays the uncorrected values of YKL40 (A) MFG-E8 (B) and the activity of catalase (C) in CSF for each clinical group (FTLD-TDP in blue and FTLD-Tau in orange). Median and interquartile range are represented * $P < 0.05$; *** $P < 0.001$. Abbreviations: n.s., nonsignificant; CSF, cerebrospinal fluid; CON, healthy nondemented controls; TDP, TAR DNA-binding protein 43; AD, Alzheimer's disease; DLB, dementia with Lewy bodies.

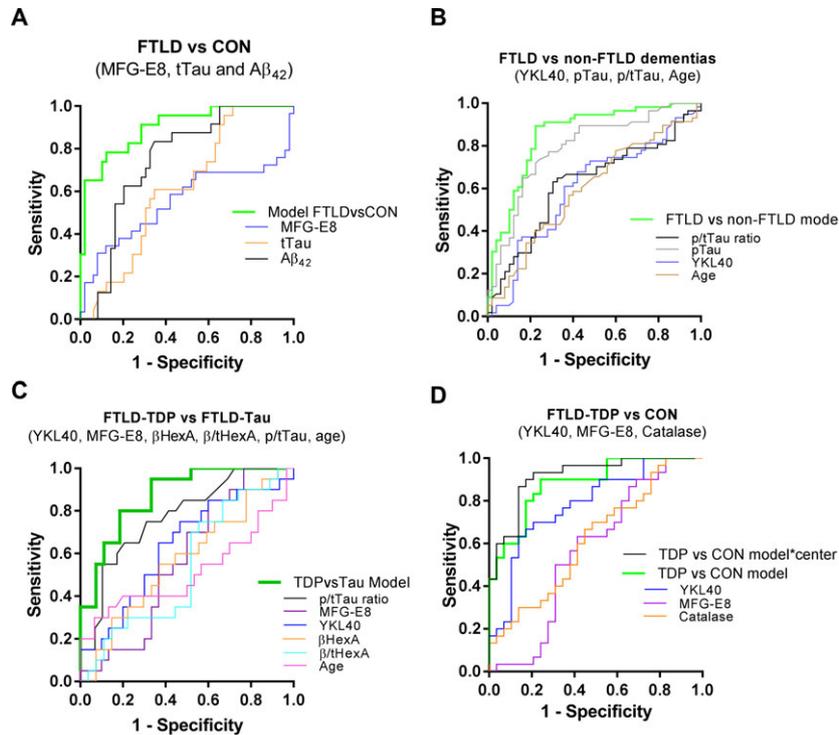


Figure 2. Receiver operating curves (ROC) of the different models discriminating specific diagnostic groups in the discovery cohort. (A) ROC curves of the individual markers or the corresponding biomarker combination (MFG-E8, tTau, and $A\beta_{42}$) discriminating FTL from CON. (B) ROC curves of the individual markers or the corresponding biomarker combination (YKL40, pTau, p/tTau, and age) discriminating FTL from non-FTL dementia (AD, DLB). (C) ROC curves of the individual markers or the corresponding biomarker combination (YKL40, MFG-E8, p/tTau ratio, β HexA activity, β /tHexA, and age) discriminating FTL-TDP from FTL-Tau. (D) ROC curves of the individual markers or the corresponding biomarker combination discriminating FTL-TDP from CON (YKL40, MFG-E8, and catalase activity) with and without center as interaction factor.

did not show enough diagnostic performance on discriminating FTL from AD.

Catalase activity in CSF was increased in FTL and FTL-TDP compared to controls ($P < 0.05$) and DLB patients ($P < 0.05$; Fig. 1C). Catalase activity could only discriminate FTL from nondemented controls with poor performance (AUC: 0.64; $P = 0.05$, Fig. 2A, Table 2).

The levels of FABP4 as well as the activity of the different lysosomal enzymes in CSF were not changed between the different diagnostic groups (Fig. S2). Differences in biomarker levels between the non-FTL dementia groups were also observed (Fig. 1A–C).

Specific CSF protein signatures discriminate FTL and its pathological subtypes

CSF biomarkers discriminating FTL from CON

Multivariate stepwise backward regression revealed that combination of MFG-E8 together with tTau and $A\beta_{42}$ could discriminate control cases from FTL patients (FTL vs. CON model) with optimal performance (AUC: 0.90, 95% CI: 0.83–0.98, $P < 0.0001$) leading to 78%

sensitivity and 83% specificity. This performance was better to that observed for any of the individual markers within the model (Fig. 2A, Table 2). These results were confirmed by multilayer perceptron analysis (AUC: 0.91).

CSF biomarkers discriminating FTL from non-FTL dementia cases

Combination of YKL-40 with pTau, the p/tTau ratio and age could discriminate FTL cases from patients with non-FTL dementia (AD and DLB) with optimal performance (AUC: 0.86, 95% CI: 0.78–0.93, $P < 0.0001$) leading to 90% sensitivity and 78% specificity. This performance was better to that observed for any of the individual markers within the model (Fig. 2B, Table 2). These results were confirmed by multilayer perceptron analysis (AUC: 0.86).

CSF biomarkers discriminating FTL-TDP from FTL-Tau

Combination of YKL40, MFG-E8, activity of β HexA, and the β HexA/tHex activity ratio together with p/tTau ratio

and age could discriminate FTLD-TDP from FTLD-Tau (TDP vs. Tau model) with an accuracy of 0.87 (95% CI AUC: 0.77–0.97, $P < 0.0001$), and a sensitivity and specificity of 80% and 81%, respectively (Fig. 2C, Table 2). Such results were confirmed by multilayer perceptron analysis (AUC: 0.9). This performance was better to that observed for the p/tTau ratio alone (AUC: 0.78; 95% CI: 0.65–0.91, $P = 0.001$, 80% sensitivity and 59% specificity, Fig. 2C, Table 2), the marker showing the strongest discrimination between FTLD-TDP and FTLD-Tau to date.^{9,10}

CSF biomarkers discriminating FTLD-TDP from CON

Combination of YKL-40, MFG-E8, and catalase activity including center as an interaction factor could discriminate control cases from FTLD-TDP patients (TDP vs. CON model) with optimal performance (AUC: 0.92, 95% CI: 0.84–0.97, $P < 0.0001$, Fig. 2D) leading to 90% sensitivity and 83% specificity. Adding tau markers, either alone or as ratio, did not improve the sensitivity or specificity of the model. Importantly, removing center as an interaction factor decreased specificity to 76% (AUC: 0.88, 95% CI AUC: 0.80–0.97, $P < 0.0001$, Fig. 2D, Table 2). These results were confirmed by multilayer perceptron analysis (AUC: 0.88).

CSF biomarker signatures discriminating CON from FTLD-Tau were not identified.

Validation of the biomarker models

We next tested how well the generated models could be validated in independent validation cohort (Table 3). We observed that the “FTLD vs. CON” model (MFG-E8, tTau, and $A\beta_{42}$) could again discriminate FTLD from CON cases with optimal performance (AUC: 0.93, 95% CI: 0.89–0.97, $P < 0.0001$; with 88% sensitivity and 85% specificity (Fig. 3A; Table 3). Using a subset of cases for which NfL measurements were available (FTLD = 92, CON = 28), we observed that the FTLD vs. CON model (AUC:0.94; $P < 0.0001$) performed similar to NfL alone (AUC:0.94 $P < 0.0001$, Fig. S3), a non-disease specific marker that optimally discriminate FTLD cases from controls.^{27,32–34}

The “FTLD vs. non-FTLD dementia” model (pTau, t/pTau ratio, YKL40 and age) could again optimally discriminate FTLD cases from non-FTLD dementia cases (AD and DLB) in the validation cohort (AUC: 0.93, 95% CI: 0.88–0.98, $P < 0.0001$) achieving 91% sensitivity and 84% specificity (Fig. 3B; Table 3). The “FTLD-TDP vs. CON” model (YKL-40, MFG-E8, and catalase activity) was also validated in the new independent cohort (AUC: 0.94, 95% CI: 0.90–0.99, $P < 0.0001$) with 90% sensitivity and 86% specificity (Fig. 3C; Table 3).

Most of the samples of the validation cohort had already undergone more than two freeze–thaw cycles, which affect the overall activity values of tHexA and β HexA.³¹ The number of samples available in which the activity of such enzymes could be optimally measured (TDP = 12, FTLD-Tau = 6) was too small and thus the “TDP vs. Tau” model could not be validated in an independent cohort. However, analysis of the discovery cohort using supporting vector machine (SVM), an alternative statistical approach that randomly split the cohort into training and testing sets, reported similar results (AUC: 0.93) with average error rates of 22.4% and 21.3% for the training and testing test.

Discussion

Biomarkers discriminating FTLD pathological subtypes are strongly needed for the selection of patients in drug trials targeting the specific proteinopathies.^{6,7} We have assessed and validated the clinical performance of novel CSF biomarkers identified previously¹² for discrimination of FTLD pathological subtypes and nondemented controls using two independent CSF cohorts coming from different centers. The main findings were the identification of four novel CSF biomarker signatures able to discriminate: (1) FTLD from non-demented controls (FTLD vs. CON model: MFG-E8, tTau, and $A\beta_{42}$), (2) FTLD from other dementia (FTLD vs. non-FTLD dementia model: YKL-40, pTau, and p/tTau ratio) and (3) the main FTLD pathological subtypes (TDP vs. Tau model: YKL40, MFG-E8, activity of β HexA, β HexA/tHex ratio, p/tTau ratio, and age).

In agreement with previous studies, CSF YKL40 was increased in FTLD and AD compared to controls.^{35–37} FTLD-TDP had the highest YKL-40 values, which were comparable to those observed in AD patients, but different to those observed in CON, FTLD-Tau, or DLB. These findings are partially in agreement with our previous study in which higher levels were also observed in the FTLD-Tau group.¹² Importantly, FTLD-Tau encompasses tauopathies with different etiologies such as FTLD-MAPT, PSP, PiD, and CBD. While our previous proteomics-based study analyzed mainly FTLD-Tau cases with MAPT mutations,¹² the current study was performed with a more heterogeneous FTLD-Tau group including also sporadic CBS and PSP cases, which may explain the observed discrepancies. Indeed, recent studies have highlighted that CSF biomarkers (e.g., pTau) can differ between familial and sporadic FTLD cases that develop the same underlying neuropathology.^{38,39} Thus, this data also highlights the impact that the heterogeneity within each FTLD subtype can have on the CSF biomarker profile. CSF YKL-40 was also increased in different acute inflammatory

Table 2. ROC analysis of CSF parameters discriminating different diagnostic groups in the discovery cohort

	Cut-off point ¹	Sensitivity (%)	Specificity (%)	AUC (95% CI)	+LR ²	LR ³	<i>p</i> value (individually)	Coefficient (B)	<i>P</i> value (within model)
CSF variables									
FTLD (<i>n</i> = 49) vs. CON (<i>n</i> = 23)									
MFG-E8	na	na	na	0.55 (0.405–0.702)	na	na	0.428	−0.0004	0.005
tTau	na	na	na	0.62 (0.487–0.747)	na	na	0.111	0.068	<0.0001
Aβ42	264	71	69	0.75 (0.632–0.859)	2.29	0.42	0.001	−0.010	<0.0001
FTLD vs. CON model ⁴	0.686	78	83	0.90 (0.827–0.976)	4.59	0.27	<0.0001		
FTLD (<i>n</i> = 49) vs. non-FTLD dementia (<i>n</i> = 57)									
YKL40	na	na	na	0.60 (0.491–0.708)	na	na	0.071	−0.004	0.021
pTau	29.1	77	67	0.80 (0.702–0.877)	2.3	0.3	<0.0001	0.044	<0.0001
p/tTau	na	na	na	0.62 (0.512–0.728)	na	na	0.034	2.33	0.008
Age	na	na	na	0.61 (0.503–0.719)	na	na	0.047	0.079	0.010
FTLD vs. non-FTLD dementia model ⁵	0.3903	90	78	0.86 (0.781–0.930)	na	na	<0.0001		
FTLD-TDP (<i>n</i> = 29) vs. FTLT -Tau (<i>n</i> = 20)									
p/t Tau ratio	0.285	80	59	0.77 (0.641–0.906)	2	0.3	0.001	7.67	0.006
YKL40	na	na	na	0.64 (0.476–0.794)	na	na	0.109	−0.009	0.049
MFG-E8	na	na	na	0.57 (0.411–0.729)	na	na	0.406	0.001	0.027
βHexA	na	na	na	0.57 (0.404–0.737)	na	na	0.414	−0.012	0.021
βHexA/tHex	na	na	na	0.54 (0.370–0.708)	na	na	0.651	44.330	0.041
Age	na	na	na	0.52 (0.336–0.696)	na	na	0.851	0.133	0.036
TDP vs. Tau model ⁶	0.4563	80	81	0.87 (0.772–0.969)	4.2	0.2	<0.0001		
FTLD-TDP (<i>n</i> = 29) vs. CON (<i>n</i> = 30)									
YKL40	25.35	80	62	0.78 (0.665–0.901)	2.1	0.3	<0.0001	0.017	0.001
MFG-E8	na	na	na	0.44 (0.283–0.589)	na	na	0.396	−0.001	0.003
Catalase	na	na	na	0.62 (0.474–0.761)	na	na	0.122	0.430	0.024
TDP vs. CON model ⁷	0.4431	90	76	0.88 (0.796–0.965)	3.8	0.1	<0.0001		

AUC, area under the curve; CI, confident interval; LR, likelihood ratio; CON, nondemented controls; FTLT, frontotemporal lobar degeneration.

n.a: not applicable due to the lack of significance.

¹Selected value of the individual biomarker or combination where the two groups of analysis could be discriminated with the reported sensitivity and specificity.

²Positive likelihood: sensitivity/100-specificity.

³Negative likelihood: 100-sensitivity/specificity.

⁴FTLD vs. CON model: $y = 2.94 - 0.0004 * \text{MFG-E8} + 0.07 * \text{tTau} - 0.01 * \text{A}\beta_{42}$.

⁵FTLD vs. non-FTLD dementia models: $y = -6.621 - 0.004 * \text{YKL40} + 0.044 * \text{pTau} + 2.33 * \text{p/tTau ratio} + 0.79 * \text{Age}$.

⁶TDP vs. Tau model: $y = -14.659 - 0.009 * \text{YKL40} + 0.001 * \text{MFG-E8} - 0.012 * \beta\text{HexA activity} + 44.33 * \beta\text{HexA/tHex activity ratio} + 7.671 * \text{p/tTau ratio} + 0.133 * \text{Age}$.

⁷TDP vs. CON model: $y = -3.193 + 0.017 * \text{YKL40} - 0.001 * \text{MFG-E8} + 0.43 * \text{catalase activity}$.

Markers/models achieving sufficient biomarker performance are highlighted in bold.

disorders indicating that YKL-40 is an inflammatory marker likely reflecting astrogliosis.^{40–44} Thus, the different levels of YKL-40 levels across different pathological groups may indicate a different inflammatory response.

We also analyzed the levels of MFG-E8, a molecule that has been shown to mediate microglia phagocytosis.⁴⁵ Despite the fact that MFG-E8 was increased in AD cases compared to both FTLT subtypes, it did not discriminate those clinical groups. Recent data has shown that Aβ can induce the release of MFG-E8,⁴⁶ and therefore, the higher levels of MFG-E8 may reflect the higher amyloid load of AD patients that is rarely seen in FTLT cases.⁴

Catalase activity in CSF was increased in FTLT-TDP compared to non-demented controls, which challenges our previous findings in which the activity of catalase was specially decreased in the FTLT group.¹² The time length of sample storage before analysis (which negatively influences CSF catalase activity) as well as the higher FTLT heterogeneity of this study may explain the discrepancy observed. Some cases within the nondemented controls or the FTLT-TDP groups showed remarkably lower values of catalase than the rest of the samples (i.e., catalase <2.5 U/L), which are likely explained by the center of collection (Milan) or time of storage rather than by a

Table 3. ROC analysis of CSF parameters discriminating different diagnostic groups in the validation cohort

	Cut-off point ¹	Sensitivity (%)	Specificity (%)	AUC (95% CI)	+LR ²	LR ³	P value (individually)	Coefficient (B)	P value (within model)
CSF variables									
FTLD (n = 90) vs. CON (n = 55)									
MFG-E8	5975	81	69	0.81 (0.734–0.884)	2.61	0.28	<0.0001	−0.001	<0.0001
tTau	262.5	71	59	0.68 (0.592–0.765)	1.73	0.49	<0.0001	0.019	<0.0001
Aβ ₄₂	886	66	65	0.67 (0.577–0.755)	1.89	0.52	0.001	−0.003	0.008
FTLD vs. CON model	0.594	88	85	0.93 (0.894–0.971)	5.87	0.14	<0.0001		
FTLD (n = 91) vs. non-FTLD dementia models (n = 37)									
YKL40	273	72	70	0.74 (0.644–0.833)	2.40	0.40	<0.0001	0.011	0.001
pTau	45.5	78	71	0.82 (0.729–0.911)	2.69	0.31	<0.0001	−0.083	<0.0001
p/tTau	0.134	70	60	0.68 (0.577–0.788)	1.75	0.50	0.001	−13.04	0.017
Age	na	na	na	0.55 (0.444–0.646)	na	na	0.423	−0.062	0.113
FTLD vs. non-FTLD dementia model	0.636	91	84	0.93 (0.884–0.975)	5.69	0.11	<0.0001		
FTLD-TDP (n = 42) vs. CON (n = 57)									
YKL40	259.93	79	70	0.78 (0.690–0.881)	2.63	0.30	<0.0001	0.016	<0.0001
MFG-E8	6069.5	80	79	0.83 (0.745–0.907)	3.81	0.25	<0.0001	−0.001	<0.0001
Catalase	4.44	70	62	0.71 (0.614–0.815)	1.84	0.48	<0.0001	0.413	0.151
TDP vs. CON model	0.297	90	86	0.94 (0.898–0.989)	6.43	0.12	<0.0001		

AUC, area under the curve; CI, confident interval; LR, likelihood ratio; CON, nondemented controls; FTLD, frontotemporal lobar degeneration, n.a, nonapplicable.

¹Selected value of the individual biomarker or combination where the two groups of analysis could be discriminated with the reported sensitivity and specificity.

²Positive likelihood: sensitivity/100-specificity.

³Negative likelihood: 100-sensitivity/specificity.

Markers/models achieving sufficient biomarker performance are highlighted in bold.

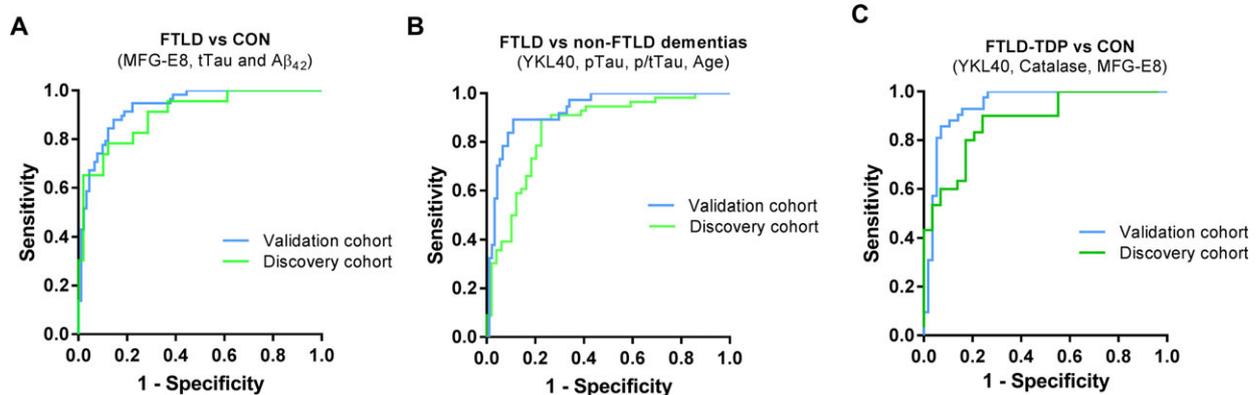


Figure 3. Receiver operating curves (ROC) of the different models discriminating specific diagnostic groups in the validation cohort. (A–C) ROC curves of the (A) FTLD vs. CON model (MFG-E8, tTau, and Aβ₄₂), (B) the FTLD vs. non-FTLD dementia models (YKL40, pTau, p/tTau, and age) and (C) the FTLD-TDP vs. CON model (YKL40, MFG-E8, and catalase activity) discriminating corresponding patients in the discovery (green line) and validation (blue line) cohorts. Abbreviations: FTLD, frontotemporal lobar degeneration; CON, healthy nondemented controls; TDP, TAR DNA-binding protein 43; AD, Alzheimer’s disease.

specific pathophysiological characteristic (i.e., TDP-ALS). Catalase is an antioxidant enzyme, and thus, the observed increase activity may reflect a compensatory mechanism to counteract the oxidative stress present in different dementia such as AD or FTLD.^{47,48}

We next assessed whether our protein dataset could reveal specific combination of markers discriminating non-demented controls and FTLD subtypes. We observed that combination of MFG-E8 together with CSF tTau and Aβ₄₂ could discriminate FTLD patients from

nondemented controls with 78% sensitivity and 82% specificity, which was validated in a larger independent cohort achieving sensitivity and specificity values >80%. Most of the potential FTLD CSF biomarkers studied to date (e.g., tau, tdp43) did not achieve enough sensitivity or specificity.^{8,49–52} In a subset of cases, we observed that the FTLD vs. CON model achieved similar performance to that observed for NfL alone. However, CSF NfL is a nonspecific disease biomarker that is upregulated in other disorders such as AD.^{27,32,34} Importantly, recent studies showed that the ratio between NfL and the soluble β fragment of amyloid precursor protein (sAPP β)⁵³ or the combination of TDP43 with p/tTau ratio⁵⁴ could optimally discriminate FTLD patients from CON in a large cohort, promising data that need to be replicated in independent cohorts. An additional challenge in clinical practice is the differential diagnosis of dementia. Noteworthy, up to 30% of FTLD cases are misdiagnosed with other disorders, especially AD.⁵⁵ Several studies have shown that ratios with AD CSF biomarkers (i.e., pTau/A β 42 or tTau/A β 42) can discriminate FTLD from AD with performances over 80%.^{38,56} In this study, we identified a model able to discriminate FTLD from a general group of non-FTLD dementia (AD and DLB) with 91% sensitivity and 84% specificity using pTau, p/tTau ratio and YKL40. Taken together, the biomarker combinations described above may aid on the optimal diagnosis of FTLD within the dementia spectrum, the first step toward diagnosing the specific FTLD subtypes.

We next identified a model able to discriminate the two main pathological subtypes of FTLD (TDP vs. Tau model: YKL40, MFG-E8, β HexA, β HexA/tHex, p/tTau ratio, and age) with a sensitivity and specificity of 80% and 82%. The p/tTau ratio has already been shown to be a reproducible biomarker discriminating both FTLD pathological subtypes with sensitivity and specificity values around 82% and 62%, respectively,^{9,10} as also observed in the current study. Interestingly, the addition of age, YKL40, and MFG-E8 together with the activity of β HexA and its ratio increased the specificity to 81%. These outcomes were not affected by the center in which samples were collected, and similar results were obtained when data were reanalyzed using SVM. These data reveal a potential biomarker model discriminating FTLD pathological subtypes with enough sensitivity and specificity values according to biomarker guidelines (>80%).⁵⁷ Such model could be highly relevant since it may facilitate the appropriate selection of cases (FTLD-TDP or FTLD-Tau) for clinical trials targeting the specific protein aggregates (TDP43 or Tau) once FTLD diagnosis is made, ultimately easing the development of disease-modifying therapies. However, it is important to highlight that the achieved sensitivity and specificity still did not reach excellent

performance (over 90%) and that validation of this model in independent cohorts remains to be performed.

We identified a model that could discriminate FTLD-TDP patients from controls with 90% sensitivity and 76% specificity, which was also validated in a larger independent cohort. Despite the clinical utility of this model might be limited, the optimal validation of the model in an independent cohort further supports the validity of the data obtained in this study. Importantly, we observed that the specificity of this model increased up to 83% when center of was included as an interaction factor, indicating a strong influence of preanalytical confounding factors (e.g., differences in freeze–thaw cycles, spinning conditions, length of storage, tube filling, brand collection tube). This data stresses the importance of unraveling and controlling for those preanalytical factors within biomarker studies.²⁵

Strikingly, some of the biomarkers that were not significantly changed between two specific diagnostic groups (i.e., MFG-E8) contributed to discriminate those patients within the predicting models. Thus, the results of this study also highlight the importance of selecting biomarker candidates based not only on the fold-change between the groups of interest but also based on their effect in combination with other markers using unbiased predicting models. These models may reflect not only changes in protein concentration but also association of different proteins to the specific phenotype within each patient, ultimately reducing interindividual variability and increasing diagnostic performance. Thus, multivariate models might be especially helpful for diagnosis of complex disorders with strong comorbidity such as neurodegenerative dementia.

Limitations

The data revealed in this study are promising but important limitations also apply. Some of the models revealed in this study are based on complex formulas including six markers and thus its final implementation in clinical practice might be challenging. However, development of highly sensitive targeted multiplex assays may facilitate the validation of such biomarkers signatures.^{58,59} Noteworthy, biomarkers outcomes may differ across different stages of the disease. Despite there is no well-established tools to optimally define the disease stage of FTLD cases yet,⁶⁰ the CSF samples used in this study were mostly collected at the same stage of the disease, within 1–3 years from symptoms onset. Thus, it would be also relevant to analyze the performance of the revealed models in cases at more advance stages of the disease. In addition, the lack of autopsy confirmation in some of the selected cases (i.e., those with clinical syndromes highly predictive of

FTLD-Tau and FTLD-TDP) may have led to the inclusion of cases with AD copathology⁵⁵ influencing the resulting diagnostic performances. However, inclusion of cases with potential AD comorbidity may provide also a more heterogeneous scenario that better resembles clinical practice. Lastly, although the cohorts analyzed in this study are relatively large compared to earlier pathology-confirmed CSF biomarker studies, we acknowledge that the sample size remains small and therefore results should still be replicated in larger cohorts, specially the biomarker model that optimally discriminated FTLD-TDP patients from FTLD-Tau. However, considering that the three other models unraveled within this study (FTLD vs. CON, FTLD vs. non-FTLD dementia, and TDP vs. CON) were replicated in a large independent cohort, we expect to further validate the TDP vs. Tau biomarker signature as soon as more samples become available. Whether combinations of the markers analyzed in this study aid in the diagnosis of non-FTLD dementia remains to be evaluated.

Conclusion

This study reveals different biomarker models based on the p/tTau ratio and a panel of different neuroinflammatory and lysosomal CSF biomarkers that can discriminate FTLD from nondemented controls and other dementia as well as the main FTLD pathological subtypes with optimal accuracy (specificity/sensitivity >80%). These models may allow appropriate selection of cases for clinical trials targeting the specific proteinopathy, thereby facilitating the development of successful therapies.

Acknowledgments

Not applicable.

Authors' Contributions

MC developed the study concept and design, and was responsible of acquisition of data, statistical analysis and interpretation, and drafting/revision of the manuscript. NE, LB, and KW carried out the immunoassay analyses, and SP carried out the enzyme activities. JvS, YP, TB, and DG participated in acquisition and interpretation data and the critical revision of the manuscript. WH participated in acquisition, analysis and interpretation of data, and in the critical revision of the manuscript. CT developed the study concept and design, and was involved in acquisition of data, analysis and interpretation, drafting/revision of the manuscript, and study supervision. All authors read and approved the final manuscript.

Conflict of Interest

MC, NE, LB, KW, SP, DG, TB, and YP report no disclosures.

WH reports grants from Alzheimer's Drug Discovery Foundation, grants from National Institute of Health, and grants from American Federation for Aging Research, during the conduct of the study; personal fees from ViveBio, LLC, personal fees from Locks Law Firm, grants from Fujirebio US, nonfinancial support from Avid Radiopharmaceutical, personal fees from Abbvie, personal fees from Hoffman LaRoche, personal fees from AARP, Inc, personal fees from Interpleader Law, outside the submitted work; In addition, Dr. Hu has a patent US No. 9,618,522 issued.

CT reports personal fees from advisory board of Fujirebio and Roche, nonfinancial support from research consumables from ADxNeurosciences, other from performed contract research or received grants from Janssen prevention center, Boehringer, EIP farma, Roche and Probiobio, Charles River, outside the submitted work.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The studies were approved by the Institutional Ethical Review Boards of each center. Informed consent was obtained from all subjects or their authorized representatives.

References

1. Ratnavalli E, Brayne C, Dawson K, Hodges JR. The prevalence of frontotemporal dementia. *Neurology* 2002;58:1615–1621.
2. Brunnström H, Gustafson L, Passant U, Englund E. Prevalence of dementia subtypes: a 30-year retrospective survey of neuropathological reports. *Arch Gerontol Geriatr* 2009;49:146–149.
3. Brodaty H, Seeher K, Gibson L. Dementia time to death: a systematic literature review on survival time and years of life lost in people with dementia. *Int Psychogeriatr* 2012;24:1034–1045.
4. Mackenzie IRA, Neumann M, Bigio EH, et al. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. *Acta Neuropathol* 2010;119:1–4.
5. Irwin DJ, Trojanowski JQ, Grossman M. Cerebrospinal fluid biomarkers for differentiation of frontotemporal

- lobar degeneration from Alzheimer's disease. *Front Aging Neurosci* 2013;5:6.
6. Irwin DJ, Cairns NJ, Grossman M, et al. Frontotemporal lobar degeneration: defining phenotypic diversity through personalized medicine. *Acta Neuropathol* 2015;129:469–491. [cited 2015 Jan 2]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25549971>
 7. International Conference on Frontotemporal Dementias 2016. Fluid NFL Shines, Tau PET Dims, in the Hunt for FTD Biomarkers|ALZFORUM. [date unknown]; Available from: <http://www.alzforum.org/news/conference-coverage/fluid-nfl-shines-tau-pet-dims-hunt-ftd-biomarkers>
 8. Oeckl P, Steinacker P, Feneberg E, Otto M. Cerebrospinal fluid proteomics and protein biomarkers in frontotemporal lobar degeneration: current status and future perspectives. *Biochim Biophys Acta – Proteins Proteomics* 2014;1854:757–768.
 9. Hu WT, Watts K, Grossman M, et al. Reduced CSF p-Tau181 to Tau ratio is a biomarker for FTLD-TDP. *Neurology* 2013;81:1945–1952.
 10. Borroni B, Benussi A, Archetti S, et al. Csf p-tau181/tau ratio as biomarker for TDP pathology in frontotemporal dementia. *Amyotroph Lateral Scler Frontotemporal Degener* 2014;16:86–91.
 11. Pijnenburg YAL, Verwey NA, van der Flier WM, et al. Discriminative and prognostic potential of cerebrospinal fluid phosphoTau/tau ratio and neurofilaments for frontotemporal dementia subtypes. *Alzheimer's Dement* 2015;1:505–512.
 12. Teunissen CE, Elias N, Koel-Simmelink MJA, et al. Novel diagnostic cerebrospinal fluid protein biomarkers for pathologic subtypes of frontotemporal dementia identified by proteomics. *Alzheimer's Dement* 2016;2:86–94.
 13. Baker M, Mackenzie IR, Pickering-Brown SM, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature* 2006;442:916–919.
 14. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011;72:257–268.
 15. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006;314:130–133.
 16. Ingelsson M, Ramasamy K, Russ C, et al. Increase in the relative expression of tau with four microtubule binding repeat regions in frontotemporal lobar degeneration and progressive supranuclear palsy brains. *Acta Neuropathol* 2007;114:471–479.
 17. Dickson DW, Bergeron C, Chin SS, et al. Office of rare diseases neuropathologic criteria for corticobasal degeneration. *J Neuropathol Exp Neurol* 2002;61:935–946.
 18. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* 2005;65:1863–1872.
 19. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 1998;51:1546–1554.
 20. Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 2011;134(Pt 9):2456–2477.
 21. Armstrong MJ, Litvan I, Lang AE, et al. Criteria for the diagnosis of corticobasal degeneration. *Neurology* 2013;80:496–503.
 22. Litvan I, Agid Y, Calne D, et al. Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. *Neurology* 1996;47:1–9.
 23. Jack CR, Albert MS, Knopman DS, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement* 2011;7:257–262.
 24. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement* 2011;7:263–269.
 25. Del Campo M, Mollenhauer B, Bertolotto A, et al. Recommendations to standardize preanalytical confounding factors in Alzheimer's and Parkinson's disease cerebrospinal fluid biomarkers: an update. *Biomark Med* 2012;6:419–430.
 26. Carecchio M, Fenoglio C, Cortini F, et al. Cerebrospinal fluid biomarkers in progranulin mutations carriers. *J Alzheimer's Dis* 2011;27:781–790.
 27. Meeter LHH, Vijverberg EG, Del Campo M, et al. Clinical value of neurofilament and phospho-tau/tau ratio in the frontotemporal dementia spectrum. *Neurology* 2018a;90:e1231–e1239.
 28. Templeton GF. A two-step approach for transforming continuous variables to normal: implications and recommendations for IS research. *Commun Assoc Inf Syst* 2011;28:41–59.
 29. Templeton GF, Burney LL. Using a two-step transformation to address non-normality from a business value of information technology perspective. *J Inf Syst* 2017;31:149–164.
 30. Strbenac D, Mann GJ, Yang JYH, Ormerod JT. Differential distribution improves gene selection stability and has competitive classification performance for patient survival. *Nucleic Acids Res* 2016;44:e119.

31. Persichetti E, Chiasserini D, Parnetti L, *et al.* Factors influencing the measurement of lysosomal enzymes activity in human cerebrospinal fluid. *PLoS ONE* 2014;9:e101453.
32. Zetterberg H, Skillbäck T, Mattsson N, *et al.* Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. *JAMA Neurol* 2016;73:60.
33. Skillbäck T, Farahmand B, Bartlett JW, *et al.* CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. *Neurology* 2014;83:1945–1953.
34. Scherling CS, Hall T, Berisha F, *et al.* Cerebrospinal fluid neurofilament concentration reflects disease severity in frontotemporal degeneration. *Ann Neurol* 2014;75:116–126.
35. Craig-Schapiro R, Perrin RJ, Roe CM, *et al.* YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry* 2010;68:903–912.
36. Alcolea D, Carmona-Iragui M, Suárez-Calvet M, *et al.* Relationship between β -secretase, inflammation and core cerebrospinal fluid biomarkers for Alzheimer's disease. *J Alzheimer's Dis* 2014;42:157–167.
37. Antonell A, Mansilla A, Rami L, *et al.* Cerebrospinal fluid level of YKL-40 Protein in preclinical and prodromal Alzheimer's disease. *J Alzheimer's Dis*. 2014;42:901–908.
38. Lleó A, Irwin DJ, Illán-Gala I, *et al.* A 2-step cerebrospinal algorithm for the selection of frontotemporal lobar degeneration subtypes. *JAMA Neurol* 2018;75:738–745.
39. Irwin DJ, Lleó A, Xie SX, *et al.* Ante mortem cerebrospinal fluid tau levels correlate with postmortem tau pathology in frontotemporal lobar degeneration. *Ann Neurol* 2017;82:247–258.
40. Cantó E, Tintoré M, Villar LM, *et al.* Chitinase 3-like 1: prognostic biomarker in clinically isolated syndromes. *Brain* 2015;138(Pt 4):918–931.
41. Comabella M, Fernandez M, Martin R, *et al.* Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. *Brain* 2010;133:1082–1093.
42. Modvig S, Degen M, Roed H, *et al.* Cerebrospinal fluid levels of chitinase 3-like 1 and neurofilament light chain predict multiple sclerosis development and disability after optic neuritis. *Mult Scler J* 2015;21:1761–1770.
43. Martinez MAM, Olsson B, Bau L, *et al.* Glial and neuronal markers in cerebrospinal fluid predict progression in multiple sclerosis. *Mult Scler J* 2015;21:550–561.
44. Cantó E, Reverter F, Morcillo-Suárez C, *et al.* Chitinase 3-like 1 plasma levels are increased in patients with progressive forms of multiple sclerosis. *Mult Scler J* 2012;18:983–990.
45. Fuller AD, Van Eldik LJ. MFG-E8 regulates microglial phagocytosis of apoptotic neurons. *J Neuroimmune Pharmacol* 2008;3:246–256. [cited 2016 Jan 25].
46. Neniskyte U, Brown GC. Lactadherin/MFG-E8 is essential for microglia-mediated neuronal loss and phagocytosis induced by amyloid β . *J Neurochem* 2013;126:312–317.
47. Swerdlow R, Burns J, Khan SM. The Alzheimer's disease mitochondrial cascade hypothesis. *J Alzheimer's Dis* 2010;20(Suppl 2):265–279.
48. Carmona M, Portero-otín M, Naudí A, *et al.* Type-dependent oxidative damage in frontotemporal lobar degeneration : cortical astrocytes are targets of oxidative damage. *J Alzheimer's Dis* 2008;67:1122–1136.
49. Suárez-Calvet M, Dols-Icardo O, Lladó A, *et al.* Plasma phosphorylated TDP-43 levels are elevated in patients with frontotemporal dementia carrying a C9orf72 repeat expansion or a GRN mutation. *J Neurol Neurosurg Psychiatry* 2013;43:1–8.
50. Hu WT, Chen-Plotkin A, Grossman M, *et al.* Novel CSF biomarkers for frontotemporal lobar degenerations. *Neurology* 2010;75:2079–2086.
51. Kuiperij HB, Versleijen AAM, Beenes M, *et al.* Tau rather than TDP-43 proteins are potential cerebrospinal fluid biomarkers for frontotemporal lobar degeneration subtypes: a pilot study. *J Alzheimer's Dis* 2017;55:585–595.
52. Meeter LH, Doppert EG, Jiskoot LC, *et al.* Neurofilament light chain: a biomarker for genetic frontotemporal dementia. *Ann Clin Transl Neurol* 2016;3:623–636.
53. Alcolea D, Vilaplana E, Suárez-Calvet M, *et al.* CSF sAPP β , YKL-40, and neurofilament light in frontotemporal lobar degeneration. *Neurology* 2017;89:178–188.
54. Bourbouli M, Rentzos M, Bougea A, *et al.* Cerebrospinal fluid TAR DNA-binding protein 43 combined with tau proteins as a candidate biomarker for amyotrophic lateral sclerosis and frontotemporal dementia spectrum disorders. *Dement Geriatr Cogn Disord* 2017;44:144–152.
55. Forman MS, Farmer J, Johnson JK, *et al.* Frontotemporal dementia: clinicopathological correlations. *Ann Neurol* 2006a;59:952–962.
56. Rivero-Santana A, Ferreira D, Perestelo-Pérez L, *et al.* Cerebrospinal fluid biomarkers for the differential diagnosis between Alzheimer's disease and frontotemporal lobar degeneration: systematic review, HSROC analysis, and confounding factors. *J Alzheimer's Dis* 2017;55:625–644.
57. The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group 123. Consensus report of the working group on: "molecular and biochemical markers of Alzheimer 's disease". *Neurobiol Aging* 1998;19:109–116.
58. Christiansson L, Mustjoki S, Simonsson B, *et al.* The use of multiplex platforms for absolute and relative protein quantification of clinical material. *EuPA Open Proteomics* 2014;3:37–47.
59. Lewczuk P, Kornhuber J, Vanderstichele H, *et al.* Multiplexed quantification of dementia biomarkers in the CSF of patients with early dementias and MCI: a multicenter study. *Neurobiol Aging* 2008;29:812–818.
60. Premi E, Gualeni V, Costa P, *et al.* Looking for measures of disease severity in the frontotemporal dementia continuum. *J Alzheimer's Dis* 2016;52:1227–1235.

Supporting Information

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

Figure S1. Effect of freeze–thaw on the concentration of YKL40 (A), MFG-E8 (B), and catalase activity (C) in CSF. Each line represents a different CSF sample. Data represents the % of change in the biomarker concentration/activity. Most of samples remained within acceptable + 20% range (gray dash lines) according to guidelines.²⁵

Figure S2. Dot plot of the levels of FABP4 (A) and the activity of HexA (B), β -HexA (C), α -GLA (D) in CSF displayed for each clinical group (FTD-TDP in blue and FTD-Tau in orange).

Figure S3. Receiver operating curves (ROC) of the CON vs. FTLD model and NfL in the validation cohort.

Table S1. Demographic data, overall CSF values and collection/storage protocols by center

Table S2. Relationship between CSF biomarker values and demographic variables calculated by linear regression

Data S1. Supplementary methods