Synaptic, axonal damage and inflammatory cerebrospinal fluid biomarkers in neurodegenerative dementias.

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Abstract

INTRODUCTION: Synaptic damage, axonal neurodegeneration, and neuroinflammation are common features in Alzheimer's disease (AD), frontotemporal dementia (FTD), and Creutzfeldt-Jakob disease (CJD).

METHODS: Unicentric cohort of 353 participants included healthy control (HC) subjects, AD continuum stages, genetic AD and FTD, and FTD and CJD. We measured cerebrospinal fluid neurofilament light (NF-L), neurogranin (Ng), 14-3-3, and YKL-40 proteins.

RESULTS: Biomarkers showed differences in HC subjects versus AD, FTD, and CJD. Disease groups differed between them except AD versus FTD for YKL-40. Only NF-L differed between all stages within the AD continuum. AD and FTD symptomatic mutation carriers presented differences with respect to HC subjects. Applying the AT(N) system, 96% subjects were positive for neurodegeneration if 14-3-3 was used, 94% if NF-L was used, 62% if Ng was used, and 53% if YKL-40 was used.

DISCUSSION: Biomarkers of synapse and neurodegeneration differentiate HC subjects from neurodegenerative dementias and between AD, FTD, and CJD. NF-L and 14-3-3 performed similar to total tau when AT(N) system was applied.

Keywords (5-15): Alzheimer's disease, Frontotemporal dementia, Creutzfeldt-Jakob disease, biomarker, cerebrospinal fluid, neurofilament light, neurogranin, YKL-40, 14-3-3, mutation carriers, AT(N) system, Preclinical AD, MCI due to AD

1. Introduction

Alzheimer's disease (AD) is a heterogeneous and pathophysiologically complex disease, polygenic in most cases (sporadic AD) with a minority of monogenic phenotypes (<0.5%, autosomal dominant AD, ADAD). Early-diagnosis as well as differential diagnosis with other neurodegenerative diseases is crucial for patient management and for potential future treatments. Cerebrospinal fluid (CSF) Amyloid β isoform 42 (Aβ42), total tau protein (t-tau) and tau protein

phosphorylated at Thr-181 (p-tau) have been accepted as core biomarkers for detecting AD neuropathological features in living individuals [1]. Aβ42 is considered indicative of amyloid pathology, t-tau of cortical axonal degeneration and p-tau of tangle pathology.

Previous guidelines for AD [2,3] have defined the preclinical and prodromal stages of AD. In those criteria, the preclinical AD stage includes subjects with normal cognition and abnormal amyloid and tau markers, while the mild cognitive impairment (MCI) stage represents the early, symptomatic, pre-dementia phase of AD, characterized by an episodic memory deficits and pathological levels of core AD biomarkers (MCI due to AD). The new NIA-AA Research Framework has proposed a biological definition of AD, using the AT(N) system (Amyloid, Tau, Neurodegeneration), where N is open to new neurodegeneration biomarkers if supported by the available evidence [4]. Subjects are classified according to their biomarker profile within the Alzheimer's continuum: Aβ biomarkers (A) determine whether or not an individual is in the Alzheimer's continuum, pathologic tau biomarkers (T) determine if someone who is in the Alzheimer's continuum has AD and neurodegeneration biomarkers (N) determine the AD stage.

Core AD biomarkers have demonstrated high diagnostic validity to differentiate AD from healthy controls (HC), but with little added value for prognosis or disease severity staging stressing the need for new biomarkers for these purposes [5]. Core AD biomarkers have also demonstrated good global accuracy in the differential diagnosis of AD with other neurodegenerative

dementias, like frontotemporal dementia (FTD) or Creutzfeldt-Jakob disease (CJD) [6,7].

Other CSF biomarkers are being studied in AD and other neurodegenerative dementias. Neurofilament light (NF-L) is a major cytoskeletal constituent of neuronal cells and can be released into the CSF, detecting significant elevations in different neurological disorders [8–14]. Neurogranin (Ng) is a calmodulin-binding post synaptic neuronal protein that is abundantly expressed in perikaryal and dendritic cytoplasm [15], involved in modulating synaptic transmission and plasticity mechanisms [16–19]. The 14-3-3 proteins are higlhy expressed in the brain and some of the 14-3-3 isoforms are particularly enriched in the pre-synapses, to regulate transmission and plasticity [20]. 14-3-3 γ protein is part of the diagnostic criteria for sCJD, and present increased levels in the CSF of sporadic CJD [21–23]. YKL-40 (also known as Chitinase 3-like 1) is a glycoprotein produced by inflammatory cells, mostly astrocytes. Its physiological role is not completely understood but YKL-40 is elevated in the brain and CSF in several neurological and neurodegenerative diseases associated with inflammatory processes [14,24–26].

In this study, we aim to analyze the diagnostic accuracy and discriminatory properties of four non-core biomarkers (NF-L, Ng, YKL-40 and 14-3-3) in an unicentric cohort with a broad spectrum of clinical diagnoses: Alzheimer's continuum (including preclinical phase and genetic cases), FTD (sporadic and genetic) and sporadic CJD. We also evaluate these markers as markers of neurodegenation and disease severity using the new AT(N) system.

2. Materials and methods

2.1. Participants

Unicentric cohort of 353 participants. All the participants underwent a complete clinical and neuropsychological examination. Longitudinal follow-up consisted in an annual clinical assessment and Mini-Mental State Examination (MMSE).

HC subjects had normal cognition defined according to the following criteria: Mini-Mental State Examination (MMSE) scores above 24, objective cognitive performance within the normal range (performance within 1.5 SD) in all tests from a specific test battery (see below), clinical dementia rating (CDR) scale score of 0, no significant psychiatric symptoms or previous neurological disease, and a CSF biomarker profile inconsistent with AD pathology. All clinical diagnosis were made in accordance with standard criteria [27–30]. Some of the Preclinical AD subjects had only Aβ42 biomaker altered, being Alzheimer's pathologic change biomarker category according to the new Research Criteria for AD [4], and some had Aβ42 and tau biomarkers altered, classified as AD biomarker profile. CSF AD core biomarkers were used as selection criteria with the following own cut-offs: 550 pg/mL for Aβ42 in CSF samples measured before February 2016 and 750 pg/mL for the CSF samples measured after February 2016; 385 pg/mL for Total-tau and 65 pg/mL for p-tau.

All the genetic participants were recruited at the Genetic counselling programme for familial dementias (PICOGEN) at the Hospital Clinic, Barcelona, Spain[31]. Participants were adult children from families with a known mutation

in one of the following genes: *PSEN1* or *APP* gene (for ADAD) and *GRN*, *MAPT* or *C9orf72* (FTD genetic cases).

The study was approved by the Ethics Committee of Hospital Clínic of Barcelona. Written informed consent was obtained from all participants.

2.2. CSF collection and protein levels determination

CSF samples were collected from all participants and centrifuged at 2000xg for 10 minutes at 4ºC and stored at -80ºC in polypropilene tubes until usage.

Core AD biomarker concentrations were measured with INNOTEST ELISAs following manufacturer's instructions (Fujirebio, Ghent, Belgium). CSF NF-L concentration was measured using a commercially available ELISA from IBL International (Hamburg, Germany) [32]. γ-14-3-3 protein ELISA kit was CircuLex 14-3-3 gamma (MBL International Corporation, Woburn, MA, USA) and a CSF sample dilution 1:5 was performed [33]. CSF YKL-40 concentration was measured with an ELISA from QUIDEL (San Diego, CA, USA), using a CSF sample dilution of 1:2.5. Ng was measured using an in-house ELISA assay based on the monoclonal antibody Ng7 (epitope including amino acids 52-65 on Ng) as described previously [16].

All analyses were performed by duplicate and experienced laboratory personnel blinded to clinical diagnosis. We are participants of the Alzheimer's Association QC program (ref: https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/23622690/), and Aβ42, t-tau and p-tau levels obtained in our lab have consistently been within mean ± 2 SD [34].

Due to CSF sample limited availability, not all the biomarkers could be studied in all the samples.

2.3. APOE genotyping

APOE genotype was determined through the analysis of two single nucleotide polymorphisms: rs429358 and rs7412, either using TaqMan genotyping technologies (Thermo Fisher Scientific, Waltham, MA, USA) or alternatively by Sanger sequencing and visualization of these two SNPs.

2.4. Statistical Analysis

All data are described using median and 25th, 75th percentiles or absolute frequency and percentages for quantitative and qualitative respectively. Estimations outcomes from statistical models described below were assessed by 95% confidence intervals (95%CI). Baseline comparisons were made using Mann-Whitney U test or Fisher's exact test and Generalized Lineal Models (GLM) for adjusted comparisons by age and sex. Generalized Estimating Equations (GEE) models, using an autoregressive model (1) (AR1) approach in order to assess intra-individual correlation matrix, were used for longitudinal analyses with baseline result of MMSE as covariate with the aim of evaluate differences between diagnosis groups with or without adjustment for each biomarker, additionally these GEE models were used to assess the evolution of MMSE in each group. GLM and GEE models were performed by non-parametrical approach by means rank-transformation of dependent variable. Accuracy for prediction of biomarkers between controls and neurodegenerative diseases were performed by estimation of area under curve (AUC) from ROC

curves. An approach to cut-off for each biomarker was assessed by Likelihood Ratio Positive (LR+) = sensitivity / 1-specificity (ratio of true positives with respect to false positives) from ROC analysis. For some of these analysis, a group of neurodegenerative patients was created, which included: MCI due to AD, AD dementia, FTD and CJD patients.

All analyses were performed in SPSS (version 25) software and where performed using a two-sided type I error of 5%. Due to methodological characteristics of this study the p-values presented were nominal and were not adjusted for multiplicity.

2.5. Comparison of the different biomarkers of neurodegeneration for the AT(N) classification system

Patients within the AD biological continuum with positive core AD biomarkers (i.e. altered Aβ42 and p-tau) were analyzed to evaluate the effect of the different neurodegeneration markers on the AT(N) classification system. Biomarkers results were dichotomized as positive or negative according to the established cut-off best discriminating between HC and AD. The percentage of positivity in CSF of the five potential biomarkers of neurodegeneration (t-tau, 14-3-3, NF-L, Ng and YKL-40) was calculated.

3. Results

3.1. Groups characteristics and biomarkers results

We studied 353 participants: Healthy controls HC (n=50), asymptomatic subjects within the AD continuum (Preclinical AD) (n= 21), MCI due to AD (MCI) (n=56), AD dementia (n=108), sporadic FTD (n=34), CJD (n=38), ADAD

cases (n=25, 9 asymptomatic, ADaMC / 16 symptomatic, ADsMC) and FTD genetic cases (n=21, 5 asymptomatic, FTDaMC / 16 symptomatic, FTDsMC). Sporadic FTD patients were further subdivided for some of the analysis in 3 subtypes: behavioral variant FTD (bvFTD, n=8), non-fluent variant Progressive Primary Aphasia (nfvPPA, n=11) and semantic variant PPA (svPPA, n=15). In some of the analysis, sporadic FTD and FTDsMC were pooled together (FTD, n=50).

Demographics, basal MMSE and biomarkers results for each group are shown in Table 1, Table 2 and Supplementary Table 1.

3.2. Gender and age effects

There were no significant differences in gender proportions within the AD continuum groups. Only when comparing HC and FTD patients, there were significant differences in gender proportions (more males in FTD group). When comparing all the participants pooled, we found statistical significant differences in NF-L between genders (male>female, *P* value <.002) and in Ng (male<female, *P* value =.015). These differences were not observed when we restricted the analysis to the HC group. Age was significantly different between all the comparisons except for: “Preclinical AD vs MCI” and “AD vs CJD”. All the pairwise comparisons have been adjusted for age and gender. However, there were no differences in the statistical significance/non-significance when adjusting for age and gender in most of the comparisons.

3.3. Group comparisons

CSF biomarkers results were compared between HC and each group of neurodegenerative diseases studied (AD dementia, FTD and CJD). All biomarkers presented statistically significant differences between HC and AD, HC and FTD (with the exception of p-tau) and HC and CJD.
When different clinical groups were compared, AD and FTD differed in all the biomarkers except for YKL-40, and CJD differed from both AD and FTD in NF-L and Ng (Table 3A; Figure 1A). The groups could be sorted according to their concentration, from lower to higher values for each non-core biomarker: NF-L: HC<AD<FTD<CJD; Ng: FTD<HC<AD<CJD; YKL-40: HC<AD = FTD and 14-3-3: HC<FTD<AD. HC showed the lowest concentration in all the biomarkers except for Ng, in which FTD showed the lowest values (Table 1).

Group comparisons were also performed in the groups within the AD continuum: HC, Preclinical AD, MCI due to AD and AD dementia. NF-L levels differed between all the stages. In contrast, the other non-core biomarkers analyzed (YKL-40, 14-3-3 and Ng), although they were different between HC and MCI and HC and AD dementia, showed no differences between HC and Preclinical AD (Table 1, Table 3B; Figure 1B).

Pairwise comparisons in genetic AD and FTD cases showed that NF-L and 14-3-3 differed between HC and symptomatic mutation carriers (sMC), with no differences with respect to asymptomatic mutation carriers (aMC), both in ADAD and genetic FTD. Ng levels differed in the comparison HC-ADsMC. No differences were found in YKL-40 levels in genetic FTD (ADsMC not analyzed).

There were significant differences for all four biomarkers between aMC and sMC in both diseases.

Sporadic FTD patients were divided in 3 subtypes for pairwise comparisons: behavioral variant FTD (bvFTD, n=8), non-fluent variant Progressive Primary Aphasia (nfvPPA, n=11) and semantic variant PPA (svPPA, n=15) (Suppl.Table 1). No significant differences were found in the comparisons between bvFTD, svPPA and nfvPPA subtypes for any of the 4 biomarkers. When comparing HC with every subtype, significant comparisons were: NF-L in all the three comparisons, Ng between HC and svPPA, 14-3-3 between HC and nfvPPA and between HC and bvFTD. YKL-40 was not significant in any comparison.

3.4. ROC analyses for differential diagnosis of HC and neurodegenerative diseases

All the patients with a neurodegenerative disease in our cohort (MCI, AD dementia, FTD and CJD) were grouped to calculate a cut-off to differentiate them from HC (Table 4A).

A LR+ value >10, that indicates a good validity of the test, was obtained for all the biomarkers except for YKL-40. The best cut-off for NF-L was >1217 pg/mL, with an area under the curve (AUC) of 0.97 (0.94;0.99, 95% CI) and a sensitivity of 96% and specificity of 92%. The other biomarkers showed slightly lower AUC, with similar specificities but worst sensitivities. 14-3-3 cut-off of >3598 AU/mL had an AU 0.905 (0.86;0.95), with a sensitivity of 77.5% and a specificity of 93.3%. Ng cut-off was >250 and YKL-40 cut-off >545 (other data is shown in Table 4A).

The same ROC analyses were also performed comparing HC vs AD, HC vs FTD and HC vs CJD. For NF-L the same cut-off worked for the different disease groups (>1140 pg/mL), and for 14-3-3 a slightly different cut-offs but in the same direction were obtained. For Ng different cut-offs were necessary as FTD patients presented lower Ng levels with respect to HC, and AD and CJD higher levels. For YKL-40, a cut-off was obtained for FTD although with a not very good LR+, sensitivity and specificity, and for AD it was not possible to calculate a cut-off (Table 4B; Figure 2).

3.5. Comparison of the different biomarkers for neurodegeneration in the biological definition of Alzheimer's disease using the AT(N) system.

We used the five potential neurodegeneration CSF biomarkers to evaluate neuronal damage in AD continuum subgroup (A+T+). We observed two main clusters: one of three biomarkers, t-tau (98% of the subjects positive), 14-3-3 protein (96% positive) and NF-L (94% positive) and another cluster with Ng and YKL-40 (62% and 53% positive respectively).

All of these subjects had clinical diagnoses falling within the AD continuum (Preclinical AD, MCI due to AD and AD dementia, including AD sMC) at the baseline visit.

3.6. Model for MMSE inference

We created a model for the longitudinal MMSE value to be predicted by the group, baseline MMSE, follow-up time from baseline and interaction between

group and follow-up time. We added biomarkers levels separately to check the effect of the biomarker for the prediction of MMSE value.

For intra-group comparisons, we did not find differences in statistical significance between the crude model (without biomarker) and the other models adding biomarkers. For inter-group comparisons, changes in statistical significance were obtained in the following comparisons: AD vs FTD at baseline visit (Ng, 14-3-3, Aβ42, t-tau and p-tau biomarkers separately) and HC vs Preclinical AD at baseline visit (Aβ42 biomarker).

4. Discussion

In this study, biomarkers of synapse loss, axonal damage and astroglial inflammation differentiate HC from neurodegenerative dementias (AD, FTD and CJD). When considering their utility in the differential diagnosis between neurodegenerative diseases, NF-L and Ng showed statistically significant differences between all three groups (AD, FTD and CJD). YKL-40 showed no differences between AD and FTD and 14-3-3 protein levels showed significant differences between AD and FTD. These differences, albeit statistically significant, have to be interpreted with caution due to the existing overlap between groups. NF-L and 14-3-3 performed similar to t-tau when used as neurodegeneration marker within the AD biological continuum patients using the AT(N) system.

Similar to previously published data, we have found an increase in NF-L in all groups of patients with neurodegenerative dementia, suggesting a non-specific effect [9,13]. The analysis of the biomarkers studied within the AD continuum

shows that NF-L is the only biomarker that does not reach a plateau in the clinical phases of AD (MCI and AD dementia) and differentiates severity stages within the AD continuum, including Preclinical AD vs HC. Results obtained in the different subtypes of FTD are similar to those published before, with higher CSF NF-L levels in bvFTD, nfvPPA and svPPA in comparison with AD and HC [9,10].In the ROC analysis comparing HC vs Neurodegenerative diseases, NF-L showed the higher AUC, with a good specificity and sensitivity. NF-L levels also performed with high accuracy discriminating HC of AD, FTD and CJD.

An increase in CSF Ng concentration was reported to be specific for AD [16–18]; however, our results show that CJD patients also have an increase of CSF Ng levels. On the other hand, we observed a decrease in Ng levels in FTD patients. A significant decrease in FTD with respect to HC has not previously described, but previous studies have found a trend in this sense [18]. This difference may be due to different sample sizes or different statistical methodology. When analyzing the different FTD subtypes, the only statistically significant comparison compared with HC was the lower levels of svPPA and all the FTD subtypes presented statistically significant lower levels with respect to AD. This data is not concordant with previously published works, in which AD presented no differences with respect to svFTD [18]. In the ROC analysis of HC vs Neurodegenerative diseases, Ng was also a good predictor, with a good specificity but a poor sensitivity. A possible reason for this fact in Ng could be explained by the inverse behavior of Ng in AD and FTD as compared with HC (higher in AD and lower in FTD).

The detection of 14-3-3 protein levels was performed with a different methodology until the validation of an optimized commercial ELISA for the detection of 14-3-3 γ protein in 2015 [33]. Thus, with this methodology there is only one previous study that describes 14-3-3 levels in AD and HC but with a smaller sample size and without comparisons to a HC group [22]. To our best knowledge there are no studies of 14-3-3 levels in FTD patients, and we have reported significant differences in this biomarker levels between HC and FTD and between AD and FTD. In the ROC analysis, comparing HC and Neurodegenerative diseases, 14-3-3 resulted to be also a good predictor, with a good specificity but a slightly worst sensitivity than NF-L. For the discriminating capacity of HC-AD and HC-FTD, with a very similar cut-off, 14-3-3 showed slightly best AUC and sensibility for AD than FTD. The opposite behavior of 14-3-3 and Ng levels, both considered markers of synaptic loss although 14-3-3 is mainly presynaptic would deserve further studies.

With respect to YKL-40, in this study we have significantly increased the sample published in a previous work [24], confirming results and providing new data about FTD patients. Increased levels of YKL-40 in AD and FTD have been previously reported [25,26], although no differences between AD and FTD were observed [26]. In the ROC analysis, in the comparison HC and Neurodegenerative dementias, we found a bad predictive capacity of YKL40, and it was not possible to calculate a cut-off in the comparison HC-AD due to the high variability of the biomarker values in HC.

To date, few studies have reported results on multi-CSF biomarkers results (core AD biomarkers, NF-L, Ng, 14-3-3, YKL-40) within the same cohort, comparing biomarkers levels among groups and sorting the magnitude of the differences in each disease [35,36].We have also analyzed their performance in the application of the AT(N) biological definition of AD from the National Institute on Aging-Alzheimer's Association (NIA-AA) [4]. Our data suggests a relatively similar performance of t-tau, 14-3-3 and NF-L as neurodegeneration CSF biomarkers within the AD biological continuum, pointing out to the possibility to use either of these three biomarkers for AT(N) classification purposes. In contrast, YKL-40 and Ng showed worse results, suggesting that these biomarkers are not useful for this purpose.

In the analyses of genetic cases of AD and FTD, aMC do not show differences in any of the markers studied. However, lack of differences between HC and aMC may be due to limited sample size. When comparing the two groups of mutation carriers (asymptomatic vs symptomatic) we observed differences in NF-L, as described in FTD [11], but also in 14-3-3.

A model for the prediction of MMSE value in the AD continuum and FTD patients was assessed. In most of the cases, results indicated that the biomarkers do not explain the MMSE value better than the group classification itself. Other studies have tried to use these non-core AD biomarkers, mainly Ng, to predict future cognitive impairment or cognitive decline [16,17]. Here, we found an effect in MMSE inference when comparing AD vs FTD for Ng, 14-3-3, Aβ42, t-tau and p-tau CSF biomarkers, indicating that these biomarkers can

explain differences in MMSE found between AD and FTD patients. In the comparisons HC-Preclinical AD, Aβ42 is related with MMSE and group classification, which could be explained by the fact that Aβ42 has been used to classify subjects in this group

As a limitation for the study, the individual sample group sizes are relatively small despite being similar to other published cohorts, especially in genetic subjects and FTD subtypes and not all the biomarkers were available in all the samples due to the quantity of CSF sample available in some of them.

To sum up, biomarkers of synapse loss and axonal damage differentiate HC from neurodegenerative dementias and between AD, FTD and CJD, even if the relevant overlap observed limit their use in the differential diagnosis between different clinical phenotypes. In FTD, different neurodegeneration biomarkers showed different direction of changes, suggesting different mechanisms of change. For the application of the AT(N) system in AD continuum NF-L and 14-3-3 biomarkers performs similar to t-tau as markers for neurodegeneration (N).

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FIGURE LEGENDS

Figure 1. Pair-wise comparisons of NF-L, Ng, 14-3-3 and YKL-40 CSF biomarkers.

Scatter plots displaying CSF NF-L, Ng, 14-3-3 and YKL-40 concentrations. A) In neurodegenerative diseases. Ctrl, n=50; AD, n=108; FTD, n=50; CJD, n=38. B) In Alzheimer's disease continuum. Ctrl, n=50; Preclinical AD, n=21; MCI, n=56; AD, n=108. The middle line shows the median. The lower and upper lines correspond to interquartile range. The differences between the groups were assessed using generalized lineal models adjusting for age and gender. A p value <0.05 was considered statistically significant. *P,.05, **P,.01, ***P,.001.

Abbreviations: NF-L = Neufrofilament-light chain; Ng = neurogranin; 14-3-3= 14-3-3 g; Ctrl: Healthy controls; AD = Alzheimer's disease dementia; MCI= MCI due to AD; FTD=Frontotemporal dementia; CJD= Creutzfeldt-Jackob disease.

Figure 2. ROC curves of CSF biomarkers NF-L, Ng, 14-3-3 and YKL-40, differentiating healthy controls from: A) Alzheimer's disease: AD B) Creutzfeldt-Jackob disease: CJD C) Frontotmeporal dementia: FTD.

Table 1.

	Controls	Preclinical AD	MCI due to AD	AD dementia	FTD	CJD
Sex, n (F/M)	50 (34/16)	21 (14/7)	56 (36/20)	108 (68/40)	50 (24/26)	38 (20/18)
Age at LP (years, IQR)	57.0 (48.1; 65.4)	69.4 (62.9; 75.2)	67.0 (62.8; 74.6)	63.6 (57.7; 68.4)	62.9 (55.7; 67.2)	68.0 (61.0; 72.0)
APOE4 -/ + (E4+ %)	38/12 (24)	11/10 (47.6)	19/36 (65.5)	52/53 (50.5)	40/10 (20)	6/0 (0)
Basal MMSE	29.00 [28.00; 30.00]	28.0 [27.0; 29.0]	26.0 [24.0; 27.0]	21.50 [18.00; 25.00]	24.00 (37) [21.00; 26.00]	NA
Aβ42 (pg/mL)	789.25 [676.93; 984.28]	416 [314; 482]	386 [337; 503]	372.98 [311.90; 446.47]	683.00 [544.70; 867.45]	NE
t-tau (pg/mL)	233.05 [167.57; 267.00]	258 [147; 482]	720 [565; 941]	765.11 [543.13; 1060.65]	276.45 [208.72; 400.00]	3802.64 (28) [1303.17; 8631.83]
p-tau (pg/mL)	49.64 [40.53; 57.10]	61,3 [37,5; 86,0]	108 [93,5; 123]	98.46 [79.58; 134.44]	45.10 [34.00; 57.10]	NE
NF-L (pg/mL)	820.43 [624.80; 992.95]	1069 [857; 1941]	1885 [1487; 2258]	2041.41 [1630.55; 2546.20]	4938.70 [2878.18; 7884.00]	10091.32 [6532.43; 14228.05]
YKL-40 (ng/mL)	217.23 [153.86; 271.37]	325.14 [237.24; 363.73]	347.24 [257.71; 397.06]	305.12 [247.33; 396.56]	315.28 [213.31; 387.28]	NE
Ng (pg/mL)	173.00 [132.30; 210.62]	159 [105; 224]	250 [217; 320]	252.40 [189.80; 314.20]	135.30 [101.70; 170.80]	587.84 [335.50; 1079.17]
14-3-3 (AU)	2595.64 (45) [2306.13; 3010.51]	NE	5096 [4443; 5842]	5187.65 (45) [3973.76; 5963.26]	3833.63 [2968.15; 4758.52]	NE

CSF levels (median, IQR)

Table 2. Genetic cases

	ADaMC	ADsMC	FTDaMC	FTDsMC
Sex, n (F/M)	9 (3/6)	16 (6/10)	5 (3/2)	16 (6/10)
Age at LP (years, IQR)	37.4 [33.5; 45.0]	49.6 [45.0; 57.1]	40.9 [34.4; 58.1]	59.6 [53.7; 65.5]
APOE4 -/ + (E4+ %)	8/1 (11.1)	15/1 (6.3)	2/2 (50)	10/6 (37.5)
Basal MMSE	29.0 [29.0; 30.0]	20.5 [19.0; 24.0]	30.0 [29.0; 30.0]	24.0 [23.0; 26.0]
Aβ42 (pg/mL)	788 [609; 1268]	375 [200; 460]	882 [843; 1206]	719 [568; 1048]
t-tau (pg/mL)	233 [196; 298]	849 [464; 1532]	203 [131; 301]	414 [211; 606]
p-tau (pg/mL)	51.3 [42.8; 58.0]	118 [72.4; 172]	49.5 [31.5; 60.0]	45.6 [38.0; 58.9]
NF-L (pg/mL)	576 [512; 615]	2018 [1794; 2557]	1090 [776; 1132]	7540.71 [3349.58; 11430.80]
YKL-40 (ng/mL)	148.34 [148.34; 167.18]	NE	289.96 [140.20; 374.41]	317.77 [168.82; 380.97]
Ng (pg/mL)	148 [136; 209]	272 [142; 544]	NE	152 [113; 187]
14-3-3 (AU)	2680 [2381; 3009]	4806 [3126; 8384]	2067 [1760; 2379]	4297 [3599; 5380]

Table 3.

a)*P* values in neurodegenerative diseases

NF-L	HC	AD	FTD	CJD
HC		<0.001	<0.001	<0.001
AD	<0.001		<0.001	<0.001
FTD	<0.001	<0.001		0.030
CJD	<0.001	<0.001	0.030	
Ng	**HC**	**AD**	**FTD**	**CJD**
HC		<0.001	0.024	<0.001
AD	<0.001		<0.001	<0.001
FTD	0.024	<0.001		<0.001
CJD	<0.001	<0.001	<0.001	
YKL-40	**HC**	**AD**	**FTD**	**CJD**
HC		0.010	0.019	NE
AD	0.010		0.945	NE
FTD	0.019	0.945		NE
CJD	NE	NE	NE	
14-3-3	**HC**	**AD**	**FTD**	**CJD**
HC		<0.001	<0.001	NE
AD	<0.001		<0.001	NE
FTD	<0.001	<0.001		NE
CJD	NE	NE	NE	

b) *P* values in AD continuum

NF-L	HC	Preclinical AD	MCI	AD dementia
HC		0.003	<0.001	<0.001
Preclinical AD	0.003		<0.001	<0.001
MCI	<0.001	<0.001		0.008
AD dementia	<0.001	<0.001	0.008	
Ng	**HC**	**Preclinical AD**	**MCI**	**AD dementia**
HC		0.908	0.025	<0.001
Preclinical AD	0.908		<0.001	<0.001
MCI	0.025	<0.001		0.321
AD dementia	<0.001	<0.001	0.321	
YKL-40	**HC**	**Preclinical AD**	**MCI**	**AD dementia**
HC		0.726	0.025	0.010
Preclinical AD	0.726		0.131	0.109
MCI	0.025	0.131		0.980
AD dementia	0.010	0.109	0.980	
14-3-3	**HC**	**Preclinical AD**	**MCI**	**AD dementia**
HC		NE	<0.001	<0.001
Preclinical AD	NE		NE	NE
MCI	<0.001	NE		0.557
AD dementia	<0.001	NE	0.557	

Table 4.- ROC

a)

Variable	Group	Median (IQR)	Valid N	AUC (95% CI)	Positive if	Sensitivity	Specificity	LR(+)
Ab42	Control	789.25 [676.93; 984.28]	50	0.907 (0.873; 0.942)	<=597	0.824	0.936	12.9
	Neurodeg Dis	424.50 [340.13; 544.85]	216					
T-tau	Control	233.05 [167.57; 267.00]	50	0.923 (0.893; 0.953)	>327	0.859	0.92	10.7
	Neurodeg Dis	641.37 [431.00; 1025.00]	241					
P-tau	Control	49.64 [40.53; 57.10]	50	0.842 (0.796; 0.888)	>71	0.713	0.94	11.9
	Neurodeg Dis	92.41 [65.84; 118.28]	216					
NF-L	Control	820.43 [624.80; 992.95]	50	0.967 (0.941; 0.993)	>1217	0.96	0.92	12
	Neurodeg Dis	2377.25 [1736.87; 4938.70]	252					
Ng	Control	173.00 [132.30; 210.62]	47	0.729 (0.669; 0.79)	>250	0.477	0.957	11.1
	Neurodeg Dis	244.00 [170.20; 330.60]	239					
14-3-3	Control	2595.64 [2306.13; 3010.51]	45	0.905 (0.86; 0.95)	>3598	0.775	0.933	11.6
	Neurodeg Dis	4534.41 [3766.19; 5628.63]	138					
YKL-40	Control	217.23 [153.86; 271.37]	47	0.749 (0.669; 0.829)	>545	0.067	0.979	3
	Neurodeg Dis	310.71 [241.25; 394.79]	208					

b)

Variable	Disease	AUC (95% CI)	Positive if	Sensibility	Specificity	LR(+)
NF-L	**AD**	0.98 (0.94; 1)	>1140	1.00	0.80	5.13
	DFT	0.97 (0.93; 1)	>1140	0.96	0.81	5.02
	CJD	0.998 (0.99; 1)	>1140	1.00	0.81	5.22
Ng	**AD**	0.85 (0.77; 0.93)	>230	0.64	0.88	5.26
	DFT	0.69 (0.58; 0.8)	<83	0.11	0.98	5.11
	CJD	0.86 (0.75; 0.96)	>230	0.83	0.85	5.56
14-3-3	**AD**	0.94 (0.88; 1)	>3210	0.90	0.83	5.26
			>3400	0.90	0.88	7.36
	DFT	0.8 (0.71; 0.89)	>3410	0.63	0.88	5.30
	CJD	*NC*				
YKL-40	**AD**	0.69 (0.58; 0.81)	*NC*			
	DFT	0.69 (0.58; 0.81)	>310	0.54	0.83	3.26
	CJD	*NC*				

Supplementary Table 1. FTD subtypes

	bvFTD	nfvPPA	svPPA
Sex, n (F/M)	8 (4/4)	11 (6/5)	15 (10/5)
Age at LP (years, IQR)	59.7 [53.4; 65.0]	69.1 [63.8; 72.5]	59.3 [55.7; 68.0]
APOE4 -/ + (E4+ %)	6/2 (25)	10/1 (9.1)	14/1 (6.7)
Basal MMSE	21.0 [16.0; 26.0]	25.0 [23.0; 27.0] (2)	26.0 [24.0; 27.0]
Aβ42 (pg/mL)	615 [432; 815]	652 [530; 817]	710 [607; 887]
t-tau (pg/mL)	213 [161; 260]	345 [203; 400]	256 [218; 280]
p-tau (pg/mL)	33.2 [29.4; 45.6]	57.1 [36.3; 71.0]	38.1 [34.0; 49.9]
NF-L (pg/mL)	5831 [3306; 8442]	4062 [2501; 6406]	4319 [3026; 5003]
YKL-40 (ng/mL)	356.37 [185.56; 398.39]	315.28 [224.45; 473.19]	281.94 [252.01; 346.51]
Ng (pg/mL)	108 [102; 190]	159 [93.0; 205]	134 [86.0; 142]
14-3-3 (AU)	3974 [2634; 4572]	4121 [2364; 4872]	3280 [2865; 3540]