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Two-level diagnostic classification using CSF YKL-40 in Alzheimer's disease

Filippo Baldacci^{a,b}, Nicola Toschi^{c,d}, Simone Lista^{e,f}, Henrik Zetterberg^{g,h,i}, Kaj Blennow^{g,h},
Ingo Kilimann^j, Stefan Teipel^j, Enrica Cavedo^{b,k}, Antonio Melo dos Santos^b, Stéphane Epelbaum^b,
Foudil Lamari^l, Bruno Dubois^b, Roberto Floris^c, Francesco Garaci^{c,m},
Ubaldo Bonuccelli^a, and Harald Hampel^{b,f,*}

^a*Department of Clinical and Experimental Medicine, University of Pisa, via Savi 10, Pisa, 56126, Italy*

^b*Sorbonne Universités, Université Pierre et Marie Curie (UPMC) Paris 06, Inserm, CNRS, Institut du cerveau et de la moelle (ICM), Département de Neurologie, Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A), Hôpital Pitié-Salpêtrière, Boulevard de l'hôpital, F-75013, Paris, France.*

^c*Department of Biomedicine and Prevention, Faculty of Medicine, University of Rome "Tor Vergata", 00173 - Roma, Italy*

^d*Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, 02129 - Boston, MA, United States*

^e*IHU-A-ICM – Paris Institute of Translational Neurosciences, Pitié-Salpêtrière University Hospital, 75651 - Paris, France*

^f*AXA Research Fund & UPMC Chair, 75005 - Paris, France*

^g*Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, S-431 80 - Mölndal, Sweden*

^h*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, S-431 80 - Mölndal, Sweden*

ⁱ*Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK*

^j*Department of Psychosomatic Medicine, University of Rostock, and DZNE Rostock, 18147 - Rostock, Germany*

^k*IRCCS Istituto Centro San Giovanni di Dio-Fatebenefratelli, 25125 - Brescia Italy*

^l*AP-HP, UF Biochimie des Maladies Neuro-métaboliques, Service de Biochimie Métabolique, Groupe Hospitalier Pitié-Salpêtrière, 75651 - Paris, France*

^m*Casa di Cura "San Raffaele Cassino", 03043 - Cassino (FR), Italy*

*Corresponding author:

Harald Hampel, MD, PhD, MA, MSc
AXA Research Fund & UPMC Chair,
Sorbonne Universities, Pierre and Marie Curie University, Paris 06,
Institute of Memory and Alzheimer's Disease (IM2A),
Brain and Spine Institute (ICM) UMR S 1127,
Department of Neurology,
Pitié-Salpêtrière University Hospital,
47 Boulevard de l'Hôpital, 75651 - Paris, CEDEX 13, France
Phone: +33 1 57 27 44 81
Fax: +33 1 42 16 75 16
E-mail: harald.hampel@med.uni-muenchen.de (H. Hampel)

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Abstract

Introduction: We assessed the diagnostic accuracy of cerebrospinal fluid (CSF) YKL-40 in discriminating: a) clinical Alzheimer's disease (AD) from cognitively healthy controls (HC), and frontotemporal dementia (FTD) (Level I), b) patients stratified by different pathophysiological profiles from HC and FTD following a novel unbiased/descriptive categorization based on CSF biomarkers, independently of cognitive impairment severity (Level II).

Methods: YKL-40 was compared among HC (n=21), mild cognitive impairment (n=41), AD (n=35), FTD (n=9) (Level I); among HC (n=21), subjects AD pathophysiology (tau and amyloid- β) negative (n=15), tau-positive (n=15), amyloid- β -positive (n=13), AD pathophysiology-positive (n=33), and FTD (n=9) (Level II).

Results: Level I: YKL-40 discriminated AD from HC and FTD (AUROCs=0.69, 0.71). Level II: YKL-40 discriminated tau-positive and AD pathophysiology-positive individuals from HC, AD pathophysiology-positive patients from FTD (AUROCs=0.76, 0.72, 0.73).

Discussion: YKL-40 demonstrates fair performance in distinguishing tau-positive patients from HC, suggesting it may aid clinical diagnosis and support a biomarker-guided pathophysiological stratification.

Key words: Alzheimer's disease, biomarkers, biomarker-based diagnosis, cerebrospinal fluid, clinical diagnosis, dementia, diagnostic biomarkers, Frontotemporal dementia, mild cognitive impairment, neurodegeneration, neuroinflammation, YKL-40

Abbreviations: Alzheimer's disease (AD); amyloid- β 1 to 42 ($A\beta_{1-42}$); area under the receiver operating characteristic curve (AUROC); A/T/N system: A= $A\beta$, T= phospho-tau, N= total-tau; cerebrospinal fluid (CSF); cognitively healthy controls (HC); ^{18}F -fluorodeoxyglucose-PET (^{18}F -FDG-PET); False Discovery Rate (FDR); fronto-temporal dementia (FTD); hyperphosphorylated tau (p-tau); Institute of Memory and Alzheimer's Disease (IM2A); International working group-2 (IWG-2); Kruskal-Wallis (KW); leave-one out cross validation (LOO-CV); mild cognitive impairment (MCI); Mental-State Examination (MMSE); National Institute on Aging–Alzheimer's Association (NIA-AA); National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA); pairwise multiple comparison of mean ranks (PMCMR); total tau (t-tau)

1. INTRODUCTION

Alzheimer's disease (AD) is a genetically, biologically and clinically heterogeneous multifactorial disease [1–3]. The primary pathological hallmarks are amyloid plaques consisting of aggregated amyloid- β and neurofibrillary tangles containing hyperphosphorylated and aggregated tau protein [3]. However, in most of patients, AD related brain changes are combined with other types of pathologies [4,5]. Currently, three core, feasible cerebrospinal fluid (CSF) biomarkers have shown to track pathophysiological mechanisms *in vivo* in preclinical, prodromal and AD dementia [6,7]. In particular, (I) CSF concentrations of the amyloid- β 1 to 42 ($A\beta_{1-42}$) peptide is considered a biomarker of brain amyloid deposition, (II) total tau (t-tau) protein is considered a marker of neuronal injury in several brain diseases (not pathognomonic for AD) and (III) hyperphosphorylated tau (p-tau) protein is considered a marker reflecting hyperphosphorylation of tau leading to the formation of paired helical filaments and ultimately neurofibrillary tangles [8]. Although neuroinflammation has been consistently suggested with accumulating evidence to contribute as additional pathophysiological mechanism to AD [1,9], a clinically validated and standardized CSF inflammation biomarker for both diagnosis and as indicator of mechanism of action in trials has not yet been developed.

In particular, YKL-40, a glycoprotein belonging to the chitinase-like proteins group, represents a promising candidate inflammation biomarker in progressive clinical development for AD. However, its pathophysiological functions are not yet fully clarified [10]. YKL-40 is a differentiation marker of

macrophages [11–13] and is expressed in microglia and astroglia within the central nervous system [14].

Recently, in first clinical investigations, statistically significant elevated CSF concentrations of YKL-40 were reported in AD compared with cognitively healthy controls (HC), in agreement with reported increased concentrations at the prodromal and preclinical stages [15–28].

The main objective of this study was to assess the diagnostic accuracy of CSF YKL-40 in diagnosing and categorizing individuals with cognitive impairment.

In a first step (Level I), we tested the performance of YKL-40 in discriminating clinically diagnosed patients with AD dementia from HC subjects and patients with frontotemporal dementia (FTD).

In a second step of analysis (Level II) [4], we evaluated the classificatory performance of YKL-40 across the spectrum of AD pathology by adopting a recently published unbiased descriptive categorization system based on biomarker-guidance only, namely the “A/T/N” scheme, using AD core biomarkers. The A/T/N system comprises three binary components, A= A β pathology, T= tau pathology, N= neurodegeneration for characterizing features of AD pathology/pathophysiology (independently from the severity of cognitive impairment). To this end, we determined the discriminatory performance of CSF YKL-40 in distinguishing HC from I) AD pathology patients (presenting both decreased CSF concentrations of A β ₁₋₄₂ peptide and increased t-tau or p-tau protein [7]), II) patients showing tau pathology only, and III) patients with A β pathology only. In addition, we explored the ability of CSF YKL-40 to discriminate AD pathology patients from FTD cases.

2. METHODS

2.1. Population

A total of 135 individuals from a convenience sample were examined. Of these participants, 27 were excluded due to missing data in one or more CSF biomarkers and the remaining 108 were included in the present study. Clinical and biological data from these 108 individuals (AD= 35, FTD=

9, MCI= 41, and cognitively HC= 23) were retrospectively collected in a multi-centre cross-sectional study involving three independent academic AD research centres and expert memory clinics. Thirty-five subjects were recruited at the Institute of Memory and Alzheimer's Disease (IM2A) at Pitié-Salpêtrière University Hospital in Paris (France); 57 at the German Centre for Neurodegenerative Diseases (DZNE) in Rostock (Germany); 16 at the Institute of Neuroscience and Physiology at Sahlgrenska University Hospital in Göteborg (Sweden).

The study was conducted according to the provisions of the Declaration of Helsinki. All participants or their representatives gave written informed consent for the use of their clinical data for research purposes and the local Ethical Committees at the respective universities approved the study. We followed the STARD criteria for the reporting of diagnostic test accuracy studies (available at <http://www.equator-network.org/reporting-guidelines/stard/>).

2.2. Patient stratification

2.2.1. Level I (purely clinical diagnostic approach)

The first group was composed of 23 cognitively HC. Two individuals from the Göteborg cohort were identified as asymptomatic-at-risk of AD [7] or preclinical AD [29] showing high CSF t-tau concentrations. While tau positivity is a criterion which pertains purely to level II, we decided to exclude two asymptomatic subjects showing positivity to CSF t-tau from the HC group in order to perform both level I and level II analyses on identical populations. The second group included 41 subjects with MCI [6]. The third group included 35 patients with AD dementia [30]. Finally, the fourth group included 9 patients with FTD [31] (**Figure 1**). The clinical diagnosis of AD dementia was performed according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) consensus criteria [30]. The clinical diagnosis of MCI was made according to the MCI core clinical criteria [6]. The clinical diagnosis of FTD was made following the consensus on clinical diagnostic criteria of 1998 [31]. Cognitively HC were individuals who volunteered for lumbar puncture; the inclusion

criteria comprised absence of history of neurological or psychiatric diseases and Mental-State Examination (MMSE) scores between 27 and 30.

2.2.2. Level II (unbiased categorization based on CSF core biomarker profiles)

The categorization of AD dementia patients and individuals with MCI followed the unbiased biomarker-based descriptive classification system recently proposed by Jack and colleagues: the “A/T/N” system [4]. This classification considers 3 binary (i.e. positive or negative) categories: “A” referring to an amyloid biomarker (CSF A β ₁₋₄₂ or amyloid-PET), “T” to a tau pathology biomarker (CSF p-tau or tau-PET), and “N” to a quantitative or topographic biomarker of neurodegeneration or neuronal injury (CSF t-tau, ¹⁸F-fluorodeoxyglucose-PET (¹⁸F-FDG-PET), or structural MRI). Since each individual score is displayed as an “A \pm /T \pm /N \pm ” arrangement, eight different categories are possible [4]. The A/T/N classification system is linked to the biomarker classification frameworks of the International working group-2 (IWG-2) criteria [7] and the National Institute on Aging–Alzheimer's Association (NIA-AA) guidelines [4,29,32], and is able to chart both diagnostic classifications. For practical reasons the A/T/N system was utilized in a simplified version which employed only CSF markers and excluded imaging-methods (amyloid PET, tau PET, FDG-PET, or structural MRI) to define 5 categories (groups) which were independent from severity of cognitive impairment:

Group 1 consisted of cognitively HC (n= 21), *a priori* defined as both A β and tau negative [A-/T-/N-]; group 2 [A-/T-/N-] (n= 15) included 2 AD dementia patients and 13 MCI subjects which were both A β and tau negative; group 3 [A-/T \pm /N+ or A-/T+/N \pm] (n= 15) encompassed 6 AD dementia patients and 9 MCI subjects which were tau positive but A β negative; group 4 [A+/T-/N-] (n=13) comprised 5 AD dementia patients and 8 MCI subjects which were A β positive only; group 5 [A+/T \pm /N+ or A+/T+/N \pm] (n=33) included 22 AD dementia patients in line with the IWG-2 criteria [7] and the NIA-AA guidelines [32], and 11 prodromal AD [33] or MCI due to AD [6] cases, all of which were both A β and tau positive; group 6 comprised all FTD patients (n=9) including seven

participants which were both $A\beta_{1-42}$ and tau negative, one which was $A\beta_{1-42}$ negative and tau positive, and one which was $A\beta_{1-42}$ positive and tau negative. According to the IWG-2 criteria, this last participant should be defined as a case of FTD and not as a patient with a frontal variant of AD [7]. Of note, since the A/T/N system is not directly applicable to FTD, this last group was analysed exclusively in terms of clinical diagnosis (**Figure 1**).

2.3. CSF sampling

All CSF samples were collected in polypropylene tubes, centrifuged (1000 g, 10 minutes, +4°C (sample collected at IM2A laboratory for the Paris cohort), 1500 g, 10 minutes, +4°C (sample collected at DZNE laboratory for the Rostock cohort), 1800 g, 10 minutes, +4°C (sample collected at Mölndal Clinical Neurochemistry Laboratory for the Göteborg cohort)), and the collected supernatant was stored at –80°C pending biochemical analysis.

2.4. Immunoassays for core biomarkers

The core AD CSF biomarkers ($A\beta_{1-42}$, t-tau, and p-tau) were measured in each subject. For the Paris cohort, CSF analyses were performed at the Laboratory of Biochemistry, Unit of Biochemistry of Neurometabolic diseases, Pitié-Salpêtrière University Hospital of Paris.

For the Rostock cohort, CSF analyses were executed in two different units: the Institute of Clinical Chemistry and Laboratory Medicine, Rostock University Medical Centre, after 06/2012, and the Laboratory of Neurochemistry, Department of Neurology, Göttingen University Medical Centre, before 06/2012.

For the Gothenburg cohort, CSF analyses were executed at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Mölndal.

T-tau was measured using a sandwich ELISA (INNOTEST hTAU-Ag, Fujirebio Europe, Gent, Belgium) specifically constructed to measure all tau isoforms irrespective of phosphorylation status [34]. Tau phosphorylated at threonine 181 (P-tau₁₈₁) was measured using a sandwich ELISA

(INNOTEST Phospho-Tau[181P], Fujirebio Europe, Gent, Belgium) constructed to specifically measure tau protein phosphorylated at the amino acid threonine 181 [35]. $A\beta_{1-42}$ was measured using a sandwich ELISA (INNOTEST β -AMYLOID(1-42), Fujirebio Europe, Gent, Belgium), specifically constructed for the quantitative determination of $A\beta_{1-42}$ [36]. All analysis were performed by board-certified laboratory technicians blinded to clinical information.

CSF biomarkers abnormalities were defined based on reference threshold cut-off values currently used in each memory clinic: at IM2A in Paris, $A\beta_{1-42} < 500$ pg/mL, t-tau > 450 pg/mL, p-tau₁₈₁ > 60 pg/mL; at DZNE in Rostock, $A\beta_{1-42} < 567$ pg/mL, t-tau > 512 pg/mL, p-tau₁₈₁ > 66 pg/mL for the CSF samples measured before 06/2012 and $A\beta_{1-42} < 450$ pg/mL, t-tau > 450 pg/mL, p-tau₁₈₁ > 62 pg/mL for the CSF sampels measured after 06/2012; at Mölndal Clinical Neurochemistry Laboratory, $A\beta_{1-42} < 550$ pg/mL, t-tau > 400 pg/mL, p-tau₁₈₁ > 80 pg/mL.

2.5. Immunoassay for YKL-40

All CSF YKL-40 analyses were performed at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Mölndal, Sweden, using a commercial available ELISA kit (R&D Systems, Minneapolis, MN, US), according to manufacturer instructions. The measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Intra-assay coefficients of variation were below 10%. All samples were well within the linear range of the assay.

2.6. Statistical Analysis

Associations between sex and diagnostic group were assessed by Fisher's exact test, and the associations between age and diagnostic group was assessed through a nonparametric Kruskal-Wallis (KW) test. Subsequently, as a preprocessing step, all YKL-40 values were adjusted for age, sex and site employing nonparametric regression to enable age-, sex- and site- independent assessment of the diagnostic potential of YKL-40 while foregoing assumptions of normality. Correlations of YKL-40

with core biomarkers in the entire sample were executed using Spearman's rank-order correlation test. Hence, we conducted group-wise comparisons of YKL-40 values through nonparametric KW tests followed by pairwise post-hoc comparison (Conover's-test for multiple comparisons) whenever the result of the KW test was statistically significant ($p < 0.05$). Results of post-hoc testing were corrected for multiple comparisons using a False Discovery Rate (FDR) procedure ($\alpha = 0.05$).

We then evaluated the diagnostic potential of YKL-40 using logistic regression within a leave-one out cross validation (LOO-CV) approach in the following *a priori* comparisons: HC vs. AD and AD vs. FTD (Level I), HC vs. group 3 [A-/T±/N+ or A-/T+/N±], HC vs. group 4 [A+/T-/N-], HC vs. group 5 [A+/T±/N+ or A+/T+/N±] (Level II). In this analysis, the age-, sex-, and site adjusted YKL-40 values were entered as predictors and the diagnostic group was entered as the dependent variable. After model fitting, we calculated the area under the receiver operating characteristic curve (AUROC) and its associated confidence intervals using a bootstrap procedure (100000 bootstraps) [37] by pooling predictions computed on the test sets from each train-test split in the LOO-CV procedure. The discriminatory ability of YKL-40 to correctly allocate participants to diagnostic groups was classified as follows: excellent (AUROC 0.90-1.00), good (AUROC 0.80-0.89), fair (AUROC 0.70-0.79), poor (AUROC 0.60-0.69), or fail/no discriminatory capacity (AUROC 0.50-0.59) [38].

All statistical analyses were performed in the R statistical environment version 3.2.3 (available at <https://www.R-project.org/>) under a Linux environment using the nonparametric kernel smoothing methods for mixed data types package (np package) [39], partial ROC (pROC) package [37], and the pairwise multiple comparison of mean ranks (PMCMR) package [40]. Two-tailed P values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Correlations of CSF YKL-40 with core biomarkers in the entire sample

YKL-40 significantly correlated with p-tau ($\rho_s = 0.574$, $P < 0.001$) and t-tau ($\rho_s = 0.554$, $P < 0.001$) but not with A β ($\rho_s = 0.002$, $P = 0.980$) in the entire population after adjusting for age, sex and site (**Supplementary materials**).

3.2. CSF YKL-40 concentrations in the population categorized according to Level I

Table 1 summarizes the concentrations of all analytes, combined with the demographic and clinical data of the population classified in line with Level I classification. Cognitively HC were slightly but significantly younger than MCI, AD, and FTD patients. MMSE scores were significantly lower in AD compared with cognitively HC and MCI. Compared with the HC group, CSF YKL-40 concentrations were significantly increased in AD ($P = 0.032$) and FTD patients ($P = 0.049$) (**Figure 2A**).

3.3. CSF YKL-40 concentrations in the population categorized according to Level II

Table 2 summarizes the concentrations of all analytes, combined with the demographic and clinical data of the population classified in line with Level II criteria. Cognitively HC (group 1) and patients belonging to group 2 [A-T-N-] were significantly younger than all the other groups (Table 1). Compared with group 1 (HC), CSF YKL-40 concentrations were significantly increased in group 3 [A-/T \pm /N+ or A-/T+/N \pm] ($P = 0.002$) and group 5 [A+/T \pm /N+ or A+/T+/N \pm] ($P = 0.002$). Group 3 [A-/T \pm /N+ or A-/T+/N \pm] and group 5 [A+/T \pm /N+ or A+/T+/N \pm] patients presented substantially higher CSF YKL-40 concentrations compared with group 4 [A+/T-/N-] ($P < 0.001$ for both) patients as well as compared with those belonging to the FTD group ($P = 0.006$ and $P = 0.007$, respectively); group 3 [A-/T \pm /N+ or A-/T+/N \pm] patients presented higher CSF YKL-40 concentrations compared with group 2 [A-/T-/N-] patients ($P = 0.033$), (**Figure 2B**).

3.4. Diagnostic value of CSF YKL-40 in the population at Level I

CSF YKL-40 differentiated HC from AD patients with an AUROC of 0.69 (95% CI, 0.55-0.84) (**Figure 3A**). CSF YKL-40 discriminated AD from FTD patients with an AUROC of 0.71 (95% CI, 0.51-0.91) (**Figure 3B**).

3.5. Discriminative value of CSF YKL-40 in the population at Level II

CSF YKL-40 discriminated cognitively HC from group 3 [A-/T±/N+ or A-/T+/N±], group 4 [A-/T±/N+ or A-/T+/N±], group 5 [A+/T±/N+ or A+/T+/N±] with AUROCs=0.76, (95% CI, 0.58-0.94), 0.52 (95% CI, 0.29-0.74), and 0.72 (95% CI, 0.58-0.87) (**Figure 4A-C**), respectively. CSF YKL-40 differentiated group 5 [A+/T±/N+ or A+/T+/N±] from FTD patients with AUROC= 0.73 (95% CI, 0.54-0.92) (**Figure 4D**).

4. DISCUSSION

In the applied diagnostic Level I approach, using clinical diagnostic criteria, CSF YKL-40 concentrations were significantly increased in clinically diagnosed AD patients compared with HC (**Figure 2A**). Moreover, the corresponding AUROC was poor/borderline fair in discriminating the two groups (**Figure 3A**). These findings partly confirm previous diagnostic studies [16,18,20,25,26,28] and data from a recent meta-analysis [14]; in contrast, one study showed no differences between AD and HC [22]. Importantly, AD patients showed higher concentrations of CSF YKL-40 compared with FTD; indeed, CSF YKL-40 exhibits a fair performance in distinguishing between the two groups (**Figure 3B**). In the literature, very few studies evaluated the diagnostic accuracy of CSF YKL-40 in discriminating between AD and FTD patients, and reported conflicting results. In particular, Craig-Shapiro and colleagues documented higher concentrations of CSF YKL-40 in FTD compared with mild AD [18]; conversely, two other studies found no significant differences between AD and FTD [15,19].

In the applied Level II approach, CSF YKL-40 concentrations were shown to be significantly increased in patients who were tau-positive only and in those with AD pathophysiology *versus* HC

(group 1) (**Figure 2B**). We found that YKL-40 exhibited a fair performance in discriminating tau-positive and AD pathophysiology-positive patients from HC (**Figure 4A and 4C**), but not in discriminating A β -positive only patients from HC (**Figure 4B**). These results are generally in agreement with studies indicating that CSF YKL-40 is more associated to tau protein pathology than to A β pathology [15,17,19,20,23,24]; this is confirmed in our results in terms of a positive correlation of YKL-40 with p-tau and t-tau. Tau-positive patients revealed higher CSF concentrations of YKL-40 compared with patients AD pathophysiology-negative, patients A β -positive only, and FTD patients. Similarly, AD pathophysiology-positive patients showed higher CSF concentrations of YKL-40 compared with patients A β -positive only, FTD patients, and a trend towards higher concentrations of YKL-40 *versus* AD pathophysiology-negative patients. In particular, the AUROC related to the discrimination between AD pathophysiology and FTD patients was fair (**Figure 4D**), i.e. equivalent to what we found in Level I analysis. Several explanations support the fact that FTD patients can display lower concentrations of CSF YKL-40 compared with those detected in AD patients. In particular, FTD patients may have underlying mechanisms related to neurodegeneration, not associated with tau protein [41]. This mechanistic pathophysiological variability possibly reflects the common finding that clinically diagnosed FTD is a heterogeneous biological and clinical syndrome with different and overlapping phenotypes and endophenotypes. Further studies conducted in large samples of demented patients are needed to systematically assess how CSF YKL-40 could differentiate AD from other neurodegenerative dementias including not only FTD but also dementia with Lewy body, Parkinson's disease dementia, atypical parkinsonisms, and, additionally, vascular dementia.

CSF YKL-40 can be considered as a biomarker of a specific pathophysiological mechanism, allowing for *in vivo* measurement of neuroinflammation that may be complementary to the core, feasible CSF AD biomarkers A β ₁₋₄₂, t-tau, and p-tau. The importance of having a dynamic early biomarker of neuroinflammation in AD is intriguing not only for diagnostic purposes (given that neuroinflammation is probably involved in a number of other neurodegenerative diseases [42]) but

also because it can be predictive as an outcome of response to novel anti-inflammatory drugs. In fact, epidemiological studies indicate that non-steroidal anti-inflammatory drugs (NSAIDs) may lower the risk of AD [43,44], although a number of trials reported negative results [9]. However, anti-inflammatory treatments may not be efficacious when administered during the dementia stage of AD. Notably, the naproxen trial in AD initially reported negative results; conversely, longer-term follow-up results suggested that naproxen may exert a protective role in asymptomatic subjects at baseline, thus reducing the conversion rate to AD [45,46]. Therefore, the discovery and validation of a reliable inflammation biomarker in prodromal AD as well as in the preclinical stage, aiming at tracking the response to an anti-inflammatory drug in the respective target population, may be supportive for developing novel therapeutic strategies for AD.

To our knowledge, in our study we apply YKL-40 for the first time as a diagnostic CSF biomarker for AD founded on the unbiased biomarker-based classification scheme [4].

Some limitations of our study need to be mentioned. First, in Level II, the categorization of our patients was only based on CSF biomarkers, i.e. the A/T/N system was used without considering additional information derived from neuroimaging methods. Moreover, this dataset allows for a cross-sectional study and longitudinal data are not yet available. In particular, we cannot differentiate potentially stable MCI subjects from those progressing and converting to dementia, or to provide data about a potential differing prognosis and rate of disease progression. Furthermore, the diagnosis of MCI was made in a routine clinical setting and additional extensive and/or homogeneous psychometric data were not available. Moreover, given the limited number of patients, and possible resulting lack of power, we were not able to test CSF YKL-40 concentrations in all possible (eight) categories originally reported by Jack and colleagues [4]; a comprehensive investigation of all these categories would have significantly higher numerosities. In this regard, further studies are also needed to determine the prevalence, currently largely unknown, of some of the A/T/N system categories (e.g. A-/T+/N- category) within the MCI and clinical AD populations. Nonetheless, in this respect, we adopted the following rationale in merging some of the A/T/N subcategories: groups of subjects with

both decreased CSF levels of A β and increased t-tau, or decreased CSF A β and increased p-tau, or decreased CSF A β and increased p-tau and t-tau were merged in one group since they show all the pathophysiological features necessary for AD diagnosis [7,32]. On the other hand, the positivity to only CSF A β , or only t-tau, or only p-tau, or both p-tau and t-tau but not A β is presently not accepted for AD diagnosis [7,32]; therefore, these four groups disclosing uncomplete AD pathophysiological features were merged in 2 largest groups (A β -positive only and tau-positive only groups, respectively).

Furthermore, we merged MCI subjects with AD dementia patients on the basis of core biomarkers characterization, without considering the degree of severity of cognitive impairment. Actually, the clinical distinction between MCI and dementia is not very precise and time dependent; in this regard, the IWG-2 criteria consider MCI subjects with AD pathophysiology as AD in its prodromal phase [7]. Finally, with the exception of YKL-40, the measurements of the core CSF AD biomarkers were performed in different academic expert laboratories and, while we controlled for center effects in our statistical analysis by integrating CSF biomarkers values adjusted for site, additional inter-laboratory variability cannot be completely ruled out.

In conclusion, our results indicate that CSF YKL-40 diagnostic performance is poor to borderline fair. Therefore, CSF YKL-40 analysis does not satisfactorily support the diagnosis of AD dementia patients from cognitively HC based on clinical categorization. CSF YKL-40, however, delivers a fair performance in discriminating between clinical AD dementia and FTD patients. Based on unbiased core biomarker classification, CSF YKL-40 concentrations fairly distinguishes HC individuals from cognitively impaired patients with both A β and tau pathology and cognitively impaired patients with tau pathology only, and patients with both A β and tau pathology from FTD patients. In contrast, CSF YKL-40 concentrations were not useful in distinguishing between HC and cognitively impaired patients who were A β -positive only. Overall, CSF YKL-40 does not sufficiently support the differentiation between AD dementia patients and patients with AD pathophysiology from HC subjects or patients with FTD. However, our results confirm that CSF YKL-40 can be considered as

a candidate biomarker of neuroinflammation potentially related to neurodegenerative processes associated with increased tau protein, thus suggesting a supportive role in clinical diagnosis and patient stratification.

In perspective, large longitudinal studies, possibly enriched with confirmatory post-mortem data, one may investigate the AD pathophysiological spectrum by applying the unbiased A/T/N classification system which can be considered an adaptive and flexible “open source” approach, based on a pattern of established biomarkers which, however, can be potentially expanded to integrate novel emerging validated biological markers, genetic and epigenetic factors [47] as well as indicators connecting different systems dimensions of pathology and pathophysiology, such as MRI-derived grey matter atrophy or functionally relevant burden of white matter damage [48]. Moreover, there is sufficient evidence in the literature of a number of other potentially complementary promising candidate biomarkers of inflammation that deserve further development, validation and standardization, such as the interleukin-6 and 12, tumor necrosis factor alpha receptor components as well as components of the complement system pathways in AD [1,49].

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Simone Lista has received lecture honoraria from Roche.

Kaj Blennow and Henrik Zetterberg are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg.

Kaj Blennow has served as a consultant or at advisory boards for IBL International, Roche Diagnostics, Eli Lilly, Fujirebio Europe, and Novartis.

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Bruno Dubois has served as a consultant or at advisory boards from Eli Lilly, Cytos Ltd and Boehringer-Ingelheim.

Harald Hampel reports no conflict of interest with the present manuscript. He serves as Senior Associate Editor for the journal *Alzheimer's & Dementia*; he has been a scientific consultant and/or speaker and/or attended scientific advisory boards of Axovant, Anavex, Eli Lilly and company, GE Healthcare, Cytos Ltd, Jung Diagnostics GmbH, Roche, Biogen Idec, Takeda-Zinfandel, Oryzon Genomics; and receives research support from the Association for Alzheimer Research (Paris), Pierre and Marie Curie University (Paris), Pfizer & Avid (paid to institution); and has patent applications, but receives no royalties.

CAPTIONS TO THE FIGURES

Figure 1. Stratification of the cohort according to the 2 levels of classification. Level I of categorization followed only a clinical diagnostic approach. It included HC, MCI, ADD, and FTD participants.

Level II of categorization followed a simplified version of the biomarker-based descriptive classification model recently proposed by Jack and colleagues: the "A/T/N" system [4]. It included: Group 1, HC; Group 2, [A-/T-/N-]; Group 3, [A-/T±/N+ or A-/T+/N±]; Group 4, [A+/T-/N-]; Group 5; [A+/T±/N+ or A+/T+/N±]; Group 6, FTD.

The figure shows how the participants have been stratified from Level 1 to Level II, according to the A/T/N classification scheme.

Abbreviations: ADD= Alzheimer's disease dementia; FTD= frontotemporal dementia; HC= cognitively healthy controls; MCI=mild cognitive impairment.

A= amyloid biomarker (A β ₁₋₄₂); T= tau pathology biomarker (p-tau); N= biomarker of neurodegeneration or neuronal injury (t-tau).

Figure 2. Boxplots showing CSF YKL-40 concentrations (adjusted for sex, age and site) in AD patients, MCI patients, FTD patients, and cognitively HCs (Level I) (A). Boxplots showing CSF YKL-40 concentrations (adjusted for sex, age and site) in all 6 groups (Level II) (B). The lower, upper, and middle lines correspond to the 25th centile, 75th centile, and median, respectively. The whiskers extend to the minimum and maximum YKL-40 data points. Dark circles represent outliers. Group-wise comparisons of YKL-40 values were conducted through nonparametric KW tests followed by pairwise comparison (Conover's-test for multiple comparisons).

Abbreviations: AD= Alzheimer's disease; CSF= cerebrospinal fluid; FTD= frontotemporal dementia; HC= healthy controls; KW= Kruskal-Wallis; MCI= mild cognitive impairment.

A= amyloid biomarker ($A\beta_{1-42}$); T= tau pathology biomarker (p-tau); N= biomarker of neurodegeneration or neuronal injury (t-tau).

Figure 3. The AUROC curves result from fitting a logistic regression model within a LOO-CV scheme to YKL-40 data adjusted for age, sex, and site in the following binary classification problems: HC *versus* AD (A, left) and AD *versus* FTD (B, right)

Abbreviations: AD= Alzheimer's disease; AUC= Area under the ROC curve. C.I.= confidence intervals (computed using a bootstrap procedure with 10000 bootstraps); CSF= cerebrospinal fluid; FTD= frontotemporal dementia; HC= healthy controls; LOO-CV= leave-one out cross validation; MCI= mild cognitive impairment.

A= amyloid biomarker ($A\beta_{1-42}$); T= tau pathology biomarker (p-tau); N= biomarker of neurodegeneration or neuronal injury (t-tau).

Figure 4. The AUROC curves result from fitting a logistic regression model within a LOO-CV scheme to neurogranin data adjusted for age, sex, and site in the following binary classification problems: Group 1 *versus* Group 3 (A, top left), Group 1 *versus* Group 4 (B, top right), Group 1 *versus* Group 5 (C, bottom left), Group 5 *versus* Group 6 (D, bottom right).

Abbreviations: AD= Alzheimer's disease; AUC= Area under the ROC curve. C.I.= confidence intervals (computed using a bootstrap procedure with 10000 bootstraps); CSF= cerebrospinal fluid; LOO-CV= leave-one out cross validation.

A= amyloid biomarker ($A\beta_{1-42}$); T= tau pathology biomarker (p-tau); N= biomarker of neurodegeneration or neuronal injury (t-tau).

LEVEL I

HC= 21

MCI= 41

ADD= 35

FTD= 9

LEVEL II

Group 1
(HC)
N= 21

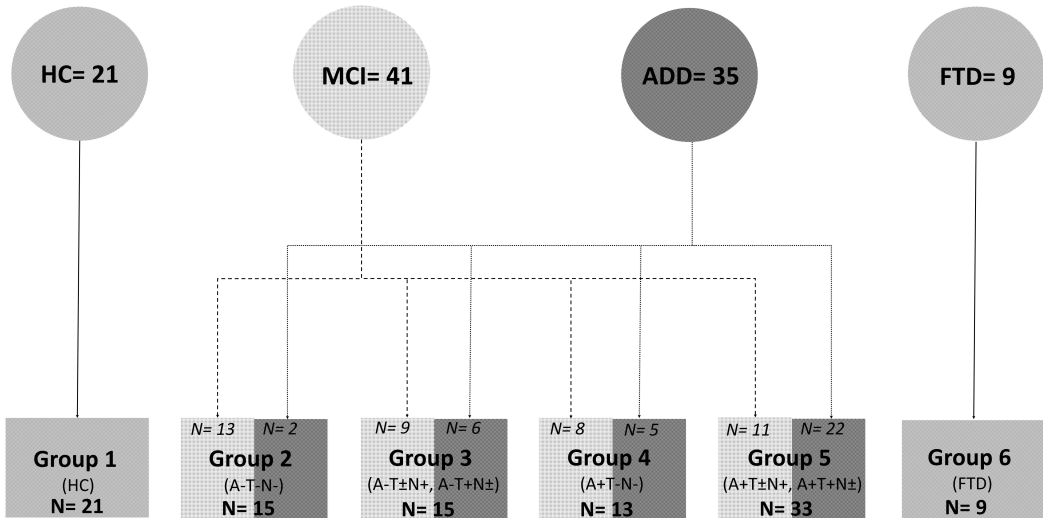
N= 13 N= 2
Group 2
(A-T-N-)
N= 15

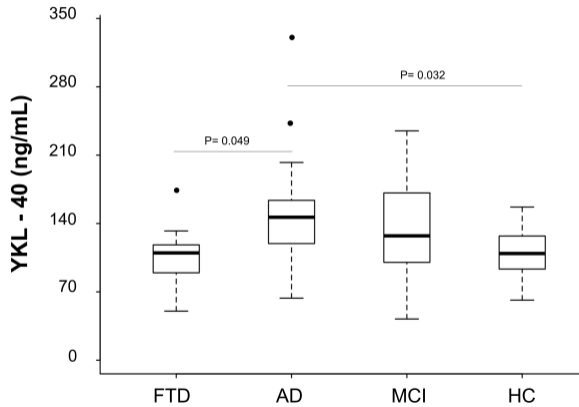
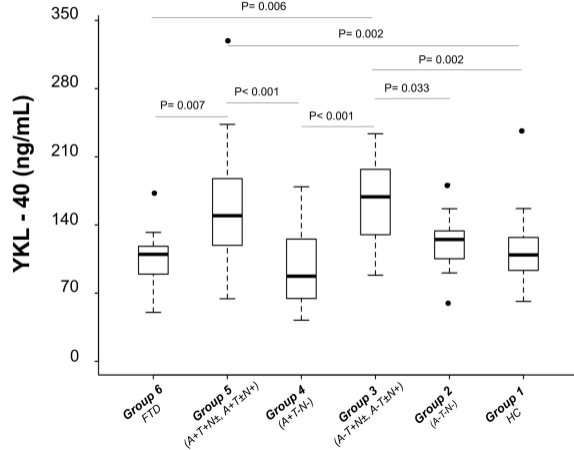
N= 9 N= 6
Group 3
(A-T±N+, A-T+N±)
N= 15

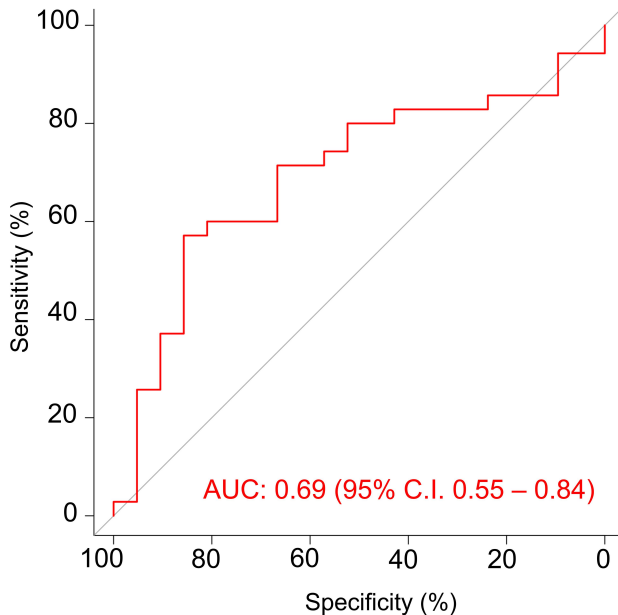
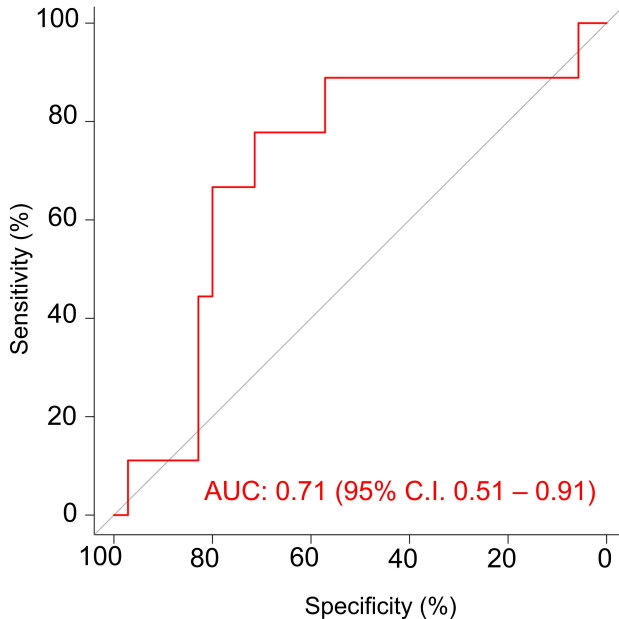
N= 8 N= 5
Group 4
(A+T-N-)
N= 13

N= 11 N= 22
Group 5
(A+T±N+, A+T+N±)
N= 33

Group 6
(FTD)
N= 9



A**B**

A**HC vs AD****B****AD vs FTD**

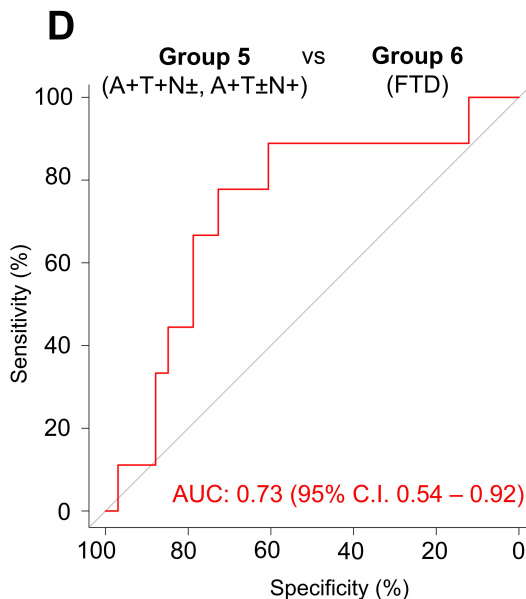
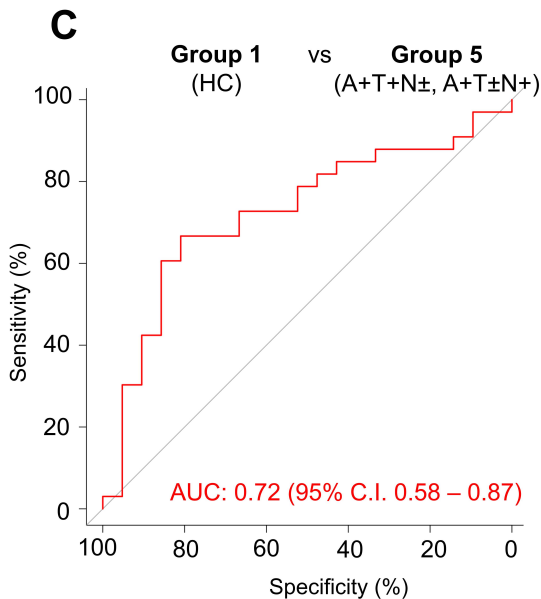
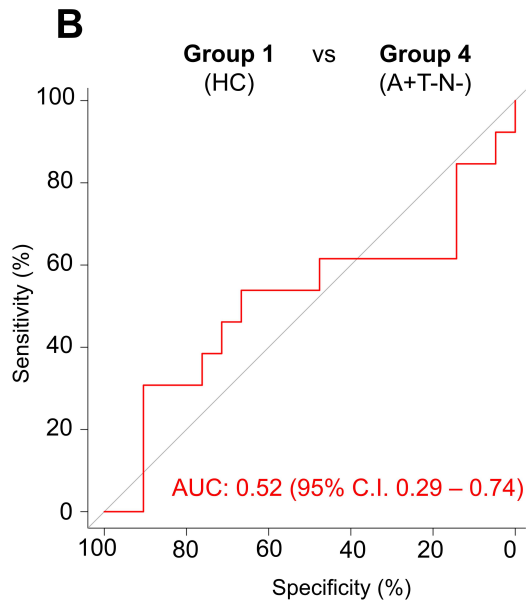
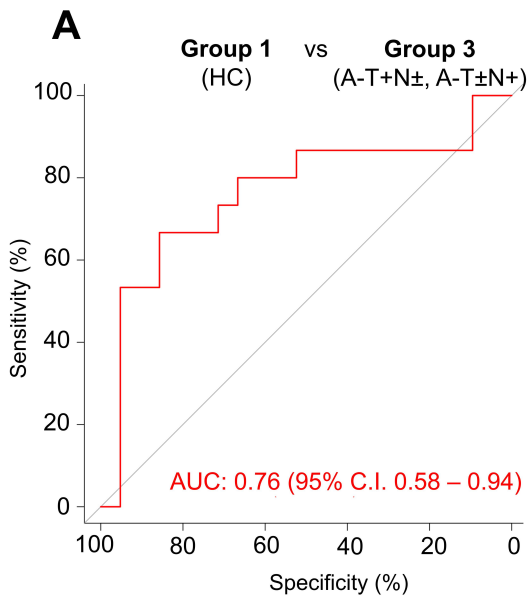


Table 1. Summary of the demographic, clinical, and biomarker data of the population (Level I).

	LEVEL I			
	HC	MCI	AD	FTD
Sex, n (F/M)	21 (13/8)	41 (14/27)	35 (24/11)	9 (5/4)
Age at LP (y)	64 (59-59)	72 (65-75)#	73 (68-76)#	73 (70-74)*
MMSE at LP (/30)	30 (29-30)	26 (24-28)	23 (19-26)*†	23 (19-26)
CSF YKL-40 (ng/mL)	98 (90-110)	128 (98-184)	146 (119-177)*§	114 (98-120)
CSF A β ₁₋₄₂ (pg/mL)	910 (785-996)	540 (411-911)*	424 (374-503) §#¶	652 (530-823)
CSF t-tau (pg/mL)	201 (127-243)	261 (189-452)	496 (360-764)†§#	208 (161-340)
CSF p-tau (pg/mL)	44 (35-48)	60 (44-80)	83 (64-126)*†§	31 (27-53)

Abbreviations: A β ₁₋₄₂, 42-amino acid-long amyloid beta peptide; AD, Alzheimer's disease; CSF, cerebrospinal fluid; HC, cognitively healthy controls; F, female; FTD, frontotemporal dementia; LP, lumbar puncture; M, male; MCI, mild cognitive impairment; MMSE, mini-mental state examination; p-tau, hyperphosphorylated tau; t-tau, total tau.

NOTE. All data are median values with 25th and 75th quartiles, except for n.

For statistical comparisons, the above MMSE, YKL-40, A β ₁₋₄₂, t-tau, p-tau comparisons were adjusted for age, sex, and site.

* $P < 0.05$ vs HC; † $P < 0.05$ vs MCI; ‡ $P < 0.05$ vs AD; § $P < 0.05$ vs FTD.

$P < 0.001$ vs HC; ¶ $P < 0.001$ vs MCI.

Table 2. Summary of the demographic, clinical, and biomarker data of the population (Level II).

	LEVEL II					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	HC	[A-T-N-]	[A-T+N±, A-T±N+]	[A+T-N-]	[A+T+N±, A+T±N+]	FTD
Sex, n (F/M)	13/8	4/11	8/7	7/6	19/14	5/4
Age at LP	64 (59-69)	65 (56-71)	75 (69-77) ^{†**}	72 (68-75) ^{*†}	74 (69-76) ^{†**}	73 (70-74) ^{*†}
MMSE	30 (29-30)	28 (26-29)	25 (20-27)	25 (23-27)	24 (19-26)	23 (19-26)
CSF YKL-40 (ng/mL)	98 (90-110)	109 (80-137)	182 (122-215) ^{*†}	87 (74-135) ^{‡‡}	155 (128-196) ^{*¶§§}	114 (98-120) [‡]
CSF Aβ1-42 (pg/mL)	910 (785-996)	872 (803-1243)	886 (586-1025)	377 (293-432) ^{**††‡‡¶¶}	406 (366-448) ^{**††‡‡¶¶}	652 (530-823) [†]
CSF t-tau (pg/mL)	201 (127-243)	170 (141-215)	461 (390-537) ^{**††§§¶¶}	195 (168-240)	519 (433-764) ^{**††§§¶¶}	208 (161-340)
CSF p-tau (pg/mL)	44 (35-48)	44 (43-52)	80 (68-117) ^{**††§§¶¶}	44 (30-51)	93 (79-126) ^{**††§§¶¶}	31 (27-53)

Abbreviations: Aβ₁₋₄₂, 42-amino acid-long amyloid beta peptide; AD, Alzheimer's disease; CSF, cerebrospinal fluid; HCs, cognitively healthy controls; F, female; FTD, frontotemporal dementia; LP, lumbar puncture; M, male; MCI, mild cognitive impairment; MMSE, mini-mental state examination; p-tau, hyperphosphorylated tau; t-tau, total tau.

NOTE. All data are median values with 25th and 75th quartiles, except for n.

For statistical comparisons, the above MMSE, YKL-40, Aβ1-42, t-tau, p-tau comparisons were adjusted for age, sex, and site.

**P* < 0.05 vs HCs; †*P* < 0.05 vs Group 2; ‡*P* < 0.05 vs Group 3; §*P* < 0.05 vs Group 4; #*P* < 0.05 vs Group 5; ¶*P* < 0.05 vs Group 6.

***P* < 0.001 vs HCs; ††*P* < 0.001 vs Group 2; ‡‡*P* < 0.001 vs Group 3; §§*P* < 0.001 vs Group 4; ###*P* < 0.001 vs Group 5; ¶¶*P* < 0.001 vs Group 6.