Alzheimer's disease biomarker-guided diagnostic workflow using the added value of six combined cerebrospinal fluid candidates: $A\beta_{1-42}$, total-tau, phosphorylated-tau, NFL, neurogranin, and YKL-40

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Abbreviations: 10-fold CV, 10-fold cross validation; Aβ, Alzheimer Precision Medicine Initiative; APMI, Alzheimer Precision Medicine Cohort Program; APMI-CP, amyloid-beta; $A\beta_{1-42}$, 42-amino acid-long amyloid beta peptide; AD, Alzheimer's disease; ADD, Alzheimer's disease dementia; AUROC, area under the receiver operating characteristic curve; C.I., confidence intervals; CoU; Context of Use, CSF, cerebrospinal fluid; FDR, False Discovery Rate; FTD, frontotemporal dementia; HC, healthy controls; KW, Kruskal-Wallis; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NFL, neurofilament light chain; NINCDS–ADRDA, National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association; p-tau, hyperphosphorylated tau; PMCMR, pairwise multiple comparison of mean ranks; t-tau, total tau.

ABSTRACT

Introduction: We investigated the diagnostic and classificatory performance of multivariate combinations of 6 gold-standard core and novel pathophysiological cerebrospinal fluid (CSF) candidate biomarkers of neurodegeneration-related mechanisms.

Methods: Core Alzheimer's disease (AD) CSF biomarkers included the 42-amino acid-long amyloid beta (Aβ) peptide ($A\beta_{1-42}$), total tau (t-tau), and phosphorylated tau (p-tau) proteins. Novel candidate pathophysiological biomarkers in development included the neurofilament light chain (NFL) protein, neurogranin, and YKL-40. The diagnostic and classificatory performances of all possible combinations of the six candidate pathophysiological CSF biomarkers were compared among individuals with mild cognitive impairment (MCI) (n=41), AD dementia (ADD) (n=35), frontotemporal dementia (FTD) (n=9), and cognitively healthy controls (HC) (n=21).

Results: We identified the combinations that ranked in the top 10 according to diagnostic accuracy in the classification of HC from MCI, HC from ADD, HC from FTD, MCI from ADD, and ADD from FTD. Notably, novel biomarkers alone or in combination appeared in the top 10 in all comparisons. The single biomarkers or biomarker combinations generating the best AUROCs were: [$A\beta_{1-42}$+p-tau+NFL] for distinguishing between ADD and HC (AUROC=0.86), t-tau for distinguishing between ADD and FTD (AUROC=0.82), t-tau for distinguishing between FTD and HC (AUROC=0.78), [$A\beta_{1-42}$+NFL] for distinguishing between ADD and MCI (AUROC = 0.71), and $A\beta_{1-42}$ for distinguishing between MCI and HC (AUROC=0.62).

Discussion: The biomarker combination signature [$A\beta_{1-42}$+p-tau+NFL] differentiated ADD patients from HC with good diagnostic accuracy. The diagnostic performances of CSF t-tau in distinguishing FTD patients from ADD and HC were good and fair, respectively. CSF $A\beta_{1-42}$+NFL differentiated ADD from MCI with fair diagnostic accuracy. CSF $A\beta_{1-42}$ discriminated MCI subjects from HC with poor diagnostic accuracy.

KEY WORDS: Alzheimer's disease dementia; diagnostic biomarkers; biomarker combination; cerebrospinal fluid; neurofilament light chain; neurogranin; YKL-40; pathophysiology; neurodegeneration, neuroinflammation; clinical diagnosis; cognitive aging; mild cognitive impairment; frontotemporal dementia; precision medicine.

1. INTRODUCTION

Polygenic Alzheimer's disease (AD) is a pathophysiologically complex and clinically heterogeneous neurodegenerative disease [1]. The extracellular deposition of accumulated amyloid beta (Aβ) peptide into amyloid plaques and the intracellular accumulation of neurofibrillary tangles are considered pathophysiological hallmarks of AD. Cerebrospinal fluid (CSF) concentrations of the 42-amino acid-long Aβ peptide ($A\beta_{1-42}$), total tau (t-tau), and hyperphosphorylated tau (p-tau) proteins, which represent pathophysiological biomarkers of amyloid pathology, cortical axonal degeneration, and tangle pathology, respectively, have been validated as core, feasible [2,3] biomarkers of AD pathophysiology [4]. Recently emerging evidence highlighted the presence of additional molecular pathophysiological pathways – such as axonal disintegration [5], synaptic pathology [6], innate immune response and neuroinflammation [5,7,8] – throughout the different stages of AD [1,7,9–11].

A growing number of discovery stage biomarker studies have been conducted aimed to identify, develop and validate additional molecular pathophysiological pathways in AD, including different target populations, such as AD dementia (ADD), mild cognitive impairment (MCI) due to AD (also called prodromal AD), as well as the asymptomatic preclinical stages [12–15]. Among those, CSF neurofilament light chain (NFL) [5], neurogranin [6], and YKL-40 [5,8] proteins have reached an advanced clinical validation stage and represent innovative pathophysiological candidate biomarkers which may complement and optimize the biomarker-guided *in vivo* detection of AD-associated pathophysiological pathways (for identifying treatable mechanisms for targeted therapy development). In other relevant contexts of use (CoU), they may complement and enhance the developing biomarker-guided detection and diagnostic algorithm to identify AD patients at various disease stages in the clinic, as established in recently refined international diagnostic criteria [16], and for clinical trials (as biomarker stratified or enriched target populations).

Specifically, NFL is a primary structural component of the neuronal cytoskeleton [17] and a marker of large-caliber axonal disintegration [17,18]. Neurogranin is a postsynaptic protein

predominant in dendritic spines of neurons within associative cortical areas, and is involved in modulating synaptic transmission and plasticity mechanisms [19]. Finally, YKL-40, a glycoprotein expressed in both microglia and astroglia in the central nervous system, represents a relevant candidate biomarker of neuroinflammation and/or astrocytic/microglial activation [8]. Interestingly, a recent meta-analysis showed that both NFL and YKL-40 proteins are promising biomarkers useful to differentiate AD patients from cognitively healthy control (HC) individuals [5]; furthermore, increased CSF neurogranin concentrations were found to be related to AD-characteristic pathophysiology [6,20].

Only a limited number of available studies have assessed the diagnostic accuracy of CSF core AD biomarkers in combination with two out of these three novel mechanistic biomarkers of AD pathophysiology [20–22]. To our knowledge, no previous study examined the CSF concentrations of the three biomarkers in combination with the core AD biomarkers in cohorts of patients with AD or other primary neurodegenerative diseases.

To our knowledge for the first time, we assessed the diagnostic and classificatory performance of three novel CSF pathophysiological biomarkers at advanced validation stages – NFL, neurogranin, and YKL-40 – as single biomarkers or in combination with the traditional core biomarkers, using an international academic expert multicenter cohort of individuals with cognitive impairment and dementia. We explored the diagnostic performance in differentiating HC individuals from subjects with MCI, patients with ADD, and patients with frontotemporal dementia (FTD). In addition, we determined the diagnostic accuracy in discriminating MCI subjects from ADD patients and ADD from FTD cases. For each of the above-mentioned group comparisons, we implemented exhaustive searches to assess which combination of the panel of 6 biomarkers – both novel and core biomarkers – provided the best classification performance.

2. METHODS

2.1. Study participants

This multicenter cross-sectional study was conducted retrospectively in a convenience series from three independent European academic expert memory clinics. A total of 135 participants were examined; out of these, 27 were excluded due to missing data regarding one or more CSF biomarkers and the remaining 108 were included in the present study. Specifically, 35 participants were recruited from the Institute for Memory and Alzheimer's Disease (*Institut de la Mémoire et de la Maladie d'Alzheimer, IM2A*) – a sub-cohort of the Alzheimer Precision Medicine Initiative Cohort Program (APMI-CP) [23] – at the Pitié-Salpêtrière University Hospital in Paris (France), 57 from the German Center for Neurodegenerative Diseases (DZNE) in Rostock (Germany), and 16 from the Institute of Neuroscience and Physiology at Sahlgrenska University Hospital in Mölndal (Sweden).

The study complied with the tenets of the Declaration of Helsinki and was approved by the local Ethical Committees at each participating university center. All participants or their representatives gave written informed consent for the use of their clinical data for research purposes.

2.2. Clinical diagnoses

The clinical diagnosis of ADD 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 [24]. The clinical diagnosis of MCI was based on the MCI core clinical criteria [25]. The clinical diagnosis of FTD was performed according to the consensus on clinical diagnostic criteria published in 1998 [26]. Cognitively HC were individuals who: I) volunteered for lumbar puncture, II) showed a negative of neurological or psychiatric diseases, and III) had a Mini-Mental State Examination (MMSE) scores between 27 and 30. Of the 23 cognitively HC, two individuals from the Gothenburg cohort showed CSF t-tau concentrations higher than the established cut-off value. Being asymptomatic-at-risk of AD [16] or preclinical AD [27], they were

excluded from additional analyses. The group clinically defined as MCI included 41 participants [25]. Finally, 35 ADD [24] and 9 FTD [26] patients were included.

2.3. CSF sampling

A diagnostic lumbar puncture was performed in all participants. All CSF samples included in the three study cohorts were collected in polypropylene tubes, centrifuged at 1000 g for 10 minutes at +4°C (samples collected at the IM2A in Paris), 1500 g for 10 minutes at +4°C (samples collected at the DZNE in Rostock), 1800 g for 10 minutes at +4°C (samples collected at the Clinical Neurochemistry Laboratory in Mölndal). The collected supernatant was stored at –80°C pending biochemical analysis.

2.4. Immunoassays for CSF core biomarkers

For the Paris cohort, CSF analyses of the biomarkers $A\beta_{1\text{-}42,}$ t-tau, and p-tau 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 conducted in two different units: the Institute of Clinical Chemistry and Laboratory Medicine, Rostock University Medical Centre, as of June 2012, and the Laboratory of Neurochemistry, Department of Neurology, Göttingen University Medical Center, before June 2012. For the Gothenburg cohort, CSF analyses took place at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Mölndal. CSF $A\beta_{1\text{-}42}$, t-tau, and tau phosphorylated at threonine 181 ($p\text{-}tau_{181}$) concentrations were measured using established sandwich ELISA methods, INNOTEST β-AMYLOID(1-42) [28], INNOTEST hTAU-Ag [29], and INNOTEST Phospho-Tau[181P] [30] (Fujirebio Europe NV, Gent, Belgium), respectively. All analyses were performed by experienced laboratory technicians blinded to clinical information. All laboratories participate in the Alzheimer's Association Quality Control Program for CSF biomarkers and the Global Biomarker Standardization Consortium (ref: Carrillo MC et al., Alzheimers Dement. 2013 Mar;9(2):137-40). Pathologic CSF biomarker

concentrations were defined based on reference threshold cut-off values currently established in each memory clinic: at IM2A in Paris, $A\beta_{1-42}$ < 500 pg/mL, t-tau > 450 pg/mL, p-tau_{181} > 60 pg/mL; at DZNE in Rostock, $A\beta_{1-42}$ < 567 pg/mL, t-tau > 512 pg/mL, p-tau_{181} > 66 pg/mL (for the CSF samples measured before June 2012) and $A\beta_{1-42}$ < 450 pg/mL, t-tau > 450 pg/mL, p-tau_{181} > 62 pg/mL (for the CSF samples measured after June 2012); at Clinical Neurochemistry Laboratory in Mölndal, $A\beta_{1-42}$ < 550 pg/mL, t-tau > 400 pg/mL, p-tau_{181} > 80 pg/mL.

2.5. Immunoassays for CSF NFL, neurogranin, and YKL-40

All CSF NFL, neurogranin and YKL-40 analyses were performed at the studies central laboratory, the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Mölndal, Sweden. CSF NFL protein concentrations were measured with a sensitive sandwich ELISA method (NF-light ELISA kit; UmanDiagnostics AB, Umeå, Sweden), as described by the manufacturer. The lower limit of quantification for this assay was 50 ng/L; CSF neurogranin analyses were performed using a previously described analytical methodology [31]. In brief, CSF neurogranin was measured using an in-house ELISA assay based on the monoclonal antibody Ng7 (epitope including amino acids 52–65 on neurogranin) for capture, a polyclonal neurogranin anti-rabbit antibody (ab23570; Upstate Biotechnology, Lake Placid, NY, USA) for detection, and full-length neurogranin protein as calibrator. The detection limit of the assay was 125 pg/mL. The intra- and inter-assay coefficients of variations were 6% and 9%, respectively. CSF YKL-40 protein concentrations were measured using a commercial available ELISA kit (R&D Systems, Minneapolis, MN, US), according to manufacturer instructions. Intra-assay coefficients of variation were below 10%. All analyses were performed on one occasion in a randomized fashion by board-certified laboratory personnel blinded to clinical data to avoid bias.

2.6. Statistical Analysis

The associations between participant groups and sex as well as age were assessed with Fisher's exact test and nonparametric Kruskal-Wallis (KW) tests, respectively. Before further analysis, both core and novel candidate biomarker values were adjusted for age, sex, and site using nonparametric regression. This step allowed age-, sex-, and site- independent assessment of the discriminatory performance of all biomarkers while foregoing assumptions of normality. Bivariate associations between all biomarkers in the entire study cohort were explored with Spearman's correlation coefficients with correction for multiple comparisons. Additionally, multivariate associations (i.e. independent contributions of any five biomarkers to the variability of the remaining novel biomarker) in the entire study cohort were examined by employing NFL, neurogranin, and YKL-40 as dependent variables in three distinct multivariate regression models in which all remaining biomarkers were used as regressors.

We conducted group-wise comparisons of biomarker 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 potential diagnostic and classificatory performance of all possible combinations of both traditional core and novel biomarkers (from any single biomarker to a total of 6 biomarkers) using logistic regression within a 10-fold cross validation (10-fold CV) approach in the following *a priori* comparisons: HC *versus* MCI, HC *versus* ADD, HC *versus* FTD, MCI *versus* ADD, and ADD *versus* FTD. In this analysis, age-, sex-, and site- adjusted values of all biomarkers employed in any particular combination 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 (AUROC) curve and its associated confidence intervals using a bootstrap procedure (100000 bootstraps) [32] by pooling predictions computed on the test sets from each train-test split in the 10-fold CV procedure. For each combination of biomarkers, the ability 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) [33].

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) (available at https://www.jstatsoft.org/article/view/v027i05), partial ROC (pROC) package, and the pairwise multiple comparison of mean ranks (PMCMR) package (available at https://cran.r-project.org/web/packages/PMCMR/vignettes/PMCMR.pdf) [32,34]. Two-tailed *P* values < 0.05 were considered statistically significant.

3. RESULTS

3.1. CSF biomarkers concentrations

Table 1 summarizes the concentrations of all analytes, both core and novel candidate biomarkers, combined with demographic and clinical data of the population. KW tests showed a significant effect of group on age ($P < 0.001$), MMSE ($P = 0.002$), and all CSF biomarkers ($A\beta_{1-42}$, $P < 0.001$; p-tau, $P < 0.001$; t-tau, $P < 0.001$; NFL, $P = 0.004$; neurogranin, $P = 0.002$; YKL-40, $P = 0.0156$). *Post-hoc* testing determined that cognitively HC were significantly younger than MCI subjects, AD, and FTD patients. MMSE scores were significantly lower in AD compared with HC and MCI. CSF $A\beta_{1-42}$ concentrations were significantly lower in ADD *versus* HC, MCI, and FTD ($P < 0.001$, $P < 0.001$, $P = 0.003$, respectively) and in MCI *versus* HC ($P = 0.029$). Compared with HC, both CSF t-tau and p-tau concentrations were significantly higher in MCI ($P < 0.001$ and $P = 0.002$, respectively), ADD ($P = 0.003$ and $P = 0.007$, respectively), and FTD ($P = 0.001$ and $P = 0.014$, respectively) (**Table 1**). CSF NFL concentrations were significantly higher in ADD *versus* HC and MCI ($P = 0.004$ and $P = 0.013$, respectively) (**Figure 1A**). CSF neurogranin concentrations were significantly higher in ADD *versus* HC and FTD ($P = 0.004$ for both comparisons) (**Figure**

1B). YKL-40 concentrations were significantly higher in ADD *versus* HC and FTD ($P = 0.032$ and $P = 0.049$, respectively) (**Figure 1C**).

3.2. Diagnostic accuracies of CSF biomarkers

Table 2 summarizes – in descending order in terms of AUROC values – the 10 biomarker combinations which yielded the best diagnostic accuracies in distinguishing HC from MCI, ADD, FTD, as well as MCI from ADD, and ADD from FTD. In particular, the combination [$A\beta_{1-42}$+p-tau+NFL] differentiated ADD from HC with an AUROC of 0.86 (95% CI, 0.83–0.89). T-tau and [$A\beta_{1-42}$+p-tau+YKL-40] discriminated ADD from FTD with an AUROC of 0.82 (95% CI, 0.78–0.86) and 0.81 (95% CI, 0.77–0.85), respectively. T-tau and [p-tau+YKL-40] distinguished FTD from HC with an AUROC of 0.78 (95% CI, 0.73–0.83) and 0.73 (95% CI, 0.67–0.79), respectively. The combination [$A\beta_{1-42}$+NFL] differentiated ADD from MCI with an AUROC of 0.71 (95% CI, 0.67–0.75). $A\beta_{1-42}$ and [$A\beta_{1-42}$+YKL-40] discriminated MCI from HC with an AUROC of 0.62 (95% CI, 0.58–0.67) and 0.61 (95% CI, 0.57–0.66), respectively (**Table 2**).

3.3. Correlations between all CSF biomarkers in the whole study cohort

Table 3 shows the correlation matrix between all biomarkers, in the whole study cohort, after correction for multiple comparisons. All biomarkers were significantly correlated with each other, except for the $A\beta_{1-42}$ peptide, which was only correlated with p-tau and t-tau proteins. In the multivariate regression models, YKL-40 resulted to be a significant contributor ($P < 0.001$) in explaining the variability in NFL; t-tau ($P < 0.001$) contributed significantly to neurogranin variability; p-tau ($P = 0.002$), NFL ($P = 0.0175$), and neurogranin ($P = 0.0250$) contributed significantly to YKL-40 variability.

DISCUSSION

Our results showed that CSF NFL concentrations were significantly higher in ADD patients *versus* HC and MCI subjects. These outcomes are consistent with a recent analysis reporting the association between CSF NFL concentration and cognitive impairment [35]. Both CSF neurogranin and YKL-40 concentrations were significantly higher in ADD *versus* HC, thus confirming earlier reported data [6,8]. Furthermore, we demonstrated significantly higher concentrations of CSF neurogranin in ADD compared with FTD; this finding corroborates previous data indicating a selective increase in CSF neurogranin in individuals showing AD pathophysiology [6]. We also found higher concentrations of CSF YKL-40 in ADD compared with FTD. In this regard, a non-AD specific increase of CSF YKL-40 *versus* HC was described [8,20,36,37]. Prior investigations reported both higher and similar CSF YKL-40 concentrations in FTD compared to ADD patients [8,20]. As expected, CSF $A\beta_{1-42}$ concentrations were significantly lower in ADD patients compared with HC, MCI, and FTD as well as in MCI compared to HC individuals [1,4,5]; moreover, compared with HC, both CSF t-tau and p-tau concentrations were significantly increased in MCI, ADD, and FTD patients [1,4,5]. In bivariate correlation analyses, both novel and core biomarkers were significantly associated with each other, with the exception of the $A\beta_{1-42}$ peptide, which was only correlated with p-tau and t-tau proteins. In particular, the association was strong among neurogranin, t-tau, and p-tau and moderate among NFL, neurogranin, and YKL-40. In addition, NFL and YKL-40 showed a moderate correlation with both t-tau and p-tau. Notably, in the multivariate regression models, CSF YKL-40 concentrations resulted to be a significant independent contributor in explaining the variability in NFL; this is in agreement with earlier data showing that CSF YKL-40 values are positively related to NFL in both asymptomatic preclinical AD and PD subjects [38]. Therefore, CSF NFL concentrations contributed significantly to explaining CSF YKL-40 variability. Furthermore, we disclosed that CSF t-tau contributed significantly to explaining the variation of CSF neurogranin concentrations; such a finding was also documented in two other studies on AD [20,22]. Moreover, in accordance with our results, previous

investigations reported that CSF NFL, neurogranin, and YKL-40 demonstrated more robust associations with CSF tau proteins than with CSF $A\beta_{1-42}$ [6,8,39,40]. Finally, separate studies highlighted an association of CSF NFL with both YKL-40 [8,38,41] and neurogranin [6,8,39]. Interestingly, a possible link between neurogranin and YKL-40 has not yet been examined.

To our knowledge, this is the first study scrutinizing the diagnostic contribution and added value of the novel pathophysiological CSF candidate biomarker panel – NFL, neurogranin, and YKL-40 – both as single markers and in combination – in the biomarker-guided diagnosis of AD [5,20], following the application of a diagnostic workflow for the selection of the best performing biomarker combinations (**Figure 2**). In particular, we describe – for the first time in a multicenter cohort of participants with cognitive impairment – the correlations between the three biomarkers and explore their association with tau protein-dependent pathophysiological mechanisms.

Importantly, any combination of biomarkers (standard core or novel) differentiated HC from MCI with a poor diagnostic accuracy. When distinguishing HC from ADD, both CSF NFL and neurogranin appeared within the top 10 ranked biomarker combinations in conjunction with standard core biomarkers, and all combinations delivered good diagnostic accuracy, which was comparable to the one delivered by e.g. $A\beta_{1-42}$ alone. When distinguishing HC from FTD, YKL-40 combined with p-tau and t-tau delivered fair diagnostic accuracy, which was comparable to the one delivered by e.g. t-tau alone. When distinguishing MCI from ADD, various combinations of all three novel biomarkers with the core biomarkers delivered fair diagnostic accuracy, which was comparable to the one afforded by e.g. t-tau alone or t-tau and $A\beta_{1-42}$. When distinguishing ADD from FTD, various combinations of the 3 novel candidate biomarkers with the standard core ones delivered good diagnostic accuracy that was, however, not superior to the diagnostic accuracy achieved by the core biomarkers alone ($A\beta_{1-42}$, p-tau, t-tau). In summary, as mentioned above, no combination including the 3 novel candidate biomarkers was superior to e.g. $A\beta_{1-42}$ in distinguishing HC from MCI and ADD, neither to t-tau in differentiating HC from FTD, nor to $A\beta_{1-42}$, p-tau, and t-tau in discriminating ADD from FTD as well as MCI from ADD.

The introduction of innovative pathophysiological CSF biomarkers, which reflect distinct biochemical and molecular mechanisms – axonal disintegration, synaptic pathology, innate immune response and neuroinflammation –meaningfully complements the pathways associated with polygenic AD. It will further complement the evolving biomarker-guided diagnostic workflow, such as the diagnostic model proposed by the International Working Group (IWG) [16]. Here, and at any stage of the disease, the diagnosis of "typical AD" relies on the presence of traditional core pathophysiological biomarker signature: low CSF $A\beta_{1-42}$ concentrations and elevated CSF t-tau (or p-tau) concentrations or positivity to amyloid-positron emission tomography (PET) (i.e., high retention of amyloid tracer) [16]. Recently, Ewers and colleagues (2015) [42], in a large-scale international multicenter study tested the diagnostic and classificatory performance of standard CSF core biomarkers to discriminate ADD from other clinically relevant dementia disorders. They reported that CSF $A\beta_{1-42}$ alone or combined with the CSF p-tau_{181}/$A\beta_{1-42}$ ratio differentiated ADD from FTD but exhibited a large overlap between ADD and other dementia disorders. This outcome, therefore, highlighted the limited diagnostic usefulness of the exclusive use of standard core biomarkers in the classification of ADD from a variety of other relevant neurodegenerative diseases and dementia disorders. We hypothesized, that the integration of complementary pathophysiological biomarker candidates covering additional key AD mechanisms will likely result in an incremental performance optimization for the detection, diagnosis and differential diagnosis of primary neurodegenerative diseases and dementia disorders, including AD and FTD.

The combined use of all six mostly validated and matured candidate biomarkers here, allows the for an extension of the proposed "agnostic nomenclature", the "A/T/N" scheme, an unbiased biomarker-driven model of stratification, as recently reported by Jack and colleagues (2016) [43]. This model comprises three binary (positive/negative) categories: "A" referring to an amyloid biomarker (CSF $A\beta_{1-42}$ 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, ^{18}F-Fluorodeoxyglucose (FDG)-PET, or structural magnetic resonance imaging (MRI)). Owing

to its agnostic and unbiased nature, the use of this dissection model is potentially appropriate in any context of diagnostic criteria [9]. Here, we aim at proposing additional candidates to logically expand the A/T/N biomarker panel in integrating (I) biomarkers of other existing pathophysiological mechanisms – such as axonal disintegration, synaptic pathology, and neuroinflammation – characterizing the dimensional *spectrum* of age-related proteinopathies/neurodegenerative diseases and (II) genetic/epigenetic factors [44]. This descriptive approach will enable to look as focussed but as complimentary as possible at any relevant pathophysiological alterations underlying older cognitively impaired individuals in an agnostic fashion. This will allow to identify subsets of AD patients with distinct pathophysiological patterns, as requested by the emerging theoretical concepts of precision medicine and precision neurology [9,23,45]. Additional analyses using a composite array including the three presented innovative biomarkers are necessary in order to achieve a more accurate stratification of patients' cohorts according to different AD-related pathophysiological pathways [8,9,23,45]. This strategy might help provide the basis to accelerate the development of effective targeted therapeutic approaches, namely "molecularly" or biomarker-guided targeted or customized therapies [9,23,45]. As a result, focussed therapeutic interventions are expected to be developed for the treatment of the individual patient's biological makeup, with expected higher efficacy.

An intriguing feature of the results that warrants additional studies is the moderate to strong correlations between biomarkers that should represent rather distinct pathological processes. This could either mean that their concentrations are influenced by common factors, such as CSF turnover, or that the pathological processes they are thought to represent often occur in synchrony. The latter hypothesis would fit well with the recently proposed revised model in which AD pathogenesis is described as a long, complex cellular phase consisting of feedback and feedforward responses of astrocytes, microglia, and vasculature to Aβ, tau and potentially other pathologies (ref: De Strooper B and Karran E. Cell. 2016 Feb 11;164(4):603-15).

There are some limitations of our study. As our dataset did not include longitudinal follow-up, it was not possible to distinguish between stable MCI and MCI subjects progressing to dementia. Furthermore, more extensive psychometric evaluations were not available, thus precluding the analysis of the concentrations of the novel biomarkers in relation to different cognitive domains. Moreover, the quantification of the standard core CSF AD biomarkers was not performed in one central reference laboratory and, while we controlled for center effects – as well as for age and sex – in the statistical analysis, additional inter-laboratory variability could not be fully controlled. Longitudinal studies need to be designed to evaluate the potential role of the three novel biomarker candidates – both alone and in combination – in the prediction of progression from prodromal MCI to the dementia stage. In addition, since the sample size of our cohort was relatively small, especially in the FTD group, it was not possible to evaluate in detail the diagnostic accuracy of the three emerging CSF biomarkers in differentiating AD from non-AD neurodegenerative diseases. Therefore, future studies should be directed towards increasing the statistical power by collecting larger, multisite cohorts.

In summary, we found that none of the multivariate combinations performed superior to the gold-standard core, feasible biomarkers in the classification of HC from MCI, HC from ADD, HC from FTD, MCI from ADD, and ADD from FTD. Future independent validation of our findings in larger multicenter cohorts, including sufficient numbers of patients with other neurodegenerative diseases, is needed to confirm and expand on our data. Particularly, longitudinal analyses are warranted in asymptomatic preclinical at risk for AD individuals to investigate whether components of the biomarker panel may be valuable predictors (surrogates) of disease progression and conversion to clinical milestones, such as prodromal MCI and dementia. To this aim, we are currently in the process of conducting these longitudinal studies using a unique large-scale a monocentric cohort (INSIGHT-preAD) – within the framework of the APMI and as part of the APMI-CP [23] – including amyloid-PET stratified preclinical asymptomatic individuals at risk for AD in order to elucidate the temporal dynamics of all six, and more, pathophysiological biomarkers

and test their potential correlation with genomics and multi-modal neuroimaging and EEG data, throughout disease progression commencing at the preclinical stage. The ultimate aim is to identify disease trajectories through space and time using integrated disease modelling (IDM) that may serve as more precise guideposts for detecting the disease at earliest possible preclinical stages as well as initiating treatment interventions of distinct pathophysiological mechanisms through the biomarker-guided targeted therapy trials of the future.

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CONFLICTS OF INTERESTS

Dr. Henrik Zetterberg and Dr. Kaj Blennow are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. Dr. Stéphane Epelbaum received lecture honoraria from Roche and participated on scientific advisory boards of GE Healthcare and Eli Lilly, Dr. Bruno Dubois reports personal fees from Eli Lilly and company. Dr. Harald Hampel reports no conflict of interest with the content of 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, Cytox Ltd, Jung Diagnostics GmbH, Roche, Biogen Idec, Takeda-Zinfandel, Oryzon Genomics, Qynapse; and he receives research support from the Association for Alzheimer Research (Paris), Pierre and Marie Curie University (Paris), Pfizer & Avid (paid to institution); and he has patents, but receives no royalties. Dr. Simone Lista received lecture honoraria from Roche. Dr. Nicola Toschi, Dr. Filippo Baldacci, Dr. Ingo Kilimann, Dr. Stefan J. Teipel, Dr. Enrica Cavedo, Dr. Antonio Melo dos Santos, Dr. Foudil Lamari, Dr. Robert Nisticò, Dr. Roberto Floris, and Dr. Francesco Garaci declare no conflicts of interest.

REFERENCES

[1] Scheltens P, Blennow K, Breteler MMB, de Strooper B, Frisoni GB, Salloway S, et al. Alzheimer's disease. Lancet Lond Engl 2016. doi:10.1016/S0140-6736(15)01124-1.

[2] Hampel H, Bürger K, Teipel SJ, Bokde ALW, Zetterberg H, Blennow K. Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc 2008;4:38–48. doi:10.1016/j.jalz.2007.08.006.

[3] Frank RA, Galasko D, Hampel H, Hardy J, de Leon MJ, Mehta PD, et al. Biological markers for therapeutic trials in Alzheimer's disease. Proceedings of the biological markers working group; NIA initiative on neuroimaging in Alzheimer's disease. Neurobiol Aging 2003;24:521–36.

[4] Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol 2010;6:131–44. doi:10.1038/nrneurol.2010.4.

[5] Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 2016. doi:10.1016/S1474-4422(16)00070-3.

[6] Lista S, Hampel H. Synaptic degeneration and neurogranin in the pathophysiology of Alzheimer's disease. Expert Rev Neurother 2016:1–11. doi:10.1080/14737175.2016.1204234.

[7] Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol 2015;14:388–405. doi:10.1016/S1474-4422(15)70016-5.

[8] Baldacci F, Lista S, Cavedo E, Bonuccelli U, Hampel H. Diagnostic function of the neuroinflammatory biomarker YKL-40 in Alzheimer's disease and other neurodegenerative diseases. Expert Rev Proteomics 2017;14:285–99. doi:10.1080/14789450.2017.1304217.

[9] Baldacci F, Lista S, Garaci F, Bonuccelli U, Toschi N, Hampel H. Biomarker-guided classification scheme of neurodegenerative diseases. J Sport Health Sci n.d. doi:10.1016/j.jshs.2016.08.007.

[10] Reitz C. Toward precision medicine in Alzheimer's disease. Ann Transl Med 2016;4:107. doi:10.21037/atm.2016.03.05.

[11] Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc 2012;8:1–13. doi:10.1016/j.jalz.2011.10.007.

[12] Ghidoni R, Benussi L, Paterlini A, Albertini V, Binetti G, Emanuele E. Cerebrospinal fluid biomarkers for Alzheimer's disease: the present and the future. Neurodegener Dis 2011;8:413–20. doi:10.1159/000327756.

[13] Lista S, Emanuele E. Role of amyloid β1-42 and neuroimaging biomarkers in Alzheimer's disease. Biomark Med 2011;5:411–3. doi:10.2217/bmm.11.50.

[14] Hampel H, Lista S, Khachaturian ZS. Development of biomarkers to chart all Alzheimer's disease stages: the royal road to cutting the therapeutic Gordian Knot. Alzheimers Dement J Alzheimers Assoc 2012;8:312–36. doi:10.1016/j.jalz.2012.05.2116.

[15] Hampel H, Frank R, Broich K, Teipel SJ, Katz RG, Hardy J, et al. Biomarkers for Alzheimer's disease: academic, industry and regulatory perspectives. Nat Rev Drug Discov 2010;9:560–74. doi:10.1038/nrd3115.

[16] Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol 2014;13:614–29. doi:10.1016/S1474-4422(14)70090-0.

[17] Liu Q, Xie F, Siedlak SL, Nunomura A, Honda K, Moreira PI, et al. Neurofilament proteins in neurodegenerative diseases. Cell Mol Life Sci CMLS 2004;61:3057–75. doi:10.1007/s00018-004-4268-8.

[18] Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci 2005;233:183–98. doi:10.1016/j.jns.2005.03.015.

[19] Díez-Guerra FJ. Neurogranin, a link between calcium/calmodulin and protein kinase C signaling in synaptic plasticity. IUBMB Life 2010;62:597–606. doi:10.1002/iub.357.

[20] Janelidze S, Hertze J, Zetterberg H, Landqvist Waldö M, Santillo A, Blennow K, et al. Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer's disease. Ann Clin Transl Neurol 2016;3:12–20. doi:10.1002/acn3.266.
[21] Hellwig K, Kvartsberg H, Portelius E, Andreasson U, Oberstein TJ, Lewczuk P, et al. Neurogranin and YKL-40: independent markers of synaptic degeneration and neuroinflammation in Alzheimer's disease. Alzheimers Res Ther 2015;7:74. doi:10.1186/s13195-015-0161-y.
[22] Mattsson N, Insel PS, Palmqvist S, Portelius E, Zetterberg H, Weiner M, et al. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. EMBO Mol Med 2016. doi:10.15252/emmm.201606540.
[23] Hampel H, O'Bryant SE, Durrleman S, Younesi E, Rojkova K, Escott-Price V, et al. A Precision Medicine Initiative for Alzheimer's disease: the road ahead to biomarker-guided integrative disease modeling. Climacteric 2017;0:1–12. doi:10.1080/13697137.2017.1287866.
[24] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–44.
[25] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc 2011;7:270–9. doi:10.1016/j.jalz.2011.03.008.
[26] Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998;51:1546–54.
[27] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc 2011;7:280–92. doi:10.1016/j.jalz.2011.03.003.
[28] Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buyse MA, Andreasen N, et al. Standardization of measurement of beta-amyloid(1-42) in cerebrospinal fluid and plasma. Amyloid Int J Exp Clin Investig Off J Int Soc Amyloidosis 2000;7:245–58.
[29] Blennow K, Wallin A, Agren H, Spenger C, Siegfried J, Vanmechelen E. Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? Mol Chem Neuropathol Spons Int Soc Neurochem World Fed Neurol Res Groups Neurochem Cerebrospinal Fluid 1995;26:231–45. doi:10.1007/BF02815140.
[30] Vanmechelen E, Vanderstichele H, Davidsson P, Van Kerschaver E, Van Der Perre B, Sjögren M, et al. Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization. Neurosci Lett 2000;285:49–52.
[31] Kvartsberg H, Duits FH, Ingelsson M, Andreasen N, Öhrfelt A, Andersson K, et al. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc 2015;11:1180–90. doi:10.1016/j.jalz.2014.10.009.
[32] Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-C, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011;12:77. doi:10.1186/1471-2105-12-77.
[33] Xia J, Broadhurst DI, Wilson M, Wishart DS. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics Off J Metabolomic Soc 2013;9:280–99. doi:10.1007/s11306-012-0482-9.
[34] Nonparametric Econometrics: The np Package | Hayfield | Journal of Statistical Software n.d. https://www.jstatsoft.org/article/view/v027i05 (accessed May 26, 2016).
[35] Zetterberg H, Skillbäck T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, et al. Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression. JAMA Neurol 2016;73:60–7. doi:10.1001/jamaneurol.2015.3037.
[36] Baldacci F, Toschi N, Lista S, Zetterberg H, Blennow K, Kilimann I, et al. Two-level diagnostic classification using cerebrospinal fluid YKL-40 in Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc 2017. doi:10.1016/j.jalz.2017.01.021.

[37] Teunissen CE, Elias N, Koel-Simmelink MJA, Durieux-Lu S, Malekzadeh A, Pham TV, et al. Novel diagnostic cerebrospinal fluid biomarkers for pathologic subtypes of frontotemporal dementia identified by proteomics. Alzheimers Dement Amst Neth 2016;2:86–94. doi:10.1016/j.dadm.2015.12.004.

[38] Hall S, Surova Y, Öhrfelt A, Swedish BioFINDER Study, Blennow K, Zetterberg H, et al. Longitudinal Measurements of Cerebrospinal Fluid Biomarkers in Parkinson's Disease. Mov Disord Off J Mov Disord Soc 2016;31:898–905. doi:10.1002/mds.26578.

[39] Landqvist Waldö M, Frizell Santillo A, Passant U, Zetterberg H, Rosengren L, Nilsson C, et al. Cerebrospinal fluid neurofilament light chain protein levels in subtypes of frontotemporal dementia. BMC Neurol 2013;13:54. doi:10.1186/1471-2377-13-54.

[40] Lista S, Toschi N, Baldacci F, Zetterberg H, Blennow K, Kilimann I, et al. Diagnostic accuracy of CSF neurofilament light chain protein in the biomarker-guided classification system for Alzheimer's disease. Neurochem Int 2017. doi:10.1016/j.neuint.2017.05.010.

[41] Melah KE, Lu SY-F, Hoscheidt SM, Alexander AL, Adluru N, Destiche DJ, et al. Cerebrospinal Fluid Markers of Alzheimer's Disease Pathology and Microglial Activation are Associated with Altered White Matter Microstructure in Asymptomatic Adults at Risk for Alzheimer's Disease. J Alzheimers Dis JAD 2016;50:873–86. doi:10.3233/JAD-150897.

[42] Ewers M, Mattsson N, Minthon L, Molinuevo JL, Antonell A, Popp J, et al. CSF biomarkers for the differential diagnosis of Alzheimer's disease: A large-scale international multicenter study. Alzheimers Dement J Alzheimers Assoc 2015;11:1306–15. doi:10.1016/j.jalz.2014.12.006.

[43] Jack CR, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology 2016. doi:10.1212/WNL.0000000000002923.

[44] Lista S, Garaci FG, Toschi N, Hampel H. Imaging epigenetics in Alzheimer's disease. Curr Pharm Des 2013;19:6393–415.

[45] H. Hampel, S.E. O'Bryant, J.I. Castrillo, C. Ritchie, K. Rojkova, K. Broich, et al. PRECISION MEDICINE - The Golden Gate for Detection, Treatment and Prevention of Alzheimer's Disease 2016. doi:10.14283/jpad.2016.112.

Table 1. Summary of the demographic, clinical, and biomarker data of the population

	HC	MCI	ADD	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)[e]	73 (68-76)[e]	73 (70-74)[a]
MMSE at LP (/30)	30 (29-30)	26 (24-28)	23 (19-26)[a,b]	23 (19-26)
CSF neurogranin (pg/mL)	180 (125-273)	331 (215-484)	468 (300-692)[a,d]	125 (125-192)
CSF NFL (pg/mL)	609 (516-773)	1046 (793-1767)	1483 (1180-1844)[a,b]	1022 (693-1435)
CSF YKL-40 (ng/mL)	98 (90-110)	128 (98-184)	146 (119-177)[a,d]	114 (98-120)
CSF $A\beta_{1-42}$ (pg/mL)	910 (785-996)	540 (411-911)[a]	424 (374-503)[d,e,f]	652 (530-823)
CSF t-tau (pg/mL)	201 (127-243)	261 (189-452)	496 (360-764)[b,d,e]	208 (161-340)
CSF p-tau (pg/mL)	44 (35-48)	60 (44-80)	83 (64-126)[a,b,d]	31 (27-53)

Abbreviations: $A\beta_{1-42}$, 42-amino acid-long amyloid beta peptide; ADD, Alzheimer's disease dementia; 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; NFL, neurofilament light chain protein; p-tau, hyperphosphorylated tau; t-tau, total tau.

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

P values for MMSE, neurogranin, NFL, YKL-40, $A\beta_{1-42}$, t-tau, p-tau were adjusted for age, sex and site.

[a]*P*< 0.05 vs HC; [b]*P*< 0.05 vs MCI; [c]*P*< 0.05 vs AD; [d]*P*< 0.05 vs FTD.

[e]*P*< 0.001 vs HC; [f]*P*< 0.001 vs MCI.

Table 2. The first 10 best ranked diagnostic accuracies of the CSF core and novel pathophysiological biomarkers, alone or in combination, in differentiating HC from MCI, HC from ADD, HC from FTD, MCI from ADD, and ADD from FTD are reported. Results are shown starting from the highest to the lowest AUROC value for every group comparison.

Best 10 predictors	**Group comparisons**		**AUROC**	**AUROC C.I. low**	**AUROC C.I. high**
$A\beta_{1-42}$	HC	MCI	62.37	57.95	66.78
$A\beta_{1-42}$ + YKL-40	HC	MCI	61.54	57.00	66.08
YKL-40	HC	MCI	60.93	56.45	65.41
$A\beta_{1-42}$ + NFL	HC	MCI	60.28	55.88	64.68
$A\beta_{1-42}$ + t-tau + YKL-40	HC	MCI	60.15	55.63	64.67
$A\beta_{1-42}$ + NFL + YKL-40	HC	MCI	60.03	55.50	64.56
$A\beta_{1-42}$ + t-tau	HC	MCI	59.89	55.42	64.36
$A\beta_{1-42}$ + neurogranin + YKL-40	HC	MCI	59.08	54.46	63.69
Neurogranin + YKL-40	HC	MCI	58.55	53.95	63.14
Neurogranin	HC	MCI	58.46	53.97	62.95
$A\beta_{1-42}$ + p-tau + NFL	HC	ADD	86.41	83.48	89.35
$A\beta_{1-42}$ + p-tau + t-tau + NFL	HC	ADD	86.18	83.21	89.15
$A\beta_{1-42}$	HC	ADD	86.12	82.85	89.39
t-tau + NFL	HC	ADD	85.83	82.90	88.76
$A\beta_{1-42}$ + neurogranin + NFL	HC	ADD	85.69	82.52	88.86
$A\beta_{1-42}$ + p-tau + NFL	HC	ADD	85.50	82.46	88.53
$A\beta_{1-42}$ + t-tau	HC	ADD	85.03	81.85	88.21
$A\beta_{1-42}$ + t-tau + neurogranin + NFL	HC	ADD	84.33	81.10	87.56
$A\beta_{1-42}$ + p-tau + t-tau	HC	ADD	84.21	80.96	87.45
p-tau + t-tau + NFL	HC	ADD	84.10	80.96	87.24
t-tau	HC	FTD	77.90	72.61	83.20
p-tau + YKL-40	HC	FTD	72.65	66.69	78.60

t-tau + YKL40	HC	FTD	68.85	62.76	74.93
p-tau + neurogranin + YKL-40	HC	FTD	64.70	57.97	71.43
p-tau	HC	FTD	62.84	56.32	69.37
$A\beta_{1-42}$ + t-tau + YKL-40	HC	FTD	61.83	54.98	68.67
p-tau + neurogranin	HC	FTD	61.35	54.39	68.32
$A\beta_{1-42}$ + p-tau + neurogranin + YKL-40	HC	FTD	61.16	54.58	67.74
p-tau + t-tau	HC	FTD	59.97	52.28	67.67
$A\beta_{1-42}$ + p-tau + neurogranin	HC	FTD	59.36	52.58	66.14
$A\beta_{1-42}$ + NFL	MCI	ADD	71.01	67.21	74.81
$A\beta_{1-42}$ + t-tau	MCI	ADD	70.97	67.19	74.75
$A\beta_{1-42}$ + t-tau + NFL	MCI	ADD	70.46	66.71	74.21
$A\beta_{1-42}$ + t-tau + neurogranin	MCI	ADD	69.89	66.17	73.61
t-tau	MCI	ADD	69.27	65.38	73.16
$A\beta_{1-42}$ + t-tau + NFL + YKL40	MCI	ADD	69.09	65.31	72.86
$A\beta_{1-42}$ + t-tau + YKL40	MCI	ADD	69.08	65.24	72.91
$A\beta_{1-42}$ + p-tau + t-tau	MCI	ADD	69.05	65.21	72.89
t-tau + YKL40	MCI	ADD	68.90	65.07	72.73
$A\beta_{1-42}$ + NFL + YKL40	MCI	ADD	68.76	64.90	72.62
t-tau	ADD	FTD	82.23	78.47	85.99
$A\beta_{1-42}$ + p-tau + YKL-40	ADD	FTD	81.30	77.22	85.37
p-tau + t-tau	ADD	FTD	80.92	77.02	84.82
$A\beta_{1-42}$ + p-tau + t-tau	ADD	FTD	80.28	75.98	84.59
$A\beta_{1-42}$ + p-tau	ADD	FTD	80.28	75.85	84.70
$A\beta_{1-42}$ + neurogranin + YKL-40	ADD	FTD	80.20	75.10	85.29
$A\beta_{1-42}$ + t-tau	ADD	FTD	80.10	75.89	84.32
$A\beta_{1-42}$ + p-tau + NFL	ADD	FTD	79.60	75.01	84.19
t-tau + NFL	ADD	FTD	79.59	75.49	83.69
$A\beta_{1-42}$ + p-tau + t-tau + YKL-40	ADD	FTD	79.56	75.34	83.78

Abbreviations: Aβ1-42, 42-amino acid-long amyloid beta peptide; ADD, Alzheimer's disease dementia; AUROC, area under the receiver operating characteristic curve; C.I., confidence intervals; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; HC, cognitively healthy controls; MCI, mild cognitive impairment; NFL, neurofilament light chain protein; p-tau, hyperphosphorylated tau; t-tau, total tau. NOTE. The AUROC curves result from fitting a logistic regression model within a 10-fold cross validation scheme to biomarkers data adjusted for age, sex, and site.

Table 3. Correlations between cerebrospinal fluid biomarkers in the study cohort.

	p-tau	**t-tau**	**NFL**	**YKL-40**	**Neurogranin**
Aβ1-42	*-0.305**	*-0.339***	-0.180	0.002	-0.171
p-tau		*0.900****	*0.461****	*0.574****	*0.808****
t-tau			*0.553****	*0.554****	*0.830****
NFL				*0.619****	*0.387****
YKL-40					*0.539****

*$P \leq 0.05$
**$P \leq 0.01$
***$P \leq 0.001$
Data are derived from Spearman's rank-order correlation test after adjusting for age, sex, and site. *P*-values were corrected for multiple comparisons.
Abbreviations: $A\beta_{1\text{-}42}$, 42-amino acid-long amyloid beta peptide; NFL, neurofilament light chain protein; p-tau, hyperphosphorylated tau; t-tau, total tau.

FIGURE CAPTIONS

Figure 1. CSF NFL, neurogranin, and YKL-40 concentrations according to diagnostic categories.

Boxplots showing the CSF concentrations of **(A)** NFL, **(B)** neurogranin, and **(C)** YKL-40 (adjusted for sex, age, and site) in ADD patients, FTD patients, MCI subjects, and cognitively HC. The lower, upper, and middle lines correspond to the 25th centile, 75th centile, and median, respectively. The whiskers extend to the minimum and maximum data points for NFL, neurogranin, and YKL-40. Dark circles represent outliers. Groupwise comparisons of NFL, neurogranin, and YKL-40 values (adjusted for sex, age, and site) were conducted through nonparametric Kruskal-Wallis tests followed by pairwise comparison (Conover's-test for multiple comparisons).

Abbreviations: ADD, Alzheimer's disease dementia; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; HC, healthy controls; MCI, mild cognitive impairment; NFL, neurofilament light chain.

Figure 2. Diagnostic workflow for the selection of the best performing biomarker combinations.