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Featured Article

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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Abstract

Introduction: The diagnostic and classificatory performances of all combinations of three core (amyloid β peptide [i.e., $A\beta_{1–42}$], total tau [t-tau], and phosphorylated tau) and three novel (neurofilament light chain protein, neurogranin, and YKL-40) cerebrospinal fluid biomarkers of neurodegeneration were compared among individuals with mild cognitive impairment (n = 41), Alzheimer's disease dementia (ADD; n = 35), frontotemporal dementia (FTD; n = 9), and cognitively healthy controls (HC; n = 21), using 10-fold cross-validation.

Methods: The combinations ranking in the top 10 according to diagnostic accuracy in differentiating between distinct diagnostic categories were identified.

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Results: The single biomarkers or biomarker combinations generating the best area under the receiver operating characteristics (AUROCs) were the following: the combination [amyloid β peptide + phosphorylated tau + neurofilament light chain] for distinguishing between ADD patients and HC (AUROC = 0.86), t-tau for distinguishing between ADD and FTD patients (AUROC = 0.82), and t-tau for distinguishing between FTD patients and HC (AUROC = 0.78).

Conclusions: Novel and established cerebrospinal fluid markers perform with at least fair accuracy in the discrimination between ADD and FTD. The classification of mild cognitive impairment individuals was poor.

Keywords: Alzheimer's disease; Alzheimer's disease dementia; Diagnostic biomarkers; Biomarker combination; Cerebrospinal fluid; Neurofilament light chain; Neurogranin; YKL-40; Pathophysiological pathways; Neurodegeneration; Clinical diagnosis; Cognitive aging; Mild cognitive impairment; Frontotemporal dementia; Precision medicine

1. Introduction

Polygenic Alzheimer's disease (AD), especially the late-onset form, is a pathophysiologically complex and clinically heterogeneous neurodegenerative disease [1–4]. The extracellular deposition of the accumulated amyloid β (Aβ) peptide (i.e., 42 amino acid–long Aβ peptide [$A\beta_{1-42}$]) into amyloid plaques and the intracellular accumulation of neurofibrillary tangles are considered pathophysiological hallmarks of AD [5]. Cerebrospinal fluid (CSF) concentrations of $A\beta_{1-42}$, 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 [6,7] biomarkers of AD pathophysiology [8]. Recently emerging evidence highlighted the presence of additional molecular pathophysiological pathways—such as axonal disintegration [9], synaptic dysfunction and degeneration [10], and innate immune response and neuroinflammation [9,11,12]—throughout the different stages of AD [1,11,13–15].

A growing number of discovery stage biomarker studies have been conducted that 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), as well as the asymptomatic preclinical stages [16–19]. Among those, CSF neurofilament light chain (NFL) [9], neurogranin [10], and YKL-40 [9,12] proteins have reached an advanced clinical validation stage and represent innovative pathophysiological candidate biomarkers that 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, 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 [20,21], and for clinical trials (as biomarker-stratified or biomarker-enriched target populations).

Specifically, NFL is a primary structural component of the neuronal cytoskeleton [22] and a marker of large-caliber axonal disintegration [22,23]. 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 [24]. 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 [12]. Interestingly, a recent meta-analysis showed that both NFL and YKL-40 proteins are promising biomarkers useful to differentiate AD patients from cognitively healthy controls (HC) [9]; furthermore, increased CSF neurogranin concentrations were found to be related to AD-characteristic pathophysiology [10,25].

Only a limited number of available studies have assessed the diagnostic accuracy of CSF core feasible AD biomarkers in combination with two of these three novel mechanistic biomarkers of AD pathophysiology [25–27]. To our knowledge, no previous study examined the CSF concentrations of the three biomarkers in combination with the three 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 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 aforementioned group comparisons, we implemented exhaustive searches with cross-validation to assess which combination of the panel of six 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; of these, 27 were excluded because of 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 subcohort of the Alzheimer Precision Medicine Initiative Cohort Program (APMI-CP) [28]—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 consensus criteria [29]. The clinical diagnosis of MCI was based on the MCI core clinical criteria [30]. The clinical diagnosis of FTD was performed according to the consensus on clinical diagnostic criteria published in 1998 [31]. Cognitively HC were individuals who (1) volunteered for lumbar puncture; (2) were negative for neurological or psychiatric diseases; and (3) had a Mini–Mental State Examination (MMSE) score between 27 and 30. Of the 23 cognitively HC, two individuals from the Gothenburg cohort showed CSF t-tau concentrations higher than the established cutoff value. Being asymptomatic-at-risk of AD [20] or preclinical AD [32], they were excluded from additional analyses. The group clinically defined as MCI included 41 participants [30]. Finally, 35 ADD [29] and 9 FTD [31] patients were included.

2.3. CSF sampling

CSF sampling was performed by lumbar puncture 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), and 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 for pending biochemical analysis.

2.4. Immunoassays for CSF core biomarkers

For the Paris cohort, CSF analyses of the biomarkers $A\beta_{1–42}$, 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 Center, 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 $A\beta_{1–42}$, t-tau, and tau phosphorylated at threonine 181 ($p\text{-}tau_{181}$) concentrations were measured using established sandwich enzyme-linked immunosorbent assay (ELISA) methods, INNOTEST β-AMYLOID(1–42) [33], INNOTEST hTAU Ag [34], and INNOTEST Phospho-Tau(181P) [35] (Fujirebio Europe N.V., Ghent, 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 [36] and the Global Biomarker Standardization Consortium [37]. Pathologic CSF biomarker concentrations were defined based on reference threshold cutoff values currently established in each memory clinic: at IM2A in Paris, $A\beta_{1–42} < 500$ pg/mL, t-tau > 450 pg/mL, and $p\text{-}tau_{181} > 60$ pg/mL; at DZNE in Rostock, $A\beta_{1–42} < 567$ pg/mL, t-tau > 512 pg/mL, and $p\text{-}tau_{181} > 66$ pg/mL (for the CSF samples measured before June 2012) and $A\beta_{1–42} < 450$ pg/mL, t-tau > 450 pg/mL, and $p\text{-}tau_{181} > 62$ pg/mL (for the CSF samples measured after June 2012); and at Clinical Neurochemistry Laboratory in Mölndal, $A\beta_{1–42} < 550$ pg/mL, t-tau > 400 pg/mL, and $p\text{-}tau_{181} > 80$ 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), following recommendations 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 [38]. 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 Inc., Lake Placid, NY, USA) for detection, and full-length neurogranin protein for calibration. 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, USA), 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 the Fisher's exact test and nonparametric Kruskal-Wallis (KW) tests, respectively. Before further analysis, both core feasible biomarkers 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. In addition, 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 using 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 comparisons (Conover's test for multiple comparisons), whenever the result of the KW test was statistically significant ($P < .05$). Results of post hoc testing were corrected for multiple comparisons using a false discovery rate 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 six biomarkers) using logistic regression within a 10-fold cross-validation approach in the following *a priori* comparisons: HC versus MCI, HC versus ADD, HC versus FTD, MCI versus ADD, and ADD versus FTD patients. In this analysis, age-, sex-, and site-adjusted values of all biomarkers used 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 (CIs) using a bootstrap procedure (100000 bootstraps) [39] by pooling predictions computed on the test sets from each train-test split in the 10-fold cross-validation 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) [40].

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 package, and the pairwise multiple comparison of mean ranks package (available at https://cran.r-project.org/web/packages/PMCMR/vignettes/PMCMR.pdf) [39,41]. Two-tailed P values $< .05$ were considered statistically significant.

3. Results

3.1. CSF biomarkers concentrations

Table 1 summarizes the concentrations of all analytes, both core feasible biomarkers and novel candidate biomarkers, combined with demographic and clinical data of the population. KW tests showed a significant effect of group on age ($P < .001$), MMSE ($P = .002$), and all CSF biomarkers ($A\beta_{1–42}$, $P < .001$; p-tau, $P < .001$; t-tau, $P < .001$; NFL, $P = .004$; neurogranin, $P = .002$; and YKL-40, $P = .0156$). *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 ($P < .001$, $P < .001$, $P = .003$, respectively) patients and in MCI versus HC ($P = .029$) patients. Compared with HC, both CSF t-tau and p-tau concentrations were significantly higher in MCI ($P < .001$ and $P = .002$, respectively), ADD ($P = .003$ and $P = .007$, respectively), and FTD ($P = .001$ and $P = .014$, respectively; Table 1) patients. CSF NFL concentrations were significantly higher in ADD versus HC and MCI patients ($P = .004$ and $P = .013$, respectively; Fig. 1A). CSF neurogranin concentrations were significantly higher in ADD versus HC and FTD ($P = .004$ for both comparisons; Fig. 1B). YKL-40 concentrations were significantly higher in ADD versus HC and FTD patients ($P = .032$ and $P = .049$, respectively; Fig. 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, and FTD; MCI from ADD; and ADD from FTD patients. In particular, the combination [$A\beta_{1–42}$ + p-tau + NFL] differentiated ADD patients from HC with an AUROC of 0.86 (95% CI, 0.83–0.89). Total tau and [$A\beta_{1–42}$ + p-tau + YKL-40] combination discriminated ADD from FTD patients with an AUROC of 0.82 (95% CI, 0.78–0.86) and 0.81 (95% CI, 0.77–0.85), respectively. Total tau and [p-tau + YKL-40] combination distinguished FTD patients from HC with an AUROC of

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 (years)	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 neurogranin (pg/mL)	180 (125–273)	331 (215–484)	468 (300–692)*,‡	125 (125–192)
CSF NFL (pg/mL)	609 (516–773)	1046 (793–1767)	1483 (1180–1844)*,†	1022 (693–1435)
CSF YKL-40 (ng/mL)	98 (90–110)	128 (98–184)	146 (119–177)*,‡	114 (98–120)
CSF $A\beta_{1–42}$ (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: $A\beta_{1–42}$, 42 amino acid–long amyloid β peptide; ADD, Alzheimer's disease dementia; CSF, cerebrospinal fluid; HC, 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 25th and 75th quartiles, except for n.

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

*$P < .05$ versus HC.

†$P < .05$ versus MCI.

‡$P < .05$ versus FTD.

§$P < .001$ versus HC.

¶$P < .001$ versus MCI.

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 patients with an AUROC of 0.71 (95% CI, 0.67–0.75). $A\beta_{1–42}$ and [$A\beta_{1–42}$ + YKL-40] combination discriminated MCI patients 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 $A\beta_{1–42}$, which was only correlated with p-tau and t-tau proteins. In the multivariate regression models, YKL-40 resulted to be a significant contributor ($P < .001$) in explaining the variability in NFL; t-tau ($P < .001$) contributed significantly to neurogranin variability; and p-tau ($P = .002$), NFL ($P = .0175$), and neurogranin ($P = .0250$) contributed significantly to YKL-40 variability.

4. 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 neurodegeneration [42–46]. Both CSF neurogranin and YKL-40 concentrations were significantly higher in ADD patients versus HC, thus confirming earlier reported data [10,12]. Furthermore, we demonstrated significantly higher concentrations of CSF neurogranin in ADD compared with FTD patients; this finding corroborates previous data indicating a selective increase in CSF neurogranin in individuals showing AD pathophysiology [10]. We also found higher concentrations of CSF YKL-40 in ADD compared with FTD patients. In this regard, a non–AD specific increase of CSF YKL-40 versus HC was described [12,25,47,48]. Prior investigations reported both higher and similar CSF YKL-40 concentrations in FTD compared with ADD patients [12,25]. As expected, CSF $A\beta_{1–42}$ concentrations were significantly lower in ADD patients compared with HC, MCI, and FTD patients as well as in MCI patients compared with HC [1,8,9]; moreover, compared with HC, both CSF t-tau and p-tau concentrations were significantly increased in MCI, ADD, and FTD patients [1,8,9].

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 [9,25], following the application of a diagnostic workflow for the selection of the best-performing biomarker combinations (Fig. 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) showed poor ability to differentiate HC from MCI patients. When distinguishing HC from ADD patients, 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, for example, $A\beta_{1–42}$ alone. When distinguishing HC from FTD patients, YKL-40 combined with p-tau and t-tau delivered fair diagnostic accuracy, which was comparable to the one delivered by, for example, t-tau

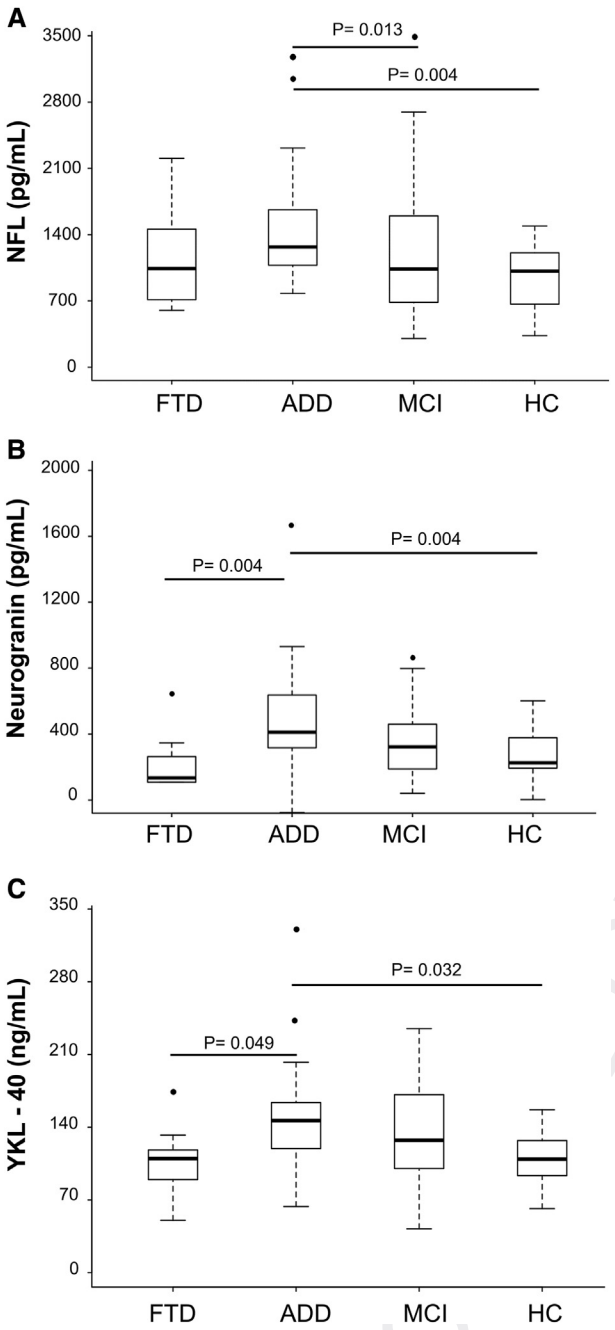

Fig. 1. CSF NFL, neurogranin, and YKL-40 concentrations according to diagnostic categories. Box plots showing the CSF concentrations of (A) NFL, (B) neurogranin, and (C) YKL-40 (adjusted for sex, age, and site) in ADD patients, FTD patients, MCI subjects, and cognitively HC. The lower, upper, and middle 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 comparisons (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.

alone. When distinguishing MCI from ADD patients, various combinations of all three novel biomarkers with the core biomarkers delivered fair diagnostic accuracy, which was comparable to the one afforded by, for example, t-tau alone or t-tau and $A\beta_{1–42}$. When distinguishing ADD from FTD patients, various combinations of the three 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, and t-tau). In summary, as mentioned previously, no combination including the three novel candidate biomarkers was superior to, for example, $A\beta_{1–42}$ in distinguishing HC from MCI and ADD patients, neither to t-tau in differentiating HC from FTD patients nor to $A\beta_{1–42}$, p-tau, and t-tau in discriminating ADD from FTD as well as MCI from ADD patients.

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 [20]. 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 (i.e., high retention of amyloid tracer) [20]. Recently, Ewers et al. (2015) [49], 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. Our data support the idea 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. Additional analyses using a composite array including the three presented innovative biomarkers are necessary to achieve a more accurate stratification of patients' cohorts according to different AD-related pathophysiological pathways [12,13,28,50]. 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 [13,28,50]. As a result, focused therapeutic interventions are expected to be

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 patients are reported

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 + YKL-40	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 + YKL-40	MCI	ADD	69.09	65.31	72.86
$A\beta_{1-42}$ + t-tau + YKL-40	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 + YKL-40	MCI	ADD	68.90	65.07	72.73
$A\beta_{1-42}$ + NFL + YKL-40	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: $A\beta_{1-42}$, 42 amino acid–long amyloid β peptide; ADD, Alzheimer's disease dementia; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; HC, 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. Results are shown starting from the highest to the lowest AUROC value for every group comparison.

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 pathophysiological processes. This could mean either that their concentrations are influenced by common factors, such as CSF turnover, or that the pathophysiological processes they

Table 3
Correlations between cerebrospinal fluid biomarkers in the study cohort

	p-tau	t-tau	NFL	YKL-40	Neurogranin
$A\beta_{1-42}$	−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‡

Abbreviations: $A\beta_{1-42}$, 42 amino acid–long amyloid β peptide; NFL, neurofilament light chain protein; p-tau, hyperphosphorylated tau; t-tau, total tau.

NOTE. Data are derived from Spearman's rank-order correlation test after adjusting for age, sex, and site. *P* values were corrected for multiple comparisons.

*$P \leq .05$.
†$P \leq .01$.
‡$P \leq .001$.

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 [51]. Alternatively, examining correlations within control and patient (MCI and AD) cohorts having different severities of the same pathophysiologies will by necessity identify such correlations. For example, it is well known that CSF concentrations of t-tau and p-tau correlate tightly within control and AD cohorts [52], which might mistakenly be taken as an indication that these biomarkers reflect the same pathophysiology, whereas in Creutzfeldt-Jakob disease [53] and stroke [54] cohorts, this correlation is lost, thus indicating that they indeed reflect different pathophysiologies.

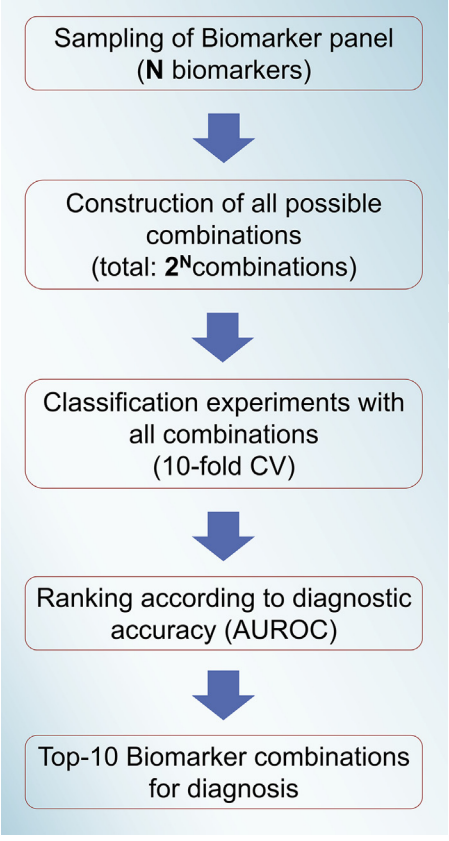

Fig. 2. Diagnostic workflow for the selection of the best performing biomarker combinations. Abbreviations: AUROC, area under the receiver operating characteristic; CV, cross-validation.

We identified several correlations between novel and traditional CSF biomarkers, whose significance and reproducibility require further scrutiny.

There are some limitations of our study. As our data set 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 interlaboratory 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, because 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 toward 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 patients. 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 stage and dementia. To this aim, we are currently in the process of conducting these longitudinal studies using a unique large-scale monocentric cohort (INSIGHT-preAD)—within the framework of the APMI and as part of the APMI-CP [28]—including amyloid positron emission tomography–stratified preclinical asymptomatic individuals at risk for AD, to elucidate the temporal dynamics of all six, and more, pathophysiological biomarkers and test their potential correlation with genomics and multimodal neuroimaging and

electroencephalography data, throughout disease progression commencing at the preclinical stage. The ultimate aim is to identify disease trajectories through space and time using integrated disease modeling that may serve as more precise guideposts for detecting the disease at the 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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RESEARCH IN CONTEXT

1. Systematic review: Besides core cerebrospinal fluid (CSF) biomarkers of neurodegeneration—including amyloid β peptide (i.e., $A\beta_{1-42}$), total tau, and phosphorylated tau—there is growing interest in novel pathophysiological markers that reflect axonal degeneration, synaptic dysfunction, and neuroinflammation—such as neurofilament light chain protein, neurogranin, and YKL-40—to distinguish between different neurodegenerative diseases.
2. Interpretation: We investigated the diagnostic and classificatory performances of all combinations of core and novel CSF biomarkers in individuals with mild cognitive impairment, Alzheimer's disease dementia, frontotemporal dementia, and cognitively healthy controls. We found that novel and established CSF markers performed with at least fair accuracy in the discrimination between Alzheimer's disease dementia and frontotemporal dementia.
3. Future directions: Longitudinal analyses are warranted in asymptomatic preclinical at risk for Alzheimer's disease individuals to investigate whether components of the biomarker panel may predict disease progression and conversion to prodromal stage and dementia.

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