

**Title: Longitudinal cerebrospinal fluid biomarker trajectories along the Alzheimer's disease continuum in the BIOMARKAPD study**

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**Abbreviations:** A $\beta$ : amyloid- $\beta$ ; AD: Alzheimer's disease; *APOE*: apolipoprotein E; CSF: Cerebrospinal fluid; FTD frontotemporal dementia; MCI: Mild cognitive impairment; MMSE: MiniMental State Examination; NfL: Neurofilament light; SCD: subjective cognitive decline; SNAP suspected non-Alzheimer's disease pathophysiology.

## **Conflict of interest statement**

AL has served at scientific advisory boards of Fujirebio Europe, Eli Lilly, Novartis and Nutricia and is the inventor of a patent on synaptic markers in CSF.

LP has received honoraria as member of advisory boards from Fujirebio Europe, IBL International, Merck, Roche and Biogen.

LF has participated in advisory boards for Allergan, Eli Lilly, Avraham Pharmaceuticals, Axon Neuroscience, Axovant, Biogen, Boehringer Ingelheim, Eisai, Functional Neuromodulation, Lundbeck, MerckSharpe & Dohme, Novartis, Pfizer, Pharnext, Roche, Schwabe and has received a research grant from Novartis.

JLM has served as a consultant or at advisory boards for Axovant, Biogen, Genentech, Eisai, Eli Lilly, IBL International, Lundbeck, Merck, Novartis, Pfizer, Roche and Roche Diagnostics,

HZ has served at scientific advisory boards of Eli Lilly, Roche Diagnostics, Wave and Celectricon, has received travel support from Teva, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

KB has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Novartis, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

**Abstract:**

**INTRODUCTION:** Within-person trajectories of cerebrospinal fluid (CSF) biomarkers in Alzheimer's disease (AD) are not well defined.

**METHODS:** We included 467 subjects from the BIOMARKAPD study with at least two serial CSF samples. Diagnoses were subjective cognitive decline (n=75), mild cognitive impairment (n=128), AD dementia (n=110) and a group of cognitively unimpaired subjects (n=154). We measured baseline and follow-up CSF levels of total tau (t-tau), phosphorylated tau (p-tau), YKL-40 and neurofilament light (NfL). Median CSF interval was 2.1 years.

**RESULTS:** Mean CSF t-tau and p-tau levels increased 2%/year in controls ( $P<.001$ ). In the AD group, mean t-tau levels decreased 1%/year ( $P<.001$ ) and p-tau levels did not change. Mean NfL and YKL-40 levels increased 2%/year in controls ( $P<.001$ ), without differences among groups. Longitudinally, only levels of NfL ( $P<.001$ ) and YKL-40 ( $P<.02$ ) increased during the study period.

**CONCLUSIONS:** All four CSF biomarkers increase with age, but this effect deviates in AD for t-tau and p-tau.

**Keywords:** Alzheimer, CSF, tau, amyloid, neurofilaments, inflammation, YKL-40.

## Highlights

- In this large longitudinal multicenter CSF study mean CSF t-tau and p-tau levels significantly increased 2% in controls with each year of increase at baseline age. Within the AD group, mean t-tau levels significantly decreased 1% and p-tau levels did not change.
- We found a significant annual increase of NfL and YKL-40 levels of 2% on average in controls without differences among groups. Longitudinally, only levels of NfL and YKL-40 significantly increased during the study in all groups.
- The pattern of change of CSF tau and NfL and YKL-40 are different in AD.

## 1. Introduction

Alzheimer's disease (AD) is characterized by a long preclinical and prodromal phase that precedes the full-blown dementia syndrome. Advances in biochemical or imaging biomarkers during the last decades have led to a conceptual transition from a clinical-pathological definition of AD to a biological framework [1]. In this new scenario, biomarkers play a major role in the characterization of different disease stages in clinical practice and in clinical trials.

Cerebrospinal fluid (CSF) biomarkers offer the possibility to detect many pathophysiological processes simultaneously at a relatively affordable cost. In AD, several studies have consistently identified a specific CSF biomarker signature consisting of low levels of  $A\beta_{1-42}$  and high levels of t-tau and p-tau ( $A\beta_{1-42}$ , t-tau and p-tau named core AD biomarkers) that reflect the main

neuropathological hallmarks of the disease [2, 3]. CSF biomarkers in AD play a major role in clinical practice by increasing diagnostic accuracy and in clinical trials by improving selection of patients in the early disease stage, and ensuring adequate drug target engagement [3, 4]. Other newer CSF biomarkers have been investigated in AD. Neurofilament light (NfL) reflects axonal damage and its levels are elevated early in the disease and correlate with disease progression and brain atrophy [3]. Different inflammatory markers have also been investigated [5]. A commonly investigated marker among inflammatory proteins is the astrocytic protein YKL-40 [6, 7]. CSF levels of YKL-40 are elevated in AD and other neurodegenerative conditions such as frontotemporal dementia (FTD) and multiple sclerosis among others [6, 8-10].

Although the pattern of change of these CSF markers in AD has been extensively described in many cross-sectional studies [5], the longitudinal trajectories of individual participants are controversial and models based on cross-sectional data have contradicted those based on longitudinal data, where serial CSF samples are taken from the same subject. It is particularly relevant to investigate neuronal injury markers in order to test whether these measures can be indicative of disease activity or can be used to predict progression at the individual level. Some studies have shown that CSF neuronal injury markers in symptomatic AD are longitudinally unchanged [11-14], increased [15-17] or decreased [18-21] along time. However, the sample size, inclusion criteria and follow-up period is highly variable in these studies. In addition, very few studies [18, 21] have investigated the pattern of longitudinal change in inflammatory markers in AD. In this study, we take advantage of a large multicenter study to investigate longitudinal CSF trajectories of two neurodegeneration markers (t-

tau, NfL), one tau pathology-associated marker (p-tau) and the astrocytic marker YKL-40 across the AD continuum.

## **2. Methods:**

### *2.1. Subjects*

We included 467 subjects from 13 participating centers from the multicenter BIOMARKAPD project ([www.biomarkapd.com](http://www.biomarkapd.com)): CITA Alzheimer, San Sebastián, Spain (n=180); VU University Medical Center, Amsterdam, Netherlands (n=86); EDAR study (n=60); Barcelona Hospital Sant Pau (n=37); Mölndal (n=29); Montreal (n=19); Perugia (n=19); Copenhagen (n=14); Nijmegen (n=7); Montpellier (n=5); Mannheim (n=4); Coimbra (n=4); and Barcelona Hospital Clínic (n=3).

The participants were cognitively unimpaired controls and patients in the AD continuum in whom at least two longitudinal CSF samples were available (444 had two serial samples, 19 had three, and 4 had four samples). Baseline diagnoses were: cognitively unimpaired controls (HC, n=154), subjective cognitive decline (SCD, n=75), mild cognitive impairment (MCI, n=128), and AD dementia (AD, n=110). The diagnosis was made at each center according to published criteria [22, 23]. The diagnosis was based on the clinical syndrome independent of the previous determinations of CSF AD biomarkers at each center. Cognitively normal subjects had no previous neurologic or psychiatric disease and had no cognitive deficits after a formal cognitive evaluation. Baseline characteristics of a subset of these participants had been previously published [9,2-31].

### *2.1. CSF analyses*

CSF was collected at each center following international consensus recommendations [32]. Samples were aliquoted and stored in polypropylene tubes at -80°C and shipped on dry ice to the Clinical Neurochemistry Laboratory in Gothenburg for analysis. Most centers (11 out of 13) used 0.5 ml aliquots, one center used 0.25 ml aliquots and 1 center used 1.5 ml aliquots. We measured baseline CSF levels of amyloid- $\beta$  ( $A\beta$ )<sub>1-42</sub>,  $A\beta$ <sub>1-40</sub>,  $A\beta$ <sub>1-38</sub>, and baseline and follow-up levels of t-tau, p-tau, YKL-40 and NfL. Biomarker concentrations were measured using commercial assays (MSD:  $A\beta$ <sub>1-42</sub>,  $A\beta$ <sub>1-40</sub>,  $A\beta$ <sub>1-38</sub>; Fujirebio-Europe INNOTEST: t-tau and p-tau; R&D: YKL-40 and Uman Diagnostics: NfL). All CSF measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Baseline and follow-up samples were always measured side by side on the same plate. The assay repeatability was 6.5-10% for t-tau, 1.5-3.6% for p-tau, 3.6 - 5.2% for NfL and 3.1 - 5.2% for YKL-40.

### *2.3. CSF classification*

In the subset of participants with MCI and AD dementia we used local values of CSF  $A\beta$ <sub>1-42</sub> at baseline to stratify subjects in amyloid-positive ( $A\beta$ +) or amyloid-negative ( $A\beta$ -). In the subset of participants with SCD and in cognitively unimpaired controls we used local values of CSF  $A\beta$ <sub>1-42</sub>, t-tau, and p-tau to classify preclinical stages of AD according to National Institute on Aging–Alzheimer’s Association (NIA-AA) criteria [33]. Participants were classified as stage 0 (normal values of  $A\beta$ <sub>1-42</sub>, t-tau and p-tau), stage 1 (reduced values of  $A\beta$ <sub>1-42</sub>, with normal values of t-tau and p-tau), stage 2 (reduced values of  $A\beta$ <sub>1-42</sub>,

with high values of t-tau or p-tau) or stage 3 (stage 2 plus subtle cognitive decline or cognitive complaints). For the analysis, stages 2 and 3 were combined due to the low number of participants in each group. Subjects with normal  $A\beta_{1-42}$  and either elevated t-tau or p-tau were classified as having suspected non-Alzheimer's disease pathophysiology (SNAP).

#### *2.4. APOE genotype*

*APOE* genotyping was performed at each site except in two centers (Perugia, Mannheim) in which genotypes were obtained in the Alzheimer laboratory at Hospital Sant Pau using previously published methods [34].

#### *2.5. Statistical analyses*

Differences in baseline age and MMSE were assessed by ANOVA and post-hoc Tukey Honest Significant Difference test. Differences in gender and *APOE*  $\epsilon 4$  status were assessed by Chi-square test. We used generalized linear mixed models for the analysis of all biomarkers. We modeled center-specific random intercepts, subject-specific random intercept and slope, and diagnostic-specific residual errors. Baseline age, time from study entry, diagnosis, *APOE*  $\epsilon 4$  status and their interactions, together with gender and time of sample storage (time from collection to analysis) were included as fixed-effects. Outliers were detected by visual inspection of their influence on the residuals. Final models were determined by backward selection of effects based on their significance starting from those of higher order interactions. Age-centered baseline effects were calculated for time point 0.



2.6. *Standard protocol approvals, registrations, and patient consents.* All participants gave their written consent, and the local ethics committee at each center approved the study.

### **3. Results**

#### *3.1. Demographics*

We included in the study 467 participants who met the key criteria of having longitudinal CSF samples. The median Lumbar Puncture (LP) interval was 2.1 years (range 0.2-6.2 years). The demographic characteristics, *APOE* genotype and baseline CSF biomarkers are shown in Table 1.

Patients with MCI and AD were older at baseline than patients with SCD and controls (ANOVA  $F=48.3$ ;  $P<.001$ ). As expected, MMSE scores were lower in AD compared to the other groups, and in MCI compared to SCD and controls (ANOVA  $F=156.7$ ;  $P<.001$ ). We also found the expected differences in *APOE*  $\epsilon 4$  frequency ( $X^2=43$ ;  $P<.001$ ) between groups (Table 1).

#### *3.2. CSF biomarkers at baseline*

Baseline CSF biomarker levels differed between groups (Table 1). AD and MCI groups had higher levels of t-tau ( $P<.001$ ), p-tau ( $P<.001$ ), NfL ( $P<.001$ ) and YKL-40 ( $P<.001$  for AD and  $P=0.05$  for MCI) compared to SCD and controls. In addition, CSF levels of  $A\beta_{1-42}$  and the  $A\beta_{1-42/1-40}$  ratio were lower in AD and MCI compared to SCD and controls ( $P<.001$ ). No differences in  $A\beta_{1-40}$  levels were observed between groups. Levels of  $A\beta_{1-38}$  were 13% lower in AD compared to controls ( $P=.05$ ) without other significant differences between groups.

### 3.3. Effect of gender and APOE genotype

Levels of CSF NfL were 17% higher in males than females ( $P < .001$ ). No other gender differences were found between groups. APOE  $\epsilon 4$ -negative subjects had 28% (95%CI 19-39%) higher  $A\beta_{1-42}$  levels and 27% (95%CI 20-34%) higher  $A\beta_{1-42}/A\beta_{1-40}$  levels than APOE  $\epsilon 4$ -positive subjects ( $P < .001$ ). APOE  $\epsilon 4$ -negative subjects also had 16% (95%CI 8-24%) lower t-tau and 13% (95%CI 6-19%) lower p-tau levels than APOE  $\epsilon 4$ -positive subjects ( $P < .001$ ). There was no significant interaction effect between APOE and diagnosis. No effect of APOE genotype was observed for  $A\beta_{1-38}$ ,  $A\beta_{1-40}$ , NfL or YKL-40 levels.

### 3.4. Estimated effect of baseline age on CSF biomarkers

We used general linear mixed models to estimate the effect of age at baseline on biomarker levels. Each year of baseline age was associated with a +2% change in mean CSF t-tau and p-tau levels in controls ( $P < .001$ , Fig. 1A-B). This age-associated effect was different in AD patients where levels were 1% lower per year of baseline age in t-tau ( $P < .001$ ) and no change per year in p-tau levels was observed.

Each year of baseline age was associated with +2% change in mean NFL and YKL-40 levels in controls ( $P < .001$ , Fig. 1C-D) without differences between diagnostic groups.

### 3.5. Longitudinal CSF changes along the AD continuum

Next, we used general linear mixed models to estimate within-individual rates of change in CSF t-tau, p-tau, NfL and YKL-40 levels in the four groups. Results

were adjusted for baseline age, *APOE*  $\epsilon$ 4 status, gender and time of sample storage, accounting for all possible interactions.

#### *Total tau and p-tau*

Longitudinally, mean t-tau and p-tau levels did not change during the follow-up period ( $P=0.42$  and  $P=0.063$ , respectively; Fig. 2A-B), either in the whole group or within any diagnostic group.

#### *NfL*

Longitudinally, mean NfL levels increased 4% per year during the follow-up period ( $P<.001$ , Fig 2C). There were no differences among diagnostic groups.

#### *YKL-40*

On average, controls had an increase in YKL-40 levels of 1% per year in CSF during the follow-up period ( $P=.02$ , Fig 2D). There were no differences among diagnostic groups.

Next, we repeated the analyses but stratifying MCI and AD participants into  $\beta$ -amyloid-positive ( $A\beta+$ ) and  $\beta$ -amyloid-negative ( $A\beta-$ ) according to the local CSF  $A\beta_{1-42}$  values obtained at baseline at each center using local cutoffs. Demographic data, *APOE* genotype and baseline CSF biomarkers of this subset ( $n=341$ ) are shown in Supplementary Table 1. For this analysis we excluded controls with  $A\beta+$ CSF values and AD patients with  $A\beta-$  CSF values. In this subsample there was no significant effect of the amyloid status on within-individual rates of change in CSF t-tau, p-tau, NfL or YKL-40 levels (Fig 3).

### *3.6. Longitudinal CSF changes in preclinical AD*

Finally, we performed an exploratory analysis in a subset of 178 cognitively unimpaired subjects and patients with SCD that had available local core AD

CSF biomarkers. We used local cut-offs to classify these participants in preclinical stages 0 (n=142), 1 (n=13), 2-3 (n=6) or SNAP (n=17). Demographic data, *APOE* genotype and baseline CSF biomarkers of this subset are shown in Supplementary Table 2. There were expected differences in the levels of A $\beta$ <sub>1-42</sub>, t-tau and p-tau at baseline consistent with the definition of preclinical stages. Levels of NfL were 41% (95%CI 16-72%, *P*<.001) higher in stage 2 and 34% (95%CI 14-58%; *P*<.001) higher in SNAP compared to those in stage 0 and 1. YKL-40 levels were 30% (95%CI 1-69%; *P*=.05) higher in stage 2 and 46% (95%CI 24-72%; *P*<.001) higher in SNAP compared to those in stage 0 and 1. Longitudinally, none of the biomarkers investigated showed significant changes within these stages (Fig 4).

#### **4. Discussion**

This is, to our knowledge, the largest study investigating longitudinal trajectories of CSF biomarkers along the AD continuum. We found that the CSF levels of tau markers did not change longitudinally over a median LP interval of 2.1 years, while NfL levels increased in all clinical groups. All three neuronal injury markers and the astrocytic (YKL-40) protein investigated increased with age. However, the age-related effect on CSF t-tau and p-tau levels differed in controls from the AD group, in which lower levels were found with more advanced age. Therefore, the age-related pattern of tau markers in AD, but not NfL or YKL-40, deviates from the pattern observed in normal aging. Previous studies on longitudinal CSF trajectories of neuronal injury markers along the AD continuum yielded conflicting results with some studies [18-21] showing a longitudinal decrease, others [11, 12-14] showing no change and

some [15-17] showing an increase. The different results between studies are likely due to the differences in the populations included, the inclusion of biomarker-positive groups in some studies, and the different LP intervals. In addition, the inherent variability in longitudinal studies is well recognized [35]. The source of variability in longitudinal studies can be attributed to variability in between-individual and intra-individual trajectories as well as measurement errors in the biomarker analyses. The variability in between-individual trajectories can be minimized by selecting a homogeneous patient population and the measurement error can be reduced by using precise assays to analyze simultaneously all serial samples.

In a recent work [21], there was a decrease in p-tau levels longitudinally in patients with symptomatic AD ( $P \leq .0001$ ), and a decrease in t-tau that did not reach statistical significance ( $P = .095$ ). These findings mirror those observed in autosomal-dominant AD, where a similar pattern of change has been described [36]. Although in our study we could not detect a longitudinal change in t-tau and p-tau levels, the different age-associated effect in AD supports the idea of a change in the trajectories of tau markers in the dementia phase of AD.

The decreases in the CSF trajectory of tau in late clinical AD could reflect a slowing in the neurodegeneration activity in this stage, but it could be also explained by the reduction in the total number of cells that contribute to the CSF pool (with the same activity) or an abnormal CSF clearance with disease progression. The pattern of tau is different from the pattern on NfL, which increased in all groups independently of the diagnosis. NfL is found in large-caliber axons in the brain [37] and therefore has been proposed as a marker of axonal degeneration and white-matter damage in AD [38, 39]. In cross-sectional

studies higher levels of NfL are associated with higher risk of progression to AD dementia in MCI [40]. However, in this study NfL levels increased with age and during the follow-up period in all groups irrespective of the diagnosis. This is in contrast with other studies [15] in which levels of NfL decreased in MCI and AD patients. The reasons for this difference remain unclear at this point and deserve further investigation. Although there is a positive correlation between CSF levels of NFL and tau, evidence suggest that NfL levels provide information on neurodegeneration that is at least in part different from CSF tau. Tau is a protein predominantly expressed in cortical brain regions, and tau levels in CSF could be a reflection of hippocampal and cortical atrophy while NfL levels could be a reflection of subcortical damage [37, 41]. The different topographical patterns of degeneration along the course of AD could explain the different trajectories of both markers [41].

YKL-40 is a protein expressed in a subset of astrocytes in the brain [7]. YKL-40 levels in CSF are increased early in AD [6, 9, 42], and also in multiple sclerosis and FTD [8, 10, 43]. In this study we found that YKL-40 levels increased longitudinally in all groups during the study without differences between groups. In a recent study, YKL-40 levels also increased in controls and AD, but only reached significance in MCI subjects with positive amyloid Positron Emission Tomography (PET) [21, 44]. It is likely that the lack of biomarkers to stratify the MCI and AD groups in our study may explain these differences. Another study looked at the glial S-100B protein [45] and found that levels also increased in AD and DLB. The different longitudinal patterns observed in YKL-40 and tau in AD suggests that neurodegeneration and neuroinflammation follow different trajectories.

We found the expected baseline differences between groups, with higher levels of t-tau, p-tau, NfL and YKL-40 in AD and MCI compared to the other groups. This finding is in complete agreement with the previous literature that indicates that markers of neurodegeneration and astroglial activation are increased in AD dementia [5, 8, 9]. We also found an increase in NfL levels in men participants compared to women. This finding has been previously described in other CSF studies [41] and stresses the importance of adjusting for gender when investigating CSF NfL levels. Importantly, this finding has not been observed in studies looking at plasma NfL [46].

Our findings also suggest that a 2-year period may be too short for detecting significant changes in neuronal injury markers in CSF during the AD disease course and this may help to understand the results of some studies with shorter follow-up periods. These findings should be considered when using these markers as surrogate markers for neurodegeneration in clinical trials [4]. The potential explanation for the discrepancy between CSF and imaging biomarkers has recently been discussed elsewhere [1]. It is likely that CSF reflects the axonal and synaptic damage intensity or “disease activity” at a given point while imaging techniques reflect the cumulative change and therefore correlate better with cognitive or functional scales.

The main strengths of this study are the large sample size and the inclusion of a large subset of participants in the preclinical AD stages. In addition, all analyses were performed in a central laboratory with the same assay lots and long experience in CSF biomarkers.

The study has several limitations. First, the median LP interval was relatively short which limited the window to detect longitudinal changes. Second, clinical protocols were not harmonized among centers and therefore clinical measures could not be used to assess the clinical change over time. Finally, the study did not include data on common comorbidities, structural imaging or PET measures to correlate with longitudinal CSF changes.

In conclusion, this study showed that the age-related pattern of tau markers in AD deviates from the pattern observed in normal aging, while NfL and YKL-40 continue to increase. These findings are important for the interpretation of longitudinal CSF studies and for the design of clinical trials in AD.



**Table 1. Demographics and CSF biomarker levels at baseline of the participants**

	Control	SCD	MCI	AD	<i>P</i> value
N	154	75	128	110	-
Age (Years)	58.2 (7.2)	60.9 (8.1)	67 (8.4)	68.5 (8.5)	<0.001 <sup>a</sup>
Sex (Females/Males) (%Female)	86/68 (55.8%)	32/43 (42.7%)	49/79 (38.3%)	47/63 (42.7%)	0.026 <sup>b</sup>
<i>APOE</i> ε4+(/) <i>APOE</i> ε4-	47 (31.1%)/104	14 (19.4%)/58	50 (41.3%)/71	63 (64.9%)/34	<0.001 <sup>c</sup>
MMSE	28.7 (1.2)	28.5 (1.2)	27.1 (2)	22.6 (4.1)	<0.001 <sup>d</sup>
t-tau (pg/ml)	296 (136)	312 (109)	514 (362)	759 (432)	<0.001 <sup>e</sup>
p-tau (pg/ml)	42 (16)	43 (13)	66 (50)	81 (37)	<0.001 <sup>e</sup>
NfL (pg/ml)	584 (314)	646 (339)	1019 (736)	1647 (1573)	<0.001 <sup>e</sup>
YKL-40 (pg/ml)	131606 (49537)	137557 (44213)	175993 (74548)	209732 (72760)	<0.001 <sup>e</sup>
Aβ1-38 (pg/ml)	2439 (706)	2424 (599)	2384 (1046)	2242 (895)	0.688 <sup>e</sup>
Aβ1-40 (pg/ml)	5428 (1453)	5465 (1268)	5632 (1988)	5703 (1864)	0.597 <sup>e</sup>
Aβ1-42 (pg/ml)	506 (182)	491 (166)	382 (179)	316 (192)	0.006
Aβ1-42/Aβ1-40	0.093 (0.017)	0.09 (0.019)	0.07 (0.026)	0.055 (0.023)	<0.001 <sup>e</sup>
Aβ1-42/tTau	1.89 (0.64)	1.71 (0.59)	1.10 (0.76)	0.59 (0.57)	<0.001 <sup>e</sup>

Unless otherwise specified, values are expressed as mean (SD)

Abbreviations: Aβ: amyloid-β; AD: Alzheimer's disease; *APOE*: apolipoprotein E; MCI: Mild cognitive impairment; MMSE: MiniMental State Examination; NfL: Neurofilament light; SCD: subjective cognitive decline.

<sup>a</sup> ANOVA. TukeyHSD post-hoc: Control and SCD different from MCI and AD ( $p < 0.001$ )

<sup>b</sup> Chi-squared test. Controls different from MCI (uncorrected  $p = 0.005$ ) and AD (uncorrected  $p = 0.048$ ).

<sup>c</sup> Chi-squared test. SCD different from MCI (uncorrected  $p = 0.003$ ). AD different from Control (uncorrected  $p < 0.001$ ), SCD (uncorrected  $p < 0.001$ ) and MCI (uncorrected  $p < 0.001$ ).

<sup>d</sup> ANOVA. TukeyHSD post-hoc: Control and SCD significantly different from MCI and AD ( $p < 0.001$ ). MCI significantly different from AD ( $p < 0.001$ )

<sup>e</sup> Linear mixed-models. See text for detailed group-by-group comparisons.

## Supplementary material

**Supplementary table 1: Demographics and CSF biomarker levels at baseline of the subset of participants with MCI and AD stratified as A $\beta$ <sup>+</sup> or A $\beta$ <sup>-</sup> according to local cutoffs for A $\beta$ <sub>1-42</sub>.**

	Control	SCD	MCI	AD	<i>P</i> value
N	111	70	101	59	
Age (years)	57 (6.8)	60.5 (8.1)	67.2 (8.8)	66.1 (8.3)	<0.001 <sup>a</sup>
Females/Males	58 / 53	30 / 40	38 / 63	25 / 34	0.19 <sup>b</sup>
<i>APOE</i> ε4+(/) <i>APOE</i> ε4-	26 (23.6) / 84	13 (18.8) / 56	35 (36.8) / 60	37 (66.1) / 19	<0.001 <sup>c</sup>
A $\beta$ positive (%)/A $\beta$ negative	0 (0) / 111	11 (15.7) / 59	51 (50.5) / 50	59 (100) / 0	<0.001 <sup>d</sup>
MMSE	28.8 (1.1)	28.5 (1.2)	27 (2.1)	22.2 (4.1)	<0.001 <sup>e</sup>
t-tau (pg/ml)	287.9 (122.8)	309.3 (109.9)	506.1 (334.5)	837.7 (461.9)	<0.001 <sup>f</sup>
p-tau (pg/ml)	40.2 (13.7)	42.3 (13.4)	63.1 (32.4)	88.4 (40.7)	<0.001 <sup>g</sup>
NfL (pg/ml)	566.6 (292.9)	626.2 (332.7)	995.8 (709.8)	1305.5 (771.5)	<0.001 <sup>h</sup>
YKL-40 (pg/ml)	128295.7 (48700.6)	134485.8 (43166.5)	172293.8 (71392.5)	201128 (63986.2)	<0.001 <sup>i</sup>

Unless otherwise specified, values are expressed as mean (SD)

Abbreviations: A $\beta$ : amyloid- $\beta$ ; *APOE*: apolipoprotein E; MMSE: MiniMental State

Examination; NfL: Neurofilament light; SCD: subjective cognitive decline.

<sup>a</sup> ANOVA. TukeyHSD post-hoc: Control different from SCD. MCI and AD different from control and SCD.

<sup>b</sup> Chi-squared test

<sup>c</sup> Chi-squared test. Controls and SCD different from MCI and AD. MCI different from AD.

<sup>d</sup> Chi-squared test. Controls different from SCD, MCI and AD. SCD different from MCI and AD. MCI different from AD.

<sup>e</sup> ANOVA. TukeyHSD post-hoc. Controls and SCD different from MCI and AD. MCI different from AD.

<sup>f</sup> Linear mixed-models. t-tau: MCI ( $p=0.016$ ) and AD ( $p<0.001$ ) significantly different from controls and SCD.

<sup>g</sup> Linear mixed-models. p-tau: MCI ( $p=0.017$ ) and AD ( $p<0.001$ ) significantly different from controls and SCD.

<sup>h</sup> Linear mixed-models. NfL: MCI ( $p<0.001$ ) and AD ( $p<0.001$ ) significantly different from controls and SCD.

<sup>i</sup> Linear mixed-models. YKL-40: AD ( $p<0.001$ ) significantly different from controls, SCD and MCI.

**Supplementary table 2. Demographics and CSF biomarker levels at baseline of the subset of participants classified according to preclinical AD stages**

	Stage 0	Stage 1	Stage 2	SNAP	P value
N	142	13	6	17	-
Baseline age (Years)	57 (6.6)	61.2 (8.4)	67.2 (5.8)	61.9 (5.8)	<0.001 <sup>a</sup>
Sex (Females/Males)	72/70	8/5	4/2	8/9	0.733 <sup>b</sup>
APOE $\epsilon$ 4+(/APOE $\epsilon$ 4-	28 (19.7%)/114	5 (38.5%)/8	4 (66.7%)/2	4 (25%)/12	0.030 <sup>c</sup>
MMSE scores	28.7 (1.2)	28.5 (1.3)	29.2 (1.6)	28.9 (1)	0.522 <sup>d</sup>
t-tau (pg/ml)	261 (83)	240 (110)	553 (71)	488 (67)	<0.001 <sup>e</sup>
p-tau (pg/ml)	37 (9)	33 (11)	73 (13)	64 (9)	<0.001 <sup>e</sup>
NfL (pg/ml)	532 (245)	687 (576)	736 (103)	872 (468)	<0.001 <sup>e</sup>
YKL-40 (pg/ml)	122614 (41269)	117682 (38126)	178503 (35233)	185494 (53693)	<0.001 <sup>e</sup>

Unless otherwise specified, values are expressed as mean (SD)

Abbreviations: A $\beta$ : amyloid- $\beta$ ; *APOE*: apolipoprotein E; MMSE: MiniMental State Examination; NfL: Neurofilament light; SCD: subjective cognitive decline.

SNAP: Suspected non-amyloid pathology

<sup>a</sup> ANOVA. TukeyHSD post-hoc: Stage 0 different from Stage 2 ( $p=0.002$ ) and SNAP ( $p=0.023$ )

<sup>b</sup> Chi-squared test

<sup>c</sup> Chi-squared test. Stage 0 different from Stage 2 (uncorrected  $p=0.026$ )

<sup>d</sup> ANOVA

<sup>e</sup> Linear mixed-models. See text for detailed group-by-group comparisons.

## Figure legends

**Figure 1.** Estimated age-related change in biomarker levels for t-tau (A), p-tau (B), NfL (C) and YKL-40 (D).

**Figure 2.** Estimated longitudinal change in biomarker levels for t-tau (A), p-tau (B), NfL (C) and YKL-40 (D).

**Figure 3.** Estimated age-related change in biomarker levels for t-tau (A), p-tau (B), NfL (C) and YKL-40 (D) in the subset of participants stratified into A $\beta$ + or A $\beta$ -.

**Figure 4.** Estimated longitudinal change in biomarker levels for t-tau (A), p-tau (B), NfL (C) and YKL-40 (D) in participants classified according to preclinical AD stages.

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