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# Longitudinal decreases in multiple cerebrospinal fluid biomarkers of neuronal injury in symptomatic late onset Alzheimer's disease

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## Abstract

**Introduction:** Individuals in early stages of Alzheimer's disease are a targeted population for secondary prevention trials aimed at preserving normal cognition. Understanding within-person biomarkers) change over time is critical for trial enrollment and design.

**Methods:** Longitudinal cerebrospinal fluid samples from the Alzheimer's Disease Neuroimaging Initiative were assayed for novel markers of neuronal/synaptic injury (visinin-like protein 1, Ng, and SNAP-25) and neuroinflammation (YKL-40) and compared with β amyloid 42, tau, and phospho-tau181. General linear mixed models were used to compare within-person rates of change in three clinical groups (cognitively normal, mild cognitive impairment, and Alzheimer's disease) further defined by β amyloid status.

**Results:** Levels of injury markers were highly positively correlated. Despite elevated baseline levels as a function of clinical status and amyloid-positivity, within-person decreases in these measures were observed in the early symptomatic, amyloid-positive Alzheimer's disease group.

**Discussion:** Knowledge of within-person biomarker change will impact interpretation of biomarker outcomes in clinical trials that are dependent on disease stage.

### Keywords

Longitudinal biomarkers; Cerebrospinal fluid; Neuronal injury



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Supplementary data

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## 1. Introduction

Clinical trials of potential disease-modifying therapies for Alzheimer's disease (AD) have failed to slow down cognitive decline in patients who have dementia or milder cognitive symptoms (e.g., mild cognitive impairment [MCI]) [1]. Since AD pathology begins to develop ~20 years before cognitive decline (preclinical AD) [2,3], it is possible that trial participants were too far along in the disease process for such therapies to impact cognition. Therefore, individuals at earlier stages, including the asymptomatic and preclinical stage (defined by biomarkers), are now receiving intense focus for secondary prevention trials aimed at preserving normal cognitive function. Understanding the patterns of biomarker(s) change over time, both in asymptomatic and early symptomatic stages, is critical for defining where individuals fall along the pathologic disease cascade.

Cross-sectional studies indicate that β amyloid (Aβ)-related biomarkers become abnormal first, followed by markers of tau-related neuronal injury, both during the preclinical period [4]. Elevated injury markers in the presence of amyloid-positivity then become a strong predictor of subsequent cognitive decline [5]. Interestingly, while regional brain atrophy then ensues, with abnormality increasing with symptomatic progression [6], arecent, albeit small, study of individuals (n = 37) from families at risk for developing autosomal-dominant AD reported longitudinal decreases in cerebrospinal fluid (CSF) levels of neuronal injury markers including total tau (tTau), phospho-tau181 (pTau181), and visinin-like protein 1 (VILIP-1) in symptomatic mutation carriers [7], suggesting a slowing of acute neurodegenerative processes and/or a decrease in the number of viable neurons contributing to the pools of these markers in this later stage of the disease. Regardless of the mechanism, if confirmed in an independent cohort of persons developing late onset AD, such a pattern will likely have an impact on interpretation of biomarker outcomes in clinical trials that is dependent on the disease stage. To this end, the present study evaluated the patterns of within-person longitudinal change in a variety of standard (tTau and pTau181) and novel (VILIP-1, neurogranin [Ng], and synaptosomal-associated protein 25 [SNAP-25]) CSF neuronal injury biomarker levels in individuals spanning the full range of AD, including normal, preclinical AD, MCI due to AD, and symptomatic AD, and a comparison of these changes with regional brain atrophy and cognitive decline.

## 2. Methods

### 2.1. Alzheimer's Disease Neuroimaging Initiative study design

CSF Aβ42, tTau, and pTau181 demographic, imaging, and cognitive data were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adniloni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. ADNI participants have been recruited from more than 50 sites across the USA and Canada. Regional ethical committees of all institutions approved of the study, and all

participants provided written informed consent. For up-to-date information, see www.adni-info.org.

### 2.2. Study participants

The ADNI cohort in the present study consisted of all cognitively normal (CN) participants and those with MCI or AD dementia (AD) with available CSF samples from at least two visits as of April 2012. This cohort included 152 individuals across ADNI1, ADNI GO, and ADNI2 (n = 56 CN, n = 73 MCI, and n = 17 AD). Demographic and cognitive data were downloaded in August 2015 and were collected as described (adni.loni.usc.edu/methods/documents/). By definition, individuals in the CN group all had a clinical dementia rating (CDR) score of 0 at the time of lumbar puncture (LP) and a Mini–Mental State Examination (MMSE) score ≥ 24. Individuals with MCI also scored ≥24 on the MMSE but exhibited subjective memory loss (> 1 standard deviation [SD] below the normal mean of the delayed recall of the Wechsler Memory Scale Logical Memory II), received a CDR of 0.5, and preserved activities of daily living and the absence of dementia. The AD group met the definition of probable AD according to the criteria established by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [8] and had MMSE scores of 20–26 and CDRs of 0.5 or 1. Groups were designated by clinical diagnosis at the time of initial available CSF sample in the longitudinal cohort (defined herein as baseline).

### 2.3. ADNI clinical, CSF and imaging data

Scores for MMSE and Alzheimer's Disease Assessment Scale-cognitive 11 (ADAS11) and ADAS13 were downloaded from the LONI site in August 2015 via ADNIMerge R Package. Values for CSFAβ42 (INNO-BIA AlzBio3;Fujirebio, Ghent, Belgium) were downloaded at the same time from two data sets (UPENNBIOMK4 and UPENNBIOMK6) and were used to define amyloid-positivity based on a published, autopsy-confirmed cutoff value (<192 pg/mL) [9]. For statistical analyses, values for Aβ42, tTau, and pTau181 generated by a single lot number of the novel, fully automated, electrochemiluminescent Elecsys® immunoassays (Roche Diagnostics, Basel, Switzerland) were downloaded from the LONI site in March 2017 from a single data set (UP-ENNBIOMK9). The Elecsys® system aims to offer a fully automated CSF biomarker test for AD capable of achieving *In Vitro* Diagnostic capability and offers some improvements over current Research Use Only assays including the following: reduction in manual steps, improved precision and accuracy both within labs and between labs, and improved lot-to-lot reagent performance. The Elecsys® Aβ42 immunoassay in use is not a commercially available *In Vitro* Diagnostic assay. It is an assay currently under development and used only for investigation purposes. The measuring range of the assay is 200 (lower technical limit)—1700 (upper technical limit) pg/mL. The performance of the assay beyond the upper technical limit has not been formally established. Therefore, values above the upper technical limit have been truncated at 1700 pg/mL. In the present study, baseline analyses excluded these data. Longitudinal statistical analyses were run with and without these truncated values and performed nearly identically.

MRI data for the left and right hippocampal (HP) volume (white matter parcellation) and left and right entorhinal cortex (EC) thickness, two regions known to be affected early in AD,

were also analyzed. EC thickness and HP volume were downloaded in November 2016 from the file UCSFFSL_02_01_16. Acquisition of 1.5 Tesla MRI and data processing methods are as described (adni.loni.usc.edu/methods/mri-analysis/). Data were processed using FreeSurfer, version 4.4, and only values that passed all quality control standards were included in the analyses. Values for left and right HP and EC thickness were added together to create a value for "total" HP volume and EC thickness. In analyses evaluating potential effects of ventricular volume on CSF biomarker concentrations, we created a variable termed " total ventricular volume" by summing left [ST37SV] and right [ST96SV] lateral ventricle, left [ST30SV] and right [ST89SV] inferior lateral ventricle, and third ventricle [ST127SFV] from the ADNI data set so to best capture ventricular volume in its entirety.

### 2.4. Novel CSF analytes

Samples were analyzed for YKL-40 (also known as chitinase 3-like 1, a marker of gliosis/neuroinflammation) [10], VILIP-1 (a neuronal calcium sensor protein and marker of neuronal injury) [11], Ng (a postsynaptic protein and marker of synaptic dysfunction) [12], and SNAP-25 (a presynaptic protein and marker of synaptic dysfunction) [13]. YKL-40 was measured with a plate-based enzyme-linked immunoassay (MicroVue ELISA; Quidel, San Diego, CA) [14]. VILIP-1 [15,16], Ng [17,18], and SNAP-25 were measured using microparticle-based immunoassays using the Singulex (now part of EMD Millipore; Alameda, CA) Erenna system, and employed antibodies developed in the laboratory of Dr. Jack Ladenson at Washington University. All samples (each on the same freeze/thaw cycle) were run in triplicate on a single lot number for VILIP-1, SNAP-25, and Ng and in duplicate for YKL-40. Within-person longitudinal samples were run on the same assay plate to reduce interplate and intraplate variability. Quality control for VILIP-1, SNAP-25, and Ng included analysis of three internal standard CSF pools run on each plate and two internal pools for YKL-40. See Supplementary Text for assay details.

### 2.5. Statistical analysis

Because the study intent was to compare baseline biomarker levels and their longitudinal change over time in individuals who span the AD continuum (from no disease [normal] to preclinical AD, to MCI due to AD, and to AD), participants in the three diagnostic categories (CN, MCI, and AD) were further stratified into β amyloid-positive (Aβ+) versus β amyloid-negative (Aβ−) at baseline based on the published ADNI CSF Aβ42 cutoff of < 192 pg/mL [9]. Baseline characteristics for the five resultant groups (CN−, CN+, MCI−, MCI+, and AD+) were summarized as mean (SD) for continuous variables or number (percentage) for categorical variables. Group differences among the various measures were assessed using one-way analysis of variance and post hoc Tukey tests. Correlations between measures were assessed via Spearman correlation.

Biomarker concentrations, cognitive performance, and MRI measures within individuals over time were compared among the five groups (all AD individuals were Aβ+) by general linear mixed models with random intercepts/slopes at the subject level to allow estimation and comparison of within-person rates of change [19]. In addition to the mean intercept and slope for each group (unadjusted models), covariates including age at baseline, apolipoprotein E (*APOE*) ε4 carriage, sex, education, and ventricular volume, their

interactions with subject groups on the intercepts and slopes, were also included as fixed effects (see Supplementary Text). All general linear mixed models assumed a subject-level random effect on intercept and slope and were fitted using the maximum likelihood method. Statistical tests were based on the approximate F or t-tests with denominator degrees of freedom approximated by the Satterthwaite methods [13]. All analyses were performed using SAS software, version 9.4 (SAS Institute Inc.), with statistical significance defined as $P < .05$.

## 3. Results

### 3.1. Demographics

Of the 152 ADNI participants who met the criteria for having longitudinal CSF samples (range 2–7 LPs over 1–7 years of follow-up [mean (SD) = 4.0 (1.62)] and a mean [SD] LP interval of 16 [8.6] months), four were omitted from the data set due to missing values for CSF Aβ42 (via AlzBio3) required to define baseline amyloid status (Aβ+ vs. Aβ−). Participants in the final data set of n = 148 were 38% female, between 58 and 90 years of age at the time of initial LP (mean [SD] = 75 [7.13]), and 68% were *APOE* ε4-positive (Table 1). All individuals in the MCI group were classified by ADNI as “late MCI”. As expected, baseline HP volume and EC thickness were different among the groups (CN > MCI > AD) ($P < .0001$). Performances on MMSE, ADAS11, and ADAS13 were also as expected, with the MCI and AD groups performing worse than the CN group ($P < .0001$).

When the clinical groups were dichotomized into Aβ+ and Aβ− [9], neuronal injury/inflammation biomarker levels were higher (more AD-like) in the Aβ+ than those in the Aβ− groups, both among and within each clinical group (Table 2). Positive correlations were observed among the injury markers at baseline, strongest among tTau, VILIP-1, and Ng (Spearman r = 0.798–0.853) (Supplementary Table 1). SNAP-25 was moderately correlated with the other injury markers (r = 0.619–0.720), and as expected, tTau and pTau exhibited the highest positive correlation (r = 0.975). Elecsys Aβ42 was positively correlated with AlzBio3 Aβ42 (r = 0.869) and negatively correlated with tTau, pTau, and SNAP-25 (r = −0.214, −0.324 and −0.240, respectively). YKL-40 was significantly, but weakly, correlated with the injury markers (r = 0.307–0.422) but not Aβ42.

### 3.2. Patterns of neuronal injury and neuroinflammatory markers

Participant-level CSF biomarker trajectories were plotted for each of the five amyloid-defined clinical groups (see Supplementary Fig. 1 for spaghetti plots). General linear mixed models (with random intercepts/slopes at the subject level) were then used to estimate and compare baseline biomarker levels and within-person rates of change in the five groups. Results adjusting for sex, *APOE* ε4 status, education, baseline age, and total ventricular volume are provided in the Supplementary Text.

### 3.3. Elecsys® tTau

Baseline tTau levels were significantly elevated in the AD+ group compared with all other groups (all $P \geq .01$) and the MCI+ compared with the MCI− and CN− ($P < .0001$) and CN+ groups ($P = .02$) (Table 2). Longitudinally, tTau levels significantly increased in both CN

(both $P < .05$) and the MCI+ groups ($P < .0001$) (Fig. 1, Table 2) tTau levels decreased longitudinally in the AD+ group, but this change did not reach statistical significance ($P = .095$).

### 3.4. Elecsys® pTau

pTau levels at baseline were significantly elevated in the AD+ compared with all other groups (all $P < .01$), MCI+ compared with MCI− and CN− (both $P < .0001$) and CN+ groups ($P = .02$), and the CN+ compared with the MCI− and CN− groups (both $P < .03$) (Table 2). Longitudinally, pTau levels significantly increased in the CN+ ($P = .001$) and trended toward increase in the MCI+ group ($P = .055$). Strikingly, pTau levels significantly declined in the AD+ group ($P \leq .0001$) (Fig. 1, Table 2), with rate of change greater than the change in all other groups ($P < .001$).

### 3.5. VILIP-1

Levels of baseline VILIP-1 were significantly higher in the MCI+ and AD+ compared with both the MCI− and CN− groups (all $P \leq .01$) (Table 2). The amyloid-positive groups did not differ from one another (all $P > .05$). Longitudinally, as with pTau, VILIP-1 levels strongly and significantly decreased in the AD+ group ($P = .006$), whereas no significant changes were observed in the other groups (Fig. 1, Table 2).

### 3.6. SNAP-25

SNAP-25 values at baseline were significantly higher in the AD+ and MCI+ compared with the CN− (both $P < .0003$), CN+ ($P = .001$ and $P = .01$, respectively), and MCI− groups (both $P < .0001$) (Table 2). Longitudinally, SNAP-25 levels declined significantly in the AD+ group ($P = .05$), whereas no significant changes were observed in the other groups (Fig. 1, Table 2).

### 3.7. Ng

Baseline levels of Ng were significantly higher in the AD+ group than the CN− ($P = .003$), CN+ ($P = .02$), and MCI− ($P = .0006$) groups, although not between the MCI+ and AD+ groups ($P = .10$) (Table 2). Levels were also higher in the MCI+ compared with the CN− ($P = .004$) and MCI− ($P = .02$) groups. Longitudinally, Ng markedly and significantly decreased in the AD+ group ($P < .0001$), whereas no significant changes were observed in the other groups (Fig. 1, Table 2).

### 3.8. YKL-40

In contrast to the markers of neuronal injury, baseline levels and longitudinal patterns of change in the neuroinflammatory marker, YKL-40, exhibited a large degree of within-group variability. Baseline YKL-40 was significantly higher in the AD+ compared with the MCI− ($P = .04$) but not the other groups (Table 2). Longitudinally, all groups showed an increase in mean levels over time, but this increase was statistically significant only in the MCI+ group ($P = .03$) (Fig. 1, Table 2), perhaps due to less variability (smaller SD) within that group.

### 3.9. Elecsys® Aβ42

Although CSF Aβ42 (as measured in ADNI by AlzBio3) was used *a priori* to define amyloid status in the clinical groups, we were also interested in evaluating the patterns of this biomarker using the novel Elecsys® platform. As expected, baseline Aβ42 levels (via Elecsys®) were significantly lower in all Aβ+ than those in Aβ42− groups (all $P < .0001$) (Table 2). Longitudinally, levels decreased in all groups (and at similar rates), although only the AD+ and CN− groups reached statistical significance ($P = .04$ and $P = .0004$, respectively) (Fig. 1, Table 2).

### 3.10. Cognitive measures

As expected, cognitive performance differed with clinical diagnosis, particularly in the Aβ+ symptomatic groups. Furthermore, Aβ+ individuals exhibited longitudinal changes in MMSE and ADAS11/13 that are consistent with a worsening of cognitive performance and often at a faster rate than the Aβ− groups. See Supplementary Fig. 2 for spaghetti plots.

### 3.11. MMSE

Baseline MMSE was lower (indicative of worse performance) in the AD+ group than any other group (all $P < .0001$), lower in the MCI+ compared with the MCI− ($P = .03$) and both CN groups (both $P < .0001$), and in the MCI− compared with both CN groups (both $P < .03$) (Table 3). In the AD+ and MCI+ groups, MMSE was decreasing significantly (both $P < .0001$) and at a faster rate in the AD+ compared with the MCI + group ($P < .0001$) (Table 3).

### 3.12. ADAS11 and ADAS13

At baseline, ADAS11 was significantly elevated (indicating worse performance) in the AD+ compared with both CN groups (both $P < .0001$), both MCI groups compared with both CN groups (both $P < .02$), and in the AD+ compared with both MCI groups (both $P < .0001$) (Table 3). Longitudinally, ADAS11 score significantly increased in the AD+ and MCI+ groups (both $P < .0001$) and at a significantly faster rate in the AD+ versus the MCI+ group ($P < .0001$) (Table 3).

Baseline ADAS13 performance was similar to ADAS11 except that the MCI+ group was also significantly elevated (worse performance) compared with the MCI− group ($P = .05$) (Table 3). Longitudinally, ADAS13 was significantly increasing in all three Aβ+ groups (all $P < .004$), at a faster rate in the AD+ compared with the MCI+ ($P = .0005$) and CN+ ($P < .0001$) groups, and at a faster rate in the MCI+ than the CN+ group ($P = .02$) (Table 3).

### 3.13. Volumetric MRI measures

As expected, HP volume and EC thickness were smaller at baseline in the AD+ than those in the other groups. However, all but the CN− group exhibited significant atrophy over time, albeit at different rates. See Supplementary Fig. 3 for spaghetti plots.

### 3.14. HP volume

HP volume at baseline was significantly smaller in the AD+ compared with all other groups ($P < .001$ for both CN groups; $P = .03$ for both MCI groups) and in both MCI groups

compared with the CN groups (MCI− vs. CN− [$P = .003$] and CN+ [$P = .01$]; MCI+ vs. CN − and CN+ [both $P \leq .0007$]) (Table 3). Longitudinally, all groups exhibited significant HP shrinkage over time (all $P \leq .0001$) (Table 3). Volume in the AD+ and MCI+ groups decreased at a significantly faster rate than in both CN groups ($P \leq .003$ and $P \leq .001$, respectively) and the MCI− group ($P = .04$ and $P = .003$, respectively). The rate of atrophy in the MCI− group was faster than the CN− group ($P = .0009$) and in the CN+ compared with the CN− group ($P = .03$).

### 3.15. Entorhinal cortex thickness

At baseline, EC thickness was significantly smaller in the AD+ compared with all other groups ($P \leq .0003$), in the MCI+ compared with the CN groups ($P = .0004$ for CN− and $P = .01$ for CN+) (Table 3). MCI− was also significantly thinner than the CN− group ($P = .03$) and at the significance level compared with the CN+ group ($P = .05$). Longitudinally, EC thickness was declining in all but the CN− group (all $P \leq .0003$) and at a faster rate in the AD+ compared with the CN+ ($P = .005$) and MCI− ($P = .007$) groups (Table 3). The EC in the MCI+ group was also shrinking more quickly than the CN+ and MCI− groups (both $P \leq .0001$).

## 4. Discussion

Our primary finding is the decrease over time in the concentration of several different CSF markers of neuronal injury (Tau, pTau, VILIP-1, SNAP-25, and Ng) in individuals who had symptomatic AD. In contrast, elevations in tTau, but not the other injury markers, were observed at earlier stages (amyloid-positive MCI and CN groups). Importantly, these findings replicate similar longitudinal patterns (for tTau, pTau, and VILIP-1) reported in a small cohort of individuals with autosomal-dominant AD [7], thus supporting a commonality in neuropathologic processes in sporadic and genetic forms of the disease. Interestingly, reductions in Aβ42 were observed in the CN− group, potentially indicating amyloid deposition in the very earliest stage of disease; other studies have shown that levels of CSF Aβ42 begin to decrease before amyloid being detectable by positron emission tomography and before changes in CSF tau(s) [20,21]. The findings are also similar to the first published study on longitudinal (up to 2 years) Aβ42, tTau, and pTau in ADNI, which showed longitudinal changes in pTau after changes in Aβ42 [20]. Knowledge of such within-person patterns of change has important implications for clinical trials in MCI and early stage AD in terms of the use of biomarker concentrations as pathologic endpoints in determining treatment efficacy for neuronal integrity and is being studied concurrently in related groups such as individuals with Down Syndrome [22]. Furthermore, the combination of CSF biomarkers and other modalities may be of use, even in the preclinical stages of disease, as significant changes in ADAS 13 were seen in the CN+ group.

While all the injury markers decreased over time in the AD+ group, the reduction in Ng was especially robust. Ng is a calmodulin-binding postsynaptic neuronal protein [23,24] thought to be involved in activity-dependent synaptic plasticity and long-term potentiation [25]. Levels are reduced in AD brain [26,27] and elevated in AD CSF [12,28], with high levels predictive of progression from MCI to AD dementia [18,29–31]. Because elevations in CSF

Ng are associated with brain atrophy [18,31] and reduced brain glucose uptake [31], it is considered a marker of synaptic dysfunction/loss.

Although less is known about SNAP-25 (a presynaptic t-SNARE molecule that plays a crucial role in calcium-dependent exocytosis of synaptic vesicles) in AD, like Ng, levels are reduced in brain [32] and elevated in CSF [33] compared with controls. Although both synaptic markers were decreasing longitudinally in the AD+ group, Ng was dropping at more than twice the rate as SNAP-25 (annual decreases of 6.9% vs. 2.5%, respectively) and the other markers (1.8% tTau, 3.9% pTau, and 3.4% VILIP). Interestingly, Aβ42 was also significantly decreasing annually by 5% in the early AD+ group but less so in the other groups. Although levels of Aβ42 are known to drop early in the disease and then plateau as amyloid continues to accumulate [3], 63% (10/16) of individuals in the current AD group were at very early symptomatic stages (CDR 0.5). Baseline levels of YKL-40, an astrocyte-derived protein with presumed neuroinflammatory properties [34], also increased with clinical severity as reported previously [35], but we observed a high level of within-group variability in longitudinal patterns. It is likely that YKL-40 reflects neuroinflam-matory components not specifically due to AD. Interestingly, levels appeared to increase with age in the AD+ group (Supplementary Fig. 1) as has also been observed in CN middle-aged individuals [14]. Further studies regarding the role of YKL-40 in neurodegenerative diseases are warranted [36,37].

Despite the fact that there were strong positive correlations among levels of the various injury markers, consistent with previous reports [18,38], discordance in patterns of longitudinal change over time for tTau was observed in the amyloid-positive MCI group (robust increases in tTau but no statistical change in the other markers, including pTau). CSF tTau levels are known increase in response to acute neuronal death as occurs in response to stroke, traumatic brain injury, and Creutzfeldt-Jakob disease [39], thus suggesting a robust phase of neuronal death and/or alterations in the normal metabolism of tau at the very early (MCI) symptomatic stage of AD, the time during which the first signs of cognitive impairment are evident. The reason(s) for a lack of within-person increase in these other injury markers remains unclear but may have something to do with the relatively short follow-up time in the current cohort (mean 4.0 ± 1.61 years) and/or the lack of information regarding how long a given individual had been in their designated clinical group at the time of baseline LP (i.e., where in the natural progression of the disease), or could conceivably be influenced by the older age of the ADNI cohort (mean baseline age of ~75 years for all groups), as some CSF biomarkers do appear to be age related [14]. Alternatively, such discordance may indicate that these markers reflect different processes associated with synaptic dysfunction and/or neuronal injury [38]. A full understanding of biomarker trajectories will require serial samples being collected from an independent and larger cohort over a long period of time as individuals progress from one disease stage to the next.

The biological reason(s) for reductions in CSF injury markers over time in early AD is unclear. In fact, very little is known about the normal metabolism of these markers that would lead to their appearance in the CSF in both normal and pathological settings. Although it is conceivable that such reductions reflect a dilution of CSF analytes that would come with increasing ventricular volume associated with overall brain atrophy, reductions

were still observed after controlling for ventricular volume (see Supplementary Text). It is possible that longitudinal reductions from an elevated baseline during early AD reflect a slowing of acute neurodegenerative processes with symptomatic disease progression and/or neuronal death, leading to a smaller number of neurons that remain and contribute to the pool in CSF. Unlike structural MRI and amyloid (and tau) positron emission tomography imaging measures that reflect cumulative change over the course of the disease, CSF measures reflect a snapshot in time, thus measuring different things. Indeed, HP and EC atrophy continued over the course of the disease even in the face of decreasing levels of injury markers in the CSF. It is therefore not unexpected that there exists some discordance when defining biomarker positivity (and notably for neuronal injury), as a function of imaging versus CSF [40]. This issue is important to consider when selecting biomarker modalities (CSF and/or imaging) for use in screening and/or outcome measures in clinical trials.

This study is not without limitations. The cohort with longitudinal CSF samples available for analysis was relatively small which, when divided into five groups, may limit statistical power to detect longitudinal changes, especially in the pre-clinical and early symptomatic AD groups, as well as influence the large variability seen when modeling longitudinal slopes. The distribution of males and females was also skewed in this cohort, with roughly 62% of participants being male. Although serial LP follow-up was longer than that in some previous longitudinal ADNI CSF studies [41,42], it was still relatively short (3–5 years). Also, despite the groups being dichotomized as amyloid-positive versus -negative to ascertain plaque status in the clinical groups, there was considerable overlap in clinical and biomarker patterns between individuals, especially in the MCI and AD groups. Finally, due to the small numbers of individuals in the clinical/biomarker groups and the unique biological traits captured by the different biomarkers that may contribute independently to the overall disease process, statistical models were not adjusted rigorously for multiple comparisons. However, this approach could potentially result in inflated type I errors, so interpretation should be made with caution.

## 5. Conclusions

The present results underscore the importance of evaluation of true longitudinal, serial measures of CSF biomarkers from individuals as they progress through the normal course of the disease as opposed to the more traditional approach of inferring longitudinal change by comparing cross-sectional data from groups of individuals at different disease stages. Indeed, concentrations of each of the markers have been reported to be elevated in AD compared with MCI and CN controls [35]. While we also observed such elevations in baseline levels of these injury markers among the different clinical/amyloid groups, the within-person patterns of change over time were different. For clinical trial purposes, given the stage-specific differences in the direction of true longitudinal change in these biomarkers, a “positive” biomarker outcome would be different depending on the characteristics of the trial cohort. For example, a slowing of the course of neuronal injury may be indicated by a slowing of the rate of increase in CSF tau in individuals who are early in the disease process (MCI), but perhaps a stabilization or even a slowing or reversal of the downward trajectory later in the disease (mild AD), potentially reflected as a longitudinal

increase or as no decrease in this marker. Such possibilities warrant consideration in clinical trial design.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of interest: C.L.S., L.M., and E.M.H. report no conflicts. C.H. is supported by NIH grants including P50AG005681, P01AG003991, P01AG026276, R01AG034119, and R01AG053550. He reports no conflicts. J.H.L. is named on patents related to biomarkers for Alzheimer's disease. These patents and any resulting licenses are being administered by Washington University in accordance with University policies. D.M.H. is supported by NIH grants including P50AG005681, P01AG003991, and P01AG026276. He is on the scientific advisory board of C2N Diagnostics. He is a consultant over the last year for Genentech, AbbVie, Neurophage, Denali, and Eli Lilly. He reports no conflicts. A.M.F. is supported by the NIH grants, including P50AG005681, P01AG003991, P01AG026276, and UF01AG03243807. She is on the Scientific Advisory Boards for Roche Diagnostics, IBL International, and AbbVie and consults for Biogen, DiamiR, LabCorp, and Araclon Biotech/Grifols. She reports no conflicts.

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## RESEARCH IN CONTEXT

1. 1. Systematic review: The authors reviewed the literature using PubMed. Alzheimer's disease biomarkers have been instrumental in understanding Alzheimer's disease as a continuum in which pathologies begin to develop 10–20 years before dementia onset. As clinical trials of potential disease-modifying therapies are focusing on early disease stages, elucidating within-person biomarker change over time is critical for defining where individuals fall along the disease cascade. Crosssectional studies report increases in neuronal injury markers in cerebrospinal fluid with increasing symptom severity, assessments of longitudinal change within individuals are scarce.
2. 2. Interpretation: Our findings of within-person reductions over time in several neuronal injury markers in early symptomatic Alzheimer's disease will likely have an impact on interpretation of biomarker outcomes in clinical trials, and thus, should be considered in trial design.
3. 3. Future directions: Evaluation of within-person change in cerebrospinal fluid biomarkers in a larger, independent cohort that has longer follow-up is needed to confirm our findings.

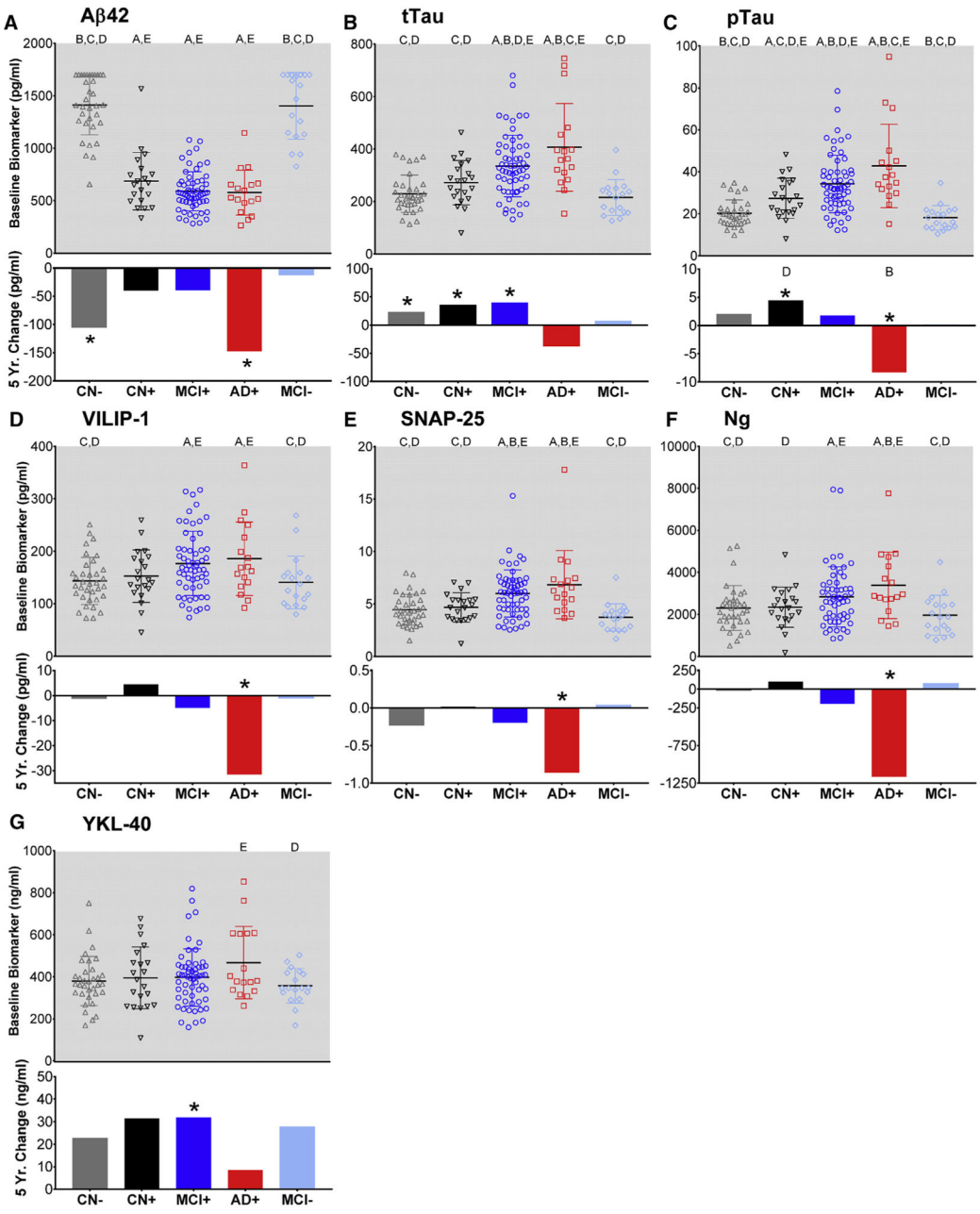


**Fig. 1.**

Baseline concentrations and estimated within-person 5-year change in CSF biomarkers. Baseline biomarker concentrations (top, gray panel) and estimated group slopes (bottom, white panel) for Aβ42 (A), tTau (B), pTau (C), VILIP-1 (D), SNAP-25 (E), Ng (F), and YKL-40 (G). Baseline is shown for each individual, estimated group slopes of average annual change in five bins defined by diagnostic group and amyloid status are extrapolated to show 5 years of change. [A] Different from CN− group, [B] Different from CN + group, [C] Different from MCI + group, [D] Different from AD + group, [E] Different from MCI− group, * Different from 0. Abbreviations: Aβ; β amyloid; tTau, total tau; pTau, phospho-tau;

VILIP-1, visinin-like protein 1; SNAP-25, synaptosomal-associated protein 25; Ng, neurogranin; YKL-40, chitinase-3 like-1.

## Table 1

Study demographics

| | CN | | MCI | | AD |
|---|---|---|---|---|---|
| **Characteristics** | **Aβ−** | **Aβ+** | **Aβ−** | **Aβ+** | **Aβ+** |
| N | 35 | 21 | 18 | 58 | 16 |
| Baseline age, mean (SD) | 76 (5.7) | 76 (3.7) | 77 (7.3) | 74 (6.5) | 74 (6.7) |
| Gender, F/M (%F) | 14/21 (40) | 10/11 (48) | 4/14 (22) | 18/40 (31) | 11/6 (65) |
| Education, mean (SD), y | 16 (3.1) | 16 (3.4) | 17 (1.8) | 16 (2.8) | 15 (3.0) |
| *APOE* ε4 allele, ± (%+) | 3/32 (9) | 9/12 (43) | 0/18 (0) | 40/18 (69) | 13/4 (77) |
| # CDR 0/0.5/1, n | 35/0/0 | 21/0/0 | 0/18/0 | 0/57/1 | 0/10/6 |
| CDR-SB, mean (SD) | 0.029 (0.12) *,†,‡ | 0.024 (0.11) *,†,‡ | 1.25 (0.55) ‡,§,¶ | 1.61 (0.85) ‡,§,¶ | 4.24 (1.49) *,‡,§,¶ |
| MMSE, mean (SD) | 29.1 (1.1) *,†,‡ | 29.4 (0.9) *,†,‡ | 27.6 (1.8) ‡,§,¶ | 26.8 (1.8) ‡,§,¶ | 23.7 (1.7) *,‡,§,¶ |
| ADAS11, mean (SD) | 5.3 (2.2) *,†,‡ | 7.1 (3.3) †,‡ | 9.9 (4.1) ‡,§ | 11.7 (5.1) ‡,§,¶ | 18.7 (6.1) *,‡,§,¶ |
| ADAS13, mean (SD) | 8.4 (3.5) *†,‡ | 10.5 (3.9) †,‡ | 15.5 (5.9) ‡,§ | 19.5 (7.1) ‡,§,¶ | 28.9 (7.4) *,‡,§,¶ |
| # LP's 2/3/4/5/6/7, n | 0/15/7/8/5/0 | 0/8/6/4/3/0 | 0/5/10/2/1/0 | 2/26/18/5/6/1 | 1/9/5/1/0/0 |
| LP interval, mean (SD), mo | 17.01 (9.44) ‡ | 17.55 (10.40) ‡ | 16.92 (8.98) | 15.90 (7.92) | 12.73 (2.86) §,¶ |
| LP follow-up, mean (SD, range), mo | 52.9 (19.7, 23-86) ‡ | 55.0 (17.0, 26-85) ‡ | 49.8 (17.9, 24-87) ‡ | 45.0 (18.9, 16-86) ‡ | 30.2 (10.2, 12-50) *,†,§,¶ |
| Total EC thickness, mean (SD), mm | 6.88 (0.84) *,†,‡ | 6.88 (0.95) *,†,‡ | 6.32 (0.96) ‡,§,¶ | 6.44 (0.87) ‡,§,¶ | 5.26 (0.82) *,†,§,¶ |
| Total HP volume, mean (SD), $mm^3$ | 6577 (815) *,†,‡ | 6553 (886) *,†,‡ | 5818 (978) ‡,§,¶ | 5861 (880) ‡,§,¶ | 5117 (848) *,†,§,¶ |

Abbreviations: Aβ, amyloid β status; AD, Alzheimer's disease; ADAS 11, Alzheimer's Disease Assessment Scale-cognitive test, version 11 (higher score is worse performance); ADAS 13, Alzheimer's Disease Assessment Scale-cognitive test, version 13 (higher score is worse performance); *APOE*, apolipoprotein E; CDR, Clinical Dementia Rating score; CDR-SB, CDR sum of boxes; CN, cognitively normal; EC, entorhinal cortex; HP, hippocampus; LP, lumbar puncture; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination.

*Significantly different from MCI Aβ−.

†Significantly different from MCI Aβ+.

‡Significantly different from AD Aβ+.

§Significantly different from CN Aβ−.

¶Significantly different from CN Aβ+.

## Table 2

Baseline CSF biomarker levels and estimated within-person annual change over time

| | CN | | MCI | | AD |
|---|---|---|---|---|---|
| **Characteristics** | **Aβ−** | **Aβ+** | **Aβ−** | **Aβ+** | **Aβ+** |
| N | 35 | 21 | 18 | 58 | 16 |
| Baseline CSF Biomarkers | | | | | |
| Elecsys Aβ42, mean (SD), pg/mL | 1413 (284)[*,†,‡] | 687 (274)[§,¶] | 1404 (318)[*,†,‡] | 590 (187)[§,¶] | 578 (214)[§,¶] |
| Elecsys tTau, mean (SD), pg/mL | 230 (70.8)[†,‡] | 272 (84.9)[†,‡] | 215 (68.2)[†,‡] | 331 (117.5)[*,‡,§,¶] | 407 (167.5)[*,‡,§,¶] |
| Elecsys pTau, mean (SD), pg/mL | 20.3 (6.30)[*,†,‡] | 27.4 (9.56)[†,‡,§,¶] | 18.1 (5.83)[*,†,‡] | 33.7 (13.62)[*,‡,§,¶] | 42.8 (19.90)[*,‡,§,¶] |
| VILIP-1, mean (SD), pg/mL | 143.3 (44.9)[†,‡] | 152.6 (49.8) | 140.5 (50.2)[†,‡] | 176.7 (61.0)[§,¶] | 185.6 (70.1)[§,¶] |
| SNAP-25, mean (SD), pg/mL | 4.45 (1.5)[†,‡] | 4.66 (1.4)[†,‡] | 3.72 (1.3)[†,‡] | 6.01 (2.2)[*,§,¶] | 6.84 (3.3)[*,§,¶] |
| Ng, mean (SD), pg/mL | 2302 (1066)[†,‡] | 2339 (953)[‡] | 1962 (945)[†,‡] | 2836 (1426)[§,¶] | 3383 (1576)[*,§,¶] |
| YKL-40, mean (SD), ng/mL | 384.1 (20.08) | 399.6 (19.4) | 361.6 (19.4)[‡] | 401.3 (17.87) | 471.9 (41.86)[¶] |
| CSF Biomarker Estimated Annual Slope | | | | | |
| Elecsys Aβ42, pg/mL (SE) | **−20.91 (5.6)** | −7.96 (7.27) | −2.38 (8.4) | −7.82 (5.17) | **−29.48 (14.2)** |
| *P* value | **.0004**[//] | .28 | .78 | .13 | **.039**[//] |
| Elecsys tTau, pg/mL (SE) | **4.29 (2.1)**[‡] | **6.75 (2.7)**[‡] | 1.10 (3.1) | **7.55 (1.8)**[‡] | −7.11 (4.2)[*,†,§] |
| *P* value | **.048**[//] | **.015**[//] | .72 | <**.0001**[//] | .095 |
| Elecsys pTau, pg/mL (SE) | 0.39 (0.2)[‡] | **0.88 (0.3)**[‡,¶] | 0.028 (0.3)[*,‡] | 0.35 (0.2) | **−1.65 (0.4)**[*,†,§,¶] |
| *P* value | .69 | **.013**[//] | .93 | .055 | <**.0001**[//] |
| VILIP-1, pg/mL (SE) | −0.23 (1.0)[‡] | 0.89 (1.2)[‡] | −0.21 (1.4)[‡] | −0.96 (0.9)[‡] | **−6.31 (2.3)**[*,†,§,¶] |
| *P* value | .81 | .48 | .88 | .27 | **.006**[//] |
| SNAP-25, pg/mL (SE) | −0.0453 (0.042) | 0.00279 (0.053) | 0.00715 (0.060) | −0.0387 (0.037) | **−0.172 (0.088)** |
| *P* value | .28 | .96 | .91 | .29 | **.05**[//] |
| Ng, pg/mL (SE) | −2.74 (26.1)[‡] | 19.88 (33.6)[‡] | 15.5521 (38.2)[‡] | −38.6334 (23.6)[‡] | **−232.43 (58.9)**[*,†,§,¶] |
| *P* value | .92 | .56 | .68 | .10 | <**.0001**[//] |
| YKL-40, ng/mL (SE) | 4.51 (3.5) | 6.29 (4.3) | 5.54 (4.9) | **6.37 (3.0)** | 1.68 (7.1) |
| *P* value | .20 | .15 | .26 | **.035**[//] | .81 |

Abbreviations: Aβ, amyloid β status; AD, Alzheimer disease; CN, cognitively normal; MCI, mild cognitive impairment; Ng, neurogranin; pTau, phosphotau181; SNAP-25, synaptosomal-associated protein 25; tTau, total tau; VILIP-1, visinin-like protein 1.

Bold–Slope that is statistically different from zero.

NOTE. All significance at least $P < .05$.

[*] Significantly different from CN Aβ+.

[†] Significantly different from MCI Aβ+.

[‡] Significantly different from AD Aβ+.

[§] Significantly different from CN Aβ−.

[¶] Significantly different from MCI Aβ−.

[//] Statistically significant slope.

## Table 3

Baseline cognitive performance and imaging measures and estimated within-person annual change over time

| | CN | | MCI | | AD |
|---|---|---|---|---|---|
| **Characteristics** | **Aβ−** | **Aβ+** | **Aβ−** | **Aβ+** | **Aβ+** |
| N | 35 | 21 | 18 | 58 | 16 |
| Baseline Cognitive and Imaging Biomarkers | | | | | |
| MMSE, mean (SD) | 29.1 (1.1) *,†,‡ | 29.4 (0.9) *,†,‡ | 27.6 (1.8) †,‡,§,¶ | 26.8 (1.8) *,‡,§,¶ | 23.7 (1.7) *,†,§,¶ |
| ADAS 11, mean (SD) | 5.3 (2.2) *,†,‡ | 7.1 (3.3) *,†,‡ | 9.9 (4.1) ‡,§,¶ | 11.7 (5.1) ‡,§,¶ | 18.7 (6.1) *,†,§,¶ |
| ADAS 13, mean (SD) | 8.4 (3.5) *,†,‡ | 10.5 (3.9) *,†,‡ | 15.5 (5.9) †,‡,§,¶ | 19.5 (7.1) *,‡,§,¶ | 28.9 (7.4) *,†,§,¶ |
| Total EC thickness, mean (SD), mm | 6.88 (0.84) *,†,‡ | 6.88 (0.95) *,†,‡ | 6.32 (0.96) ‡,§,¶ | 6.44 (0.87) ‡,§,¶ | 5.26 (0.82) *,†,§,¶ |
| Total HP volume, mean (SD), $mm^3$ | 6577 (815) *,†,‡ | 6553 (886) *,†,‡ | 5818 (978) ‡,§,¶ | 5861 (880) ‡,§,¶ | 5117 (848) *,†,§,¶ |
| Cognitive and Imaging Estimated Annual Slope | | | | | |
| MMSE, points (SE) | −0.051 (0.2) †,‡ | −0.22 (0.2) †,‡ | −0.039 (0.2) †,‡ | **−1.26 (0.1)** *,‡,§,¶ | **−2.49 (0.3)** *,†,§,¶ |
| *P* value | .76 | .30 | .87 | **<.0001** // | **<.0001** // |
| ADAS 11, points (SE) | 0.20 (0.3) †,‡ | 0.75 (0.4) †,‡ | 0.30 (0.4) †,‡ | **2.06 (0.3)** *,‡,§,¶ | **4.74 (0.6)** *,†,§,¶ |
| *P* value | .52 | .06 | .50 | **<.0001** // | **<.0001** // |
| ADAS 13, points (SE) | 0.37 (0.3) †,‡ | **1.25 (0.4)** †,‡ | 0.53 (0.5) †,‡ | **2.43 (0.3)** *,‡,§,¶ | **4.98 (0.7)** *,†,§,¶ |
| *P* value | .27 | **.0042** // | .27 | **<.0001** // | **<.0001** // |
| Total EC thickness, mm (SE) | −0.0401 (0.022) *,†,‡,¶ | **−0.118 (0.023)** †,‡,§ | **−0.118 (0.031)** †,‡,§ | **−0.261 (0.018)** *,§,¶ | **−0.295 (0.057)** *,§,¶ |
| *P* value | .069 | **<.0001** // | **.0003** // | **<.0001** // | **<.0001** // |
| Total HP volume, $mm^3$ (SE) | **−59.4 (14.5)** *,†,‡,¶ | **−111.2 (18.2)** †,‡,§ | **−145.9 (20.5)** †,‡,§ | **−216.3 (11.9)** *,§,¶ | **−230.8 (36.0)** *,§,¶ |
| *P* value | **.0001** // | **<.0001** // | **<.0001** // | **<.0001** // | **<.0001** // |

Abbreviations: Aβ, amyloid β status; AD, Alzheimer disease; ADAS 11; Alzheimer's Disease Assessment Scale-cognitive test, version 11 (higher score is worse performance); ADAS 13, Alzheimer's Disease Assessment Scale-cognitive test, version 13 (higher score is worse performance); CN, cognitively normal; EC, entorhinal cortex; E-pTau, ElecsyspTau181; HP, hippocampal; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination (0-30, with 30 as perfect score).

Bold–Slope that is statistically different from zero.

NOTE. All significance at least $P < .05$.

*Significantly different from MCI Aβ−.

†Significantly different from MCI Aβ+.

‡Significantly different from AD Aβ+.

§Significantly different from CN Aβ−.

¶Significantly different from CN Aβ+.

//Statistically significant slope.