Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 2 (2016) 27-38

Alzheimer's & Dementia

CSF Biomarkers

Cerebrospinal fluid ratios with $A\beta_{42}$ predict preclinical brain β-amyloid accumulation

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Abstract

Introduction: Biomarkers are urgently needed for the critical yet understudied preclinical stage of Alzheimer's disease (AD).

Methods: Cerebrospinal fluid (CSF) collection, [C-11]Pittsburgh compound B (PiB) amyloid imaging, and magnetic resonance imaging were acquired in 104 cognitively healthy adults enriched with risk for sporadic AD. Image-derived cerebral β-amyloid (Aβ) burden, measured concurrently and longitudinally, was regressed on CSF measures of Aβ, neural injury, and inflammation, as well as ratios with $A\beta_{42}$. Linear mixed-effects regression was used to model the effect of the CSF measures that predicted longitudinal brain amyloid accumulation on longitudinal cognitive decline, measured by memory test scores.

Results: At baseline, $A\beta_{42}/A\beta_{40}$ and all CSF ratios to $A\beta_{42}$ were associated with PiB binding in AD-vulnerable regions. Longitudinally, $A\beta_{42}/A\beta_{40}$ and ratios of total tau (t-tau), phosphorylated-tau (p-tau), neurofilament light protein, and monocyte chemoattractant protein-1 to $A\beta_{42}$ were associated with increased Aβ deposition over 2 years, predominantly in lateral parietal and temporal cortex. However, these CSF ratios were not significantly associated with cognitive decline, and the effect seems to be largely driven by $A\beta_{42}$ in the denominator.

Discussion: These results corroborate previous findings that t-tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ are the strongest candidate biomarkers during the preclinical time frame. They support a framework in which neural injury and amyloid deposition are likely occurring simultaneously. It may be that neurodegenerative processes influence progressive amyloid accumulation, even in the preclinical

H.A.R. has a pending patent for an magnetic resonance imaging pulse sequence. K.B. reports personal fees associated with Advisory Boards from IBL International, Eli Lilly, and Roche Diagnostics. S.A. reports grants during the conduct of the study from NIH/NIA, which support the Alzheimer's Disease Research Center, and grants from Merck Pharmaceutical and ADCS/Toyoma Phramaceutical for activities outside the submitted work. All other authors have no conflicts of interest to disclose.

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http://dx.doi.org/10.1016/j.dadm.2015.11.006

time frame. CSF biomarkers for nonspecific axonal injury and inflammation may provide more information at more advanced stages of the preclinical time course.

Keywords: Amyloid; PiB; PET; Cerebrospinal fluid; Biomarkers; Preclinical AD; Alzheimer's disease; Biological parametric mapping; Aβ42; Tau; NFL; MCP-1; YKL-40; Linear mixed-effects

1. Introduction

Although it is widely accepted that Alzheimer's disease (AD) pathology, including β-amyloid (Aβ) deposition in plaques and microvessels and tau pathology in the form of neurofibrillary tangle formation, begins decades before symptom onset [1–4], there is a paucity of longitudinal research in the late mid-life time frame [1] necessary to establish this effect empirically. Greater understanding of biomarkers associated with these hallmark features of AD, including tau and Aβ proteins in cerebrospinal fluid (CSF) and in-vivo neuroimaging measures of Aβ burden, during preclinical stages is important for early detection and future treatment and prevention efforts [1,2,5].

The most widely accepted model of AD etiology proposes that amyloid deposition in the brain is an early and critical step in driving the pathophysiological processes of AD that in turn initiate neurodegeneration, in the form of synaptic failure and neuronal death, and eventual symptom manifestation [1,6,7]. Increasing evidence suggests that amyloid may be necessary—although even this is contested by some [8]—but not sufficient for developing AD [6,8–11]. Aβ may be neither the primary nor the only neurotoxin that causes AD; but it is likely the key initiator of many complex, often tau-dependent, pathologic changes in the brain culminating in neurodegeneration years later [12]. In accordance with this theory, recent in vitro and in vivo work demonstrates a dynamic positive feed-forward mechanism whereby Aβ drives the disease pathway through tau, and tau further increases Aβ levels [13]. Aβ imaging by itself is not sufficient for a clinical prognosis in the preclinical time frame, and it may be that the most promising AD biomarkers will encompass multiple aspects of the disease, including amyloid and tau-mediated neural injury.

Because of the molecular exchange of metabolites between the brain and CSF, CSF analytes reflect biochemical and pathologic processes in the brain [5,14,15], thereby providing means for examining multiple indicators of disease processes occurring in the central nervous system. CSF Aβ and tau have high diagnostic accuracy for AD [16], but their reliability for the detection of possible preclinical AD is still unclear.

To examine features associated with spatial amyloid binding and longitudinal accumulation in the preclinical time frame, we conducted a multimodal study with Pittsburgh compound B (PiB) positron emission tomography (PET) imaging and CSF biomarkers in a late middle-aged, cognitively healthy sample enriched with the risk factors of apolipoprotein E ε4 (*APOE* ε4) genotype and parental history of sporadic AD from the Wisconsin Registry for Alzheimer's Prevention (WRAP; [17]). Our study had three aims as follows: (1) investigate relationships between amyloid pathology in the brain and concurrent CSF biomarkers associated with amyloid deposition, neuronal injury, and inflammation; (2) determine whether CSF biomarker levels at baseline lumbar puncture (LP) predict changes in PiB amyloid deposition over a subsequent 2-year period; and (3) investigate relationships between CSF predictors of brain Aβ and longitudinal cognitive decline.

We hypothesized that CSF biomarkers of AD pathology including amyloid burden (lower $A\beta_{42}$), tangle pathology (elevated phosphorylated-tau [p-tau]), axonal injury (elevated total-tau [t-tau] and neurofilament light protein [NFL]), and microglial activation/inflammation (elevated monocyte chemoattractant protein 1 [MCP-1] and chitinase-3–like protein [YKL-40]) would be associated with greater amyloid deposition at baseline and greater longitudinal amyloid accumulation over 2 years. We further hypothesized that $A\beta_{42}$ ratios (e.g., t-tau/$A\beta_{42}$) would most likely be associated with cognitive decline, as ratios reflect multiple AD-related pathologies simultaneously, suggesting greater risk for imminent disease.

2. Material and methods

Extended materials and methods are included as Supplementary Material.

2.1. Participants

Participants in this study were recruited from WRAP [17] if they had participated in biomarkers substudies. For this analysis, subjects were included if they had one or more amyloid and magnetic resonance imaging (MRI) scans, and CSF collected at the time of the first amyloid scan, resulting in a sample of 104. The University of Wisconsin Institutional Review Board approved all study procedures, and each subject provided signed informed consent before participation.

2.2. Biomarker collection

All participants underwent baseline MRI, [C-11]PiB PET, and LP as described previously [18,19]. A total of 78 additionally underwent a second PiB scan approximately 2 years later. The first visit at which amyloid scans, MRI

scans, and CSF were obtained is referred to as baseline or visit 1; the second imaging visit is referred to as follow-up or visit 2. PiB distribution volume ratio (DVR) maps were used as the amyloid variable for all voxel-wise analyses. A composite measurement of global amyloid derived from eight bilateral regions of interest (ROIs) was calculated for visit 1 and visit 2 as described previously [20] and is henceforth referred to as PiB burden.

CSF measures were selected based on their ability to detect known pathology in AD including amyloid deposition ($A\beta_{42}$), neurofibrillary tangles (p-tau), neuronal damage (t-tau and NFL), and inflammation (MCP-1 and YKL-40). We also examined the most commonly described CSF ratios of p-tau/$A\beta_{42}$, t-tau/$A\beta_{42}$, and $A\beta_{42}/A\beta_{40}$ as well as more novel ratios of NFL, MCP-1, and YKL-40 to $A\beta_{42}$. To ensure that these ratios truly reflect cumulative pathology, we additionally examined the inverse of $A\beta_{42}$ ($1/A\beta_{42}$) to mimic the effect of CSF $A\beta_{42}$ in the denominator without a marker of pathology in the numerator.

2.3. Cognitive data measures and collection

At each WRAP visit, participants completed a comprehensive neuropsychological battery. To investigate the relationship between CSF biomarkers and episodic memory performance, we selected the delayed memory scores from the Rey auditory verbal learning test (RAVLT) and the Wechsler memory scale-revised (WMS-R) [21–24]. For RAVLT, which was initiated at the first wave of WRAP neuropsychology data collection, one participant had two time points, 25 had three time points, 52 had four time points, and 25 had five time points. For WMS-R, which was initiated at the second wave, 25 participants had two time points, 52 had three time points, and 25 had four time points.

2.4. Statistical analyses

Separate models were run for each CSF measure unless otherwise stated. Covariates always include age at LP, sex, parental family history (FH), and *APOE* ε4.

For models run in Statistical Package for the Social Sciences (SPSS) 22, significance is inferred at $P < .05$, adjusted for multiple comparisons with false discovery rate (FDR) correction [25]. Regressions were hierarchical such that the model's first step includes only covariates and the second step adds the individual CSF measure. R^2 change is calculated as the difference between the R^2 of the first and second steps of the model. Effect sizes were calculated using Cohen's f^2 for hierarchical multiple regression; effect sizes of 0.02, 0.15, and 0.35 are interpreted as small, medium, and large, respectively. Results are visualized using partial regression plots, which are scatter plots of residuals from regressing PiB burden and the CSF variable on all other predictors.

2.4.1. Concurrent CSF measures and amyloid

2.4.1.1. PiB burden

PiB burden at the initial scan was entered as the dependent variable in a multiple regression model in SPSS 22, and individual CSF measures were entered as the independent variable of interest, along with covariates.

To minimize effects of multicollinearity among CSF measures, a separate model was run for each CSF analyte or ratio, adjusting for covariates. However, AD is a multifaceted disease and ratios are only able to capture two pathologic features. Therefore, we also examined a comprehensive regression model with all CSF biomarkers with the exception of p-tau because it was highly correlated with t-tau (Spearman's $\rho = .881$). Using baseline PiB burden as the outcome, we performed hierarchical regression with z-scores of CSF t-tau, MCP-1, YKL-40, NFL, and $A\beta_{42}$ in addition to the standard covariates to determine the additional predictive power of each biomarker to the overall model. For each of these CSF measures, a model would include all covariates and four of the five CSF variables as the first step and then the fifth CSF measure would be added as the second step. Standardized rather than unstandardized β-coefficients are reported for easier comparison of contributions of each variable to the model.

2.4.1.2. Regional Aβ

A multiple regression framework in statistical parametric mapping (SPM) 12 (http://www.fil.ion.ucl.ac.uk/spm/) was used to assess relationships between CSF markers associated with AD pathology and amyloid in the brain. Baseline PiB DVR images were entered as the dependent variable, and individual CSF measures were input as the independent variable of interest in addition to the standard covariates. Analyses were restricted to the cerebral gray matter using a template-based mask. Significance was inferred at the voxel peak-level when $\alpha < 0.05$ with multiple comparisons by family-wise error (FWE) correction and a cluster extent > 100 voxels.

2.4.2. Baseline CSF measures and longitudinal amyloid

Although there are many ways to approach longitudinal data analysis with two time points, we chose a standard approach of regressing a follow-up variable on the baseline variable and covariates [26,27]. This method statistically controls for variance in the baseline state, enabling the interpretation of variables affecting follow-up state independent of baseline.

2.4.2.1. PiB burden

PiB burden at visit 2 was entered as the dependent variable in a multiple regression model in SPSS 22. In addition to the standard set of covariates, we also controlled for PiB burden at visit 1 and then looked at the effect of the CSF analyte. By controlling for amyloid load at visit 1 and looking at amyloid load at visit 2 as the outcome, the coefficient on an individual CSF measure is interpreted as the effect of that CSF measure on longitudinal amyloid load, controlling

for baseline amyloid load. Amyloid scans occurred about 2 years apart (mean = 25.47 months; standard deviation = 2.37 months; range = 21–33 months) because the study was designed to keep this interval uniform, and variation was relatively symmetrical, it was not included as a covariate in the regression models.

We repeated the hierarchical regression model, which included the five CSF measures (t-tau, MCP-1, YKL-40, NFL, and $A\beta_{42}$) simultaneously, for longitudinal PiB burden. Each model included the standard covariates as well as baseline PiB burden, and four of the five CSF variables as the first step; then the fifth CSF measure was added as the second step. Standardized rather than unstandardized β-coefficients are reported for easier comparison of contributions of each variable to the model.

2.4.2.2. *Regional Aβ*

To gain spatial resolution on findings from the mentioned analyses, regressions using biological parametric mapping, an SPM5 toolbox for multimodal image analysis based on a voxel-wise use of the general linear model [28], were performed on CSF measures that were associated with longitudinal PiB burden. The PiB DVR scan from the second visit was the dependent variable and the PiB DVR scan from the first visit was used as an imaging covariate in addition to five nonimaging covariates: age, sex, *APOE* ε4, FH, and CSF biomarker level. Because visit 1 amyloid burden was such a strong predictor of visit 2 amyloid burden, we chose a more moderate threshold for the resultant longitudinal statistical maps of $\alpha < 0.001$ (uncorrected) together with a cluster extent >250 voxels; however, our primary inference was still at the peak voxel where significance was again evaluated at α(FWE) <0.05.

2.4.3. *CSF measures and cognitive decline*

Linear mixed-effects regression was used to model the effect of the CSF biomarkers that predicted longitudinal Aβ accumulation on longitudinal cognitive decline measured by tests of delayed recall. First, unconditional means models adjusting for random effects were examined using unstructured covariance structure. Next, conditional models were run which included significant random effects plus fixed effects of sex, *APOE* ε4, FH, interval between first cognitive evaluation and LP (months), literacy (time 1 Wide Range Achievement Test III reading scores), CSF biomarker level, time (age at each visit), and the interaction of time × CSF measure (slope).

3. Results

3.1. *Sample characteristics*

Sample characteristics are summarized in Table 1.

Table 1
Sample characteristics

Demographics	Range		Mean (SD) or %
Age at LP (years)	49.04–70.96		61.15 (5.58)
Sex (% female)			66%
APOE ε4* (% positive)			38%
FH (% positive)			77%
Education (years)	12–21		16.63 (2.47)
PiB burden	**Range**	**n**	**Mean (SD)**
PiB visit 1	0.995–1.68	104	1.17 (0.15)
PiB visit 2	0.991–1.83	78	1.17 (0.17)
PiB burden change from visit 1 to visit 2†	−0.12 to 0.20	78	0.0047 (0.059)
CSF measures (ng/L)	**n PiB visit 1, n PiB visit 2**		**Mean (SD)‡**
Amyloid			
$A\beta_{42}$	103, 78		735.91 (205.06)
$A\beta_{42}/A\beta_{40}$	104, 78		0.096 (0.018)
$1/A\beta_{42}$	101, 76		0.0014 (0.0004)
Neural injury			
T-tau	103, 77		317.50 (112.53)
P-tau	102, 76		43.09 (13.77)
NFL	102, 77		597.21 (185.06)
T-tau/$A\beta_{42}$	100, 75		0.440 (0.191)
P-tau/$A\beta_{42}$	99, 75		0.060 (0.024)
NFL/$A\beta_{42}$	99, 76		0.844 (0.317)
Inflammation			
MCP-1	104, 78		558.27 (131.56)
YKL-40	102, 76		139,470.69 (41,248.72)
MCP-1/$A\beta_{42}$	101, 76		0.810 (0.317)
YKL-40/$A\beta_{42}$	99, 74		194.11 (60.91)
Intervals	**Range**		**Mean (SD)**
PiB visit 1 to visit 2 (months)	21–33		25.47 (2.37)
PiB visit 1 to LP (days)	−57 to 68		−1.35 (18.32)
PIB visit 1 to NP testing wave 1 (months)	0–107		70.24 (19.88)
First RAVLT testing (wave 1) to LP (months)	36–109		70.29 (19.88)
First WMS-R testing (wave 2) to LP (months)	−11 to 58		20.67 (17.04)

Abbreviations: SD, standard deviation; LP, lumbar puncture; *APOE* ε4, possession of apolipoprotein E ε4 allele; FH, parental family history; PiB, Pittsburgh compound B amyloid imaging; CSF, cerebrospinal fluid; Aβ, beta-amyloid; t-tau, total tau; p-tau, phosphorylated-tau; NFL, neurofilament light protein; MCP-1, monocyte chemoattractant protein 1; YKL-40, chitinase-3–like protein; NP, neuropsychology; RAVLT, Rey auditory verbal learning test; WMS-R, Wechsler memory scale-revised.

**APOE* ε4 allele breakdown: 38% *APOE* ε4; n = 13 ε2/ε3, n = 52 ε3/ε3, n = 3 ε2/ε4, n = 35 ε3/ε4, and n = 1 ε4/ε4.

†PiB burden change is displayed for descriptive purposes only, it was not used in any statistical analyses.

‡CSF was only collected once, mean and SD values are for all subjects at PiB visit 1.

3.2. *Concurrent CSF measures and amyloid*

3.2.1. *PiB burden*

CSF t-tau, $A\beta_{42}$, $A\beta_{42}/A\beta_{40}$, $1/A\beta_{42}$, t-tau/$A\beta_{42}$, p-tau/$A\beta_{42}$, NFL/$A\beta_{42}$, MCP-1/$A\beta_{42}$, and YKL-40/$A\beta_{42}$ all significantly predicted baseline PiB burden at P(FDR) <.05 (Supplementary Table 2, Fig. 1). Additionally, p-tau (P = .04) was significant at an uncorrected threshold of P < .05. $A\beta_{42}/A\beta_{40}$, $1/A\beta_{42}$, t-tau/$A\beta_{42}$, and p-tau/$A\beta_{42}$

had large effect sizes; CSF $A\beta_{42}$, NFL/$A\beta_{42}$, MCP-1/$A\beta_{42}$, and YKL-40/$A\beta_{42}$ had medium effect sizes; and t-tau and p-tau had small effect sizes.

When CSF measures of amyloid, neural injury, and inflammation were simultaneously included as predictors in the model, only t-tau and $A\beta_{42}$ significantly accounted for variance in baseline PiB burden. Inclusion of CSF $A\beta_{42}$ (R^2 change = 0.229; f^2 = 0.495) and t-tau (R^2 change = 0.104; f^2 = 0.225) significantly improved the model. $A\beta_{42}$ (standard $\beta = -0.582$, $P < .001$) and t-tau (standard $\beta = 0.385$, $P < .001$) were the strongest predictors of baseline brain amyloid burden.

3.2.2. Regional Aβ

CSF $A\beta_{42}$, $A\beta_{42}/A\beta_{40}$, and all ratios of individual CSF markers to CSF $A\beta_{42}$ were consistently related to a spatial pattern of amyloid in the brain in regions commonly affected in AD including posterior cingulate, lateral parietal cortex, precuneus, and medial prefrontal cortex (Fig. 2). Supplementary Table 3 provides statistical details and anatomic locations for the peaks that survived *P*(FWE) <.05. Although tau alone did not survive FWE at the voxel-level, tau/$A\beta_{42}$ showed a significant positive relationship with amyloid burden. These data support a model where amyloid burden is more severe in the presence of neurofibrillary pathology. The statistical map of the inverse of $A\beta_{42}$ ($1/A\beta_{42}$) shows a significant relationship that is consistent but not identical with the overall pattern of other CSF measures.

3.3. Baseline CSF measures and longitudinal amyloid

3.3.1. PiB burden

T-tau/$A\beta_{42}$, p-tau/$A\beta_{42}$, and NFL/$A\beta_{42}$ were significantly associated with PiB burden at visit 2 at *P*(FDR) <.05 (Supplementary Table 4, Fig. 3). $A\beta_{42}/A\beta_{40}$ (P = .017) and MCP-1/$A\beta_{42}$ (P = .03) were additionally associated with PiB burden at visit 2 at *P*(uncorrected) <.05. All results were in the expected direction (CSF/$A\beta_{42}$ were positive, whereas $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ were negative). These results suggest that a composite CSF measurement, here in the form of a ratio, which is sensitive to both amyloid ($A\beta_{42}$) and neural injury (tau, p-tau, or NFL) or inflammation (MCP-1), predicts amyloid deposition over time. All f^2 effect sizes for CSF markers were small because visit 1 PiB burden is a very strong predictor of visit 2 PiB burden. T-tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ had the largest effect sizes (f^2 >0.12) of the CSF biomarkers.

When CSF measures of amyloid, neural injury, and inflammation were simultaneously included as predictors in the longitudinal model, only t-tau significantly accounted for variance in amyloid accumulation (R^2 change = 0.007; f^2 = 0.08). Besides amyloid load at visit 1 (standard β = 0.794, $P < .001$), $A\beta_{42}$ (standard $\beta = -0.107$, P = .053) and t-tau (standard β = 0.113, P = .034) were the strongest predictors of longitudinal amyloid accumulation.

3.3.2. Regional Aβ

T-tau/$A\beta_{42}$, p-tau/$A\beta_{42}$, NFL/$A\beta_{42}$, and MCP-1/$A\beta_{42}$ were positively associated and $A\beta_{42}/A\beta_{40}$ was negatively associated with longitudinal amyloid deposition in the eight ROIs (Fig. 4). Supplementary Table 5 provides statistical details and anatomic locations for the peaks that survived *P*(FWE) <.05. Ratios were primarily associated with amyloid increases in the frontal and lateral parietal and temporal lobes, and p-tau/$A\beta_{42}$ and MCP-1/$A\beta_{42}$ were additionally associated with amyloid increases in the precuneus.

3.4. CSF measures and cognitive decline

A significant relationship between the CSF ratios which predicted longitudinal Aβ accumulation (CSF $A\beta_{42}/A\beta_{40}$, t-tau/$A\beta_{42}$, p-tau/$A\beta_{42}$, NFL/$A\beta_{42}$, and MCP-1/$A\beta_{42}$) and slope of cognitive decline was not detected.

4. Discussion

The etiology of AD involves multiple pathologic processes occurring decades before disease onset, with the two primary forms of pathology consisting of amyloid plaques and neurofibrillary tangles. AD is also associated with inflammation [29] and changes in white matter structural integrity [30]. The aim of the present study was to better characterize early coincident preclinical changes by examining the relationship between CSF biomarkers covering different aspects of AD neuropathology and PET-PiB amyloid deposition in a relatively younger cognitively healthy adult sample. Our overarching hypothesis, that CSF markers of neural injury would predict both baseline and longitudinal Aβ burden, was supported. CSF ratios to $A\beta_{42}$ were spatially related to amyloid deposition in regions commonly affected in AD at baseline, and CSF ratios of tau to $A\beta_{42}$ were related to an increase in global Aβ load and to a spatial pattern of Aβ in the brain in AD-sensitive ROIs longitudinally assessed over a 2-year time span. Although an array of CSF biomarkers have been shown to be related to amyloid longitudinally in a similar cohort [31], this is the first known study to provide spatial specificity with voxel-wise analyses, and to investigate novel ratios reflecting simultaneous gliosis and Aβ deposition.

Voxel-wise analyses showed CSF markers had a highly conserved relationship to amyloid within regions commonly labeled as the default mode network (DMN) including lateral parietal cortex, precuneus, and medial prefrontal cortex. It has been suggested that susceptibility to amyloid deposition in these regions may be due to high activity or metabolism [32–34] and relatively greater extent of

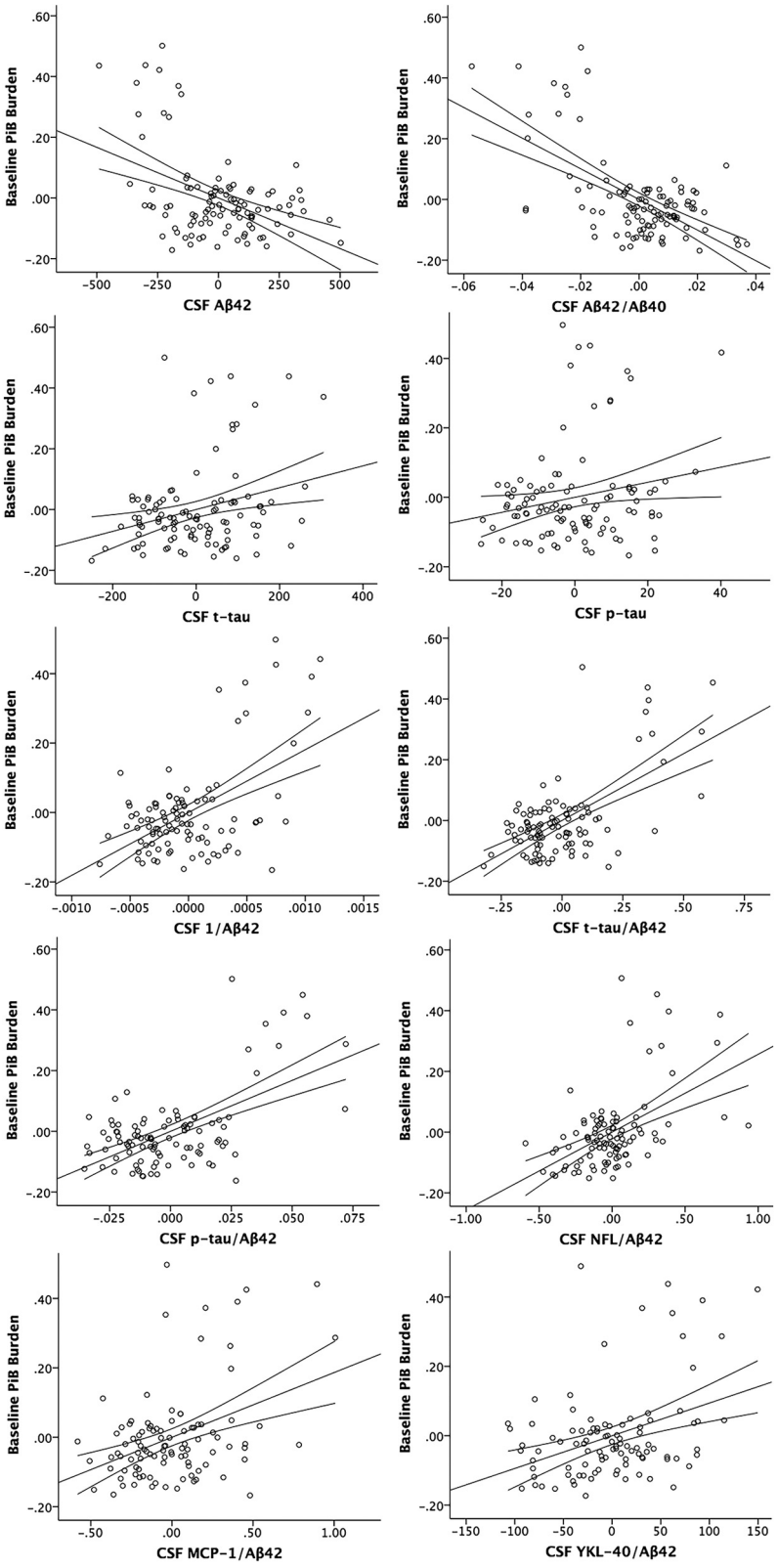

Fig. 1. Partial regression plots of CSF measures and baseline PiB burden. Y-axis: DVR of baseline PiB burden adjusted for all other predictors in the model (age, sex, *APOE* ε4, and FH). X-axis: CSF measure adjusted for all other predictors in the model. 95% confidence intervals for the regression line are displayed. Abbreviations: PiB, Pittsburgh compound B; CSF, cerebrospinal fluid; DVR, distribution volume ratio; Aβ, beta-amyloid; NFL, neurofilament light protein; t-tau, total tau; p-tau, phosphorylated-tau; MCP-1, monocyte chemoattractant protein 1; YKL-40, chitinase-3–like protein; FH, parental family history.

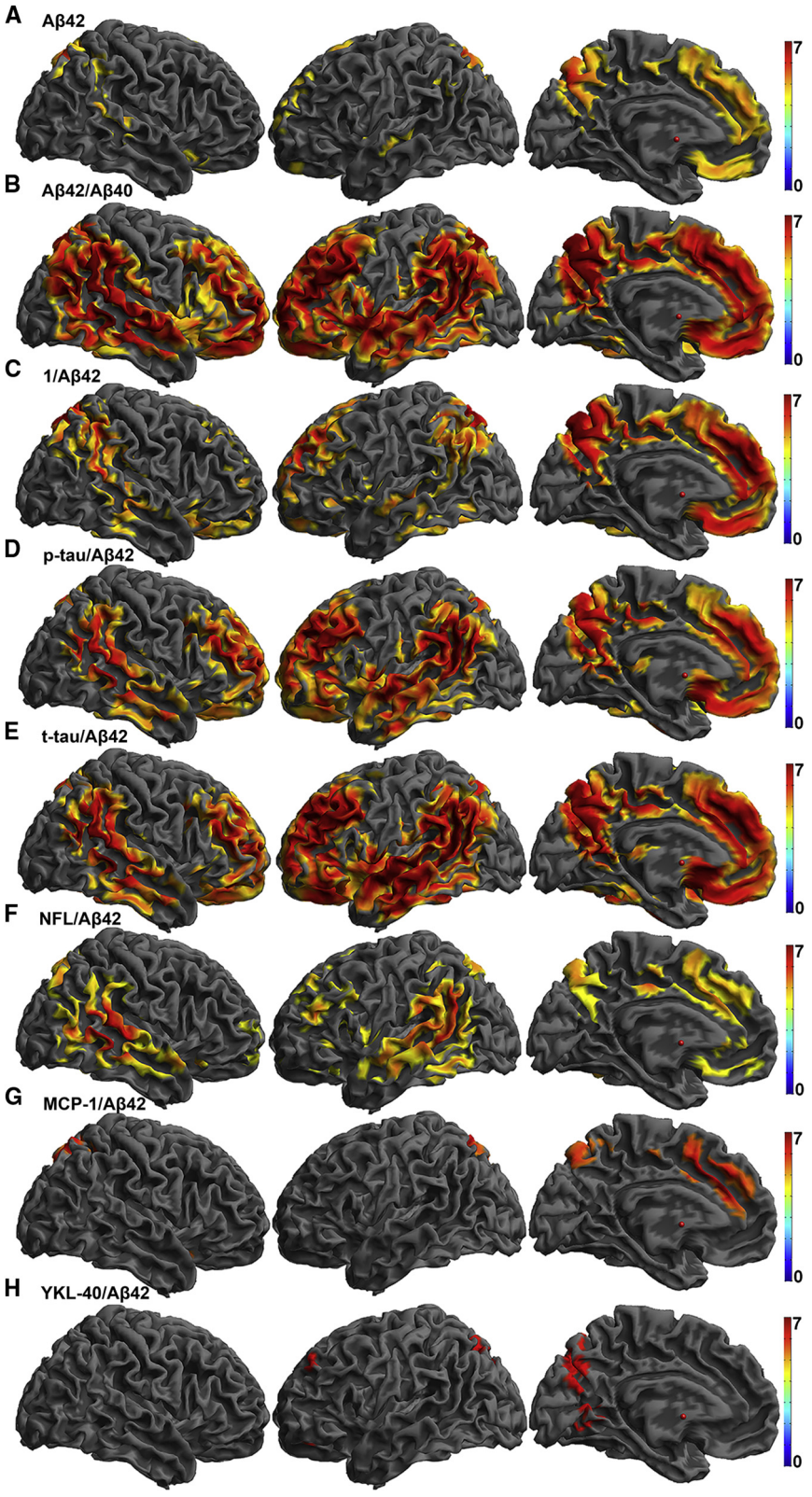

Fig. 2. Heat maps of T-statistics for voxel-wise regressions of baseline CSF measures on baseline PiB images rendered onto a template brain for (A) $A\beta_{42}$, (B) $A\beta_{42}/A\beta_{40}$, (C) $1/A\beta_{42}$, (D) p-tau/$A\beta_{42}$, (E) t-tau/$A\beta_{42}$, (F) NFL/$A\beta_{42}$, (G) MCP-1/$A\beta_{42}$, and (H) YKL-40/$A\beta_{42}$. The negative contrast is displayed for $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$, and the positive contrast is displayed for all other ratios. Color bars representing t-statistics from 0 to 7 are on the right. All voxels are significant at $P(\text{FWE}) < .05$. Abbreviations: CSF, cerebrospinal fluid; Aβ, beta-amyloid; PiB, Pittsburgh compound B; p-tau, phosphorylated-tau; t-tau, total tau; NFL, neurofilament light protein; MCP-1, monocyte chemoattractant protein 1; FWE, family-wise error; YKL-40, chitinase-3–like protein.

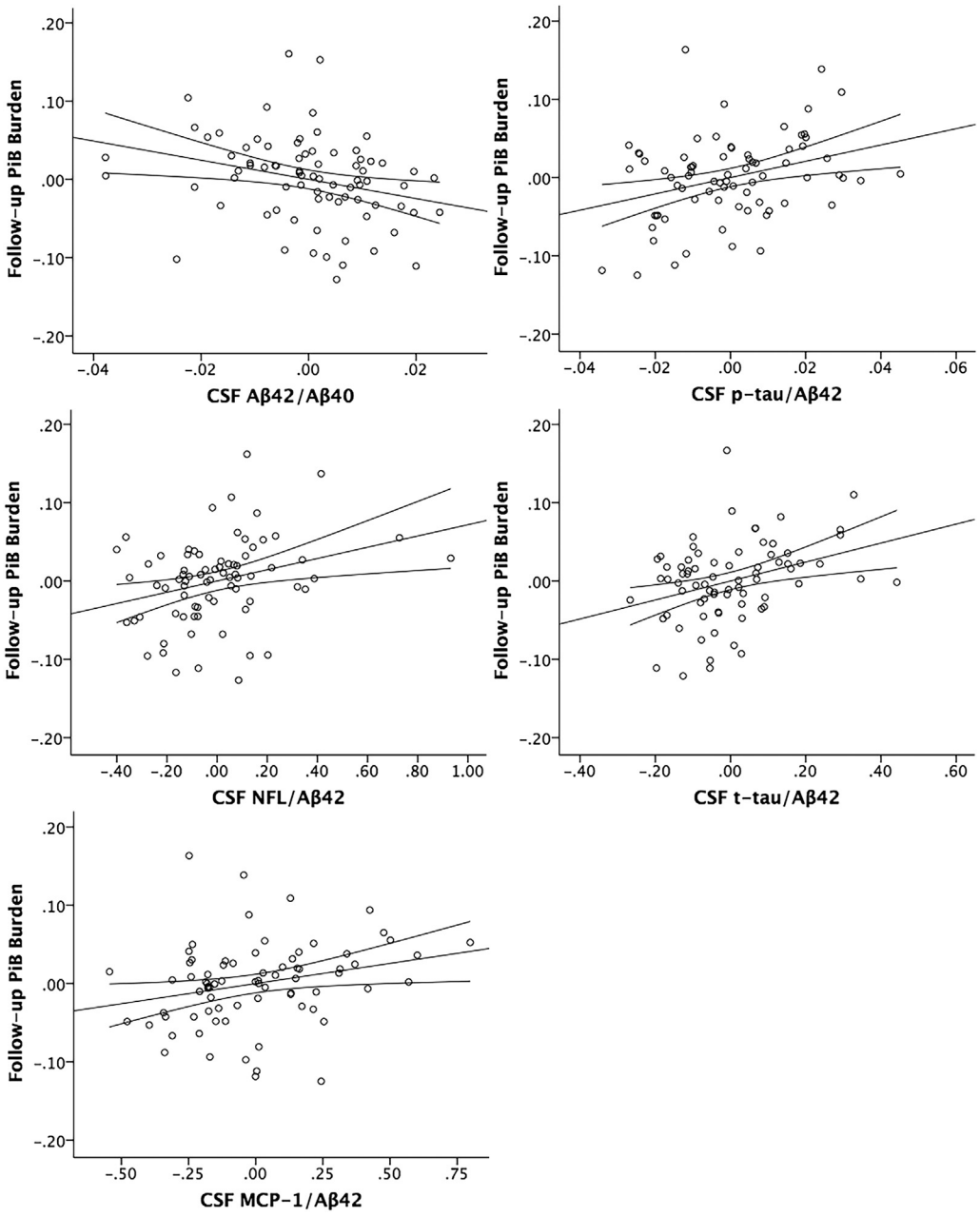

Fig. 3. Partial regression plots of CSF measures and follow-up PiB burden. Y-axis: DVR of follow-up PiB burden adjusted for all other predictors in the model (age, sex, *APOE* ε4, FH, and baseline PiB burden). X-axis: CSF measure adjusted for all other predictors in the model. 95% confidence intervals for the regression line are displayed. Abbreviations: CSF, cerebrospinal fluid; PiB, Pittsburgh compound B; DVR, distribution volume ratio; Aβ, beta-amyloid; p-tau, phosphorylated-tau; t-tau, total tau; NFL, neurofilament light protein; MCP-1, monocyte chemoattractant protein 1; FH, parental family history.

neuroplasticity over the life span [35,36]. Buckner et al. [37] (2005) found convergence of five different in vivo imaging methods on the DMN in young adults and older adults with AD, postulating that lifetime cerebral metabolism in the DMN predisposes these cortical regions to AD-related changes including amyloid deposition, metabolic disruption, and atrophy.

CSF ratios with $A\beta_{42}$ were consistently significantly associated with PiB amyloid burden in the brain at baseline and 2-year follow-up. Correspondingly, previous literature has demonstrated that $A\beta_{42}/A\beta_{40}$ is decreased in AD [5,38] and improves accuracy of distinguishing prodromal AD and dementia compared with $A\beta_{42}$ alone [39]. The ratio has been suggested to normalize the $A\beta_{42}$ concentration to a measure of overall amyloidogenic processing by amyloid precursor protein, making it possible to detect low $A\beta_{42}$ in high Aβ producers and vice versa [40]. Similarly, studies have shown that combinations of tau and $A\beta_{42}$ improve sensitivity and specificity [5,41,42], predict subjective cognitive decline [43], and predict conversion from a clinical dementia rating of 0 (cognitively normal) to 1 (mild dementia; [44]) and from mild cognitive impairment to AD [45].

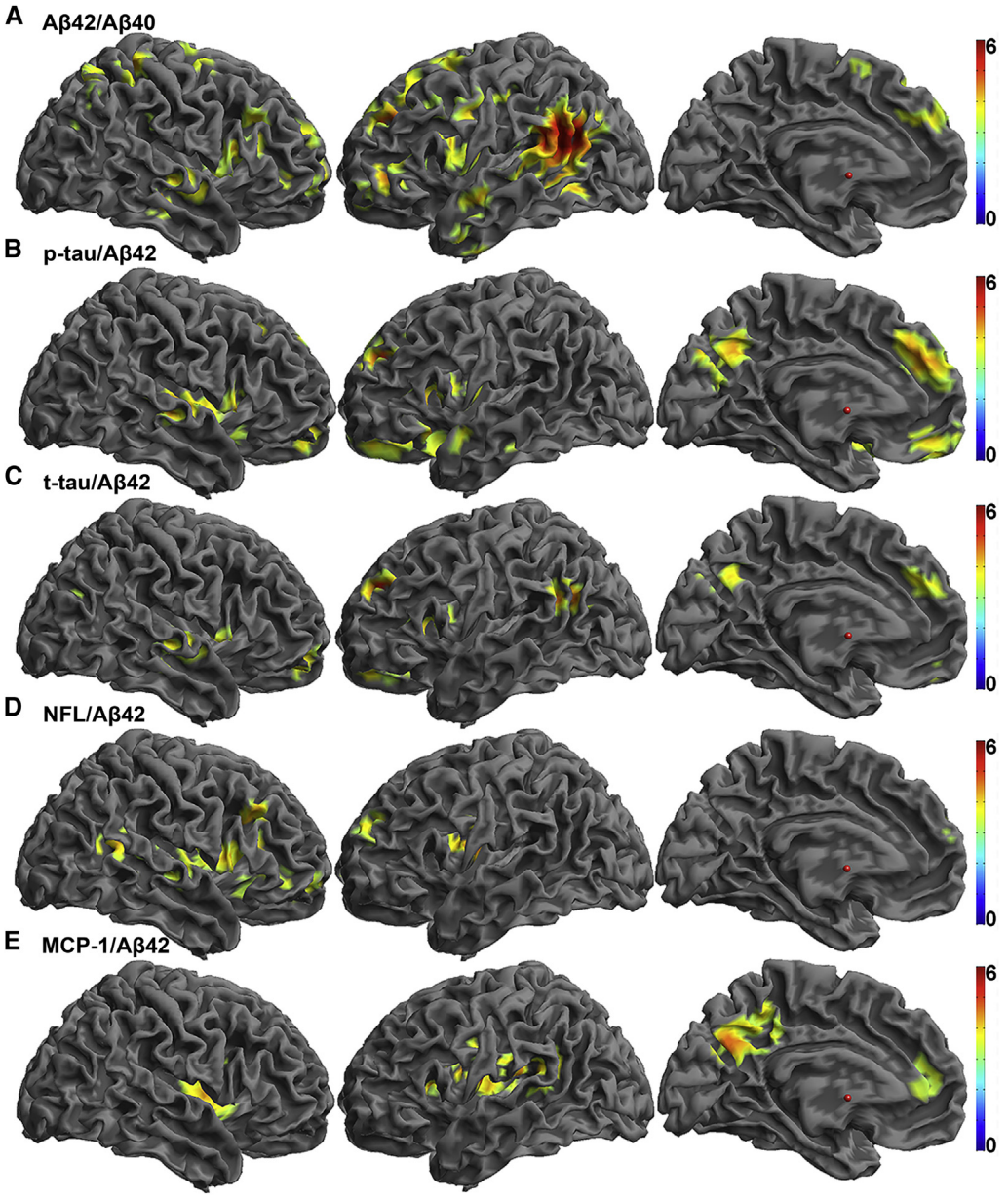

Fig. 4. Heat maps of T-statistics for voxel-wise BPM regressions of CSF measures on longitudinal PiB rendered onto a template brain for (A) $A\beta_{42}/A\beta_{40}$, (B) p-tau/$A\beta_{42}$, (C) t-tau/$A\beta_{42}$, (D) NFL/$A\beta_{42}$, and (E) MCP-1/$A\beta_{42}$. The negative contrast is displayed for $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$, and the positive contrasts are displayed for all other ratios. BPM statistical maps are thresholded at $P < .001$ with cluster extent >250 voxels. All clusters are significant at P(FWE) <.05. Color bars representing t-values from 0 to 6 are on the right. Abbreviations: BPM, biological parametric mapping; CSF, cerebrospinal fluid; PiB, Pittsburgh compound B; Aβ, beta-amyloid; FWE, family-wise error; p-tau, phosphorylated-tau; t-tau, total tau; NFL, neurofilament light protein; MCP-1, monocyte chemoattractant protein 1.

This is likely because they encompass not only strong amyloid effects but also more subtle disease-relevant tau changes, with simultaneous pathology more indicative of disease. This is particularly interesting in light of recent and compelling evidence showing that tau increases Aβ production [13].

The NFL/$A\beta_{42}$ findings further support a theory of coincident axonal degeneration and amyloid pathology. Interestingly, the ratio of the inflammatory marker of MCP-1 to $A\beta_{42}$ was also related to both baseline and longitudinal amyloid, but YKL-40/$A\beta_{42}$ only predicted baseline amyloid. MCP-1 has been found near amyloid plaques [46], is upregulated in CSF of AD patients [47], and predicts future conversion to AD and the rate of cognitive decline [48]. Although these findings and ours suggest a role for coincident Aβ and microglial/inflammatory processes in AD pathogenesis, other studies have shown mixed results for the diagnostic value of these CSF markers [49]. More studies are needed to validate these CSF analytes, and their ratios to $A\beta_{42}$, as AD biomarkers.

Our results indicated that 1/$A\beta_{42}$ was a strong predictor of baseline Aβ in a consistent but not identical pattern to other

CSF measures. This suggests not only that $A\beta_{42}$ in the denominator is not solely driving results but also that $1/A\beta_{42}$ may even be more sensitive to amyloid in the brain than $A\beta_{42}$ alone, possibly because the inverse transformation corrects for positive skewness. However, $1/A\beta_{42}$ did not survive significance thresholds in the longitudinal frameworks, suggesting that ratios with markers of neural injury truly reflect cumulative pathology rather than a mathematical phenomenon. Effect sizes further support the theory that ratios provide more information than $1/A\beta_{42}$ alone. For baseline analyses, t-tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ had larger effect sizes than $1/A\beta_{42}$ (Supplementary Table 2). Coincident markers seem to be even more relevant for predicting longitudinal amyloid accumulation, which showed larger effect sizes for all ratios with $A\beta_{42}$ except YKL-40/$A\beta_{42}$ compared with $1/A\beta_{42}$, which was not significant (Supplementary Table 4). Therefore, at a single time point, CSF $A\beta_{42}$ (or $1/A\beta_{42}$) may be sufficient to detect brain Aβ, but CSF ratios indicative of simultaneous pathology predict progressive accumulation over time, which is a stronger indicator of disease progression than stable brain amyloid levels. However, a supplemental analysis suggests that although t-tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ account for unique variance above and beyond $1/A\beta_{42}$, the story is less clear for MCP-$1/A\beta_{42}$ and NFL/$A\beta_{42}$ (Supplementary Table 6). These remain important biomarkers to investigate, and may prove to be better predictors of other AD-relevant outcomes, or more informative at more advanced ages and stages of the preclinical AD time course. This will be ascertained with a future study.

The majority of our baseline and longitudinal analyses indicate that CSF measures of amyloid alone but not tau alone corresponded with amyloid load in the brain, consistent with previous findings [50]. This is likely because our sample is relatively young and, thus, may show considerable amyloid pathology while only beginning to show changes in tau. Consistent with this theory, Buchhave et al. [51] (2009) found that among AD patients, $A\beta_{42}$ levels did not change over time but tau increased 16% over 2 years, with higher levels associated with faster conversion. This suggests that $A\beta_{42}$ levels increase earlier in the disease stage and then plateau, whereas tau biomarkers may increase gradually closer to symptom onset [45]. Similarly, findings from Kanai et al. [52] suggest that although CSF $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ may make the most significant changes at early AD stages, CSF tau likely increases with clinical progression of dementia. The data here suggest that at the earliest stages of increasing tau pathology—likely at an intermediary stage of amyloid accumulation—tau may interact to accelerate brain amyloid accumulation. The comprehensive model with all CSF biomarkers highlights the important contribution of tau to preclinical amyloid accumulation. This finding that tau significantly contributes to a model when $A\beta_{42}$ is also included in the model in addition to findings that tau/$A\beta_{42}$ but not tau alone was significant in voxel-wise models further corroborates our theory that tau's impact on AD pathogenesis is dependent on the presence of amyloid.

Although there are likely subtle cognitive changes occurring during the preclinical time period [1,2,53], we did not find a significant association between CSF biomarkers and slopes of episodic memory. However, because of the relatively young age and cognitive health of our sample, associations between CSF biomarkers and cognitive decline are likely to be subtle. These findings do not preclude the likely possibility that CSF ratios are related to preclinical cognitive decline. Furthermore, others have found that change in CSF levels may be more closely related to cognition [31,54]. Longitudinal CSF collection and ongoing neuropsychological testing is underway in this cohort and will be critical to understanding the relationship of CSF changes over time to disease progression, cognitive decline, and an interacting cascade between tau and amyloid.

There are limitations to our study that deserve mentioning. First, CSF from LP is only an indirect measure of pathology; however, it is a common clinical and research technique that is currently much less expensive and more widely available than PET imaging. Second, although this cohort is enriched for risk factors for AD, our sample's relatively younger age and lack of significant clinical symptoms restrict our clinical interpretation of the results. Third, the correlational design of this study prevents us from describing causal mechanisms between CSF analytes and AD pathology or symptoms. Fourth, serial CSF was not available, further preventing this study from ascertaining whether amyloid and tau are interacting in a reciprocal positive feedback loop, as recent work suggests [13]. Fifth, CSF analytes and ratios were correlated, making interpretation of a model with multiple CSF analytes less interpretable, even though neural injury, inflammation, and amyloid likely interact to impact amyloid accumulation. Although they are interpreted with this caution, the findings from the cumulative model suggest that tau and $A\beta_{42}$ are the strongest CSF predictors and statistically improve modeling of amyloid accumulation, and normal tolerance and variance inflation factors suggest that each variable was contributing unique variance.

This study adds important breadth to the investigation of biomarkers for AD by first, examining a spread of biomarkers spanning neural injury, inflammation, and gliosis in addition to the traditional measures of tau and $A\beta_{42}$; second, recruiting participants who are enriched for AD risk factors but are still in late-middle age and are cognitively normal; and third, longitudinally assessing both brain amyloid and cognitive decline. Follow-up longitudinal studies investigating clinical outcomes are necessary to determine the diagnostic accuracy of these biomarkers.

Acknowledgments

This research was supported by NIH grants AG021155 (S.C.J.), AG000213 (S.A.), AG027161 (S.C.J.), AG037639 (B.B.B.), and P50 AG033514 (S.A.); by P30 HD003352, by

a Clinical and Translational Science Award (UL1RR025011) to the University of Wisconsin, Madison; by NSF Award SES 0849122, and by the Swedish Research Council, the Swedish Brain Foundation and Torsten Söderberg's Foundation to the University of Gothenburg. Portions of this research were supported by the Helen Bader Foundation, Northwestern Mutual Foundation, Extendicare Foundation, and from the Veterans Administration including facilities and resources at the Geriatric Research Education and Clinical Center of the William S. Middleton Memorial Veterans Hospital, Madison, WI. The authors gratefully acknowledge Sharon Lu, Nancy Davenport-Sis, Amy Hawley, Sandra Harding, Jennifer Bond, Chuck Illingworth, Erika Starks, and the support of researchers and staff at the Waisman Center, University of Wisconsin-Madison for their assistance in recruitment, data collection, and data analysis. Above all, the authors thank their dedicated volunteers for their participation in this research.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dadm.2015.11.006.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed and appropriately cited the literature using traditional online sources and data presented at relevant conferences. There is estimable analysis on Alzheimer's disease (AD) fluid and imaging biomarkers in older adults who are normal or cognitively impaired, but considerably less information is available regarding the important preclinical late-midlife time frame.
2. Interpretation: These findings support a theory whereby presence of β-amyloid (Aβ) in addition to other pathologic features of AD including neural injury, neurofibrillary tangles, and gliosis predict amyloid accumulation in the preclinical time frame.
3. Future directions: Longitudinal analysis of biomarkers is critical for understanding their utility, especially when collection and analysis begins in the preclinical time frame which by definition and design implies a lack of clinical outcomes initially. This work, therefore, sets the groundwork for baseline and earliest change, and future research will investigate clinical end points, longitudinally measured cerebrospinal fluid biomarkers, and longitudinal multimodal imaging.

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