Cerebrospinal Fluid tau/β-Amyloid$_{42}$ Ratio as a Prediction of Cognitive Decline in Nondemented Older Adults

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Objectives: To investigate the ability of cerebrospinal fluid (CSF) and plasma measures to discriminate early-stage Alzheimer disease (AD) (defined by clinical criteria and presence/absence of brain amyloid) from nondemented aging and to assess whether these biomarkers can predict future dementia in cognitively normal individuals.

Design: Evaluation of CSF β-amyloid$_{40}$ (Aβ$_{40}$), Aβ$_{42}$, tau, phosphorylated tau$_{181}$, and plasma Aβ$_{40}$ and Aβ$_{42}$ and longitudinal clinical follow-up (from 1 to 8 years).

Setting: Longitudinal studies of healthy aging and dementia through an AD research center.

Participants: Community-dwelling volunteers (n = 139) aged 60 to 91 years and clinically judged as cognitively normal (Clinical Dementia Rating [CDR], 0) or having very mild (CDR, 0.5) or mild (CDR, 1) AD dementia.

Results: Individuals with very mild or mild AD have reduced mean levels of CSF Aβ$_{42}$ and increased levels of CSF tau and phosphorylated tau$_{181}$. Cerebrospinal fluid Aβ$_{42}$ level completely corresponds with the presence or absence of brain amyloid (imaged with Pittsburgh Compound B) in demented and nondemented individuals. The CSF tau/Aβ$_{42}$ ratio (adjusted hazard ratio, 5.21; 95% confidence interval, 1.58-17.22) and phosphorylated tau$_{181}$/Aβ$_{42}$ ratio (adjusted hazard ratio, 4.39; 95% confidence interval, 1.62-11.86) predict conversion from a CDR of 0 to a CDR greater than 0.

Conclusions: The very mildest symptomatic stage of AD exhibits the same CSF biomarker phenotype as more advanced AD. In addition, levels of CSF Aβ$_{42}$, when combined with amyloid imaging, augment clinical methods for identifying in individuals with brain amyloid deposits whether dementia is present or not. Importantly, CSF tau/Aβ$_{42}$ ratios show strong promise as antecedent (preclinical) biomarkers that predict future dementia in cognitively normal older adults.

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ithout truly effective therapies, Alzheimer disease (AD) will soon become a public health crisis. Biological markers are essential to aid in the development of disease-modifying therapies and identify individuals at high risk or in the earliest clinical stages. Alzheimer disease pathologic abnormalities (amyloid plaques, neurofibrillary tangles) appear to begin 10 to 20 years before cognitive symptoms or significant neuronal loss. Thus, it is critical to identify affected individuals while they are still cognitively normal (preclinical AD), prior to overt synaptic and neuronal loss, so new therapies have a chance to preserve normal brain function.

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yntheses in cerebrospinal fluid (CSF) have shown promise in discriminating individuals with AD from nondemented aging. Mean levels of CSF β-amyloid$_{42}$ (Aβ$_{42}$) (forming amyloid plaques) typically decrease in AD whereas levels of CSF tau (forming neurofibrillary tangles) increase. However, overlap between demented and nondemented groups has limited the diagnostic potential of these markers. This overlap may represent biological variability, inaccurate diagnostic classification, or both. Reductions in CSF Aβ$_{42}$ may result from a “sink” effect of Aβ$_{42}$-containing plaques. Currently, a definitive diagnosis of AD is possible only at autopsy. Some individuals with clinical diagnoses of AD may be misdiagnosed and have high levels of CSF Aβ$_{42}$ because they lack amyloid deposition. Some who are cognitively normal may have low CSF Aβ$_{42}$ levels due to the presence of amyloid prior to dementia (preclinical AD).
Abbreviations: APOE, apolipoprotein E; CDR, Clinical Dementia Rating; LP, lumbar puncture; MMSE, Mini-Mental State Examination.

*Statistically different from CDR 0, (P < .02).
†Statistically different from CDR 0 (P < .01) and CDR 0.5 (P < .01) (F1,98 = 6.36, P = .02).
‡Statistically different from CDR 0, P < .001 (F2,98 = 97.56, P < .001).
§Statistically different from CDR 0.5, P < .001 (F2,98 = 97.56, P < .001).
¶Statistically different from CDR 0 (F2,98 = 11.93, P = .003).
εDue to concerns about potential ceiling effects, differences in MMSE scores across the CDR groups were also tested using the nonparametric Kruskall-Wallis test. This test confirmed significant differences between the CDR 0 and the CDR 0.5 (χ2 = 48.9, P < .001) and CDR 1 (χ2 = 43.6, P < .001) groups as well as between the CDR 0.5 and CDR 1 groups (χ2 = 13.6, P = .002).

To address these issues, we investigated the ability of CSF and plasma markers to discriminate early-stage AD cohorts defined by clinical criteria as well as the presence or absence of brain amyloid (via positron emission tomographic imaging of Pittsburgh Compound B [PIB]) from nondemented aging. We also assessed whether these biomarkers could predict future dementia in cognitively normal elders.

METHODS

PARTICIPANTS

Participants were community-dwelling volunteers enrolled in longitudinal studies of healthy aging and dementia through the Washington University Alzheimer Disease Research Center. Participants were 60 to 91 years of age and in good general health; they had no other neurological, psychiatric, or systemic medical illness that could contribute importantly to dementia nor medical contraindication to lumbar puncture (LP). Cognitive status was determined annually in accordance with standard protocols and criteria. A Clinical Dementia Rating (CDR) of 0 indicated no dementia whereas CDR 0.5 and CDR 1 indicated very mild and mild AD dementia, respectively, based on criteria from the National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer’s Disease and Related Disorders Association. The rate of postmortem confirmation of AD clinical diagnosis in our center is 93%, including the CDR 0.5 stage. Studies were approved by the human studies committee at Washington University, and written informed consent was obtained from all participants. Apolipo-

protein E (APOE) genotypes were provided by the Alzheimer Disease Research Center Genetics Core (A. Goate, DPhil, core leader).

CSF AND PLASMA COLLECTION, PROCESSING, AND ASSESSMENT

Cerebrospinal fluid (20-30 mL) was collected at 8 AM after overnight fasting. Samples were gently inverted to avoid possible gradient effects, briefly centrifuged at low speed, and aliquoted into polypropylene tubes prior to freezing at −84°C. Fasted blood was obtained at the time of LP and collected into polypropylene tubes containing ethylenediaminetetraacetic acid. Plasma was prepared by standard centrifugation methods prior to aliquoting and freezing at −84°C.

Cerebrospinal fluid samples were analyzed for total tau, phosphorylated tau181 (ptau181), and Aβ42 by enzyme-linked immunosorbant assay (Innotest; Innogenetics, Ghent, Belgium). Cerebrospinal fluid Aβ40 and plasma Aβ40 and Aβ42 were assayed by enzyme-linked immunosorbant assay.

IN VIVO AMYLOID IMAGING WITH PIB

Fifty of 139 participants underwent in vivo amyloid imaging via PIB positron emission tomography within 2 years of LP as described. Presence of cortical amyloid was defined by a mean cortical PIB binding potential of 0.2 or more (averaging prefrontal cortex, precuneus, lateral temporal cortex, and gyrus rectus).

STATISTICAL ANALYSES

Analyses were performed by the Alzheimer Disease Research Center Biostatistics Core (P. Miller, AB, core leader) using SAS version 9.1 for Sun OS (SAS Inc, Cary, NC). General linear models examined whether biomarker values differed by CDR. Clinical Dementia Rating, age, sex, education, APOE genotype (presence vs absence of an ε4 allele), and all possible 2- and 3-factor interaction terms were included in the models. Independent t tests, single-factor analysis of variance, or χ² analyses tested whether unadjusted biomarker and demographic variables differed among groups. Receiver operating characteristic curve analyses assessed biomarker sensitivity and specificity for discriminating clinical groups. To assess predictors of future dementia in nondemented participants, Cox proportional hazards models tested the effect of demographic and biomarker variables on the rate of receiving a CDR greater than 0 in individuals who had a CDR of 0 at the time of LP and had 1 or more follow-up clinical assessments. Follow-up times for participants retaining a CDR greater than 0 were considered statistically censored on the date of their last assessment. Statistical significance was defined by P < .05.

DEMOGRAPHICS OF NONDEMENTED AND EARLY AD COHORTS

We compared CSF biomarker data from cognitively normal subjects (CDR 0) with those of subjects with very mild (CDR 0.5) or mild (CDR 1) AD. Demographic characteristics of 139 participants are described in Table 1. The groups did not differ significantly with regard to age at LP. However, they did differ in (1) mean educational level, with CDR 1 participants having fewer years of education com-
pared with the CDR 0 and 0.5 groups; (2) mean Mini-Mental State Examination scores, with the CDR 0.5 group lower than the CDR 0 group and the CDR 1 group lower than both the CDR 0 and 0.5 groups; and (3) sex distribution, with women composing 69% of the CDR 0 group and 42% and 50% of the CDR 0.5 and 1 groups, respectively. The CDR 0.5 and 1 groups together form the early-stage AD group with an APOE ε4 allele frequency of 57%, a proportion comparable with studies of later-stage AD.15

CSF AND PLASMA MARKERS AS A FUNCTION OF CLINICAL DIAGNOSIS

We examined whether levels of candidate markers (Aβ40, Aβ42, tau, and ptau181) differed among the CDR groups. In unadjusted comparisons, CSF levels of Aβ42 were significantly lower, and levels of tau and ptau181 were significantly higher, in each of the early-stage AD groups (CDR 0.5 and 1) compared with the nondemented group (Figure 1 and Table 2). In addition, mean CSF Aβ40/Aβ42, tau/Aβ42, and ptau181/Aβ42 ratios were significantly higher in the CDR 0.5 and 1 groups compared with the CDR 0 group. Thus, mean levels of CSF Aβ42, tau, and ptau181 (and related ratios), but not CSF Aβ40, discriminate early-stage AD from nondemented aging comparable with studies of later-stage AD.6

Because sex, education, and APOE genotype distributions are different among the CDR groups (Table 1), we next adjusted for and simultaneously tested the effect of these demographic variables together with CDR on each of the biomarker measures using general linear models. Education and APOE genotype were not associated with any of the biomarker measures in this elderly cohort. Sex, however, related to some of the biomarker effects. We also observed several complex 2- and 3-way interactions (between CDR, sex, and APOE genotype). The reliability and/or biological significance of these sex effects and complex interactions, if any, remain to be determined. Age and APOE genotype effects on CSF Aβ42 levels in nondemented individuals have recently been reported16; however, differences in the mean ± SD age of subjects in that study compared with the present study (50 ± 20 years vs 73.3 ± 8.4 years, respectively) make it difficult to compare the 2 results. Of note, in analyzing a cohort of cognitively normal individuals including those of younger ages (45-90 years), we too observe a decrease in the CSF Aβ42 level in individuals with the APOE ε4 allele as seen by Peskind et al16 (A.M.F. and D.M.H., unpublished data, 2006). We observed no differences in the mean levels of plasma Aβ40 and Aβ42 (Figure 1) or the Aβ40/Aβ42 ratio among the CDR groups (Table 2), which is consistent with results from a previous study.17

Cerebrospinal fluid tau/Aβ42 and ptau181/Aβ42 ratios, the most promising potential discriminators of clinical diagnosis, were assessed for their sensitivity and specificity. Receiver operating characteristic curves assessed the accuracy of these measures in classifying the presence (CDR 0.5 or CDR 1) vs absence (CDR 0) of dementia. The areas under the curve in these analyses were 0.79 (95% confidence interval [CI], 0.71-0.87) for the tau/Aβ42 ratio and 0.73 (95% CI, 0.65-0.82) for the ptau181/Aβ42 ratio, values considered to be acceptable but not impressive.

UTILITY OF CSF Aβ42 LEVELS AND TAU/Aβ42 AND PTAU181/Aβ42 RATIOS FOR IDENTIFYING INDIVIDUALS WITH CORTICAL AMYLOID DEPOSITION

We used the new technique of in vivo brain amyloid imaging via PIB positron emission tomography7,8 to evaluate the ability of Aβ42-related CSF measures to discriminate individuals with amyloid plaques from those without plaques, regardless of clinical diagnosis. We recently reported an inverse relation between CSF Aβ42 level and in vivo brain amyloid load in a small cohort of non-demented and mildly demented individuals.7 We now have 50 individuals with both PIB and CSF measures in the present cohort. Individuals with positive binding (PIB+) displayed low levels of CSF Aβ42 within their clinical group (Figure 2A) and high ratios of tau/Aβ42 (Figure 2B) and ptau181/Aβ42 (Figure 2C) compared with PIB-negative (PIB−) individuals, regardless of clinical diagnosis. Every subject in this cohort with CSF Aβ42 levels lower than 457 pg/mL was PIB+, and every subject with CSF Aβ42 of 457 pg/mL or greater was PIB−. In contrast, plasma Aβ42 levels did not accurately identify PIB+ vs PIB− individuals (Figure 2D).

ABILITY OF CSF MEASURES TO PREDICT FUTURE DEMENTIA IN NONDEMENTED ELDERS

Cerebrospinal fluid levels of tau, ptau181, and Aβ42 appear useful in predicting further cognitive decline in individuals with mild cognitive impairment.18-20 To our knowledge, the ability of these or other candidate markers to predict future cognitive decline or dementia in individuals who are still cognitively normal has not been assessed. We investigated whether demographic or biomarker measures influenced the rate of conversion from cognitively normal (CDR 0) to very mildly or mildly demented (CDR > 0). Imaging with PIB was not performed on these individuals because this technique has become available only recently. For this analysis, we included data from cognitively normal elders (≥60 years old) for whom there were 1 or more follow-up annual cognitive assessments. Of the 61 participants meeting these criteria, 13 (21%) had 1 or more CDRs of 0.5 or greater at follow-up, which averaged 3 to 4 years. This rate of dementia development in nondemented elders is consistent with population-based reports.21 Individuals who went from CDR 0 to CDR greater than 0 were classified as converters, and those remaining CDR 0 at follow-up were considered nonconverters. Group demographics are described in Table 3. Demographic variables did not differ between the 2 groups, except converters had significantly fewer years of formal education than nonconverters, and a greater percentage of women than men converted during follow-up in this small cohort.

Cox proportional hazard models revealed that education and CSF tau/Aβ42 and ptau181/Aβ42 measures significantly predicted conversion from CDR 0 to CDR greater than 0 (Table 4). These findings were subsequently confirmed in adjusted models (adjusting for age, sex, education, and APOE genotype); participants with higher CSF tau/Aβ42 (adjusted hazard ratio, 5.21; 95% CI, 1.58-17.22) or ptau181/Aβ42 (adjusted hazard ratio,
4.39; 95% CI, 1.62-11.86) had a faster rate of conversion than those with low ratios. Consistent with other reports, participants with more formal education had a slower rate of conversion than those with less formal education (adjusted hazard ratio, 0.73; 95% CI, 0.58-0.93, adjusting for tau/\(A_\beta\))42; adjusted hazard ratio, 0.68; 95% CI, 0.53-0.88, adjusting for ptau181/\(A_\beta\)).

For illustrative purposes, Kaplan-Meier estimates of rate of conversion from CDR 0 to CDR greater than 0 using tau/\(A_\beta\) and ptau181/\(A_\beta\) ratios as predictors are shown in Figure 3. Individuals with high tau/\(A_\beta\) ratios (≥1.15, corresponding to the top 15% of all values) were faster to display cognitive impairments (ie, CDR > 0) compared with the remainder of the cohort (<1.15, corresponding to the bottom 85% of tau/\(A_\beta\) values) (Figure 3A). A similar pattern was observed for the CSF ptau181/\(A_\beta\) ratio (using ≥0.214 as the 15% cut-off) (Figure 3B). Thus, CSF tau/\(A_\beta\) and ptau181/
Aβ42 show strong promise as predictive biomarkers for future cognitive impairment in cognitively normal older adults. In addition, the finding that ratios of CSF tau/Aβ42 and ptau/αβ42, but not Aβ42 levels alone, predicted conversion from CDR 0 to CDR greater than 0 over this short period suggests that increases in CSF tau levels occur after decreases in CSF Aβ42 levels and herald the onset of dementia.

Increased life expectancy, coupled with potential disease-modifying therapies currently in clinical trials, has shifted the goal of AD biomarker discovery from simply confirming a probable clinical diagnosis to identifying individuals with preclinical AD prior to any cognitive symptoms.
The 3 main findings of this study reflect this change in focus and may have important implications for the diagnosis and ultimate treatment of affected individuals. First, individuals at the very mildest symptomatic stage of AD (elsewhere often termed mild cognitive impairment) exhibit the same CSF biomarker phenotype as those in more advanced stages (≥CDR 1). This finding is consistent with clinicopathologic evidence of well-established AD pathologic abnormalities in many individuals who die at a very early stage of cognitive impairment and suggests that a clinical diagnosis of AD at the early CDR 0.5 stage can be as accurate as at later stages (CDR=1). Second, combin-
hort size and treatment duration required to ascertain therapeutic efficacy in clinical trials, thus moving the field closer toward the ultimate goal of delaying or preventing AD.

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Author Contributions: Dr Fagan had full access to all data in the study and takes responsibility for the integrity of the data and accuracy of data analysis. Study concept and design: Fagan, Morris, and Holtzman. Acquisition of data: Fagan, Roe, and Mintun. Analysis and interpretation of data: Fagan, Roe, Xiong, Mintun, Morris, and Holtzman. Drafting of the manuscript: Fagan, Roe, and Holtzman. Critical revision of the manuscript for important intellectual content: Fagan, Roe, Xiong, Mintun, Morris, and Holtzman. Statistical analysis: Roe and Xiong. Obtained funding: Fagan, Mintun, Morris, and Holtzman. Administrative, technical, and material support: Fagan, Mintun, Morris, and Holtzman. Study supervision: Fagan.

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