OPEN

Neurology®

The most widely read and highly cited peer-reviewed neurology journal
The Official Journal of the American Academy of Neurology

Neurology Publish Ahead of Print
DOI: 10.1212/WNL.0000000000200040

Cerebrospinal Fluid Biomarkers in Autopsy-Confirmed Alzheimer Disease and Frontotemporal Lobar Degeneration

Author(s):

Niklas Mattsson-Carlsson, MD, PhD^{1,2,3}; Lea T Grinberg, MD, PhD^{4,5}; Adam Boxer, MD, PhD⁴; Rik Ossenkoppele, PhD^{1,6}; Magnus Jonsson, MD, PhD⁷; William Seeley, MD^{4,5}; Alexander Ehrenberg^{4,8}; Salvatore Spina, MD, PhD⁴; Shorena Janelidze, PhD¹; Julio Rojas-Martinez, MD, PhD⁴; Howard Rosen, MD⁴; Renaud La Joie, PhD⁴; Orit Lesman-Segev, MD, MMedSc⁴; Leonardo Iaccarino, PhD⁴; Gwendlyn Kollmorgen, PhD⁹; Peter Ljubenkov, MD⁴; Udo Eichenlaub, PhD⁹; Maria Luisa Gorno-Tempini, MD, PhD⁴; Bruce Miller, MD, PhD⁴; Oskar Hansson, MD, PhD^{1,10}; Gil Dan Rabinovici, MD^{4,11}

Equal Author Contributions:

Dr Hansson and Rabinovici contributed equally to this work

Corresponding Author:

Niklas Mattsson-Carlsson
niklas.mattsson@med.lu.se

This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.

Affiliation Information for All Authors: 1. Clinical Memory Research Unit, Faculty of Medicine, Lund University, Lund, Sweden; 2. Department of Neurology, Skåne University Hospital, Lund University, Lund, Sweden; 3. Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden; 4. Memory & Aging Center, Department of Neurology, University of California San Francisco, San Francisco, CA, USA; 5. Department of Pathology, University of California San Francisco, San Francisco, CA, USA; 6. Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands; 7. Department of Clinical Chemistry, Skåne University Hospital, Malmö, Sweden; 8. Department of Integrative Biology, University of California, Berkeley; 9. Roche Diagnostics GmbH, Penzberg, Germany; 10. Memory Clinic, Skåne University Hospital, Malmö, Sweden; 11. Department of Radiology & Biomedical Imaging, University of California San Francisco, San Francisco, CA, USA

Contributions:

Niklas Mattsson-Carlsson: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data

Lea T Grinberg: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Additional contributions: carried out neuropathological assessments

Adam Boxer: Drafting/revision of the manuscript for content, including medical writing for content

Rik Ossenkoppele: Drafting/revision of the manuscript for content, including medical writing for content

Magnus Jonsson: Drafting/revision of the manuscript for content, including medical writing for content

William Seeley: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Additional contributions: carried out neuropathological assessments

Alexander Ehrenberg: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Additional contributions: assisted with the analysis of neuropathological data

Salvatore Spina: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Additional contributions: carried out neuropathological assessments

Shorena Janelidze: Drafting/revision of the manuscript for content, including medical writing for content

Julio Rojas-Martinez: Drafting/revision of the manuscript for content, including medical writing for content

Howard Rosen: Drafting/revision of the manuscript for content, including medical writing for content

Renaud La Joie: Drafting/revision of the manuscript for content, including medical writing for content

Orit Lesman-Segev: Drafting/revision of the manuscript for content, including medical writing for content

Leonardo Iaccarino: Drafting/revision of the manuscript for content, including medical writing for content

Gwendlyn Kollmorgen: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Additional contributions: provided assays and organized the Elecsys biomarker measurements

Peter Ljubenkov: Drafting/revision of the manuscript for content, including medical writing for content

Udo Eichenlaub: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Additional contributions: provided assays and organized the Elecsys biomarker measurements

Maria Luisa Gorno-Tempini: Drafting/revision of the manuscript for content, including medical writing for content

Bruce Miller: Drafting/revision of the manuscript for content, including medical writing for content

Oskar Hansson: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Additional contributions: supervised the work

Gil Dan Rabinovici: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Additional contributions: supervised the work

Number of characters in title: 109

Abstract Word count: 223

Word count of main text: 4500

References: 49

Figures: 6

Tables: 1

Search Terms: [26] Alzheimer's disease, [29] Frontotemporal dementia, [322] Class II, [319] Spinal cord infection

Study Funding: This work was partially funded by Roche Diagnostics (assay kits, materials and employee salaries for U.E. and G.K). ELECSYS is a registered trademark of Roche. The Elecsys CSF Abeta42, Abeta40, pTau, and tTau immunoassays are not yet cleared or approved for clinical use in the US. The NeuroToolKit robust prototype assays are for investigational purposes only and are not approved for clinical use. All other product names and trademarks are the property of their respective owners. Work at the authors' lab is funded by Knut and Alice Wallenberg Foundation, the Medical Faculty at Lund University, Region Skåne, the European Research Council, the Swedish Research Council, the Strategic Research Area MultiPark at Lund University, the Swedish Alzheimer Foundation, the Swedish Brain Foundation, The Parkinson foundation of Sweden, The Parkinson Research Foundation, the Skåne University Hospital Foundation, the Swedish federal government under the ALF agreement, and the Bundy Academy. Work at UCSF was supported by NIH/NIA grants (P30-AG062422, P01-AG019724, R01-AG038791, K08-AG052648, R01-NS050915, P50 AG023501, R01 AG045611, U19AG063911, K24053435, U54NS092089, R01AG031278, K99AG065501).

Disclosures: O. Hansson has acquired research support (for the institution) from Roche, Pfizer, GE Healthcare, Biogen, Eli Lilly and AVID Radiopharmaceuticals. In the past 2 years, he has received consultancy/speaker fees (paid to the institution) from Biogen and Roche. G.D. Rabinovici receives research support from AVID Radiopharmaceuticals, Eli Lilly, Life Molecular Imaging, GE Healthcare. In the past 2 years, he has received consultancy/speaker from Axon Neurosciences, Eisai, Merck, GE Healthcare, Johnson & Johnson. He is an Associate Editor for JAMA Neurology. N. Mattsson-Carlgren is an Associate Editor for "Alzheimer's Dementia: Diagnosis, Assessment & Disease Assessment". A. Ehrenberg is a Scientific Editor for "Alzheimer's & Dementia: The Journal of the Alzheimer's Association" and has received compensation for consulting work done with Epiodyne, Inc. A.L. Boxer has been a consultant (past 2 years) for AGTC, Alector, Arkuda, Arvinas, Asceneuron, AZTherapies, Bioage, Humana, Lundbeck, Ono, Roche, Samumed, Sangamo, Stealth, Third Rock, Transposon, UCB and Wave; and has research support from, Biogen, Eli Lilly, and Eisai. L.T. Grinberg has acquired research support (for the institution) from Eli Lilly and AVID Radiopharmaceuticals. She received compensation for consulting work with CuraSen Inc. S. Spina has received compensation for consulting work with Precision Xtract and Acel Health. W.W. Seeley receive consulting fees from GLG Council, Guidepoint Global LLC, and Corcept Therapeutics. G. Kollmorgen and U. Eichenlaub are employees of Roche Diagnostics. J. C. Rojas is site PI for clinical trials sponsored by Eli-Lilly and Eisai. His research is supported by NIH/NIA K23AG059888. Dr. Ljubenkova is a clinical site primary investigator for clinical trials sponsored by Alector, AbbVie, and Woolsey. Dr. Ljubenkova serves as an advisor for Retrope. Dr. Ljubenkova receives research and salary support from the NIH-NIA and the Alzheimer's Association- Part the Cloud Partnership. Howard Rosen has been a consultant for Biogen, Ionis, Alector, Wave, Takeda, and Otsuka. The remaining authors report no disclosures relevant to the manuscript.

Abstract

Objective: To determine how fully automated Elecsys® CSF immunoassays for β -amyloid ($A\beta$) and tau biomarkers, and an ultrasensitive Simoa assay for neurofilament light chain (NFL), correlate with neuropathological changes of Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD).

Methods: We studied 101 patients with antemortem CSF and neuropathology data. CSF samples were collected a mean of 2.9 years prior to death (range 0.2-7.5 years). CSF was analyzed for $A\beta_{40}$, $A\beta_{42}$, total tau (T-tau), tau phosphorylated at amino acid residue 181 (P-tau), P-tau/ $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ ratios, and NFL. Neuropathology measures included Thal phases, Braak stages, CERAD scores, Alzheimer's disease Neuropathological Change (ADNC), and primary and contributory pathological diagnoses. Associations between CSF biomarkers and neuropathological features were tested in regression models adjusted for age, sex, and time from sampling to death.

Results: CSF biomarkers were associated with neuropathological measures of $A\beta$ (Thal, CERAD), tau (Braak) and overall AD neuropathologic change (ADNC). The CSF P-tau/ $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ ratios had high sensitivity, specificity and overall diagnostic performance for intermediate-high ADNC (area under the curve [AUC] range: 0.95-0.96). Distinct biomarker patterns were seen in different FTLD-subtypes, with increased NFL and reduced P-tau/T-tau in FTLD-TDP, and reduced T-tau in progressive supranuclear palsy compared to other FTLD variants.

Conclusions: CSF biomarkers, including P-tau, T-tau, $A\beta_{42}$, $A\beta_{40}$ and NFL support in vivo identification of AD neuropathology and correlate with FTLD neuropathology.

Classification of Evidence: This study provides Class II evidence that distinct CSF biomarker patterns, including for P-tau, T-tau, A β 42, A β 40 and NFL are associated with AD and FTLN neuropathology.

ACCEPTED

Introduction

Alzheimer's disease (AD) and frontotemporal lobe degeneration (FTLD) are common causes of dementia. AD is characterized by β -amyloid ($A\beta$) and tau aggregation¹ while FTLD is most often associated with tau (FTLD-tau) or TDP-43 (FTLD-TDP) aggregation. Clinical presentations have varying correlations with neuropathology in AD and FTLD^{2,3}. Biomarkers are therefore needed to assist in diagnosis and to accelerate drug development.

Positron emission tomography (PET) and cerebrospinal fluid (CSF) biomarkers are available for neurodegenerative diseases⁴. CSF biomarkers include $A\beta$ 42 and $A\beta$ 40, total tau (T-tau), phosphorylated tau (P-tau), and neurofilament light chain (NFL). PET for $A\beta$ and tau has been validated with neuropathology^{5,6}, but few studies have reported such validation for CSF AD biomarkers, and mainly using older assays^{7,8}. CSF studies in FTLD focused on differentiation versus AD⁹⁻¹², or differences between FTLD variants¹³⁻¹⁷. Post-mortem FTLD studies are rare, with some but not all¹⁵ finding reduced P-tau^{14,16} or P-tau/T-tau^{17,18} in FTLD-TDP compared to FTLD-tau.

Older CSF assays were hampered by variability, but this has been overcome with novel, fully automated assays¹⁹. We tested associations between Elecsys[®] $A\beta$ 42, $A\beta$ 40, T-tau and P-tau, and NFL (using Simoa) assays and neuropathology. Our primary research question was whether distinct CSF biomarker patterns, including for P-tau, T-tau, $A\beta$ 42, $A\beta$ 40 and NFL were associated with AD and FTLD neuropathology. We hypothesized that AD neuropathological changes would be associated with reduced $A\beta$ 42/ $A\beta$ 40 and increased P-tau/ $A\beta$ 42, also when co-existing with other pathologies. We also expected to find reduced tau markers in progressive supranuclear palsy (PSP, a variant of FTLD-Tau)^{13,20}, and lower P-tau/T-tau in FTLD-TDP^{14,16,17}

Methods

Study group

Patients were recruited from the University of California San Francisco Memory and Aging Center's Alzheimer's Disease Research Center. They met diagnostic criteria for different FTLT syndromes, including behavioral variant frontotemporal dementia (bvFTD)²¹, corticobasal syndrome (CBS)²², non-fluent variant PPA (nfvPPA)²³, PSP²⁴, semantic variant primary progressive aphasia (svPPA)²³, or frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS)²⁵, or had probable AD-type dementia²⁶. Five patients did not meet any research diagnostic criteria (but had syndromes considered most compatible with AD, bvFTD or PSP). All subjects underwent a medical history and physical examination, a structured caregiver interview, lumbar puncture (LP) and neuropsychological testing. We did not have a criterion for maximum time difference between LP and death (the mean time was 2.9 years [standard deviation 1.8, range 0.2-7.5]). We included all eligible study participants.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from all subjects or their assigned surrogate decision-makers. The UCSF institutional review board for human research approved the study.

CSF biomarkers and cognitive testing

CSF was obtained following protocols from Alzheimer's Disease Neuroimaging Initiative (ADNI)²⁷. In short, CSF was sampled in the morning after an overnight fast, using a 20- or 24-gauge spinal needle. Samples were transferred from collection tubes into polypropylene tubes, and frozen within 1 hour from sampling. Samples were shipped frozen to Lund University and Skåne University Hospital, where they were analyzed for Elecsys CSF AD biomarkers¹⁹ and NFL by a Simoa method (NF-light Simoa Assay Advantage Kit). Aβ42 and

A β 40 have been associated with A β pathology, T-tau with axonal injury, P-tau with tau pathology in AD, and NFL with injury preferentially in large-diameter myelinated axons²⁸. Cognitive testing with mini mental examination score (MMSE, testing overall cognitive status²⁹), Trail Making Test B (testing speed of processing and executive function³⁰), and California Verbal Learning Test-II (CVLT-II, an episodic memory test³¹) was done at median 2 (inter-quartile range 1-6) days before LP.

Neuropathology

Comprehensive neuropathological assessments were performed by the UCSF Neurodegenerative Disease Brain Bank, blinded to CSF results, following previously described procedures³². Classifications of AD³³, FTLT-TDP and FTLT-tau (including PSP and CBD) followed standard neuropathological criteria^{33–35}. For each patient, a main (“primary”) pathology was defined as the pathology that was most likely to explain the clinical syndrome based on its anatomical location and degree of pathological change. For each patient, other (“contributing”) pathologies were also defined, as the pathological changes that could explain some of the symptoms in addition to the primary pathology. We also determined the AD Thal amyloid phase³⁶, indicating topographical extent of A β plaque pathology, Braak neurofibrillary tangle stage³⁷, indicating the topographical extent of tau neurofibrillary pathology, and CERAD score³⁸, indicating the density of neocortical neuritic plaques. Thal phase, Braak stage and CERAD score were aggregated in the Alzheimer’s Disease Neuropathological Change (ADNC) score³². ADNC has four levels: none, low, intermediate, or high. One of our main aims was to test associations between CSF biomarkers and AD pathology. To avoid bias from subjective interpretations, we used intermediate-to-high ADNC as an indicator of AD pathology³⁹ independent of whether the neuropathologists had identified AD as a primary or contributory pathology to the patient’s clinical syndrome

(four patients who presented clinically with PSP, CBS or bvFTD had intermediate ADNC, although AD changes were not considered primary or contributing).

Statistics

Linear regression models, adjusted for age, sex, and time between LP and death were used to test associations between biomarkers (as outcomes) and neuropathological features (as categorical predictors). The overall effect of each feature (across all levels, e.g. Thal phases 0-5) on biomarkers were tested by likelihood ratio tests comparing nested models with and without neuropathological score as a predictor. Associations between dichotomous ADNC (none-low versus intermediate-high) and dichotomous biomarker status were tested with Fisher's exact test. Receiver Operating Characteristics (ROC) analyses were used to test diagnostic performance (by area under the ROC curve, AUC) of different biomarkers for dichotomous ADNC. This used predictions from logistic regression models with ADNC as the outcome and biomarkers as predictors, with confidence intervals generated by a bootstrap procedure, with the *pROC* package⁴⁰. An internal 10-fold cross-validation of AUC estimates and confidence intervals was done using influence curves with the *cvAUC* package⁴¹.

Sensitivity and specificity were tested using a priori cut-points. When indicated, we used Akaike Information Criterion (AIC) for model selection (a difference of >2 favors the model with smaller AIC). Sensitivity analyses were done on subsets of subjects. P-values were considered significant at $P < 0.05$, two-tailed. When mentioned, multiplicity correction was done using the Bonferroni method. Statistical analyses were done in R (v 4.0.2).

Data availability

Per the UCSF Memory and Aging Center data sharing policy, data requests are available upon reasonable request via our online portal:<https://memory.ucsf.edu/research-trials/professional/open-science>.

Results

Group characteristics by primary neuropathological diagnoses (AD, FTLD-tau [CBD], FTLD-tau [PSP], FTLD-TDP, and others) are shown in Table 1. Frequencies of co-pathologies are shown in Table 1 (by primary neuropathology) and eTable 1 (by clinical diagnosis). Only three of 15 patients with a clinical AD diagnosis had a non-AD primary pathological diagnosis, and two of these three had AD as contributing diagnosis (eTable 1). Conversely, AD was the primary underlying pathology in only four of 85 with a non-AD diagnosis. Seventy-five patients had a clinical diagnosis of an FTD syndrome (PSP-Richardson's Syndrome, CBS, bvFTD, svPPA, nfvPPA or ALS [Amyotrophic lateral sclerosis, most often together with bvFTD]), and all but three of these had an FTLD neuropathological variant as primary pathological diagnosis. Most heterogeneity in underlying primary neuropathology was seen in CBS, where 10 patients had FTLD-tau (CBD), eight had FTLD-tau (PSP), one had FTLD-tau (Pick's), one had FTLD-TDP, and one lacked evidence of neurodegeneration. eFigure 1 shows CSF biomarkers by clinical diagnosis.

Frequencies of AD-related neuropathological scores, including Thal phase, Braak stage CERAD, and ADNC are shown in eFigure 2. Most patients had either CERAD "none" or "frequent", with few having intermediate CERAD levels. Among CERAD "none" patients, most had Braak stage 0-II and Thal phase 0-2. Among CERAD "frequent" patients, most had Braak stage IV-VI and Thal phase 4-5.

CSF biomarkers by ADNC class

We tested how well biomarkers differentiated between ADNC none-low (N=70) versus ADNC intermediate-high (N=28, ADNC data was missing in 3 patients due to missing CERAD [N=1] and/or Thal phase [N=2] data). These comparisons were done irrespective of other pathologies, but other pathologies were commonly reported as either primary or contributory in both ADNC none-low and ADNC intermediate-high (eTable 2). The ADNC intermediate-high group had significantly lower A β 42 and A β 42/A β 40, and higher T-tau, P-tau, P-tau/A β 42 and P-tau/T-tau compared to the ADNC none-low group (Figure 1). There were no differences in A β 40 or NFL (note that all subjects had cognitive impairment) (eFigure 3). We also tested the performance of the biomarkers in detecting ADNC intermediate-high in ROC analyses (Figure 2). The best individual biomarker was A β 42 (AUC 0.886). Both the P-tau/A β 42 and the A β 42/A β 40 ratio had very high performance (AUC 0.953-0.956) to separate ADNC none-low from ADNC intermediate-high. An internal 10-fold cross-validation of the estimates gave very similar results (eTable 3).

Time between LP and death was a possible confounder for associations between biomarkers and neuropathology (subject-level data on LP to death time are included in eFigure 4). In a sensitivity analysis, the individuals who were correctly classified by A β 42 (concordance between predicted and observed ADNC class) had slightly longer lag time from those who were misclassified (P=0.04, Mann-Whitney U test). There were no differences in lag time between correctly classified and misclassified groups for other biomarkers/ratios (P=0.14-0.89).

An a priori defined cut-point (derived in the external BioFINDER cohort, manuscript in preparation) for A β 42/A β 40 (<0.054) had high specificity for minimal AD pathology, defined as ADNC none-low (high A β 42/A β 40 was seen in 67/70 [96%] of ADNC none-low patients) and high sensitivity for significant AD pathology, defined as ADNC

intermediate-high (low A β 42/A β 40 was seen in 24/28 [86%] of ADNC intermediate-high patients). Similar results were seen for an a priori defined cut-point⁴² for P-tau/A β 42 (>0.022) with high specificity (61/66 ADNC none-low, 92%) and high sensitivity (25/28 ADNC intermediate-high, 89%) for AD neuropathology. There were no differences in time from LP to death between correctly classified and misclassified individuals using the a priori cut-points (P=0.57-0.60). In sensitivity analyses, we tested the a priori cut-points in subgroups of subjects. In patients with a clinical diagnosis of AD (N=15), both A β 42/A β 40 and P-tau/A β 42 were positive in one of the two patients with ADNC none-low. A β 42/A β 40 was positive in 12 of the 13 patients with ADNC intermediate-high, and P-tau/A β 42 was positive in all the 13 patients with ADNC intermediate-high. In patients with a non-AD diagnosis and ADNC data (N=83, P-tau data was missing in 4 of these), A β 42/A β 40 was positive in 2 of 68 patients with ADNC none-low (97% specificity) and P-tau/A β 42 was positive in 4 of 64 patients with ADNC none-low (94% specificity). Both A β 42/A β 40 and P-tau/A β 42 were positive in 12 of 15 patients with ADNC intermediate-high (80% sensitivity).

CSF biomarkers by Thal phase, Braak stage, CERAD score and ADNC

We next tested associations between biomarkers and Thal phase, Braak stage, CERAD score, and all levels of ADNC. Figure 3 shows data for A β 42/A β 40 and P-tau/A β 42 (ADNC data is shown in eFigure 5). eFigure 6 (for Thal phase, Braak stage, and CERAD score) and eFigure 7 (for ADNC) show data for A β 40, A β 40, P-tau, T-tau, P-tau/T-tau, and NFL. When testing overall associations (across all levels of the scores), lower levels of A β 42 and A β 42/A β 40, and higher levels of P-tau, T-tau, P-tau/T-tau, and P-tau/A β 42 were significantly associated with worse pathology. There were no significant associations for A β 40 or NFL.

To test the sensitivity of biomarkers for lowest possible level of pathology, we compared biomarkers between every stage of the scores to the respective reference category

(i.e. Thal phase 0, Braak stage 0, CERAD none, and ADNC none) (Figure 4). These models were corrected for age, sex, and time between LP and death. The first significant reductions in A β 42 were seen in Thal phase 4, Braak stage IV, CERAD frequent and ADNC intermediate. The first reductions in A β 42/A β 40 were seen in Thal phase 2, Braak stage IV, CERAD moderate and ADNC intermediate. The first increases in P-tau/A β 42 were seen in Thal phase 4, Braak stage V, CERAD frequent and ADNC intermediate. These biomarker changes were always consistent at higher levels of pathology (e.g. A β 42/A β 40 remained changed at Thal phase 3-5). Significant increases in P-tau, T-tau and P-tau/T-tau were only seen at the highest levels of pathology (Thal phase 5, Braak stage VI, CERAD frequent and ADNC high). For A β 40 there was an increase at Thal phase 2, but this was not seen at greater Thal phases, and there were no significant changes in NFL (eFigure 8).

We considered the possibility that associations between biomarkers and pathology were driven partly by group differences between AD and FTL. In a sensitivity analysis, we adjusted all models for AD as a primary pathology. This attenuated associations for A β 42, P-tau, T-tau and the P-tau/T-tau ratio, but associations for the A β 42/A β 40 and P-tau/A β 42 ratios with neuropathology were robust (eTable 4). The lowest pathology levels with significant biomarker changes were slightly attenuated (eFigure 9). A β 42/A β 40 was significantly reduced from Thal phase 2, Braak stage V, CERAD moderate and ADNC intermediate, while P-tau/A β 42 was significantly increased from Thal phase 5, Braak stage V, and in CERAD frequent and ADNC high.

Although we included time from LP and death as a covariate in the models, we considered the possibility that differences in lag time could still impact the findings. In another sensitivity analysis, we therefore excluded individuals with more than the median lag time (>2.67 years). This did not alter the overall associations between biomarkers and pathology (eTable 4). There was no indication that the biomarkers had greater sensitivity for

lower grade of pathology in the subset with short time from LP to death (eFigure 10). We also evaluated the interaction between pathology and time from LP to death to predict CSF biomarkers, which was generally not significant (eFigure 11).

We also considered education and level of cognitive impairment as possible confounders (although these were not significantly associated with the biomarkers). We re-fit the models when also adjusting for years of education and baseline MMSE. This did not affect associations between biomarkers and pathology (eTable 4).

CSF biomarkers by combinations of Thal, Braak and CERAD score

To clarify if biomarkers depended on one or several of Thal phase, Braak stage and CERAD, we compared models with different sets of predictors of CSF biomarkers: a basic model using only age, sex and time between LP and death (these were also included as covariates in all models below), only Thal phase, only Braak stage, only CERAD score, and combinations of two or three pathological features. Models were compared in terms of AIC and R^2 (eTable 5). For A β 40 and NFL, no pathology model was better than the basic model. The Thal phase-only model was preferred for A β 42 ($R^2=0.21$, $\Delta AIC=-18.1$ compared to the basic model). The “Thal phase and CERAD” model was preferred for A β 42/A β 40 and P-tau/A β 42 ($R^2=0.33$, $\Delta AIC=-27.9$; $R^2=0.24$, $\Delta AIC=-16.3$, respectively). The Braak stage-only model was preferred for P-tau/T-tau ($R^2=0.41$, $\Delta AIC=-34.2$).

CSF biomarkers by primary neuropathological diagnosis

We tested associations between primary neuropathological diagnosis and biomarkers. All biomarkers except A β 40 differed across groups (Figure 5 and eFigure 12). We also compared biomarkers pairwise between groups (adjusted for multiple comparisons using the Bonferroni method for 10 comparisons). These comparisons are summarized in eTable 6. AD had

significantly lower A β 42/A β 40 ratio, and higher P-tau, P-tau/A β 42, and P-tau/T-tau than all other groups. AD also had lower A β 42 than FTLD-tau (CBD), FTLD-TDP and “other”, and higher T-tau than FTLD-tau (CBD), FTLD-tau (PSP), and FTLD-TDP. FTLD-tau (PSP) patients had higher P-tau/T-tau than FTLD-TDP. FTLD-TDP had higher NFL than AD, FTLD-tau (PSP) and “other”.

Twelve non-AD patients (1 FTLD-tau [CBD], 8 FTLD-tau [PSP], 2 FTLD-TDP and 1 other) had intermediate-high ADNC (shown in red in Figure 5). When excluding these patients and repeating the pairwise comparisons (eTable 6), FTLD-TDP patients had higher NFL than FTLD-tau (CBD), in addition to the other groups. The difference in P-tau/T-tau between FTLD-tau (PSP) and FTLD-TDP was attenuated and no longer significant after correction for multiple comparisons (uncorrected $P=0.0065$).

A priori cut-points for CSF biomarkers and primary neuropathological diagnosis

We evaluated a priori cut-points for A β 42, P-tau, T-tau, A β 42/A β 40 and P-tau/A β 42 for primary neuropathological diagnosis, in terms of sensitivity for AD and specificity for non-AD. A β 42, A β 42/A β 40 and P-tau/A β 42 had 94-100% sensitivity for AD (Table 1). Overall for non-AD, A β 42 had 38% specificity, T-tau had 79% specificity, P-tau and A β 42/A β 40 had 86% specificity, and P-tau/A β 42 had 83% specificity (Table 1 shows details by individual variants).

For non-AD patients, we tested if biomarker status (positive or negative) varied with co-morbid ADNC (none-low vs intermediate-high). These analyses showed that among patients with diseases other than AD as primary pathology (“non-AD”), positive A β 42/A β 40 or P-tau/A β 42 biomarker ratios (but not individual biomarkers) were significantly associated with AD co-pathology (eTable 7).

CSF biomarkers by primary and contributory neuropathological diagnosis

Since the designation of primary neuropathological diagnosis was not blinded to the clinical syndrome, we also classified each patient as positive or negative for AD (N=28 positive), FTLD-TDP (N=32 positive), FTLD-tau (PSP) (N=26 positive), FTLD-tau (CBD) (N=19 positive), or FTLD-tau (other) (N=12 positive), independent of whether the neuropathological diagnosis was termed “primary” or “contributory” (meaning that a patient could be positive for more than one of these). Associations were tested between these classifications and biomarkers (Figure 6). AD neuropathological diagnosis was associated with significantly altered levels (in the expected directions) of all biomarkers, except A β 40 and NFL. FTLD-TDP pathology was associated with reduced P-tau/T-tau and elevated NFL. FTLD-tau (PSP) was associated with reduced T-tau.

Note that these analyses were not adjusted for co-pathologies. However, we also performed these tests for the FTLD diagnoses after removing patients with intermediate-high ADNC (eFigure 13), leaving N=24 FTLD-TDP, N=17 FTLD-tau (PSP), N=16 FTLD-tau (CBD), and 9 FTLD-tau (other). FTLD-TDP pathology remained associated with reduced P-tau/T-tau and elevated NFL, and FTLD-tau (PSP) remained associated with reduced T-tau. FTLD-tau (other) was associated with elevated P-tau/A β 42, T-tau and P-tau.

Genetic variants, CSF biomarkers, and neuropathology

Data on genetic variants were available for *APOE* ϵ 4 (positive, N=26; negative N=74, see Table 1), *C9orf72* mutations (positive N=8 [all FLTD-TDP], negative N=92), *GRN* mutations (positive N=5 [1 AD, 4 FTLD-TDP], negative N=95), *MAPT* mutations (positive N=2 [both classified as “other”, with FTD-PPA syndromes], negative N=96).

APOE ϵ 4 was associated with lower A β 42 (P=0.028) and A β 42/A β 40 (β =-0.019, P<0.001), and higher P-tau/A β 42 (β =0.017, P=0.0056). When adjusting for Thal

phase, Braak stage, and CERAD score (used together), these associations were lost ($A\beta_{42}$, $P=0.91$; $A\beta_{42}/A\beta_{40}$, $P=0.91$; $P\text{-tau}/A\beta_{42}$, $P=0.87$), supporting that *APOE* variants affect CSF biomarker levels indirectly, through neuropathology.

Among patients with FTLN primary pathologies, there were no associations between *C9orf72* mutations (N=8 positive, N=62 negative) and biomarker levels. The other mutations had too few cases for meaningful analyses.

Classification of Evidence

This study provides Class II evidence that distinct CSF biomarker patterns, including for $P\text{-tau}$, $T\text{-tau}$, $A\beta_{42}$, $A\beta_{40}$ and NFL are associated with AD and FTLN neuropathology.

Discussion

This clinicopathological CSF biomarker study in patients with AD and FTLN, which included $A\beta_{42}/A\beta_{40}$, $P\text{-tau}$ and $P\text{-tau}/A\beta_{42}$ measured with fully automated Elecsys assays, and NFL measured with an ultrasensitive Simoa assay, found that specific biomarkers were strongly correlated with AD, including when AD was present as co-pathology in patients with another primary pathology. The $A\beta_{42}/A\beta_{40}$ and $P\text{-tau}/A\beta_{42}$ ratios had very high overall accuracy (AUC 0.95-0.96) to detect significant AD pathology, defined as intermediate-high ADNC. Although most of the biomarker changes were associated with quite advanced neuropathology, group-level changes in CSF $A\beta_{42}/A\beta_{40}$ appeared already at Thal phase 2, supporting that selected CSF biomarkers may begin to be influenced already at low levels of AD pathology. However, the biomarkers did not have sensitivity for single-subject level prediction at very early stages of $A\beta$ or tau pathologies in this study. Another key finding was that autopsy confirmed FTLN variants displayed group-level biomarker patterns, including reduced CSF $T\text{-tau}$ in FTLN-tau (PSP) and reduced CSF $P\text{-tau}/T\text{-tau}$ and increased CSF NFL

in FTLD-TDP. Taken together, these findings support that CSF biomarkers can be used to identify underlying neuropathological AD changes, and they are also differentially expressed in different FTLD pathologies. This is one of few studies that combine careful characterization of neuropathological features with the use of only recently available modern assays for CSF AD biomarkers and NFL. The findings support the use of these biomarkers to characterize the underlying neuropathological changes in patients with AD and FTLD.

There were important findings for several of the individual biomarkers and ratios. In relation to A β pathology, we noted that A β 42/A β 40 levels started to change already at Thal phase 2, which may be comparable to what has been described previously for A β PET in an overlapping cohort⁴³. Previous studies directly comparing CSF A β and A β PET biomarkers have suggested that CSF A β measures may be altered before A β PET in response to altered A β metabolism⁴⁴. We note that we did not generally find that CSF biomarkers were altered in very early stages of neuropathology. This was especially true for tau accumulation, where no biomarker was significantly altered before Braak stage IV. Although this study did not directly compare CSF and PET measures versus neuropathology, our findings suggest that the CSF biomarkers studied here were not significantly altered before changes may also be detected by PET imaging. However, one possible caveat for CSF studies of very early changes with regards to neuropathology is the delay between LP and death, which may obscure the exact relationships between subtle biomarker changes and the first neuropathological changes. Another caveat is that truly quantitative data on A β pathology may be necessary, rather than the semi-quantitative data used here, to find subtle differences in when CSF and PET biomarkers start to change with respect to pathology. Furthermore, studies that find evidence of early CSF changes before changes in PET measures have typically seen such discrepancy in people without cognitive impairment, or only mild cognitive impairment, which is different from the current study population, where most

research participants had dementia. The findings for P-tau/A β 42 ratio were similar to A β 42/A β 40, with high diagnostic accuracy for AD neuropathology. We found pronounced changes in P-tau/A β 42 also when AD was a co-pathology in FTLD. Theoretically, a high P-tau/A β 42 ratio may reflect a combination of A β pathology and AD-specific axonal degeneration with tangle material. The ratio correlated with all of Thal, Braak and CERAD scores, although significant changes seemed to appear later than for A β 42/A β 40 (Thal phase 3 instead of Thal phase 2, and Braak stage V instead of Braak stage IV).

The individual CSF A β 42 measure was reduced in AD (as expected), but also in several FTLD patients, as described before in FTLD cohorts¹⁷. We found that reductions of A β 42 were mainly seen in FTLD patients with concomitant AD pathology (defined as intermediate-high ADNC), but several FTLD patients had low CSF A β 42 levels despite having none-low ADNC grade (Figure 5B). In contrast, the CSF A β 42/A β 40 ratio was very rarely reduced in FTLD patients without concomitant A β pathology (Figure 5E). This supports that CSF A β 42/A β 40 can be used a tool to detect concomitant AD pathology in FTLD, and illustrates the importance of correcting CSF A β 42 levels for a shorter A β isoform, such as A β 40 or A β 38, to detect underlying A β pathology. Hypothetically, other factors beyond A β pathology may have contributed to the reduced levels of A β 42 (without affecting the A β ratio) in FTLD patients, including white matter disease, neuroinflammation, and neuronal loss leading to reduced overall A β release.

CSF T-tau, which is non-specifically elevated in several conditions with axonal injury, did generally not reach AD-levels in the FTLD patients in this study. Low levels of T-tau were seen in PSP, which is in agreement with previous studies based on clinical diagnosis^{13,17}. We have no clear explanation for the low tau levels in PSP. Hypothetically, it could be related to involvement of cortical versus subcortical structures (where cortical abnormalities may presumably be more readily detected as CSF changes compared to

subcortical abnormalities, which dominate in PSP). The low levels of T-tau in PSP may also be related to disease-specific differences in tau fragment confirmations, or in neuronal releases of 3R versus 4R tau¹³. Hypothetically, CSF tau levels could also be reduced due to sequestration of tau in tangles in tauopathies. We note that the reduced tau levels seem to be more specific for PSP than for CBD, which is interesting given the similarities between these conditions neuropathologically. P-tau, which has been associated with buildup of AD-specific tau aggregates, did not clearly differentiate between A β plaques and neurofibrillary tangles in this cohort, since associations were seen between high P-tau and neuropathological measures both of tau and A β pathology. Soluble P-tau may be increased in response to A β -buildup, as a first indicator of altered tau metabolism in individuals who are positive on A β PET but negative on tau PET⁴⁵. However, we did not find that P-tau was altered in response to early A β pathology, which may be expected given previous PET studies. The relationship between soluble P-tau, and buildup of aggregated A β and tau fibrils is complex and warrants further studies. We focused on P-tau181, but other variants of P-tau (including P-tau217) may also be interesting to study in relation to neuropathology. P-tau levels were generally low in FTLT. Combining P-tau and T-tau in a ratio may integrate information about phosphorylation and release of tau proteins. Theoretically, a low P-tau/T-tau ratio may reflect axonal degeneration without AD-type degeneration, since T-tau (but not P-tau) is increased non-specifically due to axonal degeneration. Among the different AD pathology features, the P-tau/T-tau ratio was most closely associated with Braak stage (rather than Thal phase and CERAD score, eTable 6). The ratio increased separation between some of the groups compared to the individual tau measures. In particular, P-tau/T-tau was reduced in individuals with FTLT-TDP, which is in line with previous findings based on both clinical, neuropathological and genetic evidence^{17,18}. The FTLT-TDP group was further characterized by increased NFL, which is also in line with previous literature⁴⁶. One biomarker-pathology

study in AD and FTLD which compared CSF T-tau and NFL in a panel of biomarkers found that NFL improved diagnostic accuracy, further supporting that NFL can provide important information⁴⁷.

One strength of this study is that we had neuropathological data in all patients, combined with CSF samples, and the sample size was relatively large for an ante-mortem biomarker versus neuropathology study. A limitation is that the sample size was relatively small for some of the subgroups. A larger population will make it easier to fully disentangle associations between biomarkers and specific pathological features. Additional weaknesses include the lack of a control population for normal ageing, as well as the lack of groups with other dementias, such as Lewy body dementia or vascular dementia. Future studies may include these groups to increase the generalizability of the findings. The time interval between LP and death may obscure relationships between CSF biomarkers and neuropathological features, although we adjusted for this.

In summary, CSF A β 42/A β 40, T-tau, P-tau, P-tau/A β 42 and NFL were related to underlying neuropathological changes of AD and FTLD variants. An “AD-like” biomarker profile supports AD co-morbidity in patients with FTLD. In contrast, a “non-AD-like” biomarker profile, with reduced P-tau/T-tau and increased NFL may support FTLD-TDP rather than FTLD-tau lesions in the brain. Low T-tau may support FTLD-tau (PSP) pathology rather than other FTLD pathologies. Further work is needed to assess how these findings can be translated to blood-based biomarkers, which have shown promising results with high diagnostic accuracy for AD and underlying pathological changes^{48,49}. Further work is needed to test how these biomarker profiles perform at the single-subject level, and the findings need to be replicated in an independent sample.

Supplement 1 --- <http://links.lww.com/WNL/B777>

References

1. Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the Clinical Diagnosis of Alzheimer Disease at National Institute on Aging Alzheimer's Disease Centers, 2005–2010. *J Neuropathol Exp Neurol* 2012;71(4):266–273.
2. Harris JM, Gall C, Thompson JC, et al. Classification and pathology of primary progressive aphasia. *Neurology* 2013;81(21):1832–1839.
3. Mackenzie IRA, Neumann M, Baborie A, et al. A harmonized classification system for FTLTD-TDP pathology. *Acta Neuropathol* 2011;122(1):111–113.
4. Jack CR, Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 2018;14(4):535–562.
5. Clark CM, Pontecorvo MJ, Beach TG, et al. Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-beta plaques: a prospective cohort study. *Lancet Neurol* 2012;11:669–78.
6. Soleimani-Meigooni DN, Iaccarino L, La Joie R, et al. 18F-flortaucipir PET to autopsy comparisons in Alzheimer's disease and other neurodegenerative diseases. *Brain* 2020;143(11):3477–3494.
7. Bian H, Van Swieten JC, Leight S, et al. CSF biomarkers in frontotemporal lobar degeneration with known pathology. *Neurology* 2008;70(19 Pt 2):1827–1835.
8. Buerger K, Ewers M, Pirttilä T, et al. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain* 2006;129:3035–41.
9. Ewers M, Mattsson N, Minthon L, et al. CSF biomarkers for the differential diagnosis of Alzheimer's disease: A large-scale international multicenter study. *Alzheimer's & Dementia* 2015;11(11):1306–1315.
10. Tang W, Huang Q, Yao Y-Y, et al. Does CSF p-tau181 help to discriminate Alzheimer's disease from other dementias and mild cognitive impairment? A meta-analysis of the literature. *J Neural Transm (Vienna)* 2014;121(12):1541–1553.
11. Koopman K, Le Bastard N, Martin J-J, et al. Improved discrimination of autopsy-confirmed Alzheimer's disease (AD) from non-AD dementias using CSF P-tau(181P). *Neurochem. Int.* 2009;55(4):214–218.
12. de Souza LC, Lamari F, Belliard S, et al. Cerebrospinal fluid biomarkers in the differential diagnosis of Alzheimer's disease from other cortical dementias. *J. Neurol. Neurosurg. Psychiatr.* 2011;82(3):240–246.
13. Wagshal D, Sankaranarayanan S, Guss V, et al. Divergent CSF τ alterations in two common tauopathies: Alzheimer's disease and progressive supranuclear palsy. *J. Neurol. Neurosurg. Psychiatr.* 2015;86(3):244–250.
14. Lleó A, Irwin DJ, Illán-Gala I, et al. A 2-Step Cerebrospinal Algorithm for the Selection of Frontotemporal Lobar Degeneration Subtypes. *JAMA Neurol* 2018;
15. Goossens J, Bjerke M, Van Mossevelde S, et al. Diagnostic value of cerebrospinal fluid tau, neurofilament, and progranulin in definite frontotemporal lobar degeneration [Internet]. *Alzheimers Res Ther* 2018;10[cited 2018 Apr 24] Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5859717/>
16. Irwin DJ, Lleó A, Xie SX, et al. Ante mortem cerebrospinal fluid tau levels correlate with postmortem tau pathology in frontotemporal lobar degeneration. *Annals of Neurology* 2017;82(2):247–258.
17. Meeter LHH, Vijverberg EG, Del Campo M, et al. Clinical value of neurofilament and phospho-tau/tau ratio in the frontotemporal dementia spectrum. *Neurology* 2018;90(14):e1231–e1239.
18. Hu WT, Watts K, Grossman M, et al. Reduced CSF p-Tau181 to Tau ratio is a

biomarker for FTLT-DTP. *Neurology* 2013;81(22):1945–1952.

19. Hansson O, Seibyl J, Stomrud E, et al. CSF biomarkers of Alzheimer's disease concord with amyloid- β PET and predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement* 2018;14(11):1470–1481.
20. Hall S, Öhrfelt A, Constantinescu R, et al. Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. *Arch. Neurol.* 2012;69(11):1445–1452.
21. Rascovsky K, Hodges JR, Kipps CM, et al. Diagnostic criteria for the behavioral variant of frontotemporal dementia (bvFTD): current limitations and future directions. *Alzheimer Dis Assoc Disord* 2007;21(4):S14–18.
22. Armstrong MJ. Diagnosis and treatment of corticobasal degeneration. *Curr Treat Options Neurol* 2014;16(3):282.
23. Gorno-Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. *Neurology* 2011;76(11):1006–1014.
24. Litvan I, Agid Y, Calne D, et al. Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. *Neurology* 1996;47(1):1–9.
25. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 1998;51(6):1546–1554.
26. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939–44.
27. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* 2009;65:403–13.
28. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol* 2016;15(7):673–684.
29. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12(3):189–198.
30. Strauss E, Sherman EMS, Spreen O. A Compendium of Neuropsychological Tests. Administration, Norms, and Commentary – third edition. Oxford: Oxford University Press; 2006.
31. Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test-Second Edition (CVLT-II). San Antonio, TX: Psychological Corporation. San Antonio, TX: Psychological Corporation; [date unknown].
32. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol* 2012;123(1):1–11.
33. Hyman BT, Trojanowski JQ. Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 1997;56(10):1095–1097.
34. Cairns NJ, Bigio EH, Mackenzie IRA, et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. *Acta Neuropathol.* 2007;114(1):5–22.
35. Mackenzie IR, Rademakers R, Neumann M. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. *Lancet Neurol* 2010;9(10):995–1007.

36. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 2002;58:1791–800.
37. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991;82:239–59.
38. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991;41:479–86.
39. Hyman BT, Phelps CH, Beach TG, et al. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement* 2012;8(1):1–13.
40. Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011;12(1):77.
41. LeDell E, Petersen M, Laan M van der. Computationally Efficient Confidence Intervals for Cross-validated Area Under the ROC Curve Estimates [Internet]. U.C. Berkeley Division of Biostatistics Working Paper Series 2012; Available from: <https://biostats.bepress.com/ucbbiostat/paper304>
42. Blennow K, Shaw LM, Stomrud E, et al. Predicting clinical decline and conversion to Alzheimer's disease or dementia using novel Elecsys Aβ(1-42), pTau and tTau CSF immunoassays. *Sci Rep* 2019;9(1):19024.
43. La Joie R, Ayakta N, Seeley WW, et al. Multisite study of the relationships between antemortem [11C]PIB-PET Centiloid values and postmortem measures of Alzheimer's disease neuropathology. *Alzheimers Dement* 2019;15(2):205–216.
44. Palmqvist S, Mattsson N, Hansson O, Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid analysis detects cerebral amyloid-β accumulation earlier than positron emission tomography. *Brain* 2016;139(Pt 4):1226–1236.
45. Mattsson-Carlgen N, Andersson E, Janelidze S, et al. Aβ deposition is associated with increases in soluble and phosphorylated tau that precede a positive Tau PET in Alzheimer's disease. *Sci Adv* 2020;6(16):eaaz2387.
46. Landqvist Waldö M, Frizell Santillo A, Passant U, et al. Cerebrospinal fluid neurofilament light chain protein levels in subtypes of frontotemporal dementia. *BMC Neurol* 2013;13:54.
47. Cousins KAQ, Phillips JS, Irwin DJ, et al. ATN incorporating cerebrospinal fluid neurofilament light chain detects frontotemporal lobar degeneration. *Alzheimers Dement* 2020;
48. Schindler SE, Bollinger JG, Ovod V, et al. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology* 2019;
49. Thijssen EH, La Joie R, Wolf A, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat. Med.* 2020;26(3):387–397.

Figure legends

Figure 1. CSF biomarkers by ADNC none-low (N-L) versus intermediate-high (I-H).

Biomarkers are shown as unadjusted raw data in the groups of Alzheimer's disease Neuropathological Change (ADNC) none-low (blue) and intermediate-high (red). T-values and P-values are shown for group differences, adjusted for age, sex, and lag between lumbar puncture and death. Reference lines are shown for a priori cut-points for A β 42, T-tau, P-tau, A β 42/A β 40 and P-tau/A β 42, as defined in⁴². A β 40 and NFL biomarkers are shown in eFigure 3. ADNC, Alzheimer's disease Neuropathological Change.

Figure 2. CSF biomarkers for ADNC classification. Performance of logistic regression models for individual biomarkers (A) and biomarker ratios (B), to distinguish between ADNC none-low versus ADNC intermediate-high. The legends show overall area under the receiver operating curve characteristics (AUC) with 95% CI from a bootstrap procedure. ADNC, Alzheimer's disease Neuropathological Change.

Figure 3. CSF biomarkers by AD neuropathological scores. Biomarker ratios ($A\beta_{42}/A\beta_{40}$ and P-tau/ $A\beta_{42}$) are shown as unadjusted raw data, by neuropathological scores for spread of $A\beta$ pathology (Thal phase 0-5), tau pathology (Braak stage 0-VI), and presence/frequency of neuritic plaques (CERAD score N, none; S, sparse; M, moderate; F, frequent). See eFigure 5 for ADNC data. P-values are for the overall associations (from likelihood ratio tests of models with and without including the neuropathological score as a predictor) between the neuropathological scores and CSF biomarker levels, adjusted for age, sex, and time between lumbar puncture and death. A reference line is shown for an a priori cutpoint for $A\beta_{42}/A\beta_{40}$. Color coding refers to ADNC class (blue = ADNC none-low, red = ADNC intermediate-high, grey = missing ADNC data). ADNC, Alzheimer's disease Neuropathological Change.

Figure 4. CSF biomarker changes at different levels of pathology. This figure shows how different biomarkers are altered at different levels of pathology, compared to the lowest levels of respective pathology. Presented data are T-statistics (black font when significant at $P < 0.05$, red font when non-significant). The box colors are related to the magnitude of the T-statistics (red colors are positive and violet colors are negative). Data are presented for each biomarker (panels A-F) and the pathological scores Thal phase (categories range from Thal phase 1 to 5), Braak stage (categories range from stage I to IV), CERAD (categories range from S, sparse; M, medium; F, frequent), and ADNC (categories range from L, low; I, intermediate; H, high). For each score, biomarkers were compared between each category and the reference category (Thal phase 0, Braak stage 0, CERAD none, and ADNC no, respectively). The T-statistics are from linear regression models, adjusted for age, sex, and time between lumbar puncture and death. For example, CSF A β 42/A β 40 (panel D) was significantly reduced at ADNC intermediate, CERAD moderate, Braak stage IV and Thal phase 2 (and all greater levels of pathology). See eFigure 8 for results for A β 40 and NFL. ADNC, Alzheimer's disease Neuropathological Change.

A. CSF A β 42

B. CSF T-tau

C. CSF P-tau

D. CSFA β 42/A β 40 E. CSFP-tau/A β 42

F. CSFP-tau/T-tau

Figure 5. CSF biomarkers by primary pathological diagnosis. Biomarkers are shown as unadjusted raw data, by primary neuropathological diagnosis. P-values are shown for overall significance of neuropathological diagnosis, adjusted for age, sex, and time between lumbar puncture and death. See eFigure 12 for results for A β 40 and A β 42. See eTable 6 for pairwise comparisons between different diagnoses. Reference lines are shown for a priori cut-points for T-tau, P-tau181, A β 42/A β 40 and P-tau/A β 42, as defined in⁴². Color coding refers to ADNC class (blue = ADNC none-low, red = ADNC intermediate-high, grey = missing ADNC data). AD, Alzheimer's disease; ADNC, Alzheimer's disease Neuropathological Change; CBD, corticobasal degeneration; FTLD, frontotemporal lobar degeneration; PSP, Progressive Supranuclear Palsy; TDP, TAR DNA binding protein 43.

Figure 6. CSF biomarkers versus primary and contributory neuropathology diagnoses.

Effects are plotted for each neuropathological class, from linear regression models adjusted age, sex, and time between lumbar puncture and death. The coefficients represent the average difference in biomarkers between patients who were positive for a neuropathology (e.g. AD in panel (A), also if only present as a co-pathology) compared to the remaining patients. AD, Alzheimer's disease CBD, corticobasal degeneration; FTLD, frontotemporal lobar degeneration; PSP, Progressive Supranuclear Palsy; TDP, TAR DNA binding protein 43.

Table 1. Demographics by primary neuropathological diagnosis

		Primary neuropathological diagnosis					
		AD	FTLD -tau (CBD)	FTLD-tau (PSP)	FTLD- TDP	Other	All
<i>N</i>		16	17	26	27	15	101
<i>Clinical diagnosis</i>	<i>MCI</i>	0	0	0	2	0	2
	<i>AD-type dementia</i>	12	0	1	0	1 FTLD-Tau (Pick's) 1 ALAS	15
	<i>PD/DLB/PDD</i>	1	0	0	2	1 LBD (Braak 5)	4
	<i>CBS/CBD</i>	0	10	8	1	1 FTLD-Tau (Pick's) 1 no neurodegeneration	21
	<i>PSP-RS</i>	0	1	13	0	0	14
	<i>nvPPA</i>	0	2	1	1	1 FTLD-tau, unclassifiable 4R	5
	<i>bvFTD</i>	2	4	1	11	2 FTLD-tau/MAPT 3 FTLD-tau (Pick's) 1 AGD 1 FTLD-UPS 1 FTLD-FET (aFTLD-U)	26
	<i>svPPA</i>	0	0	0	2	0	2
	<i>ALS</i>	0	0	0	7	0	7
	<i>Other*</i>	1	0	2	1	1 FTLD-tau unclassifiable	5
<i>Age at lumbar puncture (y)</i>		66.3 (9.7)	64.1 (5.2)	71.0 (6.4)	62.7 (8.7)	60.7 (11.1)	65.3 (8.9)
<i>Age at death (y)</i>		69.8 (9.1)	66.6 (4.9)	74.1 (6.9)	65.4 (9.1)	63.9 (10.1)	68.3 (8.9)
<i>Sex (F/M)</i>		6/10	8/9	15/11	12/15	4/11	45/56
<i>APOE ε4 (-/+)</i>		6/10	14/3	21/5	21/6	12/2	74/26
<i>MMSE (points)</i>		22.9 (3.0)	23.4 (5.7)	24.2 (5.5)	23.4 (6.4)	21.5 (7.1)	23.2 (5.8)
<i>TMT-B (total time)</i>		82.7 (42.2)	104.4 (28.1)	78.0 (39.4)	65.0 (41.0)	83.0 (39.7)	80.3 (39.7)
<i>CVLT 10 min delayed recall (correct items)</i>		2.5 (2.7)	4.0 (2.5)	5.2 (3.0)	4.2 (3.2)	2.8 (2.9)	4.0 (3.0)
<i>Contributory pathological diagnoses</i>	<i>AD</i>	NA	1	6	3	2	12
	<i>FTLD-tau (CBD)</i>	2	NA	0	0	0	2
	<i>FTLD-tau (PSP)</i>	0	0	NA	0	0	0

	<i>FTLD-TDP</i>	2	2	0	NA	0	4
	<i>LBD</i>	3	3	2	1	0	9
	<i>Vascular injury</i>	4	1	3	1	1	10
	<i>Hippocampal sclerosis</i>	2	1	0	3	1	7
	<i>Other</i>	0	0	0	2	1	3
	<i>None</i>	5	11	16	19	10	61
Biomarker positivity/negativity (%positive/% negative)	<i>Aβ42, <1100 ng/L</i>	16/0 (100/0)	8/9 (47/53)	18/8 (69/31)	18/9 (67/33)	9/6 (60/40)	69/32 (68/32)
	<i>T-tau, >300 ng/L</i>	11/5 (69/31)	3/14 (18/92)	4/22 (15/85)	6/21 (22/78)	5/10 (33/67)	29/72 (29/71)
	<i>P-tau, >27 ng/L</i>	10/6 (63/37)	2/15 (12/88)	2/23 (8/92)	2/22 (8/92)	5/10 (33/67)	21/76 (22/78)
	<i>Aβ42/Aβ40, <0.054</i>	15/1 (94/6)	0/17 (0/100)	7/19 (27/73)	3/24 (11/89)	2/13 (13/87)	27/74 (27/73)
	<i>P-tau/Aβ42, >0.022</i>	16/0 (100/0)	1/16 (6/94)	7/18 (28/72)	3/21 (13/87)	3/12 (20/80)	30/67 (31/69)

Age are mean (standard deviation). Contributory pathological diagnoses were classified as specified on the neuropathological reports (some patients had more than 1 contributory diagnosis). *These patients did not reach any diagnostic research criteria. For the section on biomarker positivity, data are positive/negative cases (% positive/negative) for each biomarker. The thresholds were defined a priori, as described in⁴². For AD, the percentage positive is equal to the sensitivity of the tests when used to differentiate AD from non-AD. For all other diagnoses, the percentage negative is equal to the specificity of the test when used to differentiate AD from non-AD. AD, Alzheimer's disease; AGD, Argyrophilic grain disease; ALAS, Adult-onset leukodystrophy / leukoencephalopathy with axonal spheroids; ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant frontotemporal dementia; CBS, corticobasal syndrome; CBD, corticobasal degeneration; CVLT, California Verbal Learning Test, FTLD, frontotemporal lobar degeneration; FTLD-FET, FTLD with FET

protein accumulation; FTLN-UPS, FTLN with involvement of the ubiquitin-proteasome system; LBD, Lewy Body disease; MAPT, microtubule-associated protein tau; MCI, mild cognitive impairment; nvPPA, non-fluent variant primary progressive aphasia; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PSP, Progressive Supranuclear Palsy; PSP-RS, PSP-Richardson's syndrome; svPPA, semantic variant primary progressive aphasia; TDP, TAR DNA binding protein 43; TMT-B, Trail Making Test part B.

Neurology®

Cerebrospinal Fluid Biomarkers in Autopsy-Confirmed Alzheimer Disease and Frontotemporal Lobar Degeneration

Niklas Mattsson-Carlsson, Lea T Grinberg, Adam Boxer, et al.

Neurology published online February 16, 2022

DOI 10.1212/WNL.0000000000200040

This information is current as of February 16, 2022

Updated Information & Services	including high resolution figures, can be found at: http://n.neurology.org/content/early/2022/02/16/WNL.0000000000200040.full
Citations	This article has been cited by 1 HighWire-hosted articles: http://n.neurology.org/content/early/2022/02/16/WNL.0000000000200040.full##otherarticles
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): Alzheimer's disease http://n.neurology.org/cgi/collection/alzheimers_disease Cerebrospinal Fluid http://n.neurology.org/cgi/collection/cerebrospinal_fluid Class II http://n.neurology.org/cgi/collection/class_ii Frontotemporal dementia http://n.neurology.org/cgi/collection/frontotemporal_dementia
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions
Reprints	Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise

Neurology® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.