

## ATN status in amnestic and non-amnestic Alzheimer's disease and frontotemporal lobar degeneration

Katheryn A.Q. Cousins, PhD<sup>1</sup>, David J. Irwin<sup>1</sup>, David A. Wolk, MD<sup>1</sup>, Edward B. Lee, PhD<sup>2</sup>, Leslie M.J. Shaw, PhD<sup>2</sup>, John Q. Trojanowski, MD, PhD<sup>2</sup>, Fulvio Da Re, MD<sup>3</sup>, Garrett S. Gibbons<sup>2</sup>, Murray Grossman, MDCM, EdD<sup>1</sup>, and Jeffrey S. Phillips, PhD<sup>1</sup>

Department of Neurology<sup>1</sup>, Department of Pathology and Laboratory Medicine<sup>2</sup>

University of Pennsylvania

School of Medicine and Surgery, Milan Center for Neuroscience<sup>3</sup>

University of Milano-Bicocca, Milan, Italy

Correspondence should be addressed to Katheryn Cousins or Jeffrey Phillips.

Department of Neurology - 3 West Gates

Hospital of the University of Pennsylvania

3400 Spruce Street

Philadelphia, PA 19104-4283

Email: [kcous@pennmedicine.upenn.edu](mailto:kcous@pennmedicine.upenn.edu) or

[jefphi@pennmedicine.upenn.edu](mailto:jefphi@pennmedicine.upenn.edu)

Voice: 215-662-3361

Fax: 215-349-8464

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## Abstract

Under the ATN framework, cerebrospinal fluid analytes provide evidence of the presence or absence of Alzheimer's disease pathological hallmarks: amyloid plaques (A), phosphorylated tau (T), and accompanying neurodegeneration (N). Still, differences in cerebrospinal fluid levels across amnestic and non-amnestic variants or due to co-occurring pathologies might lead to misdiagnoses. We assess the diagnostic accuracy of cerebrospinal fluid markers for amyloid, tau, and neurodegeneration in an autopsy cohort of 118 Alzheimer's disease patients (98 amnestic; 20 non-amnestic) and 64 frontotemporal lobar degeneration patients (five amnestic; 59 non-amnestic). We calculated between-group differences in cerebrospinal fluid concentrations of amyloid- $\beta_{1-42}$  peptide, tau protein phosphorylated at threonine 181, total tau, and the ratio of phosphorylated tau to amyloid- $\beta_{1-42}$ . Results show that non-amnestic Alzheimer's disease patients were less likely to be correctly classified under the ATN framework using independent, published biomarker cutoffs for positivity. Amyloid- $\beta_{1-42}$  did not differ between amnestic and non-amnestic Alzheimer's disease, and receiver operating characteristic curve analyses indicated that amyloid- $\beta_{1-42}$  was equally effective in discriminating both groups from frontotemporal lobar degeneration. However, cerebrospinal fluid concentrations of phosphorylated tau, total tau, and the ratio of phosphorylated tau to amyloid- $\beta_{1-42}$  were significantly lower in non-amnestic compared to amnestic Alzheimer's disease patients. Receiver operating characteristic curve analyses for these markers showed reduced area under the curve when discriminating non-amnestic Alzheimer's disease from frontotemporal lobar degeneration, compared to discrimination of amnestic Alzheimer's disease from frontotemporal lobar degeneration. In addition, the ATN framework was relatively insensitive to frontotemporal lobar degeneration, and these patients were likely to be classified as having normal biomarkers or biomarkers suggestive of primary Alzheimer's disease pathology. We conclude that amyloid- $\beta_{1-42}$  maintains high sensitivity to A status, although with lower specificity, and this single biomarker provides better sensitivity to non-amnestic Alzheimer's disease than either the ATN framework or the phosphorylated-tau/amyloid- $\beta_{1-42}$  ratio. In contrast, T and N status biomarkers differed between amnestic and non-amnestic Alzheimer's disease; standard cutoffs for phosphorylated tau and total tau may thus result in misclassifications for non-amnestic Alzheimer's patients. Consideration of clinical syndrome may help improve the accuracy of ATN designations for identifying true non-amnestic Alzheimer's disease.

**Keywords:** non-amnestic Alzheimer's Disease, Frontotemporal Degeneration, cerebrospinal fluid, ATN

### Abbreviated Summary

Cousins et al. assess the 2018 ATN framework and find that non-amnestic patients with Alzheimer's disease (AD) have lower cerebrospinal fluid (CSF) phosphorylated tau and total tau than amnestic AD, while CSF amyloid- $\beta$  accurately stratifies both non-amnestic and amnestic AD from frontotemporal lobar degeneration.

## Introduction

The 2018 ATN research framework is a systematic method to determine Alzheimer's disease (AD) continuum designation, and can be applied using cerebrospinal fluid (CSF) analytes as markers of AD pathology (Jack *et al.*, 2018). Low CSF levels of amyloid- $\beta$  1–42 peptide ( $A\beta_{1-42}$ ) are associated with amyloid deposition in the brain, while high CSF levels of tau protein phosphorylated at threonine 181 (p-tau) are associated with intracellular hyperphosphorylated tau aggregation (Andreasen *et al.*, 2003; Tapiola *et al.*, 2009; Hampel *et al.*, 2018). Measures of CSF total tau (t-tau) have been interpreted as a marker of neurodegeneration that is not specific to AD; within the ATN framework, CSF t-tau can serve as a marker of disease severity (Jack *et al.*, 2016). Cut-points for CSF  $A\beta_{1-42}$ , p-tau, and t-tau determine if patients should be judged as positive or negative for pathologic amyloid (A status), phosphorylated tau (T status), and neurodegeneration (N status), respectively (Jack *et al.*, 2018); these cut-points have been established in large autopsy cohorts (Shaw *et al.*, 2009). Under the ATN framework, A status determines whether an individual is positive or negative for Alzheimer's continuum disease, while more fine-grained designations are determined by T and N status. Designations within Alzheimer's continuum disease are based on the biological definition of AD that includes both amyloid plaques and tau tangles, and observations that changes in CSF  $A\beta_{1-42}$  typically precede CSF p-tau, followed by neurodegeneration and cognitive decline (Jack *et al.*, 2013). Thus, abnormal A and T markers (A+T+N- or A+T+N+) are interpreted as AD, while abnormal A alone (A+T-N-) is interpreted as early stage Alzheimer's continuum or "Alzheimer's pathologic change" and abnormal A and N (A+T-N+) is suspected co-pathology or "Alzheimer's and suspected non-Alzheimer's pathologic change". Individuals who are A-negative are interpreted as not having Alzheimer's continuum disease, and can have either normal biomarkers (A-T-N-) or biomarkers indicative of non-AD pathologic change (A-T+N-, A-T-N+, or A-T+N+). In this way, ATN provides a framework to interpret CSF biomarkers and to obtain a diagnosis in life beyond binary AD or non-AD.

Diagnostic sensitivity and specificity to typical, amnesic AD is high for CSF  $A\beta_{1-42}$ , p-tau, and t-tau (Shaw *et al.*, 2009; Ewers *et al.*, 2015; Palmqvist *et al.*, 2015; Hampel *et al.*, 2018), and studies have shown that the ratio of p-tau to  $A\beta_{1-42}$  (p-tau/ $A\beta_{1-42}$ ) is especially accurate at stratifying patients with AD pathology from another form of neurodegenerative disease, frontotemporal lobar degeneration (FTLD) (Struyfs *et al.*, 2015; Oeckl *et al.*, 2016; Lleó *et al.*, 2018). Still, these studies demonstrate partial overlap in CSF levels between pathologic AD and FTLD. Thus, cut-points that determine ATN status still lead to a small but consequential subset of false-negative AD and false-positive FTLD cases. Such misdiagnoses can interfere with enacting appropriate clinical management strategies for patients and their families, and hinder the development of new therapeutic treatments and strategies by adding unexplained variance in a research population.

One possible source of diagnostic error is that broadly-applied cutoffs for AD pathology may better capture amnesic than non-amnesic variants of AD due to differences in CSF levels (Teng *et al.*, 2014; Paterson *et al.*, 2015; Wellington *et al.*, 2018; Pillai *et al.*, 2019). The majority of patients with AD pathology clinically present with amnesic AD, the most common form of dementia, characterized by profound loss of episodic memory (Dubois *et al.*, 2007). However, there are several non-amnesic variants of AD that instead present with visuospatial, language, or

behavioral/executive impairments (Galton *et al.*, 2000; Murray *et al.*, 2011; Dickerson *et al.*, 2017). Patients with non-amnestic AD can phenotypically mimic and are often misdiagnosed as a clinical variant of frontotemporal dementia (FTD) (Koedam *et al.*, 2010), the second most common dementia. FTD is associated with pathologic FTLN (Hodges *et al.*, 2004; Perry and Miller, 2013) and, like non-amnestic AD, is characterized by language, behavioral, executive, and/or visuospatial dysfunction with relatively spared episodic memory. Both FTD and non-amnestic AD are characterized by a younger age of symptom onset than typical, amnestic AD (Koedam *et al.*, 2010; Crutch *et al.*, 2012; Lam *et al.*, 2013; Onyike and Diehl-Schmid, 2013; Mendez, 2017). Despite common AD pathology, amnestic and non-amnestic variants have relatively distinct patterns of anatomical disease distribution and protein accumulation (Galton *et al.*, 2000; Wolk, 2013, Phillips *et al.*, 2018a, b, 2019). Differences in disease dynamics between clinical syndromes may explain part of the observed variance in CSF levels amongst patients with AD pathology (Teng *et al.*, 2014; Paterson *et al.*, 2015).

Another potential source of diagnostic error is that CSF-derived ATN status may be relatively insensitive to primary pathologies other than AD. While the ATN framework interprets positive T and/or N status in the absence of A as non-AD pathology, these designations may not represent true A-negative status (Pouclet-Courtemanche *et al.*, 2019) or successfully detect other neurodegenerative pathologies, like FTLN. Moreover, co-occurring pathologies are common in persons with dementia, as well as in our brain bank (Robinson *et al.*, 2018). While an A+T-N+ profile is associated with Alzheimer's and concomitant suspected non-Alzheimer's pathologic change, it is unknown if this profile captures the majority of co-pathologic cases with primary non-AD pathology. Thus, secondary AD pathology — resulting in positive biomarkers for AD — may obscure a primary pathology of FTLN and reduce diagnostic specificity (Toledo *et al.*, 2012).

Here we used a sample of amnestic and non-amnestic patients with postmortem pathological diagnoses of primary AD or FTLN to test whether levels of CSF  $A\beta_{1-42}$ , p-tau, and t-tau differ across amnestic and non-amnestic phenotypes, and whether these differences lead to errors in patients' classification under the ATN framework. Similarly, we assessed pathologic and phenotypic differences for p-tau/ $A\beta_{1-42}$ , a single biomarker that represents a commonly used alternative to the ATN framework for AD pathologic change and to stratify AD from FTLN (Struyfs *et al.*, 2015; Oeckl *et al.*, 2016; Lleó *et al.*, 2018). For each CSF analyte, we compared the frequency and clinical characteristics of true- and false-negative cases in non-amnestic and amnestic AD as well as true- and likely false-positives in FTLN. Receiver operating characteristic (ROC) curve assessments tested the diagnostic accuracy of CSF markers when stratifying patients with autopsy-confirmed primary AD from primary FTLN pathology. Between-group comparisons adjusted for age, sex, global cognitive impairment, APOE status, and presence of co-pathology. We compare A and T status during life to amyloid and tangle pathologic severity at autopsy, and we compare N status during life to postmortem atrophic severity. Our goals were to test the diagnostic accuracy of the ATN framework and the p-tau/ $A\beta_{1-42}$  ratio by comparing CSF markers across phenotypes and identifying likely sources of diagnostic error.

## Materials and methods

## **Participants**

Participants were 182 patients with a lumbar puncture and autopsy-confirmed primary AD or FTLD pathology identified through the Integrated Neurodegenerative Disease Biobank and Database (Xie *et al.*, 2011; Toledo *et al.*, 2014). All patients were autopsied at the University of Pennsylvania Center for Neurodegenerative Disease Research. Autopsy analyses classified the level of AD pathologic change from “not” to “high” using established “ABC” scoring methods (Montine *et al.*, 2012) for amyloid- $\beta$  (modified Thal scoring on a four-point 0-3 scale), tau neurofibrillary tangles (modified Braak scoring on a four-point 0-3 scale), and neuritic plaques (four-point CERAD scale) when available (Mirra *et al.*, 1991). For FTLD-tau cases, Braak staging was determined using mAbs GT-7 and GT-38 immunohistochemistry that is specific to both 3 and 4 microtubule-binding repeat (3R; 4R) isoforms characteristic of AD neurofibrillary tangles (Gibbons *et al.*, 2018, 2019). Thirty cases lacked scoring for amyloid- $\beta$  but had neurofibrillary tangle and CERAD neuritic plaque scores; cases with both CERAD and Braak scores of 2 or 3 were judged to have a non-zero likelihood of AD pathology, while cases with both CERAD and Braak scores of 0 or 1 were judged unlikely for AD pathologic change. For six cases, the level of AD pathologic change was unable to be estimated, and the presence of AD pathology was based on pathologists’ judgment. FTLD pathology was assessed using established histopathologic methods by accumulations of misfolded 3R or 4R tau (FTLD-Tau) associated with corticobasal degeneration, progressive supranuclear palsy, Pick’s disease, argyrophilic grain disease, and frontotemporal dementia with parkinsonism linked to chromosome 17; or transactive response DNA-binding protein of 43 kDa (TDP-43) associated with frontotemporal dementia and amyotrophic lateral sclerosis with cognitive impairment (FTLD-TDP) (Igaz *et al.*, 2008; Mackenzie *et al.*, 2010). Gross atrophic severity was visually evaluated by pathologists at autopsy on a four-point scale from none to severe.

Patients were followed clinically at the Penn Frontotemporal Degeneration Center or the Penn Memory Center and were clinically diagnosed by board-certified neurologists according to published diagnostic criteria (Gorno-Tempini *et al.*, 2011; McKhann *et al.*, 2011; Rascovsky *et al.*, 2011; Armstrong *et al.*, 2013; Höglinger *et al.*, 2017; Strong *et al.*, 2017). Participants’ consent was obtained according to the Declaration of Helsinki and approved by the University of Pennsylvania’s Institutional Review Board. Exclusion criteria included primary pathology other than AD or FTLD, patients without cognitive impairment, co-occurring neurologic conditions (e.g. stroke, hydrocephalus, chronic traumatic encephalopathy, vascular disease), and primary psychiatric disorders (e.g. depression, anxiety).

We next determined if AD and FTLD patients had mixed pathology, with primary pathological diagnosis based on pathologist’s judgment. AD patients were classified as having “mixed” pathology if they had  $\alpha$ -synuclein positive Lewy bodies/neurites or TDP-43 inclusions (1-mild to 3-severe) in one or more neocortical regions (McKeith *et al.*, 2005; Montine *et al.*, 2012). FTLD patients were classified as having “mixed” pathology if they were positive for any level of AD pathology (ABC scores of low/intermediate/high) or neo-cortical  $\alpha$ -synuclein (McKeith *et al.*, 2005; Montine *et al.*, 2012). Co-pathologic conditions included hippocampal sclerosis, limbic-predominant age-related TDP-43 encephalopathy (LATE), argyrophilic grain disease, Lewy body disease, primary age-related tauopathy (PART), and globular glial tauopathy. If no form of clinically meaningful co-pathology was detected, patients were classified as having “negligible” co-pathology. AD patients with amygdala-predominant Lewy bodies or cerebral amyloid

angiopathy were considered to have “negligible” co-pathology. Of the 118 patients with confirmed primary AD pathology, 63 were classified as having mixed pathology. Three of these mixed cases had high levels of both FTLD and AD (high ABC rating), and primary pathology could not be determined; because of their high levels of AD pathologic change, these cases were classified as AD with mixed pathology in analyses. Of 64 patients with confirmed primary FTLD pathology, 34 were classified with mixed pathology. Of these FTLD with mixed pathology cases, three had ABC scores suggesting no AD, 22 had low AD pathologic change, and four had intermediate pathologic change. Of the remaining five FTLD cases missing ABC scores, co-pathology was determined by pathologists’ assessment; two were determined to have AD, one had argyrophilic grain disease, and two had PART.

Of 118 patients with confirmed AD pathology, 98 had an amnesic phenotype at presentation (clinical diagnoses: AD, n=95; amnesic mild cognitive impairment [aMCI], n=3), and 20 had a non-amnesic phenotype at presentation (clinical diagnoses: behavioral variant AD, [bvAD] n=1; behavioral variant FTD [bvFTD], n=2; unspecified FTD, n=5; unspecified primary progressive aphasia [PPA], n=2; logopenic variant PPA [lvPPA], n=3; corticobasal syndrome [CBS], n=7). Of 64 patients with confirmed FTLD pathology, 5 had an amnesic phenotype at presentation (clinical diagnoses: AD, n=5), and 59 had a non-amnesic phenotype at presentation (clinical diagnoses: non-amnesic MCI, n=1; bvFTD, n=16; unspecified FTD, n=8; unspecified PPA, n=3; lvPPA, n=3; nonfluent/agrammatic PPA, n=3; semantic variant PPA, n=5; CBS, n=9; progressive supranuclear palsy, n=7; and ALS with FTD, n=4).

### ***Demographic Comparisons***

Across pathology and phenotype, we compared patients for number of copies of the APOE  $\epsilon 4$  allele, sex, age at CSF, age at onset (earliest reported symptom), age at death, disease duration at CSF (years from onset to CSF), disease severity (Mini-Mental State Examination [MMSE]) (Folstein *et al.*, 1975), survival (years from earliest symptom onset to death), and years of education (Table 1). A chi-squared test indicated significant differences between AD and FTLD pathology in the frequency of individuals with zero, one, or two copies of the APOE  $\epsilon 4$  allele ( $X^2(2)=35.70$ ,  $p=1.77 \times 10^{-8}$ ). Further testing indicated that the distribution of allele carriers did not differ between amnesic and non-amnesic AD groups ( $X^2(2)=4.4$ ,  $p=0.11$ ), but that  $\epsilon 4$  alleles were more frequent among the small group of amnesic FTLD patients than non-amnesic FTLD patients ( $X^2(2)=14.78$ ,  $p=0.0006$ ). There was no significant difference in sex distribution across amnesic and non-amnesic AD and FTLD groups ( $X^2(3)=1.17$ ,  $p=0.76$ ). Because quantitative demographic measures were not normally distributed, non-parametric Mann-Whitney-Wilcoxon tests compared AD and FTLD groups. AD and FTLD patients were not significantly different for years of education or disease duration (both  $p>0.1$ ). AD patients were older at CSF compared to FTLD ( $W=5067.5$ ,  $p=0.0001$ ), and had marginally lower MMSE than FTLD ( $W=2939.5$ ,  $p=0.065$ ). Kruskal-Wallis tests were used to compare across amnesic AD, non-amnesic AD, amnesic FTLD, and non-amnesic FTLD (Table 1). Patients were matched for MMSE, disease duration, education (all  $p>0.1$ ), but were significantly different for age ( $X^2(3)=19.86$ ,  $p=0.0002$ ). In pairwise comparisons using Mann-Whitney-Wilcoxon tests, amnesic AD patients were significantly older than non-amnesic FTLD ( $U=4108.5$ ,  $p=1.02 \times 10^{-5}$ ), and were marginally older than non-amnesic AD ( $U=1235$ ,  $p=0.068$ ). We observed similar differences for all other age-related variables (Table 1), including age at disease onset, age at death and survival, consistent with findings that non-amnesic variants tend to have an earlier disease onset than

amnesic variants (Koedam *et al.*, 2010).

### ***Cerebrospinal fluid (CSF) analysis***

CSF samples were collected following a standard lumbar puncture typically following an overnight fast. Samples were measured for A $\beta_{1-42}$ , p-tau, t-tau, and p-tau/A $\beta_{1-42}$  using the xMAP Luminex platform (INNO-BIA AlzBio3 for research-only reagents; Innogenetics). Analyses were performed by laboratory technicians who were blinded to clinical data. Published cut-points for all measures are based on best thresholds reported by Shaw and colleagues (2009); amyloid (A) positivity was based on an A $\beta_{1-42}$  cutoff of 192 pg/mL; tau (T) positivity was based on a p-tau cutoff of 23 pg/mL; neurodegeneration (N) positivity was based on a t-tau cutoff of 93 pg/mL; and a cutoff of 0.10 was applied to the p-tau/A $\beta_{1-42}$  ratio to determine positivity for AD. Outlier checks were performed on CSF values, and one case in the pathologic AD group was excluded for a CSF t-tau value more than five standard deviations above the sample mean.

### ***Statistical analysis***

***ATN Classification Based on CSF Biomarkers.*** For phenotypic and pathologic groups, we evaluated ATN designation of patients on the AD continuum based on positive or negative A, T, or N status and examined the profiles of cases that were likely misclassified. Patients with CSF A $\beta_{1-42} \leq 192$  pg/mL were considered A-positive; those with CSF p-tau  $\geq 23$  pg/mL were considered T-positive; and those with CSF t-tau  $\geq 93$  pg/mL were considered N-positive. In addition, we tested classification using the p-tau/A $\beta_{1-42}$  ratio; patients with p-tau/A $\beta_{1-42} \geq 0.10$  were considered positive for AD.

***Between-group comparisons of CSF.*** To elucidate the sources of misclassification within the ATN framework or the p-tau/ A $\beta_{1-42}$  ratio, analyses of covariance (ANCOVAs) compared each CSF analyte across pathology (AD, FTLD) and phenotype (amnesic, non-amnesic), covarying for number of APOE  $\epsilon 4$  alleles, co-pathology status (negligible, mixed), age at CSF, MMSE, and sex ( $\alpha = 0.05$ ). To ensure that the presence of mixed pathology was not driving differences, models were also run excluding patients with high levels of co-pathology. MMSE scores were unavailable for five participants (two non-amnesic AD, one amnesic AD, and two non-amnesic FTLD), and education data were unavailable for two participants (one non-amnesic AD and one non-amnesic FTLD). These missing data were imputed based on the mean of each patient's respective pathology and phenotype group. In initial statistical analysis of CSF measures, model residuals were not normally distributed, violating an assumption of multivariate normality; thus, a log transformation was applied to each measure. Because of the unbalanced design, type II sum of squares were calculated for ANCOVAs. CSF levels were compared through post-hoc linear contrasts.

For each marker, true- and false-negative and true- and likely false-positive cases were identified based on established cut-points (Shaw *et al.*, 2009), and Fisher's exact tests were used to test whether the likelihood of being classified as positive for each marker differed according to memory phenotype in AD. We did not compare CSF marker positivity between FTLD phenotypes due to the small number of amnesic FTLD cases (n=6).

### ***Discrimination of pathologic AD from FTLD.***

Finally, receiver operating characteristic (ROC) curve and area under the curve (AUC) analyses assessed diagnostic accuracy of each CSF marker when discriminating non-amnestic patients with AD from FTLD pathology, compared to discriminating amnestic patients with AD from FTLD pathology. Optimal cutoffs were calculated for each CSF marker on the basis of Youden's index, which maximized the sum of sensitivity and specificity in the current sample.

All statistical analyses were conducted in the R statistical environment, using the Companion to Applied Regression (car), multcomp, and partial ROC (pROC) packages (Robin *et al.*, 2011; Fox *et al.*, 2012; R Core Team, 2017).

### ***Data Availability***

All qualified investigators are welcome to view data through an established algorithm at the CNDR.

## **Results**

### ***ATN Classification Based on CSF Biomarkers***

Table 2 outlines patient classifications according to A, T, and N positivity based on published cutoffs for CSF biomarkers (Shaw *et al.*, 2009). According to the ATN framework, of the 98 amnestic patients with autopsy-confirmed AD pathology, 91 (93%) had CSF biomarkers positive for Alzheimer's continuum disease. A similarly high proportion of non-amnestic patients with AD pathology, 17 of 20 (85%), were positive for Alzheimer's continuum disease. However, the likelihood of more specific ATN designations was different across amnestic phenotype in patients with AD pathology. Fisher's tests indicated that non-amnestic AD patients were significantly less likely to be positive for all three markers than amnestic AD patients (20% vs. 52%; OR = 0.23; CI = 0.05–0.79;  $p=0.013$ ), and were significantly more likely to be negative for all three markers and classified as having normal biomarkers than amnestic AD patients (15% vs. 2%; OR = 8.23; CI = 0.88–105.4;  $p=0.034$ ).

Only ten of 118 AD cases (8%) were negative for Alzheimer's continuum disease. Of these, five had profiles indicative of non-AD pathologic change (A-T+N- or A-T+N+); all five were amnestic; two had no co-occurring pathology detected at autopsy, two had co-occurring Lewy body pathology, and one had co-occurring TDP-43 pathology. ABC scores indicated high ( $n=2$ ) or intermediate ( $n=3$ ) AD pathologic change. Another five (three non-amnestic; 2 amnestic) had normal profiles (A-T-N-); ABC scores show high ( $n=3$ ), intermediate ( $n=1$ ), and low ( $n=1$ ) levels of AD pathologic change; two patients had negligible co-pathology and three had co-occurring TDP-43 pathology.

We also examined accuracy of the A+T-N+ classification to indicate concomitant Alzheimer's and non-Alzheimer's pathologic change. Thirteen of 118 patients (11 amnestic; two non-amnestic) were classified as A+T-N+. ABC scores indicated high ( $n=12$ ) or intermediate ( $n=1$ ) AD pathologic change. Four of these 13 (31%) patients were found to have negligible levels of co-occurring pathology at autopsy and represent probable misclassifications.

Of the 64 FTLD patients, only 12 (19%; 12 non-amnestic; zero amnestic) had an ATN profile suggestive of non-AD pathologic change (A-T+N+, A-T-N+, or A-T+N-). The majority of FTLD



patients (63%) were classified as having normal ATN biomarkers. Nine (14%) FTLD patients had an A+T-N- profile, suggesting AD pathologic change only. Of these nine, two were determined to not have AD (ABC scores, n=2; pathologist, n=1); these cases represent probable misclassifications. The other six were determined to have low AD pathologic change (ABC scores, n=5; pathologist, n=1); ATN biomarkers in these cases were insensitive to patients' primary FTLD pathology. Three FTLD patients had an A+T-N+ profile, suggesting concomitant AD and non-AD pathologic change; two were found to have negligible co-pathology at autopsy, and the final patient had likely AD pathology.

Finally, we compared the ATN framework to the p-tau/A $\beta_{1-42}$  ratio in amnesic and non-amnesic AD patients. Performance was equivalent in patients with autopsy-confirmed AD pathology. Of 98 amnesic AD patients, 91 (93%) were classified as positive for Alzheimer's continuum using the ATN framework (as reported above), and 88 (90%) were classified as positive for AD using the p-tau/A $\beta_{1-42}$  ratio. Of the 20 non-amnesic patients with AD pathology, 17 (85%) were classified as positive for Alzheimer's continuum using the ATN framework (as reported above), while 13 (65%) were classified as positive for AD using the p-tau/A $\beta_{1-42}$  ratio. Comparing the false-positives in FTLD patients with negligible co-pathology (Supplementary Table 1), two of 30 FTLD patients (7%) were positive for AD pathology using the p-tau/A $\beta_{1-42}$  ratio, and four of 30 FTLD patients (13%) were positive for Alzheimer's continuum disease according to the ATN framework. While ATN identified a numerically larger proportion of non-amnesic AD cases, and the p-tau/A $\beta_{1-42}$  ratio had fewer false-positives, Fisher's tests determined these proportions were not significantly different (all  $p > 0.2$ ).

Chi-squared tests indicated no significant differences between amnesic and non-amnesic patients with AD pathology in Thal, Braak, or CERAD staging (all  $p > 0.3$ ), nor in brain atrophy ratings between patients with FTLD and AD pathology ( $p > 0.5$ ). To elucidate the sources of ATN classification differences between non-amnesic and amnesic AD, we subsequently compared levels of each CSF marker parametrically across pathology and phenotype. If CSF A $\beta_{1-42}$ , p-tau, and the p-tau/A $\beta_{1-42}$  ratio are accurate markers of likely amyloid, tangle, and AD pathology respectively, they are expected to be significantly different across pathology (AD, FTLD) but invariant to phenotype (amnesic, non-amnesic). If CSF t-tau is an accurate non-specific staging marker of N, all patient groups would be hypothesized to have a high proportion of N-positive cases; while we do not know severity of neurodegeneration in life, all patients were symptomatic at time of CSF sample (Jack *et al.*, 2013).

### ***Between-group comparisons of CSF A $\beta_{1-42}$***

We compared levels of CSF A $\beta_{1-42}$  parametrically across pathology and phenotype (Figure 1). An ANCOVA (Type II) showed that A $\beta_{1-42}$  levels differed by pathology ( $F(1,173)=56.2$ ,  $p=3.2 \times 10^{-12}$ ), with lower values in the pathologic AD group than the pathologic FTLD group. Additionally, A $\beta_{1-42}$  levels were significantly associated with APOE status ( $F(1,173)=6.1$ ,  $p=0.014$ ), with lower concentrations associated with greater numbers of the  $\epsilon 4$  allele. Phenotypic differences in A $\beta_{1-42}$  levels were non-significant ( $F(1,173)=1.1$ ,  $p=0.29$ ). Co-pathology status ( $p=0.22$ ), age ( $p=0.99$ ), sex ( $p=0.77$ ), and MMSE ( $p=0.86$ ) were likewise not significantly associated with A $\beta_{1-42}$  levels. Omnibus results when restricting the cohort to patients with negligible co-pathology also showed the effect of pathology type was significant ( $F(1,77)=32.0$ ,  $p=2.49 \times 10^{-7}$ ) while the effect of phenotype was not ( $p=0.88$ ); all other effects were non-

significant including the association with APOE (all  $p>0.31$ ).

### ***A Status***

False-negative rates for A status were low for both amnesic (seven of 98) and non-amnesic (three of 20) patients with primary AD pathology, with Fisher's test showing no difference across phenotype ( $p=0.37$ ). In the patients with primary FTLD pathology, 12 of 64 were CSF A-positive (see Supplementary Material).

### ***Between-group comparisons of CSF p-tau***

CSF p-tau levels were compared parametrically across pathology and phenotype (Figure 2). A Type II ANCOVA showed that p-tau levels differed by pathology ( $F(1,173)=28.3$ ,  $p=3.29 \times 10^{-7}$ ) and memory phenotype ( $F(1,173)=7.2$ ,  $p=0.0081$ ), with APOE status ( $p=0.45$ ), co-pathology status ( $p=0.71$ ), age ( $F(1,173)=4.2$ ,  $p=0.043$ ), sex ( $p=0.18$ ), and MMSE ( $p=0.09$ ) included as covariates. Post-hoc linear contrasts confirmed that the pathologic FTLD group had lower CSF p-tau than the pathologic AD group ( $t(173)=-5.3$ ,  $p=3.23 \times 10^{-7}$ ), and that non-amnesic AD patients had lower CSF p-tau than amnesic AD patients ( $t(173)=-3.2$ ,  $p=0.0072$ ). Non-amnesic FTLD patients had lower CSF p-tau than both amnesic AD ( $t(173)=-9.1$ ,  $p=1.67 \times 10^{-15}$ ) and non-amnesic AD ( $t(173)=-3.5$ ,  $p=0.0031$ ) patients. Amnesic FTLD patients had lower CSF p-tau than amnesic AD ( $t(173)=-4.5$ ,  $p=1.23 \times 10^{-4}$ ); no other comparisons were significant ( $p>0.11$ ). Similar results were obtained when restricting the cohort to patients with negligible secondary pathology: we observed significant effects of pathology type ( $F(1,77)=24.2$ ,  $p=4.77 \times 10^{-6}$ ), phenotype ( $F(1,77)=6.0$ ,  $p=0.016$ ), and age ( $F(1,78)=10.3$ ,  $p=0.0020$ ). There was a marginal effect of MMSE ( $F(1,77)=3.4$ ,  $p=0.070$ ), and all other effects were non-significant (all  $p>0.65$ ), and.

### ***T Status***

Fisher's test showed significantly lower rates for T-positivity in non-amnesic patients (eleven of 20; 50%) compared to amnesic patients (26 of 98; 24%) with confirmed AD pathology (OR = 0.30; CI: 0.10–0.89;  $p=0.018$ ). Five of 64 FTLD patients were T-positive (Supplementary Material).

### ***Between-group comparisons of CSF t-tau***

CSF t-tau levels were compared parametrically across pathology and phenotype (Figure 3). A Type II ANCOVA showed that CSF t-tau differed by pathology ( $F(1,173)=8.8$ ,  $p=0.0034$ ), phenotype ( $F(1,173)=9.1$ ,  $p=0.0029$ ), with APOE status ( $p=0.95$ ), co-pathology status ( $p=0.14$ ), age ( $p=0.44$ ), sex ( $F(1,173)=11.0$ ,  $p=0.0011$ ), and MMSE ( $p=0.51$ ) included as covariates. In post-hoc contrasts, the pathologic FTLD group had lower CSF t-tau than the AD group ( $t(173)=-3.0$ ,  $p=0.0034$ ). Compared to amnesic AD, we observed lower t-tau in non-amnesic AD ( $t(173)=-3.0$ ,  $p=0.016$ ) and in non-amnesic FTLD patients ( $t(173)=-6.6$ ,  $p=2.98 \times 10^{-9}$ ); all other comparisons were non-significant ( $p>0.19$ ). CSF t-tau results were similar when restricting the cohort to patients with negligible co-pathology: the effect of pathology type was significant ( $F(1,77)=5.8$ ,  $p=0.019$ ), although the effect of memory phenotype was marginal ( $F(1,77)=3.5$ ,  $p=0.063$ ); all other effects were non-significant (all  $p>0.20$ ).

### ***N Status***

Fisher's test showed significantly lower rates of N-positivity for non-amnesic patients (six of 20,

or 30%) compared to amnesic patients (65 of 98, or 66%) with confirmed AD pathology (OR = 0.22; CI: 0.06–0.68;  $p=0.005$ ). Only 13 of 64 (20%) FTLN patients were positive for N. Two (3%) FTLN cases were true-negatives and had no atrophy at autopsy. The remaining 49 cases (77%) were likely false-negatives for N, showing mild to severe atrophy at autopsy (see Supplementary Material).

### ***Between-group comparison of CSF p-tau/A $\beta_{1-42}$ levels***

The p-tau/A $\beta_{1-42}$  ratio was compared parametrically across pathology and phenotype (Figure 4). A Type II ANCOVA showed that p-tau/A $\beta_{1-42}$  levels differed by pathology ( $F(1,173)=64.7$ ,  $p=1.35 \times 10^{-13}$ ) and memory phenotype ( $F(1,173)=8.5$ ,  $p=0.0040$ ), with APOE status ( $p=0.77$ ), co-pathology status ( $p=0.95$ ), age ( $F(1,173)=3.5$ ,  $p=0.063$ ), sex ( $p=0.27$ ), and MMSE ( $p=0.14$ ) included as covariates. Post-hoc testing confirmed that pathologic FTLN group had lower CSF t-tau than the AD group ( $t(173)=-8.0$ ,  $p=1.35 \times 10^{-13}$ ). Further, non-amnesic AD patients had significantly lower p-tau/A $\beta_{1-42}$  ratios than amnesic AD patients ( $t(173)=-3.0$ ,  $p=0.017$ ). Non-amnesic FTLN patients had lower p-tau/A $\beta_{1-42}$  ratios than both amnesic AD ( $t(173)=-12.2$ ,  $p<0.00001$ ) and non-amnesic AD ( $t(173)=-6.0$ ,  $p=3.28 \times 10^{-8}$ ); amnesic FTLN patients also had lower p-tau/A $\beta_{1-42}$  ratios than both amnesic AD ( $t(173)=-4.9$ ,  $p=1.26 \times 10^{-5}$ ) and non-amnesic AD ( $t(173)=-2.7$ ,  $p=0.031$ ). No difference was observed between non-amnesic and amnesic FTLN patients ( $p=0.79$ ). Omnibus results were highly similar when restricting the cohort to patients with no co-pathology: effects of pathology type ( $F(1,77)=50.7$ ,  $p=4.91 \times 10^{-10}$ ), phenotype ( $F(1,77)=4.5$ ,  $p=0.037$ ), age ( $F(1,78)=8.4$ ,  $p=0.0050$ ), and MMSE score ( $F(1,77)=4.2$ ,  $p=0.044$ ) were significant; and effects of sex and APOE  $\epsilon 4$  allele count were non-significant (both  $p>0.76$ ).

### ***AD status***

A Fisher's test showed significantly higher false-negative rates for non-amnesic patients (seven of 20, or 35%) compared to amnesic patients (10 of 98, or 10%) with confirmed AD pathology (OR = 4.7; CI: 1.27–16.58;  $p=0.009$ ). In the pathologic FTLN group, 10 of 64 (16%) were AD positive (see Supplementary Material).

### ***Discrimination of pathologic AD from FTLN***

We computed ROCs for each CSF measure stratifying amnesic or non-amnesic AD from all patients with FTLN pathology (Table 3). In an overall analysis distinguishing all AD from FTLN patients, CSF A $\beta_{1-42}$  and the p-tau/A $\beta_{1-42}$  ratio produced similar AUCs showing excellent discrimination of both markers (0.938 vs. 0.942). Of the two measures, CSF A $\beta_{1-42}$  had the greatest sensitivity, while the p-tau/A $\beta_{1-42}$  ratio yielded higher specificity (i.e., fewer primary FTLN patients classified as AD) than all other markers.

DeLong's test for two ROC curves compared diagnostic accuracy by AD phenotype for each marker. No difference in accuracy was observed between non-amnesic and amnesic AD when A $\beta_{1-42}$  was used to discriminate groups from pathologic FTLN ( $p=0.64$ ), which maintained high accuracy for both amnesic and non-amnesic AD (AUC=0.94 vs. AUC=0.92). However, CSF p-tau ( $D(105.1)=-1.9$ ,  $p=0.057$ ) and the p-tau/A $\beta_{1-42}$  ratio ( $D(99.6)=-1.9$ ,  $p=0.059$ ) were both marginally less accurate for discriminating non-amnesic AD than amnesic AD from all FTLN patients. Although CSF t-tau is not a diagnostic marker, DeLong's test shows a significantly lower AUC for non-amnesic AD than amnesic AD patients when discriminating from all FTLN

patients ( $D(119.9)=-2.3$ ,  $p=0.025$ ).

For discrimination of amnesic AD from all FTLD patients, optimal cutoffs in the current study were close to the thresholds published by Shaw et al. (2009): for  $A\beta_{1-42}$ , 199.5 pg/mL vs. 192 pg/mL; for p-tau, 20.1 pg/mL vs. 23 pg/mL; for t-tau, 96.5 pg/mL vs. 93 pg/mL; and for the p-tau/ $A\beta_{1-42}$  ratio, 0.11 vs. 0.10. However, for discrimination of non-amnesic AD from all FTLD patients, optimal cutoffs were substantially lower than published thresholds for  $A\beta_{1-42}$  (168.0 pg/mL), p-tau (12.5 pg/mL), t-tau (66.5 pg/mL), and the p-tau/ $A\beta_{1-42}$  ratio (0.076). ROC analyses were repeated excluding cases with co-pathology and produced similar results, although the p-tau/ $A\beta_{1-42}$  ratio had a high AUC (0.93) when discriminating non-amnesic AD from FTLD patients, albeit with a much lower cutoff of 0.051 (vs. 0.10) (Supplementary Table 2).

### ***Secondary analysis of p-tau associations with age and sex***

In the current study, non-amnesic AD patients were younger at age of symptom onset and at death than amnesic AD patients, consistent with previous reports that atypical AD phenotypes are associated with a younger age of onset (Lam *et al.*, 2013; Mendez, 2017). While there was no effect for age in analyzing  $A\beta_{1-42}$  or t-tau, this demographic difference raised the question of whether lower p-tau values in the non-amnesic AD sample were simply a consequence of their younger age, which would be reflected by a positive association between age and p-tau levels among non-amnesic AD patients. In addition, models revealed significantly lower t-tau in males than females. Secondary analyses revealed that differences in CSF p-tau and t-tau between amnesic and non-amnesic AD patients could not be explained by age or sex differences between the groups (see Supplementary Material).

## **Discussion**

Recent diagnostic strategies, such as the ATN framework, have emphasized biomarkers over traditional clinical evaluations to improve antemortem predictions of AD pathology, as well as stratification from other pathologies such as FTLD. Even with the improved accuracy of antemortem predictions of AD pathology, subtle differences in CSF levels across AD variants might lead to a small number of diagnostic errors (Teng *et al.*, 2014; Paterson *et al.*, 2015; Wellington *et al.*, 2018; Pillai *et al.*, 2019). To better characterize the full spectrum of CSF profiles in AD continuum disease and evaluate diagnostic accuracy of CSF biomarkers, we compared the accuracy of CSF-based ATN classifications in AD patients with amnesic and non-amnesic clinical phenotypes, and in FTLD. In autopsy-confirmed primary AD pathology, non-amnesic AD patients were less likely than amnesic AD patients to be classified as A+T+N+, but more likely to be classified as normal (A-T-N-) according to CSF biomarkers. Parametric comparisons revealed that differences in designation are likely because non-amnesic AD patients have lower concentrations of both p-tau and t-tau in their CSF than amnesic AD. Likewise, ROC curve analyses showed that CSF p-tau and the p-tau/ $A\beta_{1-42}$  ratio have somewhat reduced AUC and lower sensitivity in non-amnesic AD than amnesic AD when discriminating from FTLD patients. While t-tau is not a diagnostic marker, it also had a lower AUC when discriminating non-amnesic AD from FTLD, emphasizing that concentrations of t-tau differ by amnesic phenotype. Unlike CSF p-tau and t-tau, CSF  $A\beta_{1-42}$  levels were equivalent between amnesic and non-amnesic AD patients, and were significantly lower in all FTLD patients. ROC curve analyses confirmed that CSF  $A\beta_{1-42}$  is a highly sensitive marker to the presence of AD in both amnesic and non-amnesic AD, and is excellent at stratifying both AD groups from FTLD

patients. Thus, our findings indicate that A status is consistent across memory phenotypes in AD, and accurately detects individuals who are likely positive for pathologic amyloid deposition and thus AD continuum disease. However, more specific designations of T and N status based on CSF p-tau and t-tau may inaccurately classify non-amnestic AD patients as negative for tau aggregation or neurodegeneration. These findings highlight the need for careful implementation of the ATN framework for *in vivo* diagnosis, as markers optimized for the most common amnestic presentations of AD may not capture the full phenotypic spectrum of the pathologic disease.

While a primary goal of the ATN framework is to identify patients with AD pathology, a secondary goal of the ATN framework may be the characterization of individuals with non-AD pathology (Jack *et al.*, 2018). Under the ATN framework, detection of non-AD pathology relies on N-positive status in the absence of either A or T, or T-positive status in the absence of A. Our results show that patients with FTLD pathology were not reliably classified by ATN; only 12 of 64 were identified as having non-AD pathology (A-T+N-, A-T-N+, A-T+N+). Three FTLD patients were identified as having suspected co-occurring AD and non-AD pathologies; however two of these had ABC scores of 0 at autopsy. Instead, a majority of FTLD patients were N-negative due to lower CSF t-tau levels, despite showing atrophy at autopsy (only two cases of FTLD were judged not to be atrophic); nine of 64 were classified as having Alzheimer's pathologic change, and the majority were classified as having normal biomarkers (40 of 64). It is noteworthy that a majority of non-amnestic AD patients were also N-negative (14 of 20). Discrepancies between t-tau results and autopsy assessments of atrophy in non-amnestic AD and in FTLD may indicate that brain structure was relatively preserved at time of lumbar puncture; alternatively, negative t-tau results may indicate a failure to detect true degeneration. While CSF t-tau is interpreted as a non-specific marker of neurodegeneration (Jack *et al.*, 2016), our results indicate that t-tau may be relatively insensitive to neurodegeneration in non-amnestic pathologic cases, both AD and FTLD.

We additionally tested discrimination based on p-tau/A $\beta_{1-42}$  ratio as an alternative to the ATN framework. This composite marker had the highest AUC (0.96) and highest specificity (0.92) when stratifying amnestic AD from pathologic FTLD, a finding in agreement with previous studies (Vergallo *et al.*, 2017; Lleó *et al.*, 2018). Indeed, this ratio score correctly identified 90% of amnestic AD patients (Table 2). Results using the ATN framework were comparable: 93% of amnestic AD patients were classified as being on the AD continuum due to A-positive status. However, the p-tau/A $\beta_{1-42}$  ratio had a lower AUC than A $\beta_{1-42}$  alone (0.87 vs. 0.93) when discriminating non-amnestic AD patients from all FTLD patients, correctly identifying only 65% cases. By comparison, ATN designated 85% of non-amnestic AD patients as being on the AD continuum, although this increase was not significant.

The optimal thresholds for CSF A $\beta_{1-42}$ , p-tau, and p-tau/A $\beta_{1-42}$  specific to our entire cohort were broadly consistent with cut-points that were established in an independent cohort to distinguish AD from healthy controls (Shaw *et al.*, 2009). However, all optimal cutoffs were lower when stratifying non-amnestic AD patients from FTLD, including CSF A $\beta_{1-42}$ . A caveat to this statement is that CSF t-tau is not intended to discriminate pathologic AD from pathologic FTLD; to calculate a true cutoff for t-tau in non-amnestic patients, we would need a reference control sample without degeneration, which is beyond the scope of this study. Still, lower optimal

cutoffs of p-tau, t-tau, and the p-tau/ $A\beta_{1-42}$  reflect the lower concentrations of p-tau and t-tau in non-amnesic AD compared to amnesic AD. Even so, the sensitivity and specificity of these adjusted cutoffs is poor, and does little to improve diagnostic specificity of these markers due to overlap with pathologic FTLD. The exception is CSF  $A\beta_{1-42}$  which maintains high sensitivity and specificity in non-amnesic AD. Unlike p-tau and t-tau, the lower optimal cutoff of CSF  $A\beta_{1-42}$  in non-amnesic AD is a more conservative cut-off than in the entire sample, and may in part be an artifact of small cohort size; changing the cutoff from 192 to 166 would change the designation of only 1 subject (see Figure 1).

Instead of adjusting CSF cutoffs, our findings suggest that selection of the appropriate marker based on phenotype (amnesic or non-amnesic) and diagnostic goals (increase sensitivity or specificity) would do more to improve diagnostic accuracy. The p-tau/ $A\beta_{1-42}$  ratio appears to perform best in a cohort of amnesic AD dementia patients, and has slightly higher specificity for excluding non-AD cases. However, it performs less well in non-amnesic AD due to their lower levels of CSF p-tau. By comparison, the ATN framework has excellent sensitivity to the presence of Alzheimer's continuum disease due in part to the reliable performance of A status as measured by  $A\beta_{1-42}$  in both amnesic and non-amnesic AD. However, it may be that more specific designations within the ATN framework incorrectly classify non-amnesic AD patients as normal or Alzheimer's pathologic change. Potential limitations of classification using the ATN framework in non-amnesic AD should be considered for candidate treatment trials. Furthermore, our results indicate that the ATN framework is relatively insensitive to non-AD diseases like FTLD, and these patients were likely to be erroneously classified as having normal biomarkers or AD pathologic change alone. Biomarkers suggestive of co-occurring pathologies (A+T-N+) were unreliable: A+T-N+ failed to capture the majority of FTLD with secondary AD pathology and two of three A+T-N+ FTLD cases had no detected level of AD (zero ABC scores). In sum, both the ATN framework and the p-tau/ $A\beta_{1-42}$  were excellent at identifying amnesic AD cases, while the single marker of CSF  $A\beta_{1-42}$  had the most consistent performance across memory phenotypes in AD, and was sensitive to both amnesic and non-amnesic AD.

The current study additionally adds to a growing body of results on sex differences in CSF. Significantly lower levels of t-tau were observed in men compared to women with FTLD, and marginally lower levels of t-tau in men compared to women with AD (see Supplementary Material). Likewise, previous research has found that females have elevated CSF t-tau (Hohman *et al.*, 2018), a stronger association of *APOE-ε4* with p-tau and t-tau (Hohman *et al.*, 2018), and higher AD pathologic burden and tau tangle density (Oveisgharan *et al.*, 2018) compared to males. Even so, phenotypic differences in CSF biomarkers could not be explained by group differences in global cognition or sex. Like sex, analysis of age effects did not support the interpretation that non-amnesic AD patients had less tau accumulation due to their younger age. On the contrary, younger patients (both amnesic and non-amnesic) in the pathologic AD group were likely to have higher levels of p-tau than older patients, in agreement with previous findings (Lleó *et al.*, 2019).

### ***Caveats and Limitations***

There are a number of caveats to the current study. First, although autopsy studies suggest that mixed pathology is highly common in dementia, no current CSF analytes are able to determine the presence of co-occurring or "mixed" pathology. To increase the relevance and application of

our findings, we examined patients who had AD and FTLD with negligible levels of secondary pathology thought to not contribute significantly to phenotype, as well as those with mixed pathology, including AD and FTLD patients with co-occurring  $\alpha$ -synuclein pathology. To ensure that mixed pathology cases were not driving diagnostic errors, analyses included co-pathology status as a covariate and were repeated in patients with negligible co-pathology. Results were consistent across models with and without co-pathology cases, indicating that differences observed across phenotypes in AD are not explained by co-pathological status. We were insufficiently powered to examine subgroups of secondary pathology or subgroups of FTLD pathology. Second, while we interpret A-negative or T-negative status in AD as likely false-negative cases, and while the accumulation of these histopathologic features is thought to begin much earlier in the presymptomatic course of disease, we cannot entirely rule out the unlikely possibility that CSF markers accurately reflect a true absence of amyloid or tau pathology that developed only later in disease course and after obtaining the CSF sample. Third, amnesic and non-amnesic patients differed in age-related factors (age at CSF, at onset, and survival). These differences are expected given the dementia population: amnesic patients tend to be older at onset than non-amnesic variants of both AD and FTLD (Koedam *et al.*, 2010; Mendez, 2017). Still, one possible explanation of the higher incidence of T-negative and/or N-negative status in non-amnesic AD patients could be an earlier point in the course of disease at the time of CSF collection than amnesic AD. However, this was not supported by our data. To account for potential differences in the course of disease at the time of lumbar puncture, age at CSF and MMSE were included as covariates in models, and secondary analyses show CSF differences are not due to younger age in the non-amnesic AD group than AD. Even so, MMSE and other non-specific measures of disease severity may not capture differences across groups (Santana *et al.*, 2016; Pillai *et al.*, 2019). Fourth, t-tau has been criticized as a marker of neurodegeneration, in part because t-tau is highly correlated with p-tau, and thus characterizations of T-status and N-status may not be truly independent (Hampel *et al.*, 2018). Moreover, recent longitudinal work shows little prognostic value to t-tau in controls (Soldan *et al.*, 2019; Vos and Duara, 2019). While imaging-based markers of neurodegeneration may provide an attractive alternative to CSF t-tau, patients in the current study were not selected based on availability of MRI or PET data; and variability in the temporal intervals between patients' scans and lumbar punctures, coupled with differences in scan acquisition parameters, might have reduced statistical power and injected noise into analysis results. However, we note that the shortcomings of t-tau as a marker of N are orthogonal to the phenotypic differences in ATN classification reported in the current study. Finally, this is a single-center study, and thus may be limited in generalizability.

In conclusion, results show that only CSF  $A\beta_{1-42}$  is consistent across amnesic and non-amnesic patients with AD pathology, and is highly accurate at discriminating AD pathology from FTLD in both memory groups. Even so, CSF  $A\beta_{1-42}$  has decreased specificity compared to other biomarkers. By comparison, CSF p-tau and t-tau levels differ across amnesic and non-amnesic patients with AD pathology, and show a much lower accuracy for non-amnesic patients. Because of these differences, more specific ATN designations within the Alzheimer's continuum may be less accurate for non-amnesic variants of AD. Findings also show that the ATN framework had poor sensitivity to detecting primary FTLD, erroneously classifying them as Alzheimer's pathologic change alone or as having normal biomarkers. Together, our results indicate that sensitivity of AD diagnoses can be improved by considering non-amnesic phenotype when making CSF-based designations of ATN status.

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### **Competing Interests**

Authors have no conflicts of interest to report.



## Supplementary Material

### *A status in FTL D*

In the patients with primary FTL D pathology, 12 of 64 were CSF A-positive (three amnesic; nine non-amnesic). Of the 12 A-positive FTL D patients, three had definitive histopathologic assessments of no AD pathology (*i.e.*, ABC scores all equal to zero), and two had CERAD scores of zero (missing Thal); these five cases thus represent false-positives and likely false-positives. Five A-positive cases had non-zero Thal staging (one, n=3; two, n=1, three, n=1); A-positive CSF in this context may accurately capture the co-occurrence of amyloid pathology.

### *T status in FTL D*

Of the five T-positive FTL D cases (five non-amnesic; one amnesic), two had postmortem Braak stage of zero and one, respectively; CSF p-tau results in these cases may thus represent false-positives. Braak staging was not performed for the remaining three T-positive cases in the FTL D group.

### *N Status in FTL D*

Of 13 N-positive cases within the pathologic FTL D group (12 non-amnesic; one amnesic), one lacked an atrophy rating, three had mild atrophy, four had moderate atrophy, and five had severe atrophy. Of the 51 (80%) N-negative FTL D cases, only two were judged at autopsy to have no atrophy; 11 had mild atrophy, 18 had moderate atrophy, 19 had severe atrophy, and one lacked an atrophy rating.

### *AD status in FTL D determined by p-tau/A $\beta$ <sub>1-42</sub>*

In the pathologic FTL D group, 10 patients had supra-threshold p-tau/A $\beta$ <sub>1-42</sub> ratios. Three of 10 were definite false-positives and had zero ABC scores, two without co-pathology detected at autopsy and one with co-occurring hippocampal sclerosis. Four had low ABC scores, and another one with missing ABC scores had AD co-pathology according to pathologist's judgment. The final two were missing ABC scores, but had co-occurring primary age-related tauopathy according to pathologist's judgment.

### *Secondary analysis of p-tau associations with age, and t-tau associations with sex*

We performed a follow-up regression analysis with p-tau values for patients in the pathologic AD group, including a term for the interaction of age and phenotype. In the amnesic AD group, age was negatively associated with p-tau levels ( $\beta=-0.021$ ,  $t=-2.9$ ,  $p=0.0051$ ); the corresponding effect of age on CSF p-tau in the non-amnesic group did not differ from the reference value, evidenced by a non-significant interaction ( $p=0.28$ ) (Supplementary Figure 1). These results indicate that the younger average age of non-amnesic AD patients did not account for the lower p-tau values observed in that group. Instead, lower age was associated with higher CSF p-tau levels in AD.

Additionally, we performed secondary analyses to clarify sex-related effects on t-tau, as revealed by models. Patient groups were well-balanced in terms of sex distributions (Table 1; Supplementary Figure 2). We performed a follow-up regression analysis with t-tau values for patients in the pathologic AD group, including a term for the interaction of sex and phenotype. In the amnesic AD group, male sex was marginally associated with lower t-tau levels ( $\beta=-0.22$ ,  $t=-$

1.95,  $p=0.053$ ); the corresponding effect of sex on CSF t-tau in the non-amnestic group did not differ from the reference value, evidenced by a non-significant interaction ( $p=0.86$ ) (Supplementary Figure 2). These results indicate that lower levels of t-tau in non-amnestic AD compared to AD cannot be attributed to differences in sex distribution.

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**Figure 1. CSF  $A\beta_{1-42}$  concentrations according to phenotype (amnesic, non-amnesic) and postmortem ratings of amyloid- $\beta$  pathology.** Left panel plots patients with primary AD pathology. Right panel plots patients with primary FTLD pathology. Horizontal line indicates CSF  $A\beta_{1-42} = 192$  pg/mL, with patients below the line designated as A+ status. Color indicates modified Thal staging of amyloid- $\beta$  pathology on a zero-to-three scale (white indicates cases with no available Thal staging). Shape indicates presence or absence of co-pathologies.

**Figure 2. CSF p-tau concentrations according to phenotype (amnesic, non-amnesic) and postmortem ratings of tau neurofibrillary tangle pathology.** Left panel plots patients with primary AD pathology. Right panel plots patients with primary FTLD pathology. Horizontal line indicates CSF p-tau = 23 pg/mL, with patients at or above the line designated as T+ status. Color indicates postmortem Braak staging of tau tangles (white indicates cases with no available Braak staging). Shape indicates presence or absence of co-pathology.

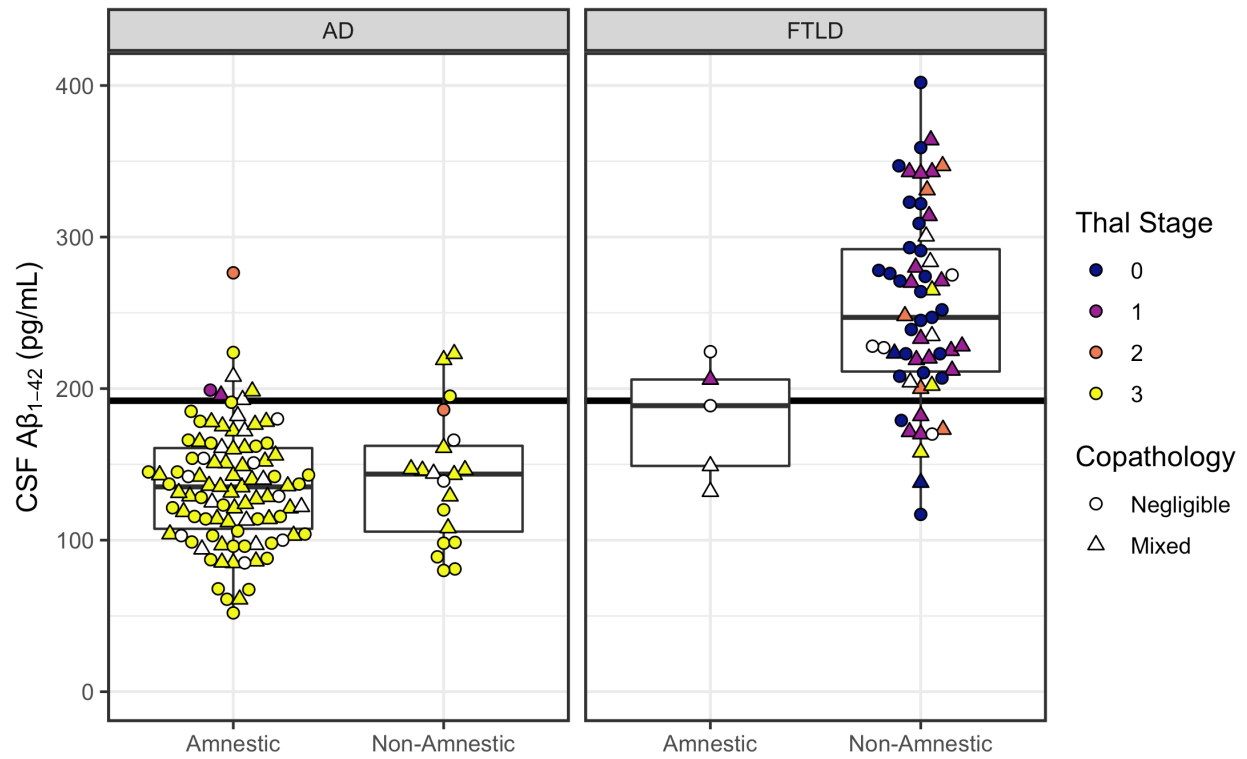
**Figure 3. CSF t-tau concentrations according to phenotype (amnesic, non-amnesic) and postmortem ratings of brain atrophy.** Left panel plots patients with primary AD pathology. Right panel plots patients with primary FTLD pathology. Horizontal line indicates CSF t-tau = 93 pg/mL, with patients at or above the line designated as N+ status. Color indicates severity of gross brain atrophy (white indicates cases with no available atrophy). Shape indicates presence/absence of co-pathology.

**Figure 4. CSF p-tau/ $A\beta_{1-42}$  according to phenotype (amnesic, non-amnesic) and postmortem ratings of AD likelihood.** Left panel plots patients with primary AD pathology. Right panel plots patients with primary FTLD pathology. Horizontal line indicates CSF p-tau/ $A\beta_{1-42} = 0.10$ , with patients at or above the line designated as positive for AD pathology. Color indicates level of AD pathologic change given by ABC score (white indicates cases with no available ABC score). Shape indicates presence/absence of co-pathology.

**Supplementary Figure 1. Cross-sectional associations of cerebrospinal fluid biomarkers with age among patients with autopsy-confirmed Alzheimer's disease.** Top panel plots values for CSF  $A\beta_{1-42}$ , middle panel plots values for CSF p-tau, and bottom panel plots values for CSF t-tau by age. Color indicates phenotype. Shape indicates presence/absence of co-pathology.

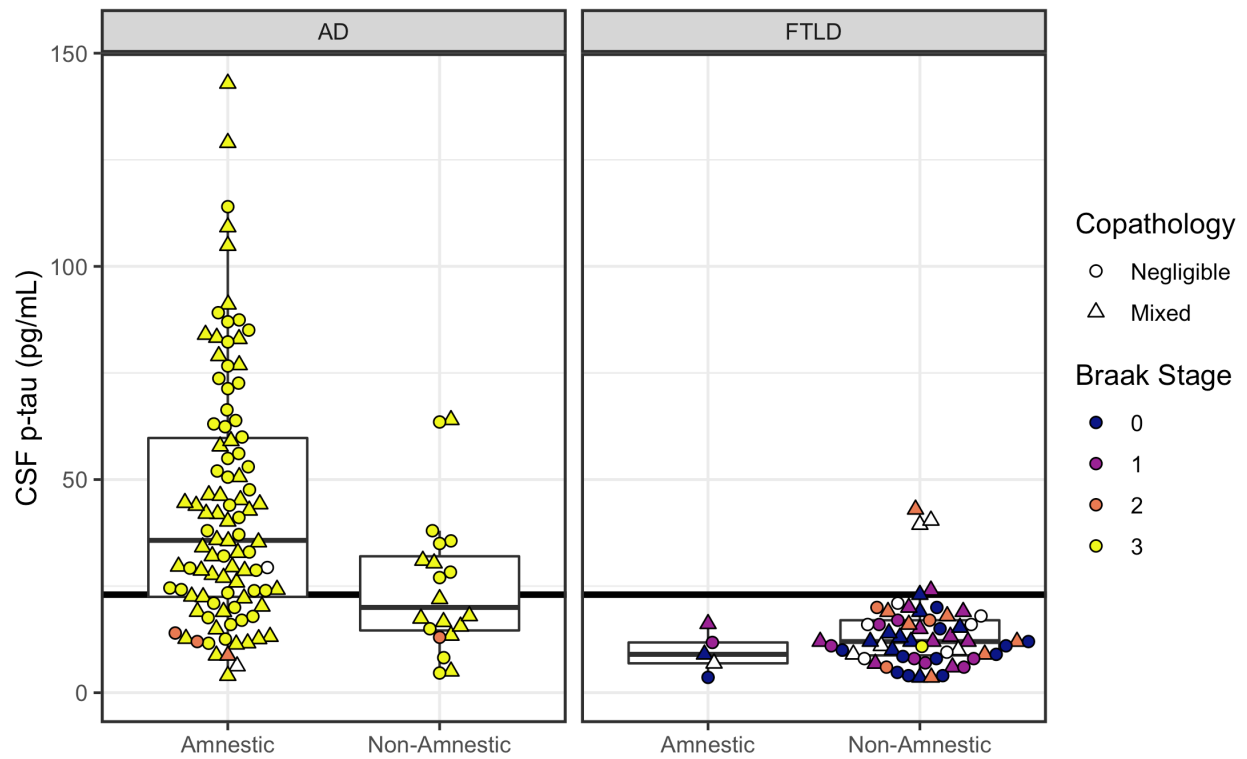
**Supplementary Figure 2. CSF p-tau concentration plotted according to phenotype and sex.** Left panel plots patients with primary AD pathology; right panel plots patients with primary FTLD pathology. Horizontal line indicates CSF p-tau = 23 pg/mL, with patients at or above the line designated as T+ status. Color indicates severity of gross brain atrophy. Shape indicates presence/absence of co-pathology.

**Figure 1**

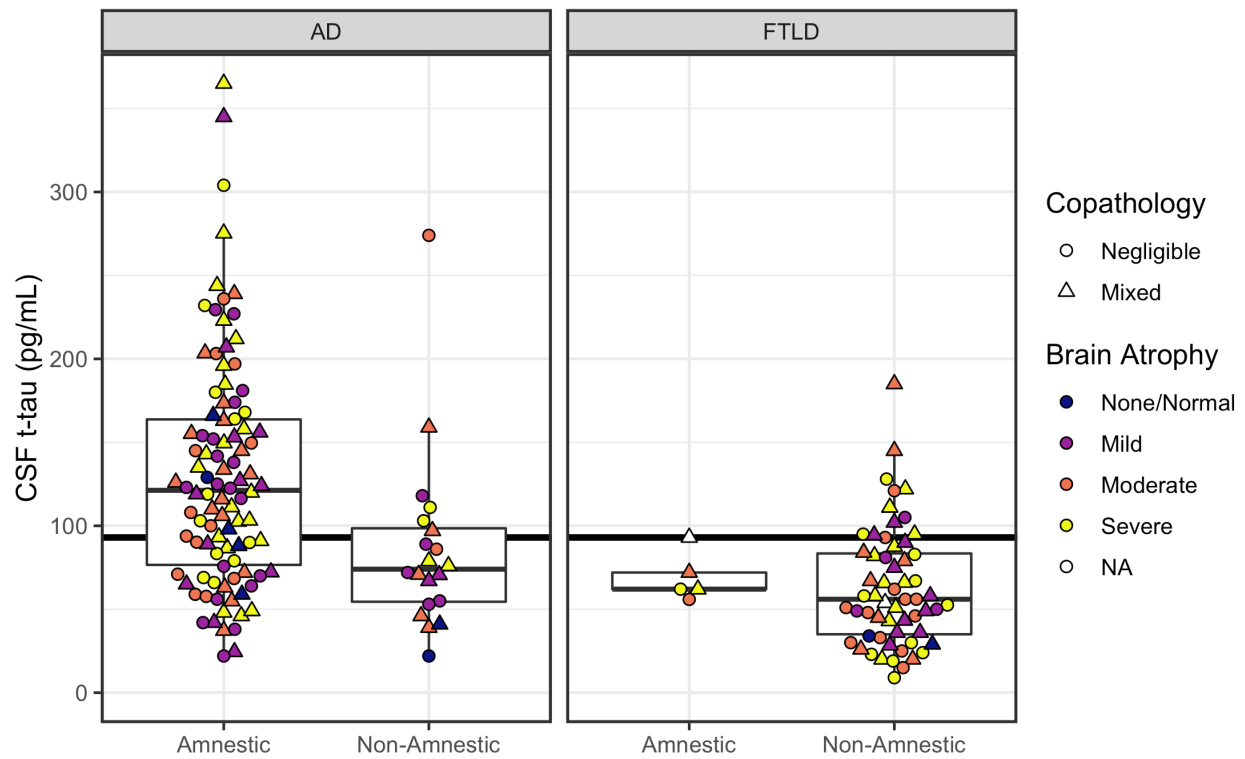




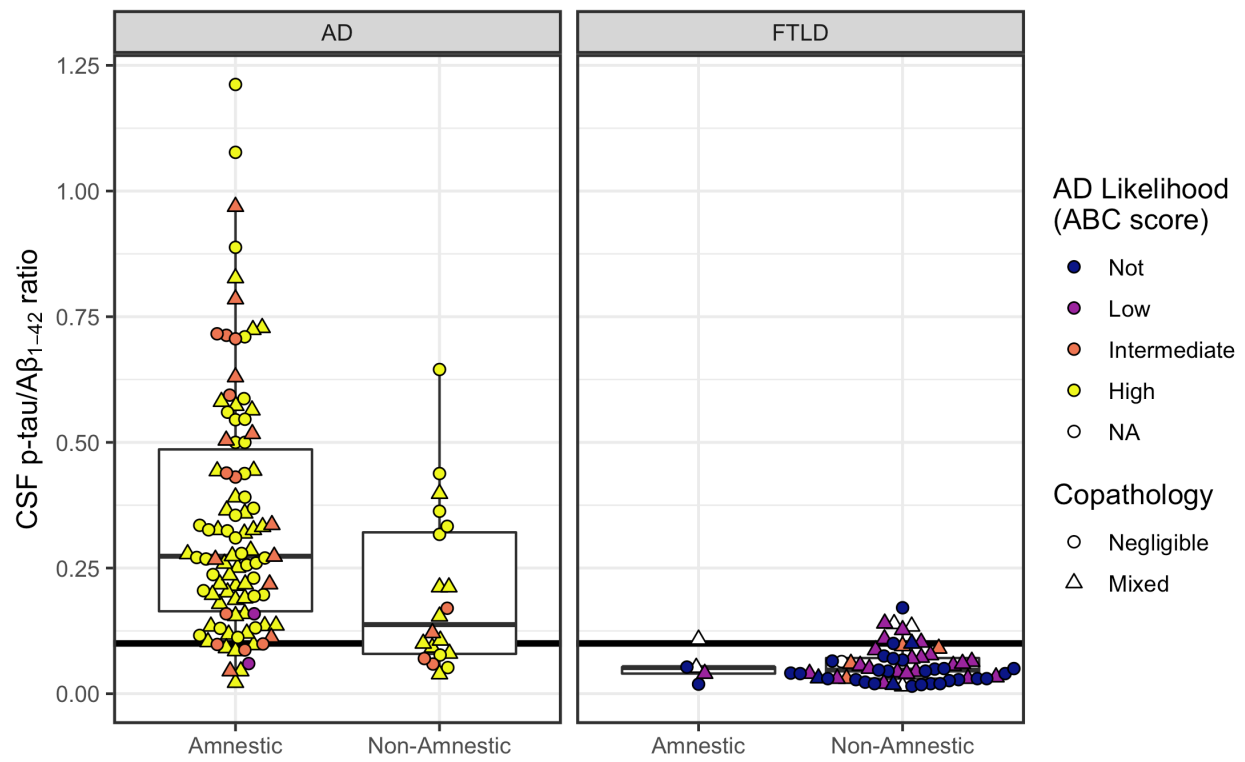
**Figure 2**



**Figure 3**



**Figure 4**



**Table 1: Sample characteristics.** Median (M), interquartile range (IQR), and sample size (n) by Pathology (Control, AD, FTLD) and Phenotype (amnesitic, non-amnesitic). For each variable,  $X^2$  and  $p$  values are reported for Chi-square or Kruskal-Wallis tests comparing the four patient groups (AD and FTLD by amnesitic and non-amnesitic).

		AD		FTLD		Chi-square/ Kruskal-Wallis	
		Amnesitic	Non-amnesitic	Amnesitic	Non-amnesitic	$X^2$	$p$
N <sub>total</sub>		98	20	5	59	—	—
Sex (% male)		48%	50%	33%	54%	1.2	0.76
APOE $\epsilon$ 4 alleles	0	32%	50%	50%	87%	47.1	<0.001
	1	49%	44%	33%	13%		
	2	19%	6%	17%	0%		
Age at CSF	M	73.5	63.5	71.0	65.0	19.9	<0.001
	IQR	14.5	13.5	4.0	10.0		
	n	98	20	5	59		
Duration at CSF	M	3.0	2.5	6.0	4.0	2.7	0.44
	IQR	3	4	3	3		
	n	98	20	5	59		
Age at Onset	M	69	61	66	61	19.8	<0.001
	IQR	15.75	13.0	6.0	11.5		
	n	98	20	5	59		
Age at Death	M	79.0	72.5	76.0	69.0	31.9	<0.001
	IQR	15.0	11.5	8.0	10.0		
	n	98	20	5	59		
Survival	M	9.0	8.5	9.0	7.0	9.5	0.02
	IQR	5	6	1	5		
	n	98	20	5	59		
Education	M	15.5	15.0	16.0	16.0	1.8	0.62
	IQR	5.0	4.0	0.0	5.75		
	n	98	19	5	58		
MMSE	M	23	19	24	25	5.6	0.13
	IQR	9	9	8	7		
	n	97	17	5	57		

**Table 2: Results of ATN classification using CSF biomarkers.** Interpretation of biomarkers using ATN framework (above the black line) and p-tau/A $\beta_{1-42}$  ratio (below the black line).

Interpretation		Biomarker profile	Amnesic AD (n=98)	Non-amnesic AD (n=20)	Amnesic FTLD (n=5)	Non-amnesic FTLD (n=59)
Alzheimer's continuum	Alzheimer's disease	A+T+N+	51 (52%)	4 (20%)	0 (0%)	0 (0%)
		A+T+N-	16 (16%)	5 (25%)	0 (0%)	0 (0%)
	Alzheimer's pathologic change	A+T-N-	13 (13%)	6 (30%)	2 (40%)	7 (12%)
	AD and concomitant suspected non-AD pathologic change	A+T-N+	11 (11%)	2 (10%)	1 (20%)	2 (3%)
Non- Alzheimer's pathologic change	A-T+N-	2 (2%)	0 (0%)	0 (0%)	2 (3%)	
	A-T-N+	0 (0%)	0 (0%)	0 (0%)	7 (12%)	
	A-T+N+	3 (3%)	0 (0%)	0 (0%)	3 (5%)	
Normal biomarkers	A-T-N-	2 (2%)	3 (15%)	2 (40%)	38 (64%)	
Alzheimer's disease	p-tau/A $\beta_{1-42}$ $\geq 0.1$	88 (90%)	13 (65%)	1 (20%)	9 (15%)	

**Table 3: ROC results for stratification of patients with AD from FTLD pathology.** Includes area under the curve (AUC), best threshold or cut-point, and sensitivity and specificity at best threshold for each CSF measure. A) Stratification of all patients with AD from all patients with FTLD pathology. B) Stratification of amnesic patients with AD pathology from FTLD pathology. C) Stratification of non-amnesic patients with AD pathology from all FTLD pathology.

<b>A) All AD from FTLD</b>				
CSF Measure	AUC	Threshold	Sensitivity	Specificity
A $\beta$ <sub>1-42</sub>	0.938	199.5 (pg/mL)	0.958	0.812
p-tau	0.863	20.1 (pg/mL)	0.737	0.906
t-tau	0.792	96.0 (pg/mL)	0.585	0.875
p-tau/A $\beta$ <sub>1-42</sub>	0.942	0.110	0.831	0.922
<b>B) Amnesic AD from FTLD</b>				
CSF Measure	AUC	Threshold	Sensitivity	Specificity
A $\beta$ <sub>1-42</sub>	0.941	199.5 (pg/mL)	0.969	0.812
p-tau	0.888	20.1 (pg/mL)	0.786	0.906
t-tau	0.822	96.5 (pg/mL)	0.643	0.875
p-tau/A $\beta$ <sub>1-42</sub>	0.958	0.110	0.888	0.922
<b>C) Non-amnesic with AD from FTLD</b>				
CSF Measure	AUC	Threshold	Sensitivity	Specificity
A $\beta$ <sub>1-42</sub>	0.925	168.0 (pg/mL)	0.80	0.922
p-tau	0.743	12.5 (pg/mL)	0.85	0.578
t-tau	0.647	66.5 (pg/mL)	0.70	0.625
p-tau/A $\beta$ <sub>1-42</sub>	0.866	0.076	0.80	0.781

**Supplementary Table 1: Results of ATN classification using CSF biomarkers, excluding cases with secondary pathology.** Interpretation of biomarkers using ATN framework (above the black line) and p-tau/A $\beta_{1-42}$  ratio (below the black line). Alzheimer's disease, AD and co-occurring non-AD pathologic change, and AD pathologic change designations are considered Alzheimer's continuum disease.

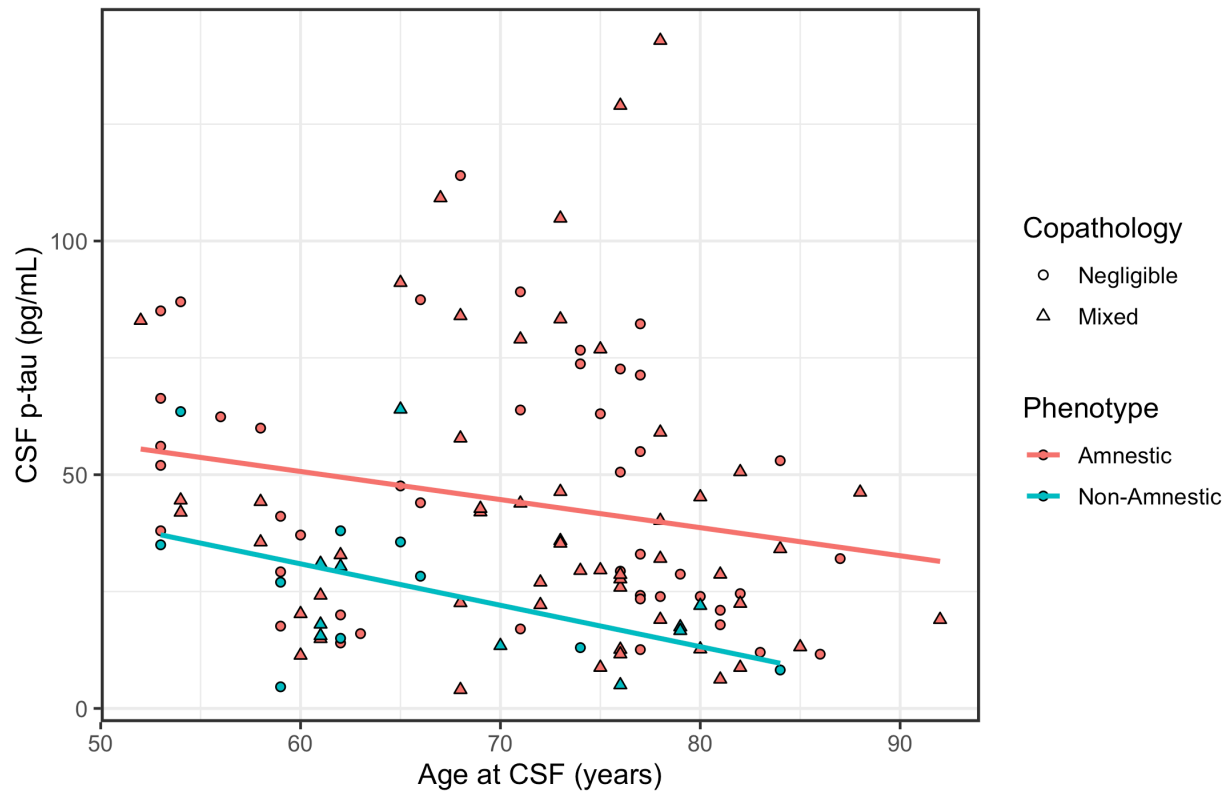
Interpretation		Biomarker profile	Amnesic AD (n=45)	Non-amnesic AD (n=10)	Amnesic FTLD (n=2)	Non-amnesic FTLD (n=28)
Alzheimer's continuum	Alzheimer's disease	A+T+N+	24 (53%)	3 (30%)	0 (0%)	0 (0%)
		A+T+N-	9 (20%)	3 (30%)	0 (0%)	0 (0%)
	Alzheimer's pathologic change	A+T-N-	6 (13%)	2 (20%)	1 (50%)	1 (4%)
	AD and concomitant suspected non-AD pathologic change	A+T-N+	3 (7%)	1 (10%)	0 (0%)	2 (7%)
Non-Alzheimer's pathologic change	A-T+N-	1 (2%)	0 (0%)	0 (0%)	0 (0%)	
	A-T-N+	0 (0%)	0 (0%)	0 (0%)	3 (11%)	
	A-T+N+	1 (2%)	0 (0%)	0 (0%)	0 (0%)	
Normal biomarkers	A-T-N-	1 (2%)	1 (10%)	1 (50%)	22 (79%)	
Alzheimer's disease	p-tau/A $\beta_{1-42}$ $\geq 0.1$	41 (91%)	6 (60%)	0 (0%)	2 (7%)	

**Supplementary Table 2: ROC results for stratification of patients with AD from FTLD pathology, excluding cases with secondary pathology.** Includes area under the curve (AUC), best threshold or cut-point, and sensitivity and specificity at best threshold for each CSF measure. A) Stratification of all patients with AD from all patients with FTLD pathology. B) Stratification of amnesic patients with AD pathology from FTLD pathology. C) Stratification of non-amnesic patients with AD pathology from all FTLD pathology.

<b>A) All AD from FTLD</b>				
CSF Measure	AUC	Threshold	Sensitivity	Specificity
A $\beta$ <sub>1-42</sub>				
p-tau				
t-tau				
p-tau/A $\beta$ <sub>1-42</sub>				
<b>B) Amnesic AD from FTLD</b>				
CSF Measure	AUC	Threshold	Sensitivity	Specificity
A $\beta$ <sub>1-42</sub>				
p-tau				
t-tau				
p-tau/A $\beta$ <sub>1-42</sub>				
<b>C) Non-amnesic with AD from FTLD</b>				
CSF Measure	AUC	Threshold	Sensitivity	Specificity
A $\beta$ <sub>1-42</sub>				
p-tau				
t-tau				
p-tau/A $\beta$ <sub>1-42</sub>				



## Supplementary Figure 1



## Supplementary Figure 2

