

CSF tau and amyloid-beta predict cerebral synucleinopathy in autopsied Lewy body disorders

David J. Irwin, MD, MTR, Sharon X. Xie, PhD, David Coughlin, MD, Naomi Nevler, MD, Rizwan S. Akhtar, MD, PhD, Corey T. McMillan, PhD, Edward B. Lee, MD, PhD, David A. Wolk, MD, Daniel Weintraub, MD, Alice Chen-Plotkin, MD, John E. Duda, MD, Meredith Spindler, MD, Andrew Siderowf, MD, MSCE, Howard I. Hurtig, MD, Leslie M. Shaw, PhD, Murray Grossman, MD, and John Q. Trojanowski, MD, PhD

Correspondence

Dr. Irwin
dirwin@pennmedicine.
upenn.edu

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Abstract

Objective

To test the association of antemortem CSF biomarkers with postmortem pathology in Lewy body disorders (LBD).

Methods

Patients with autopsy-confirmed LBD ($n = 24$) and autopsy-confirmed Alzheimer disease (AD) ($n = 23$) and cognitively normal ($n = 36$) controls were studied. In LBD, neuropathologic criteria defined Lewy body α -synuclein (SYN) stages with medium/high AD copathology (SYN + AD = 10) and low/no AD copathology (SYN – AD = 14). Ordinal pathology scores for tau, β -amyloid ($A\beta$), and SYN pathology were averaged across 7 cortical regions to obtain a global cerebral score for each pathology. CSF total tau (t-tau), phosphorylated tau at threonine₁₈₁, and $A\beta_{1-42}$ levels were compared between LBD and control groups and correlated with global cerebral pathology scores in LBD with linear regression. Diagnostic accuracy for postmortem categorization of LBD into SYN + AD vs SYN – AD or neocortical vs brainstem/limbic SYN stage was tested with receiver operating curves.

Results

SYN + AD had higher CSF t-tau (mean difference 27.0 ± 8.6 pg/mL) and lower $A\beta_{1-42}$ (mean difference -84.0 ± 22.9 g/mL) compared to SYN – AD ($p < 0.01$, both). Increasing global cerebral tau and plaque scores were associated with higher CSF t-tau ($R^2 = 0.15-0.16$, $p < 0.05$, both) and lower $A\beta_{1-42}$ ($R^2 = 0.43-0.49$, $p < 0.001$, both), while increasing cerebral SYN scores were associated with lower CSF $A\beta_{1-42}$ ($R^2 = 0.31$, $p < 0.001$) and higher CSF t-tau/ $A\beta_{1-42}$ ratio ($R^2 = 0.27$, $p = 0.01$). CSF t-tau/ $A\beta_{1-42}$ ratio had 100% specificity and 90% sensitivity for SYN + AD, and CSF $A\beta_{1-42}$ had 77% specificity and 82% sensitivity for neocortical SYN stage.

Conclusions

Higher antemortem CSF t-tau/ $A\beta_{1-42}$ and lower $A\beta_{1-42}$ levels are predictive of increasing cerebral AD and SYN pathology. These biomarkers may identify patients with LBD vulnerable to cortical SYN pathology who may benefit from both SYN and AD-targeted disease-modifying therapies.

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From Penn Frontotemporal Degeneration Center (D.J.I., N.N., C.T.M., M.G.), Alzheimer's Disease Core Center (D.A.W., J.Q.T.), Department of Neurology (D.J.I., D.C., R.S.A., C.T.M., D.A.W., D.W., A.C.-P., M.S., A.S., H.I.H., M.G.), Penn Morris K. Udall Parkinson's Disease Research Center of Excellence (D.J.I., R.S.A., C.T.M., D.W., A.C.-P., J.E.D., M.G., J.Q.T.), Center for Neurodegenerative Disease Research (L.M.S., J.Q.T.), Translational Neuropathology Research Laboratory (E.B.L.), Department of Pathology and Laboratory Medicine (E.B.L., L.M.S., J.Q.T.), and Department of Biostatistics and Epidemiology Perelman School of Medicine (S.X.X.), University of Pennsylvania; and Parkinson's Disease Research, Education and Clinical Center (D.W., J.E.D.), Michael J. Crescenz VA Medical Center, Philadelphia, PA.

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GLOSSARY

A β = β -amyloid; **AD** = Alzheimer disease; **AUC** = area under the curve; **CI** = confidence interval; **DLB** = dementia with Lewy bodies; **LBD** = Lewy body disorders; **PD** = Parkinson disease; **PDD** = Parkinson disease with dementia; **p-tau** = phosphorylated tau; **ROC** = receiver operating curve; **SYN** = α -synuclein; **t-tau** = total tau.

Roughly 40% of all patients with Lewy body disorders (LBD) (i.e., Parkinson disease [PD], PD with dementia [PDD]; dementia with Lewy bodies [DLB]) have sufficient amyloid plaque and tau tangle pathology for a concomitant diagnosis of Alzheimer disease (AD) at autopsy.^{1–6} Furthermore, increasing levels of cerebral tau and amyloid pathology in postmortem brains of patients with LBD are associated with shorter survival, rapider time to develop dementia in relation to onset of motor parkinsonism, and higher burden of cortical α -synuclein (SYN) pathology.² Thus, AD copathology has significant consequences in LBD, and antemortem identification of this patient subgroup has important implications for prognosis and clinical management in LBD.⁷ CSF levels of the protein constituents of AD neuropathology (i.e., total tau [t-tau], phosphorylated tau [p-tau], and β -amyloid [A β _{1–42}]) have been extensively studied in the context of AD,⁸ but CSF biomarker work in LBD is largely restricted to living cohorts without postmortem validation. Furthermore, neuroimaging modalities to visualize cerebral SYN pathology during life are lacking, and CSF SYN analytes are yet to be validated,⁹ so in vivo measures that predict cerebral SYN pathology are urgently needed.

Here, we test the hypothesis that AD CSF biomarkers measured during life predict postmortem cortical severity of both AD-associated tau tangles and A β plaques, as well as SYN Lewy pathology, in a cohort of 24 patients with autopsy-confirmed LBD. We find evidence of a direct association of CSF A β _{1–42} and CSF t-tau/A β _{1–42} ratio with cerebral SYN Lewy pathology.

Methods

Patients

Patients were followed up in clinical cores of the Penn Udall Center for Parkinson's Disease Research, Frontotemporal Degeneration Center, or Alzheimer's Disease Core Center and selected from the Penn Integrated Neurodegenerative Disease Database¹⁰ as of April 1, 2017, to identify patients with an antemortem clinical diagnosis of LBD (PD, PDD, DLB), autopsy confirmation through the Penn Center for Neurodegenerative Disease brain bank of SYN pathology (brainstem, limbic, or neocortical stage), and baseline antemortem CSF samples for study. We included reference cohorts with available CSF data and age-matched normal cognition (n = 36) or autopsy-confirmed AD neuropathology without SYN copathology (n = 23) (table e-1, links.lww.com/WNL/A257).

Standard protocol approvals, registrations, and patient consents

All procedures were performed with informed consent under institutional review board approval.

CSF collection and analysis

CSF was collected under standard operating procedures and analyzed with a Luminex xMAP immunoassay platform (Luminex, Austin, TX) to measure CSF t-tau, p-tau (phosphorylated at threonine-181), and A β _{1–42} as described.⁸

Neuropathologic examination

Autopsy procedures were performed as previously described¹¹ with sampling of fresh brain tissue according to a standardized atlas and fixed in 10% neutral buffered formalin or 70% ethanol with 150 mmol/L NaCl overnight, processed, and embedded in paraffin for sectioning. For neuropathologic diagnosis, 6- μ m sections were cut and stained with immunohistochemistry with established antibodies specific for hyperphosphorylated tau (PHF-1), SYN (SYN303), A β (Nab228), and phosphorylated TDP-43 (p409-410) and chemically stained with the amyloid-binding dye Thioflavin-S to detect neuritic plaques. Expert neuropathologists (E.B.L., J.Q.T.) applied currently validated diagnostic criteria^{12,13} to assign Braak tau, Thal amyloid, Consortium to Establish a Registry for Alzheimer's Disease neuritic plaque, and SYN Lewy body stages, as well as the final diagnosis for each case.

We categorized patients with medium- or high-level AD as having AD copathology (SYN + AD) and patients with no or low-level AD pathology as those without significant AD copathology (SYN – AD) according to neuropathologic criteria¹² as described.^{1,2} Alternative analyses compared patients with low/medium/high AD copathology (n = 18) to SYN with no AD (n = 6).

To obtain a continuous measure of global neuropathologic severity for tau-positive tangles, amyloid-positive plaques, and SYN-positive Lewy bodies/Lewy neurites, we calculated an average of ordinal scores (i.e., 0 = rare/none, 1 = mild, 2 = moderate, 3 = severe) obtained at autopsy using diagnostic criteria in 7 cortical regions. Briefly, the medial temporal lobe severity was calculated by averaging the ordinal scores in the amygdala, hippocampal entorhinal cortex, and cornu ammonis/subiculum regions. The global cerebral scores were derived from the average of ordinal scores in the medial temporal lobe, superior/midtemporal lobe, angular cortex, midfrontal cortex, and anterior cingulate gyrus as described.^{1,2} We also calculated a global subcortical score for SYN

pathology from sections of medulla, substantia nigra, non-nigral midbrain, pons, striatum, globus pallidus, and thalamus.

Statistical analysis

Demographics were compared between groups by use of χ^2 analysis for categorical data and cerebral neuropathology scores with the Mann-Whitney *U* test. CSF analyte measurements did not have a normal distribution and thus were natural log-transformed for analysis. Transformed CSF biomarker values and demographics at CSF collection were compared across groups with a 1-way analysis of variance with planned post hoc *t* tests for individual group comparisons. We performed analysis of covariance models to adjust for disease duration and time to autopsy at CSF collection (table e-2, links.lww.com/WNL/A257).

Linear regression with transformed CSF biomarker values as the dependent variable was used to test the independent association of the global cerebral score for each pathology. To account for demographic variables (i.e., age at CSF), time interval from onset of disease to CSF collection (years), time from CSF collection to autopsy (years), sex, and clinical diagnosis (PD/PDD vs DLB), we tested univariate models to predict each CSF analyte. Demographic variables with significant associations were added to the univariate base model including global cerebral pathology score with a stepwise approach, and bayesian information criteria¹⁴ were used to derive a final demographic-adjusted model for comparison with univariate pathology base models. Because demographic data were not influential in our models (table e-3, links.lww.com/WNL/A257), we report univariate model data (table e-4).

Diagnostic accuracy for postmortem pathology in LBD was tested with receiver operating curve (ROC) analysis to predict SYN + AD pathology (compared to SYN – AD) and neocortical SYN stage (compared to brainstem/limbic stage). To avoid overfitting, we performed a bootstrapping random sampling procedure with 1,000 bootstrap samples to generate a 95% confidence interval (CI) for the area under the curve (AUC) value for each analyte and report both the sensitivity and specificity for optimal cut points from this study and a previously cross-validated diagnostic CSF t-tau/A β_{1-42} ratio of 0.34 established in a different autopsy-confirmed neurodegenerative disease cohort (i.e., frontotemporal degeneration) sensitive and specific for AD pathology.¹⁵

All analyses were 2 tailed with $\alpha = 0.05$ and performed with SPSS version 23.0 (IBM, Chicago, IL) or STATA version 12.1 (StataCorp, College Station, TX).

Results

Table 1 gives LBD patient data and table e-1 (links.lww.com/WNL/A257) shows data for our reference cohorts of autopsy-confirmed AD and normal controls. CSF analysis finds

Table 1 Autopsy-confirmed LBD cohort clinical, demographic, and neuropathologic data

	SYN – AD (n = 14)	SYN + AD (n = 10)	<i>p</i> Value
Clinical phenotype, n			
PD	1	0	0.1
PDD	9	3	
DLB	4	7	
Sex, n			
Female	1	2	0.3
Male	13	8	
Age at onset, y	60.1 (7.2)	67.6 (6.6)	0.02
Age at death, y	76.5 (8.4)	76.1 (6.8)	0.8
Disease duration, y	16.4 (7.4)	8.5 (3.2)	0.002
Brain weight, g	1,291.2 (387.2)	1,309.5 (87.4)	0.8
Postmortem interval, h	14.9 (7.2)	14.2 (8.2)	0.8
Braak tau stage, n			
B0	1	0	<0.01
B1	7	0	
B2	6	6	
B3	0	4	
CERAD neuritic plaque stage, n			
C0	12	0	<0.001
C1	2	1	
C2	0	2	
C3	0	7	
Amyloid Thal phase, n			
A0	6	0	0.001
A1	6	1	
A2	2	2	
A3	0	7	
LBD stage, n			
Brainstem	2	0	0.09
Limbic	7	2	
Neocortical	5	8	
Global cerebral tau score	0.3 (0.2, 0.6)	1.7 (0.7, 1.9)	<0.001
Global cerebral neuritic plaque score	0 (0, 0)	2.3 (2.0, 2.5)	<0.001
Global cerebral amyloid score	0.3 (0, 0.9)	2.8 (2.5, 3.0)	<0.001

Continued

Table 1 Autopsy-confirmed LBD cohort clinical, demographic, and neuropathologic data (continued)

	SYN – AD (n = 14)	SYN + AD (n = 10)	p Value
Global cerebral SYN score	1.1 (0.9, 1.4)	2.6 (0.9, 3.0)	0.04
Global subcortical SYN score	1.9 (0.9, 2.3)	1.6 (1.3, 1.9)	0.8

Abbreviations: AD = Alzheimer disease; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; DLB = dementia with Lewy bodies; LBD = Lewy body disorders; PD = Parkinson disease; PDD = Parkinson disease with dementia; SYN = α -synuclein. Data reported as mean (SD), median (25th, 75th quartiles), or individual patient frequencies.

groupwise differences in all CSF analytes across normal controls, SYN – AD, SYN + AD, and AD (table 2 and figure 1). After adjusting for disease duration or interval to autopsy at the time of CSF collection, we found similar results of higher CSF t-tau and t-tau/ $A\beta_{1-42}$ and p-tau/ $A\beta_{1-42}$ ratios and lower CSF $A\beta_{1-42}$ in SYN + AD compared to SYN – AD (table e-2, [links.lww.com/WNL/A257](https://www.lww.com/WNL/A257)). An alternative analysis comparing patients with LBD with any level of AD copathology (low, medium, or high, n = 18) to the minority with SYN pathology in the absence of any AD copathology (n = 6) yielded similar results (table e-5).

The SYN – AD group had lower levels of CSF $A\beta_{1-42}$ compared to controls ($t = 2.3$, $df = 48$, $p < 0.05$) (figure 1), and 4 of the 6 patients with SYN pathology and no AD copathology had levels of CSF $A\beta_{1-42}$ below the mean (table 2) of our control group (range 136–274 pg/mL).

To test the direct relationship between CSF analytes and corresponding neuropathologic substrates, we used univariate

linear regression (figure 2 and table e-4, [links.lww.com/WNL/A257](https://www.lww.com/WNL/A257)). We found a mild association between increasing CSF t-tau and global cerebral tau ($R^2 = 0.15$, $\beta = 0.3$, $p = 0.04$) and with amyloid plaque scores ($R^2 = 0.16$, $\beta = 0.17$, $p = 0.05$). We also found lower CSF $A\beta_{1-42}$ to be moderately associated with increasing global cerebral tau ($R^2 = 0.43$, $\beta = -0.31$, $p < 0.001$), amyloid plaque ($R^2 = 0.49$, $\beta = -0.20$, $p < 0.001$), and SYN scores ($R^2 = 0.31$, $\beta = -0.20$, $p = 0.004$). To test the association of CSF $A\beta_{1-42}$ with SYN pathology independently from $A\beta$ pathology, we adjusted for global cerebral amyloid plaque score and found a significant independent association with the global cerebral SYN score ($\beta = -0.11$, $p < 0.05$). Furthermore, 3 of the 4 patients with SYN – AD with no cerebral amyloid pathology and low CSF $A\beta_{1-42}$ had a neocortical distribution of SYN pathology and a global cerebral pathology score ≥ 1.5 . We did not find an association of CSF $A\beta_{1-42}$ with the global subcortical SYN scores ($R^2 = 0.08$, $\beta = -0.1$, $p = 0.2$), but we did find an association with an average of all total SYN cortical and subcortical regions ($R^2 = 0.3$, $\beta = -0.2$, $p = 0.009$).

The t-tau/ $A\beta_{1-42}$ ratio was also significantly associated with increasing global cerebral tau ($R^2 = 0.47$, $\beta = 0.63$, $p < 0.001$), amyloid plaque ($R^2 = 0.46$, $\beta = 0.36$, $p < 0.001$), and SYN scores ($R^2 = 0.27$, $\beta = 0.36$, $p = 0.01$). We did not find an association of CSF p-tau with these pathologies or CSF t-tau with global cerebral SYN scores (all $p > 0.1$). We did not find a significant association of any demographic feature with CSF t-tau, p-tau, or t-tau/ $A\beta_{1-42}$ ratio (data not shown), while CSF $A\beta_{1-42}$ had significant univariate associations with years from disease onset to CSF collection and clinical diagnosis (table e-3, [links.lww.com/WNL/A257](https://www.lww.com/WNL/A257)). Covariate-adjusted models for CSF $A\beta_{1-42}$ yielded results similar to the univariate models above (table e-3).

We performed ROC analyses to assess the preliminary evidence of the predictive value of CSF AD biomarkers for both

Table 2 Autopsy-confirmed LBD CSF data

	SYN – AD (n = 14)	SYN + AD (n = 10)	AD (n = 23)	Control (n = 36)	p Value
Age at CSF, y	72.3 (9.1)	71.4 (6.2)	68.6 (10.9)	69.4 (4.6)	0.40
Disease duration at CSF, y	11.2 (7.3) ^{b,c}	3.8 (2.3)	3.4 (2.5)	NA	0.002
Interval from CSF to autopsy, y	4.2 (2.7)	4.7 (1.8)	5.2 (2.8)	NA	0.09
CSF t-tau, pg/mL	36.9 (12.1) ^{b,c}	63.9 (29.0) ^{a,b,d}	143.4 (123.0) ^{a,c,d}	44.0 (17.0) ^{b,c}	<0.001
CSF p-tau, pg/mL	15.5 (7.5) ^b	20.2 (12.0) ^b	49.8 (26.1) ^{a,c,d}	20.6 (12.2) ^b	<0.001
CSF $A\beta_{1-42}$, pg/mL	231.1 (67.2) ^{a,b,c}	147.2 (30.1) ^{a,b,d}	117.6 (37.3) ^{a,c,d}	279.7 (75.1) ^{b,c,d}	<0.001
CSF t-tau/ $A\beta_{1-42}$ ratio	0.17 (0.06) ^{b,c}	0.44 (0.20) ^{a,b,d}	1.37 (1.36) ^{a,c,d}	0.17 (0.07) ^{b,c}	<0.001
CSF p-tau/ $A\beta_{1-42}$ ratio	0.07 (0.03) ^{b,c}	0.14 (0.08) ^{a,b,d}	0.49 (0.35) ^{a,c,d}	0.08 (0.05) ^{b,c}	<0.001

Abbreviation: $A\beta = \beta$ -amyloid; AD = Alzheimer disease; LBD = Lewy body disorders; NA = not applicable (control group omitted from this analysis); p-tau = phosphorylated tau; SYN = α -synuclein; t-tau = total tau.

CSF analyte comparisons calculated from natural log-transformed values.

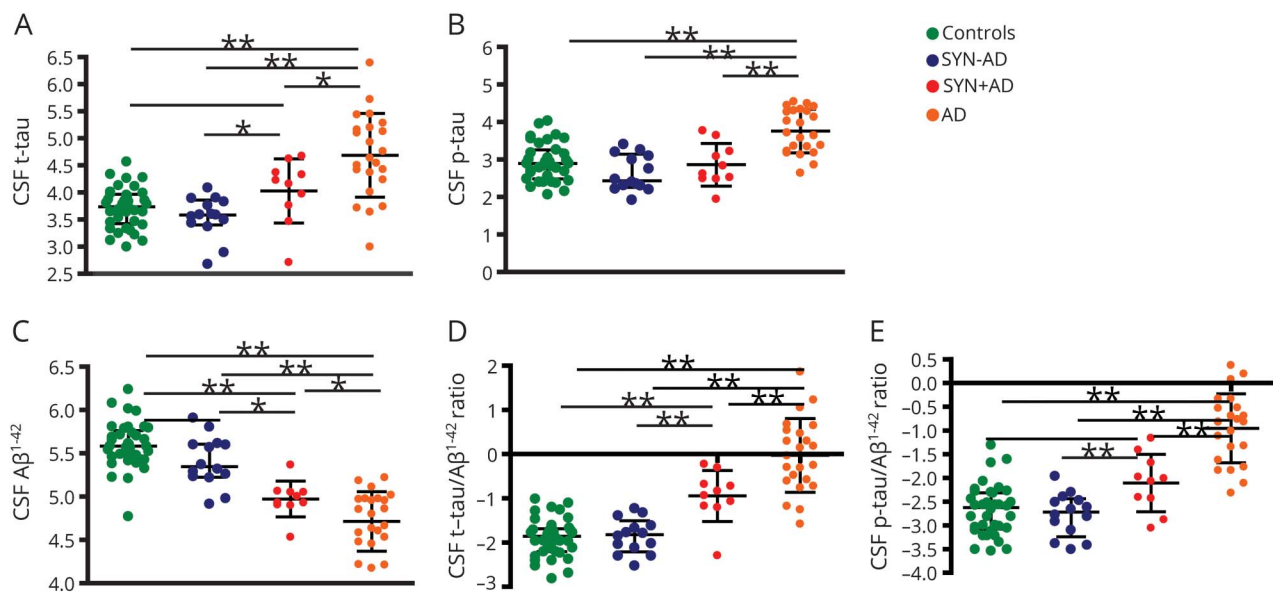
^a Individual group comparisons: $p < 0.05$ vs controls.

^b Individual group comparisons: $p < 0.05$ vs AD.

^c Individual group comparisons: $p < 0.05$ vs SYN + AD.

^d Individual group comparisons $p < 0.05$ vs SYN – AD.

Figure 1 CSF biomarkers in neuropathologic groups of LBD



Graphs depict individual data points for natural log transformed values CSF (A) t-tau, (B) p-tau, (C) Aβ₁₋₄₂, (D) t-tau/Aβ₁₋₄₂ ratio, and (E) p-tau/Aβ₁₋₄₂ ratio for the normal control (green), SYN – AD (blue), SYN + AD (red), and AD (orange) groups. Bars represent median and interquartile range. Bar denotes $p < 0.05$. * $p < 0.01$, ** $p < 0.001$ difference between groups. Aβ = β-amyloid; AD = Alzheimer disease; p-tau = phosphorylated tau; SYN = α-synuclein; t-tau = total tau.

underlying concomitant AD pathology and SYN neocortical stage in LBD (figure 3). We found the highest diagnostic AUC value for t-tau/Aβ₁₋₄₂ ratio >0.30 (AUC 0.92, 95% CI 0.67–1.0, $p < 0.001$), with 90% sensitivity and 100% specificity for SYN + AD at this cut point. To avoid overfitting, we also examined a previously validated diagnostic threshold of t-tau/Aβ₁₋₄₂ ratio of 0.34 to predict AD pathology,¹⁵ which also had high diagnostic accuracy (AUC 0.85, 95% CI 0.67–1.0, $p = 0.004$) with 70% sensitivity and 100% specificity for SYN + AD. Finally, we examined the t-tau/Aβ₁₋₄₂ ratio to predict any level of AD (i.e., low, medium, or high level) with SYN pathology compared to SYN with no AD copathology and found high diagnostic accuracy (AUC 0.87, 95% CI 0.72–1.0, $p = 0.008$) with a lower optimal cut point of 0.17 (sensitivity 78%, specificity 83%).

We found CSF Aβ₁₋₄₂ to have the highest predictive value for a neocortical stage of SYN pathology (AUC 0.76, 95% CI 0.54–0.94) with 77% sensitivity and 82% specificity using a cut point of 185 pg/mL. We did not find significant predictive value of CSF t-tau, p-tau, or p-tau/Aβ₁₋₄₂ ratio for neocortical SYN stage (AUC 0.47–0.67, $p > 0.1$ for all).

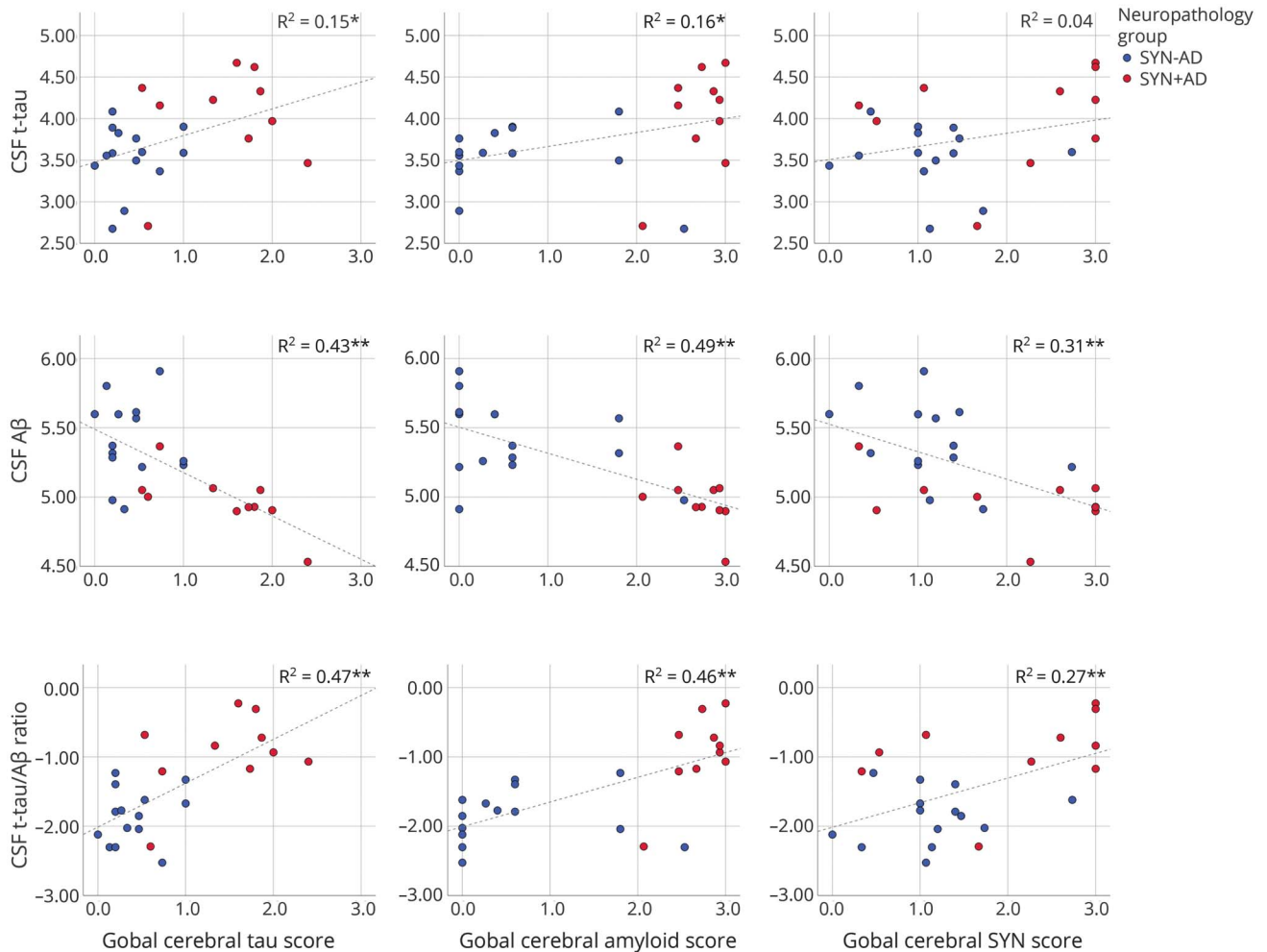
Discussion

Here, we provide tissue validation for AD CSF biomarkers in a relatively large and well-characterized autopsy-confirmed LBD cohort. Previous CSF studies in LBD focus largely on clinical samples without autopsy confirmation, which significantly limits the interpretation because of the poor clinical

diagnostic accuracy of LBD phenotypes^{16,17} and high frequency of mixed pathologies across the clinical spectrum of LBD.^{1-3,5,7} Furthermore, the few previous CSF studies in LBD that include autopsy samples¹⁸⁻²¹ examined small numbers of patients, used only categorical measures of AD pathology, and did not examine SYN pathology. We found both a robust difference in AD CSF biomarker levels between SYN + AD and SYN – AD categorical neuropathologic groups (figure 1) and direct associations of these antemortem measurements with continuous measures of postmortem AD pathology (figure 2). Furthermore, we found high diagnostic accuracy of the CSF t-tau/Aβ₁₋₄₂ ratio to distinguish patients with LBD with SYN + AD pathology from those with low/no AD copathology (figure 3). Finally, we found an association of antemortem CSF Aβ₁₋₄₂ and t-tau/Aβ₁₋₄₂ ratio with severity of postmortem cerebral SYN pathology (figure 2), which was independent of severity of cerebral amyloidosis, and high diagnostic accuracy to predict neocortical stage SYN pathology (figure 3). These data have important implications for clinical care and therapeutic trials in LBD.

AD and SYN pathology commonly coexist in LBD. Several modalities of evidence, including autopsy,^{1-5,7} genetic,²² and neuroimaging studies,²³⁻²⁵ highlight the detrimental effects of AD copathology on cognition and prognosis in LBD. Furthermore, increasing cerebral AD pathology is often associated with higher cerebral SYN pathology in LBD.^{1-3,5-7} These clinical studies mirror the growing cell^{26,27} and animal model²⁸ data that support a hypothesis of distinct strains²⁹ of pathogenic SYN that spread throughout the CNS, along with varying degrees of AD-associated tauopathy.³⁰ Thus, it is

Figure 2 Relationship between CSF biomarkers and postmortem pathology

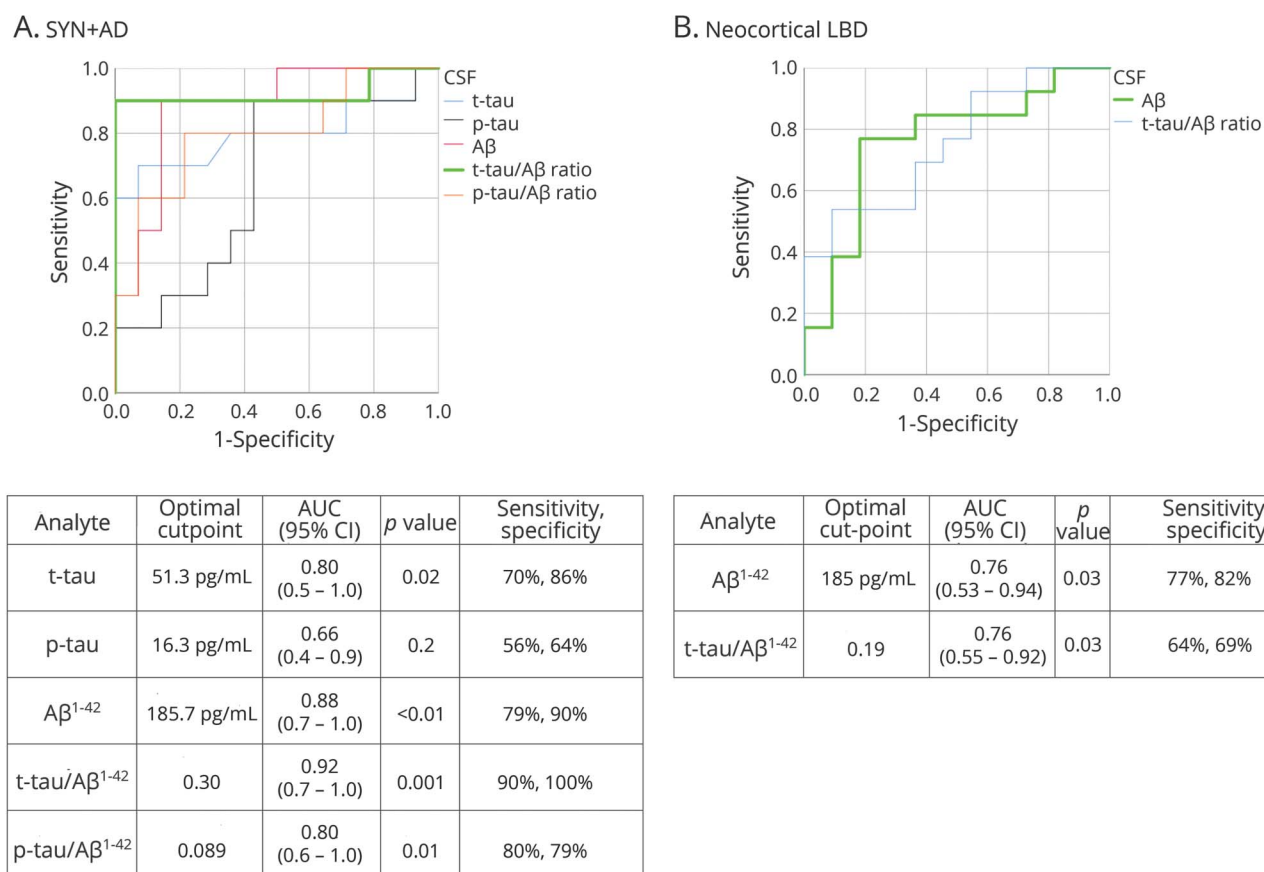


Scatterplots depict individual patient data of CSF analyte levels plotted against global cerebral pathology scores coded for neuropathologic diagnosis. Fitted lines and R^2 values derived from linear regression models predicting CSF analytes. * $p < 0.05$, ** $p \leq 0.01$. $A\beta$ = β -amyloid; AD = Alzheimer disease; p-tau = phosphorylated tau; SYN = α -synuclein.

imperative for clinical care and clinical trials in LBD to accurately detect the subset of patients with AD copathology because these patients appear to have a divergent natural history and possible altered response to therapeutics compared to those with relatively “pure” synucleinopathy.⁷ Our data highlight the potential prognostic use of AD CSF biomarkers in LBD to identify patients with AD copathology who are at risk for rapid decline. Previous work in AD has found a similar direct association of AD CSF biomarkers with both postmortem AD pathology³¹ and in vivo molecular imaging of AD pathology.³² Furthermore, AD CSF biomarkers are currently used in AD clinical trials to track target engagement for tau- and amyloid-directed therapies. While AD-targeted therapies are currently understudied in LBD, our data suggest that CSF t-tau/ $A\beta_{1-42}$ and $A\beta_{1-42}$ levels may be used in a similar manner in future LBD clinical trials targeting AD copathology.

Our cohort included patients with clinical PD, PDD, and DLB. CSF biomarker studies in living patients with LBD largely find groupwise differences in AD CSF biomarkers between these clinical LBD phenotypes (see elsewhere for comprehensive review⁹). Postmortem^{3,5} and emerging in vivo tau and amyloid molecular imaging studies^{24,25} report similar findings of variable but largely increasing levels of AD copathology across the spectrum of PD, PDD, and DLB. Despite these groupwise differences, we and others previously found that no clear pathologic or genetic substrate could clearly substantiate the clinical distinction of PDD and DLB.^{2,33} Furthermore, it is recommended that studies examining the underlying biology of synucleinopathies include the full spectrum of LBD.³⁴ Despite this strong rationale for our study design, it is possible that clinical phenotype could have influenced our results; however, when we adjusted for clinical diagnosis, we still found a significant association of CSF $A\beta_{1-42}$ with postmortem global

Figure 3 Diagnostic accuracy of CSF biomarkers for AD pathology and neocortical LBD stage in LBD



Receiver operating curves for diagnostic accuracy of CSF biomarkers to predict (A) SYN + AD pathology or (B) neocortical LBD stage pathology. Tables list optimal cut point with sensitivity, specificity, AUC, and 95% CI derived from a random sampling procedure with 1,000 bootstrap samples. A β = β -amyloid; AD = Alzheimer disease; AUC = area under the curve; CI = confidence interval; LBD = Lewy body disorders; p-tau = phosphorylated tau; SYN = α -synuclein; t-tau = total tau.

cerebral pathologies (table e-3, [links.lww.com/WNL/A257](https://www.lww.com/WNL/A257)), and clinical diagnosis did not appear to influence the other CSF biomarkers. Further study is needed in larger groups of patients identified during prodromal presymptomatic period before the onset of clinical symptoms^{35,36} and followed up to autopsy to fully elucidate the associations of CSF biomarkers across clinical phenotypes.

We did not find an association of CSF p-tau with postmortem pathology or a difference in CSF p-tau in our LBD pathology groups compared to normal controls (figure 1) in this dataset. Tau is hyperphosphorylated in AD-associated neurofibrillary pathology, and CSF levels of both t-tau and p-tau in AD reflect the severity of postmortem tau pathology,³¹ likely through release of pathogenic tau protein into the CSF from degenerating ghost tangles. In contrast, CSF t-tau can also be elevated in a range of nonneurodegenerative insults to the CNS and may reflect nonspecific neuronal damage. Some data suggest that CSF tau biomarkers may be influenced by SYN pathology in a manner that is distinct from aging and AD,⁹ but the exact nature of this interaction is currently unclear. Indeed, we find that AD without

SYN pathology had altered CSF biomarkers compared to SYN + AD (figure 1), despite similar plaque and tangle stages (table 1 and table e-1, [links.lww.com/WNL/A257](https://www.lww.com/WNL/A257)). Our CSF measurement uses an immunoassay that does not allow direct assessment of phosphorylation at each individual peptide, and we examined only 1 phospho-epitope, so it is possible that analytic factors could also contribute to this negative finding.

We found a moderate association of antemortem CSF A β ₁₋₄₂ and the CSF t-tau/A β ₁₋₄₂ ratio with postmortem global cerebral synuclein scores. Experimental model data suggest both an interaction between A β and SYN fibrils to promote synapse loss³⁷ and SYN pathology³⁸ and synergistic interactions between tau and SYN polymerization.^{26,27} Furthermore, low baseline CSF A β ₁₋₄₂ has been linked to greater cognitive decline in PD³⁹ and DLB,⁴⁰ and cerebral SYN pathology is one of the strongest correlates of dementia in PD.¹ Thus, our findings reinforce the prognostic association of CSF A β ₁₋₄₂ and t-tau/A β ₁₋₄₂ ratio in LBD and provide a link between antemortem CSF AD biomarkers and postmortem cerebral SYN pathology. Postmortem findings in LBD find strong

correlations of all 3 pathologies,^{1-3,5} and it is possible that the association between CSF A β_{1-42} and SYN pathology could be influenced by cerebral amyloidosis. However, when we included global cerebral amyloid plaque score, we found an independent association with SYN pathology. Furthermore, patients with SYN – AD had lower CSF A β_{1-42} compared to normal controls (figure 1), and the majority of this subset of patients with SYN – AD with no cerebral amyloid pathology and low CSF A β_{1-42} had significant neocortical SYN pathology, suggesting that cerebral SYN pathology alone may influence CSF A β_{1-42} levels. However, we cannot rule out very early amyloid pathologic processes that are not detectable with standard staining techniques in these patients. Our findings of high diagnostic accuracy of CSF A β_{1-42} to predict neocortical synucleinopathy in the context of the clinical LBD spectrum are important. CSF analysis is a relatively low-cost biomarker approach for LBD, and neuroimaging techniques cannot currently detect cortical synuclein pathology. Furthermore, CSF SYN assays are yet to be fully optimized, and values may be influenced by a range of confounding factors.⁹ Our findings of an association of CSF A β_{1-42} with global cerebral SYN scores and total SYN scores, but not subcortical SYN scores, suggest that low CSF A β_{1-42} is associated with increased progression of SYN pathology from the brainstem to cortical regions. While we cannot determine the topographic distribution of SYN pathology at the time of CSF collection, the association with a widespread neocortical pattern of SYN pathology at end-stage disease suggests that AD CSF biomarkers could lead to early detection of patients with LBD with AD copathology at greater risk for progression of cerebral SYN pathology.

There are additional limitations of our work to consider. Despite the rarity of autopsy-confirmed samples with antemortem CSF and the relative size of our cohort, we cannot fully assess potential clinical variables that may influence CSF analyte levels. Furthermore, while we used a bootstrapping procedure for ROC curves, these data provide proof of concept for the detection of AD copathology in LBD, and the absolute diagnostic cut points found here (figure 3) require replication in future larger autopsy-confirmed datasets. Finally, our data are retrospective and from a tertiary academic center, which may limit generalizability to the general population.

We find predictive value for AD CSF biomarkers in LBD for both AD and SYN pathology. Future work with tissue validation of CSF biomarkers from a larger group of individuals followed up prospectively with serial molecular imaging and clinical assessments will be important to further characterize the dynamic changes in tau, amyloid, and SYN pathology across the LBD spectrum toward the goal of personalized molecular therapies.

Author contributions

D.J.I.: drafting/revising manuscript, study concept/design, analysis, interpretation of data, statistical analysis, obtained funding. S.X.X.: statistical supervision, revising manuscript.

D.C. and N.N.: acquisition of data, revising manuscript. R.S.A. and C.T.M.: study concept/design, revising manuscript. E.B.L.: acquisition of data, revising manuscript. D.A.W.: acquisition of data, revising manuscript, obtained funding. D.W.: study concept/design, acquisition of data, revising manuscript. A.C.-P., J.E.D., M.S., A.S., H.I.H., and L.M.S.: acquisition of data, revising manuscript. M.G. and J.Q.T.: study concept/design, acquisition of data, revising manuscript, obtained funding.

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