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CSF α-synuclein contributes to the differential diagnosis of Alzheimer disease

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Abstract

INTRODUCTION—The ability of Alzheimer disease (AD) cerebrospinal fluid (CSF) biomarkers [amyloid beta peptide 1–42 ($A\beta_{42}$), total tau (t-tau) and phosphorylated tau (p-tau)] to discriminate AD from related disorders is limited. Biomarkers for other concomitant pathologies [e.g., CSF α-synuclein for Lewy body (LB) pathology] may be needed to further improve the differential diagnosis.

METHODS—CSF total α-synuclein, phosphorylated α-synuclein (pS129), and AD CSF biomarkers were evaluated with Luminex immunoassays in 367 participants, followed by validation in 74 different, neuropathologically-confirmed cases.

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Author Contributions: J.Z., J.Q.T., and M.S. supervised the project and designed the studies. L.T., C.G., and H.W. acquired the CSF α-syn, pS129, and hemoglobin data. J.B.T., D.W., A.S.C.-P., D.J.I., M.G., L.M., L.B.E., D.A.W., E.B.L., L.M.S., and J.Q.T. characterized human subjects and provided CSF samples as well as clinical and CSF $A\beta_{42}$, t-tau and p-tau data. P.H.J. provided antibodies for α-syn immunoassays. M.S., J.B.T., L.T., and J.Z. interpreted the data. M.S. and J.B.T. conducted statistical analysis. C.G. and P.A. provided administrative and technical supports. M.S., J.B.T., J.Z., J.Q.T., and D.W. drafted the manuscript; all authors critically reviewed the paper.

Conflicts of Interest: Nothing to report.

RESULTS—CSF total α-synuclein, when combined with $A\beta_{42}$ and either t-tau or p-tau, improved the differential diagnosis of AD vs frontotemporal dementia, LB disorders, or other neurological disorders. The diagnostic accuracy of the combined models attained clinical relevance (Area Under Curve ~0.9) and was largely validated in neuropathologically-confirmed cases.

CONCLUSIONS—Combining CSF biomarkers representing AD and LB pathologies may have clinical value in the differential diagnosis of AD.

Keywords

Alzheimer disease; Differential diagnosis; Biomarkers; Cerebrospinal fluid; α-Synuclein

1. Introduction

Investigations using biochemical measures in cerebrospinal fluid (CSF) as Alzheimer disease (AD) biomarkers have shown great promise, and such CSF biomarkers have been incorporated into recent guidelines for informed diagnosis of AD [1]. Specifically, CSF markers of core AD pathology [i.e., amyloid beta (Aβ) peptide 1–42 ($A\beta_{42}$) reflecting Aβ in plaque burden, and total tau (t-tau) and phosphorylated tau (p-tau) for assessing neurofibrillary tangles in the brain] provide both high sensitivity and specificity (80% or above) in differentiating patients with AD or mild cognitive impairment (MCI; prodromal AD) from healthy controls (HC) [2–4]. However, the diagnostic accuracy of these CSF biomarkers in the differential diagnosis of AD and other dementias is limited (40–80% sensitivity and specificity) due to substantial overlap in the CSF levels of these proteins [4–9]. A recent large-scale international multicenter study [5] suggested that the limited utility of these core CSF biomarkers to discriminate AD from a variety of related disorders could be due to overlap in the underlying primary pathologies, and introduction of additional CSF biomarkers reflecting other types of pathologies could be of value to optimize the differential diagnosis [4, 5], though reliance on clinical diagnoses might underestimate the accuracy of CSF biomarkers [10].

Among the concomitant non-AD type pathologies in AD, α-synuclein (α-syn)-positive Lewy bodies (LBs), the pathological hallmark of another family of neurodegenerative diseases including Parkinson disease (PD) and dementia with LBs (DLB), can be observed in up to 50% of familial and sporadic AD patients at autopsy [11–13]. We have reported that CSF total α-syn and phosphorylated α-syn at Ser129 (pS129) help differentiate PD from AD and other related neurodegenerative diseases [14–16]. More recently, we also found that CSF total α-syn improved the diagnostic and prognostic performance of CSF $A\beta_{42}$ and tau in AD [17, 18]. In this study, to test whether inclusion of CSF α-syn that represents brain LB pathology could improve the differential diagnosis of AD and other dementias, we further evaluated the utility of CSF total α-syn and pS129 in the differential diagnosis in a relatively large clinical cohort, followed by validating our findings in a separate cohort of neuropathologically-confirmed cases.

2. Methods

2.1 Subjects

Two cohorts of research participants were recruited at the AD Core Center (ADCC), the Penn Memory Center (PMC), the Frontotemporal Degeneration (FTD) Center, the Amyotrophic Lateral Sclerosis (ALS) Center, the Parkinson disease and Movement Disorder Clinic (PD&MDC) and the Penn Udall Center for Parkinson's Research at the University of Pennsylvania (UPenn) [19]. The clinical or discovery cohort (n=540) of clinically diagnosed participants included 165 AD, 105 MCI, 70 FTD [including 60 behavioral variant FTD (bv-FTD) and 10 corticobasal syndrome (CBS)], 79 LB disorders [LBD; including 16 DLB and 63 PD/PD with dementia (PDD)], 41 ALS, 11 progressive supranuclear palsy (PSP), and 69 HC (see Table 1 and Supplementary Table 1). The validation cohort contained 102 neuropathologically-confirmed cases, including 40 AD, 23 frontotemporal lobar degeneration with and without AD (FTLD; 17 FTLD, and 6 FTLD-AD), 30 PD/LB-related pathology with and without AD (LRP) (3 PD, 4 PD-AD, 21 LRP-AD, and 2 LRP-TDP), and 6 ALS (see below, Table 2, and Supplementary Table 2 for more details; note that 3 HC cases with an unremarkable burden of any significant brain pathology were not included in the analyses in the current study due to the small case number). The clinical diagnoses were made applying clinical diagnostic criteria for AD [1], bv-FTD [20], CBS [21], primary progressive aphasia [22], DLB [23], PD/PDD [24, 25], ALS [26], PSP [27], and HC as previously reported [19, 28, 29]. For the purposes of this study, patients diagnosed as CBS, bv-FTD, FTD-motor neuron disease, progressive non-fluent aphasia (PNFA) and semantic dementia (SD) were classified as FTD, while subjects with AD and logopenic progressive aphasia (LPA) were classified as AD. As per current conventions, the term FTD was used for the clinical diagnosis and the term FTLD for the neuropathologically-confirmed diagnoses. Informed consent to be included in research studies and to perform the autopsy was obtained in all cases from the patients or legal representatives in accordance with Pennsylvania state law. The study and all protocols were approved by the Institutional Review Boards of the UPenn and the University of Washington (UW).

2.2 CSF collection and CSF measurements

All CSF samples were obtained by lumbar puncture as described previously, and samples were immediately stored at −80°C until analysis [30]. CSF total α-syn and pS129 levels were measured at UW by using Luminex immunoassays as previously described [14, 16]. CSF data for $A\beta_{42}$, t-tau, and p-tau were obtained at UPenn by using the INNO-BIA AlzBio3™ Luminex assay reagents (Innogenetics, Ghent, Belgium) [30–32]. CSF hemoglobin levels were measured as an index of red blood cell contamination, with a human hemoglobin ELISA quantitation kit (Bethyl Lab Inc, Montgomery, TX, USA) as previously described [14].

2.3 Tissue collection and neuropathological assessment

Tissue collection procedures have been previously described [19]. Briefly, a neuropathological diagnosis of AD was assigned if the probability was intermediate or high [33]. The diagnoses of FTLD-TAU, FTLD-TDP and DLB were based on established criteria [23, 34]. FTLD-TAU cases included cases with a diagnosis of argyrophilic grain disease

(AGD), progressive supranuclear palsy (PSP), tangle predominant senile dementia (TPSD), and corticobasal degeneration (CBD). See Supplementary Methods for more details.

2.4 Statistical analysis

All analyses were performed in SPSS 18.0 (IBM, Chicago, IL, USA) or Prism 6.0 (GraphPad Software, La Jolla, CA, USA). Immunoassay data (CSF total α-syn, pS129, $A\beta_{42}$, t-tau, and p-tau) were Log10 transformed to generate a more normally distributed dataset, and the transformed data was used in all analyses. Correlations between biomarkers are reported as Pearson correlation coefficients. One way analysis of variance (ANOVA) followed by Tukey post-hoc test was used to compare group means. Receiver operating characteristic (ROC) curves for analytes, controlling for age and sex of participants, were generated to evaluate their sensitivities and specificities in distinguishing AD from HC or diseased comparison participants. Area under curve (AUC) was determined as a measure of the overall performance of a diagnostic test (the closer AUC is to 1, the better the overall diagnostic performance), which is also independent of disease prevalence since it is based on sensitivity and specificity [35]. The “optimum” cutoff value for a ROC curve was defined as the value associated with the maximal sum of sensitivity and specificity (i.e., maximizing the Youden index). Stepwise logistic regression was used to determine the best prediction models that included multiple CSF biomarkers as well as age and sex of participants. Values with $p<0.05$ were regarded as significant.

3. Results

3.1 Correlation among CSF analytes in the whole cohort

A total of 642 cases were included in the current study. As previously described [14, 15], CSF α-syn showed a strong association with CSF hemoglobin levels (an index of blood contamination in CSF; r=0.523, $p<3.8\times10^{-46}$) (Figure 1A). CSF pS129 showed a significant, although weaker, inverse association with CSF hemoglobin levels (r=−0.182, $p<3.6\times10^{-6}$) (Figure 1B).When using a cutoff of hemoglobin >500 ng/mL in this cohort to exclude blood-contaminated samples, 31.3% of the CSF samples were excluded from all further analyses (n=201), and then both CSF α-syn ($p=0.869$) and pS129 ($p=0.291$) showed no significant associations with CSF hemoglobin.

After excluding CSF samples with high hemoglobin levels (>500 ng/mL), CSF α-syn showed no association with CSF $A\beta_{42}$ (r=−0.025, $p=0.597$) (Figure 1C), but a strong positive correlation with t-tau (r=0.725, $p<1.5\times10^{-71}$) (Figure 1D) as well as a moderate positive correlation with p-tau (r=0.430, $p<3.0\times10^{-21}$) (Figure 1E). In contrast, CSF pS129 showed no association with any of the three classic AD CSF biomarkers (all $p>0.07$). CSF α-syn and pS129 were not significantly correlated with each other (r=−0.069, $p=0.15$) in this cohort (Figure 1F).

3.2 Evaluation of diagnostic and differential diagnostic values of CSF α-syn and pS129 in the clinical cohort

A cohort of 540 cases without neuropathological confirmation was used as the discovery cohort in this study (see Supplementary Table 1 for the whole cohort). As described in the

Methods, certain disease groups were combined together based on their similar underlying pathology (e.g., DLB and PD/PDD) to increase the sample size in analyses. In this cohort (n=367 subjects after excluding samples with high hemoglobin levels), CSF α-syn levels were numerically higher in AD compared to HC or LBD (DLB/PD/PDD; p=0.153) (see Table 1 and Figure 2A). However, CSF α-syn was significantly higher in AD compared to FTD (p=0.004) or ALS (p=0.014), and borderline significantly higher in AD compared to PSP (p=0.068). CSF α-syn was also significant higher in MCI compared to FTD (p=0.001), LBD (p=0.034), ALS (p=0.003), or PSP (p=0.023). CSF pS129 showed no differences between AD or MCI and HC, consistent with previous reports [16, 36], or any other diagnostic groups (Figure 2B). Use of the CSF pS129/α-syn ratio did not enhance the performance of CSF total α-syn for AD diagnosis and differential diagnosis (Figure 2C).

To further evaluate the diagnostic and differential diagnostic values of CSF biomarkers and their combinations, ROC analysis was performed to determine the sensitivities and specificities between AD and HC or patients with other neurodegenerative diseases (see Table 3 and Figure 3). For the comparison between AD and HC, although CSF α-syn alone only provided a poor differentiation and as expected, CSF $A\beta_{42}$ [AUC=0.890, 95% confidence interval (CI) 0.832–0.948; sensitivity=86.8% (95% CI 79.2–92.4%), specificity=83.3% (95% CI 69.8–92.5%)] or t-tau [AUC=0.848, 95% CI 0.783–0.912; sensitivity=77.2% (68.4–84.5%), specificity=83.3% (69.8–92.5%)] could discriminate the two groups well, the best model was the combination of CSF $A\beta_{42}$, t-tau, and α-syn, when controlling for age and sex of participants [AUC=0.931, 95% CI 0.890–0.973; sensitivity=92.1% (85.5–96.3%), specificity=85.4% (72.2–93.9%); Figure 3A].

For the comparison between AD and FTD groups, CSF α-syn alone (controlling for age and sex of participants) could provide a weak differentiation [AUC=0.760, 95% CI 0.687–0.832; sensitivity=51.8% (42.2–61.2%), specificity=89.3% (78.1–96.0%)], similar to those of CSF $A\beta_{42}$, t-tau, or p-tau alone (Table 3); a combination of CSF α-syn, $A\beta_{42}$, and p-tau differentiated AD from FTD well [AUC=0.893, 95% CI 0.845–0.941; sensitivity=80.7% (72.3–87.5%), specificity=85.7% (73.8–93.6%); Figure 3B] and was significantly more informative compared to the best individual CSF biomarker ($A\beta_{42}$; Z=2.3744, p=0.0176, DeLong's test[37]). Similarly, CSF α-syn alone could also provide a moderate differentiation for AD vs LBD (DLB/PD/PDD) [AUC=0.751, 95% CI 0.664–0.838; sensitivity=78.1% (69.4–85.3%), specificity=64.3% (48.0–78.4%)], AD vs ALS [AUC=0.858, 95% CI 0.788–0.928; sensitivity=87.7% (80.3–93.1%), specificity=68.6% (50.7–83.1%)], and AD vs PSP [AUC=0.740, 95% CI 0.539–0.940; sensitivity=70.2% (60.9–78.4%), specificity=77.8% (40.0–97.2%)], and adding CSF α-syn to $A\beta_{42}$, t-tau (or p-tau) enhanced the differential diagnosis [AD vs LBD, AUC=0.900, 95% CI 0.844–0.956, sensitivity=89.5% (82.3–94.4%), specificity=82.1% (62.5–92.5%), Figure 3C; AD vs ALS, AUC=0.947, 95% CI 0.883–1.000, sensitivity=96.5% (91.3–99.0%), specificity=88.6% (73.3–96.8%), Figure 3D; and AD vs PSP, AUC=0.915, 95% CI 0.860–0.970, sensitivity=80.7% (72.3–87.5%), specificity=100.0% (66.4–100%), Table 3].

3.3 Validation of differential diagnostic values of CSF biomarkers in the autopsy cohort

To further validate the differential diagnostic values, we measured the CSF biomarkers in a cohort of neuropathologically-confirmed cases (n=102 in total; 74 after excluding CSF samples with >500 ng/mL hemoglobin levels; see Table 2 and Supplementary Table 2). Due to the limited number of cases, the subjects were categorized into the following pathological groups: AD, FTLD (including FTLD and FTLD-AD), LRP (including PD, PD-AD, LRP-AD, and LRP-TDP), and ALS. As shown in Figure 4, consistent with the results from the clinical cohort, CSF α-syn was substantially higher in AD compared to FTLD, LRP, and ALS, while CSF pS129 didn't show significant differences among diagnostic groups.

Further ROC analysis demonstrated that CSF α-syn could differentiate AD from FTLD [AUC=0.782, p=0.002, sensitivity=58.6% (95% CI 38.9–76.5%), specificity=93.8% (69.8–99.8%); Figure 4C], LRP [AUC=0.678, p=0.033, sensitivity=79.3% (95% CI 60.3–92.0%), specificity=57.1% (34.0–78.2%); Figure 4D], and ALS [AUC=0.966, p=0.001, sensitivity=86.2% (95% CI 68.3–96.1%), specificity=100.0% (47.8–100.0%)] well, when controlling for age and sex of participants. Additionally, the combinations of CSF α-syn, $A\beta_{42}$, t-tau (or p-tau) further improved the differential diagnosis: AD vs FTLD, AUC=0.935, $p=1.67\times10^{-6}$, sensitivity=93.1% (95% CI 77.2–99.2%), specificity=87.5% (61.6–98.4%) for a model of CSF α-syn, $A\beta_{42}$, and p-tau (Figure 4E); AD vs LRP, AUC=0.767, p=0.001, sensitivity=55.2% (95% CI 35.7–73.6%), specificity=95.2% (76.2–99.9%) for a model of CSF α-syn, $A\beta_{42}$, and t-tau (Figure 4F); AD vs ALS, AUC=1.000, $p=4.23\times10^{-4}$, sensitivity=100.0% (95% CI 88.1–100.0%), specificity=100.0% (47.8–100.0%) for a model of CSF α-syn, $A\beta_{42}$, and p-tau. It should be noted that some small sample sizes (e.g., n=5 for ALS) led to wide 95% CIs.

4. Discussion

For the clinically relevant diagnosis and differential diagnosis of AD, it is essential to have a set of biomarkers that discriminate AD from other clinically relevant dementias or neurodegenerative diseases. Previous studies revealed substantial overlaps in CSF biomarker profiles ($A\beta_{42}$ and t-tau or p-tau) between AD and related disorders, and this significantly limits the utility of these core CSF biomarkers in differential diagnosis [5, 6]. In the current study, we interrogated CSF samples obtained from a relatively large, longitudinally-followed clinical cohort, and we report that higher CSF total α-syn might be relatively unique to AD, and that by combining data on CSF measures of α-syn, $A\beta_{42}$, and t-tau or p-tau we might be able to provide better diagnostic and differential diagnostic biomarker values for AD. These findings were largely confirmed in a separate cohort of participants who were longitudinally followed to autopsy for neuropathological confirmation of their diagnoses.

Although it was less apparent in the cohort included in this study, there is overlap of CSF $A\beta_{42}$ and tau values between AD and related disorders as reported in previous studies [5–9]. As discussed previously [5, 6], these observations were not that surprising, because mixed pathology is a common finding at autopsy [38–41], which may reflect converging pathophysiological mechanisms and pathways at late clinical stages [5]. For example, neuropathological and neuroimaging studies have revealed Aβ and tau pathology in LBD patients [42–44], and regional brain Aβ accumulation appears to correlate with domain-

specific cognitive performance in PD patients [45]. These results suggest that CSF $A\beta_{42}$ and tau may detect the increased levels of Aβ and tau pathology in non-AD diseases, limiting the differential diagnostic value of such biomarkers [5], particularly when used alone. Earlier studies [46, 47] reported that CSF p-tau might improve the differential dementia diagnosis (AUC 0.6–0.8), but other large-scale studies [4, 5], including the current study, found that CSF p-tau and t-tau performed largely equally.

In the current study, CSF α-syn levels tended to be higher in AD or MCI compared to related disorders in both cohorts, though the statistical significance was not achieved for AD vs HC and AD vs LBD (DLB/PD/PDD). This is in agreement with several previous large-scale studies, showing significantly higher levels of α-syn in CSF from patients with AD compared to HC [17, 18, 36] or patients with DLB/PDD [48]. The increase is perhaps due to the release of α-syn from damaged neurons during neurodegeneration, similar to what has been hypothesized for the increased levels of CSF tau in AD. However, this cannot be the entire explanation since CSF α-syn and tau do not appear to be increased in most other neurodegenerative diseases that are also associated with α-syn or tau pathology and extensive neuron loss in the brain. We have reported that CSF α-syn and tau could be transported from the central nervous system (CNS) into peripheral blood, and this potential clearance of CNS α-syn and tau via exosomes appeared to be increased in PD compared to HC [49, 50], but not in AD for tau [50] (α-syn clearance in AD has not been tested yet). Whether this is also true for CNS α-syn clearance and whether it could be a major contributor to the increase of CSF α-syn and tau in AD needs further investigation.

Most importantly, we found that the diagnostic accuracy of the combinations of these CSF biomarkers including measures of α-syn, Aβ, and tau was high enough (AUC, 0.8–0.9) to be useful in clinical settings for differentiating patients with AD from those with other related disorders. The diagnostic accuracy of these CSF proteins for differentiating AD from FTD is at least in the same order of magnitude as those obtained with advanced neuroimaging technologies [51, 52] and at a lower cost. Our findings on AD vs LBD is also in line with a previous study [48] reporting a panel of CSF biomarkers including α-syn, tau and $A\beta_{42}$ could differentiate AD from DLB and PDD with high sensitivity and specificity. It should be emphasized that these results were acquired from a retrospective study under a research setting, the biomarker accuracy and usefulness of the panel need to be further confirmed in prospective diagnostic studies under clinical settings [4]. Additionally, we used AUC as well as the Youden index-determined sensitivity and specificity in this study to assess the overall performance of candidate biomarkers for the differential diagnosis of AD, which usually has higher prevalence rate among related diseases. In some clinical settings (e.g., to screen for a certain disease of very low prevalence, a high specificity and a low false positive rate is required), the performance may need to be re-evaluated by adjusting the cutoff range or considering only a portion of the ROC curve.

However, CSF pS129 levels did not distinguish AD from any of the other diagnostic groups in this study. Previous studies have demonstrated that pS129 is the predominant post-translationally modified form of α-syn in LBs [53, 54] and have also associated CSF pS129 with PD [16, 55]. However, the role of pS129 in AD remains unclear, despite the frequent observation of LBs in AD brains [11–13]. In our previous study [16], we did not observe

significant differences in CSF pS129 levels between AD and HC when a small cohort of AD subjects was examined. This was confirmed in a more recent independent study with a much larger AD cohort [36]. In the current study, CSF pS129 and total α-syn were not significantly correlated with each other, indicating different α-syn forms in CSF might behave differently, possibly due to different transportation or clearance mechanisms. Our results also suggest that the transportation or clearance mechanisms for pS129, if different from other general α-syn species, might be less likely affected under different disease settings.

All the ROC analyses in this study were performed by controlling for age and sex of participants. Several studies have explored if demographic factors, including age, sex, and the *APOE* ε4 genotype, impact the diagnostic accuracy of CSF AD biomarkers (see review by Mattsson et al [4]). While there is no clear effect of sex on the diagnostic accuracy, age may impact the diagnostic performance of CSF AD biomarkers [4]. The genotype *APOE* ε4 is strongly associated with reduced CSF $A\beta_{42}$ in controls, but not with altered CSF t-tau or p-tau levels [4] as well as CSF α-syn levels [17]. However, because *APOE* ε4 is associated with increased amyloid pathology, rather than artificial reductions of CSF $A\beta_{42}$, it is not recommended to adjust the CSF $A\beta_{42}$ cutoff depending on the presence of *APOE* ε4 [4]. In the current study, adding *APOE* ε4 status to the models did not change the outcomes (data not shown).

One limitation of this study is that the cohort studied here did not include any subjects with vascular dementia, and thus the performance of CSF α-syn, together with $A\beta_{42}$ and t-tau or p-tau, on differentiating AD from vascular dementia remains unknown. While this needs to be further investigated in future studies, we previously reported that CSF E-selectin, a biomarker of endothelial function/vascular injury, might be a promising CSF biomarker to pursue as a potential indicator that vascular pathology is contributing to dementia [56]. Thus, CSF E-selectin should be tested in larger cohorts for its ability to differentiate AD from vascular dementia. Another potential limitation is that certain disease groups with similar underlying pathology were combined together (e.g., DLB and PD were combined into overarching LBD or LRP) to increase the sample size in the analyses, and its potential confounding effects need to be further investigated in future larger-scale studies.

In summary, CSF total α-syn, when combined with core AD biomarkers (i.e., $A\beta_{42}$, t-tau, and p-tau), improved the differential diagnosis of AD vs FTD, LBD (DLB/PD/PDD), and other neurodegenerative disease. The diagnostic accuracy of the combined models described here was high enough to be of clinical value for differentiating AD patients from patients with other related disorders in our cohort. Moreover, the diagnostic performance of these CSF biomarkers was supported by studies of a second cohort of subjects who were longitudinally followed to autopsy for neuropathological confirmation of their diagnoses. Although further validation in independent cohorts is still needed, our results indicate that CSF measures of total α-syn combined with measures of $A\beta_{42}$, t-tau, and p-tau might have clinical value in the differential diagnosis of AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in Context

1. **1. Systematic review:** The Pubmed database was searched to identify previously published research. Differential diagnosis of AD from other common geriatric dementing disorders remains unresolved. Recent studies found that the utility of the core CSF AD biomarkers ($A\beta_{42}$, t-tau, and p-tau) to discriminate AD from a variety of related disorders is limited, probably due to overlap in the underlying primary pathologies. It has been suggested that introduction of additional CSF biomarkers reflecting other types of pathologies could optimize differential diagnosis.
2. **2. Interpretation:** Our findings indicate that CSF measures of total α-synuclein combined with measures of $A\beta_{42}$, t-tau, and p-tau, representing LB and AD pathologies, respectively, likely have clinical value in the differential diagnosis of AD.
3. **3. Future directions:** Further studies are needed to include additional CSF biomarkers to reflect other comorbid pathologies, such as E-selectin for vascular pathology commonly observed at autopsy of AD brains.

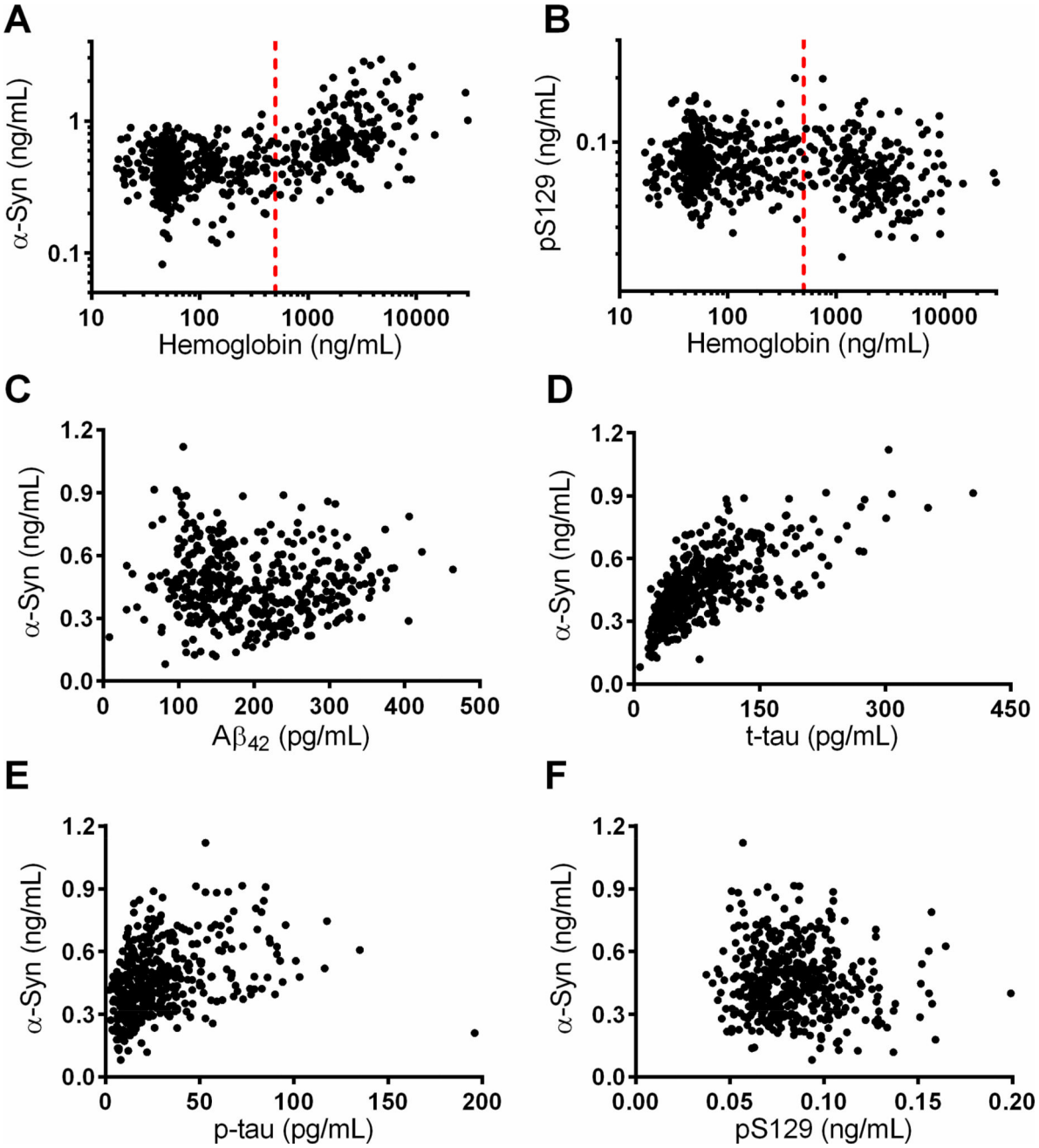

Figure 1. Association of CSF analytes

(**A and B**) Associations of CSF α-synuclein (α-syn; A) and α-syn phosphorylated at Ser129 (pS129; B) with CSF hemoglobin values in all subjects (n=642); vertical dashed red line represents the 500 ng/mL cut-off selected to exclude CSF α-syn values due to blood contamination. (**C, D, and E**) Associations of CSF α-syn with CSF amyloid beta peptide 1–42 ($A\beta_{42}$; C), total tau (t-tau; D), and phosphorylated p-tau at Thr181 (p-tau; E), after excluding subjects with >500 ng/mL of CSF hemoglobin (n=441 after exclusion). (**F**) Association of CSF α-syn and pS129 in subjects with ≤500 ng/mL hemoglobin.

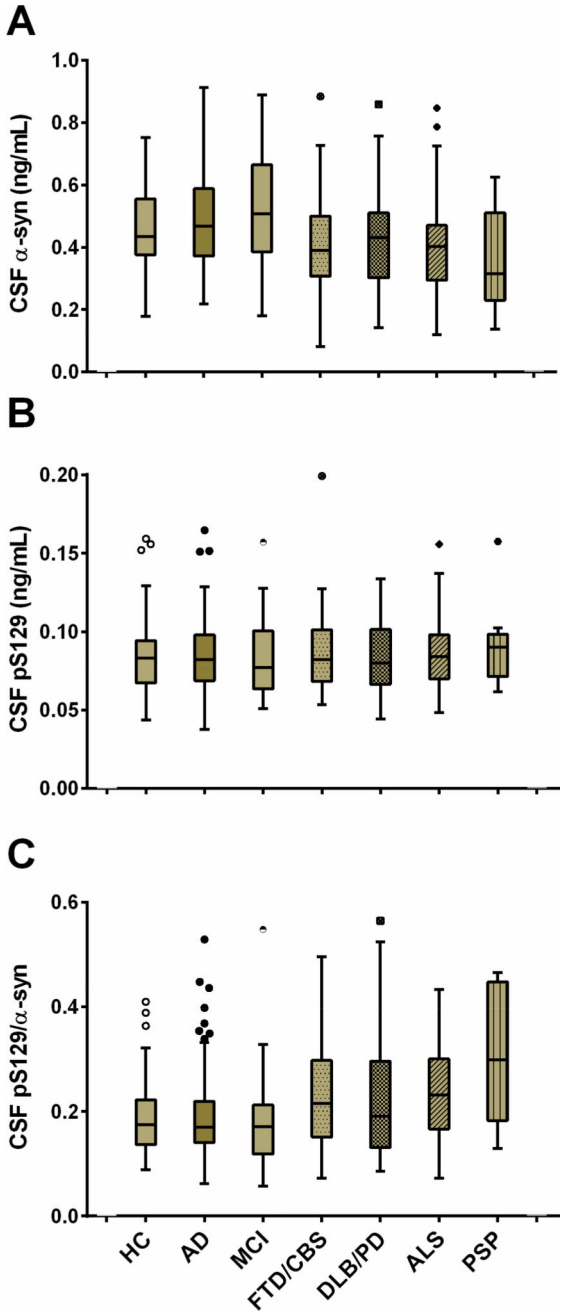

Figure 2. CSF α-syn, pS129, and the pS129/α-syn ratio stratified by clinical diagnosis in the clinical cohort

CSF total α-syn (**A**) and pS129 (**B**) concentrations were measured in the clinical cohort that includes patients with the diagnoses indicated below panel C. The ratio of pS129/α-syn is also shown (**C**). The boxes extend from the 25th to 75th percentiles. The middle dark lines indicate the medians. The whiskers extend to 1.5 times the height of the box or, if no case has a value in that range, to the minimum or maximum values. Values not included between the whiskers are plotted as outliers with corresponding symbols. No outliers were excluded from the analyses in this study.

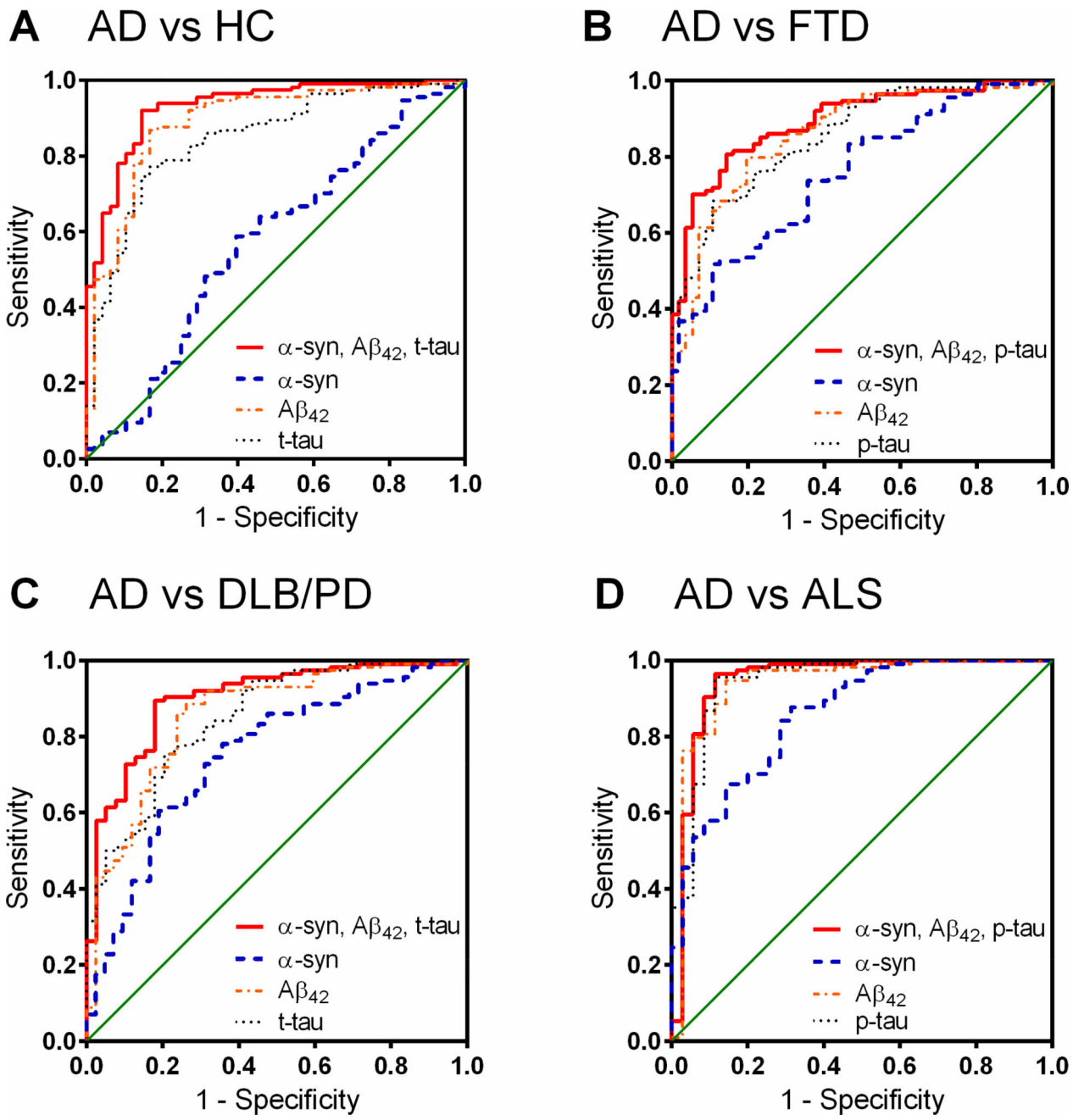

Figure 3. ROC analysis of CSF biomarkers in the clinical cohort

(**A**) Alzheimer disease (AD) vs healthy controls (HC); (**B**) AD vs frontotemporal degeneration (FTD)/corticobasal syndrome (CBS); (**C**) AD vs dementia with Lewy bodies (DLB)/Parkinson disease (PD); (**D**) AD vs amyotrophic lateral sclerosis (ALS). Blue dashed line indicates CSF α-syn alone, orange dot-dashed line indicates CSF $A\beta_{42}$, black dotted line indicates CSF t-tau or p-tau, and red solid line indicates a combined model of CSF α-syn, $A\beta_{42}$, and t-tau or p-tau.

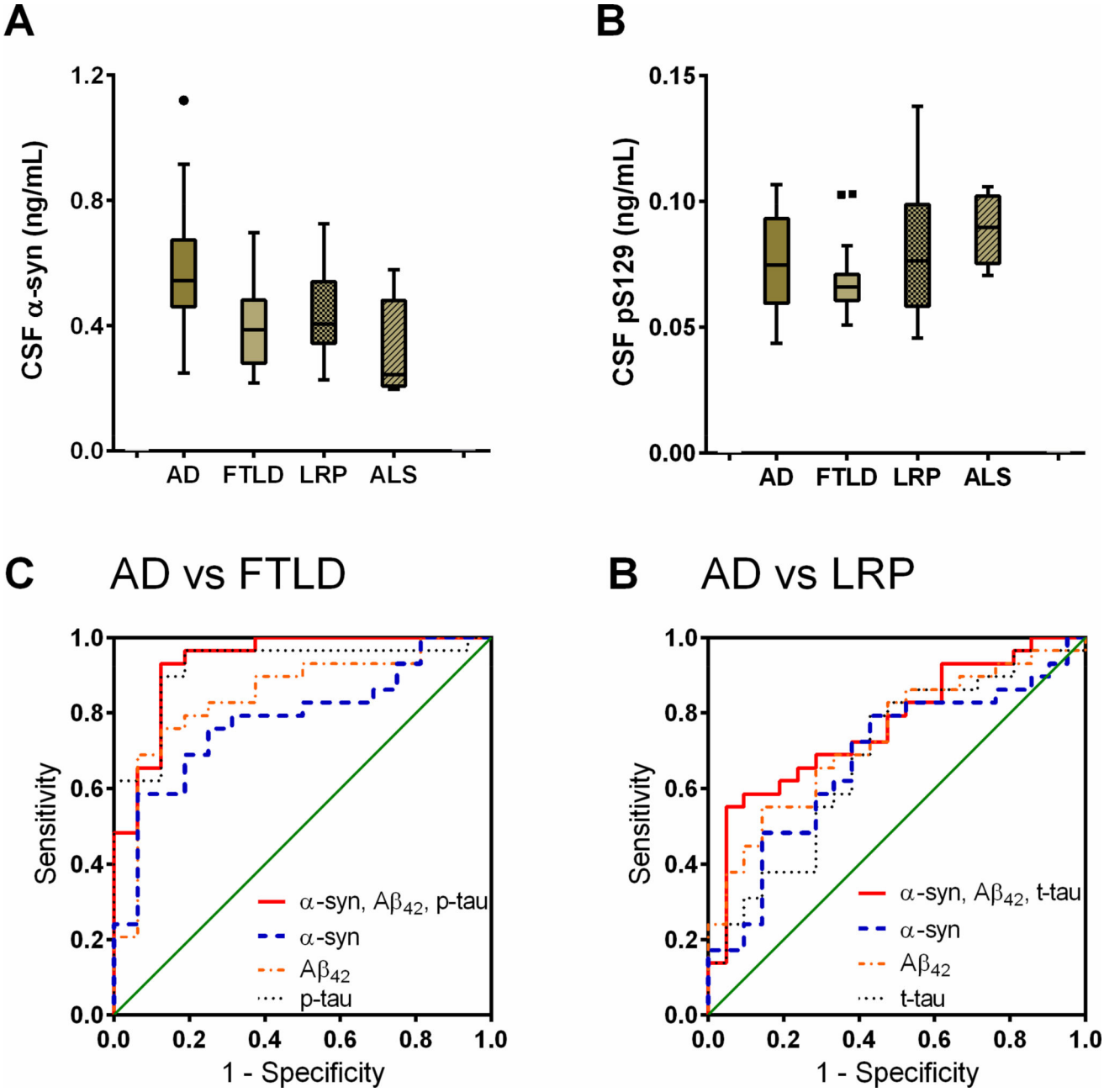

Figure 4. CSF α-syn and pS129 stratified by autopsy diagnosis and the differential diagnosis performance of CSF biomarkers in the validation (autopsy) cohort

Table 1

Characteristics of the clinical cohort (CSF hemoglobin <500 ng/mL).

	HC	AD	MCI	FTD		LBD		ALS	PSP
				bvFTD	CBS	DLB	PD		
N	48	114	63	48	8	10	32	35	9
Age at CSF sampling *	67.3 (40 – 88)	69.5 (51 – 91)	70.5 (50 – 86)	62.6 (45 – 80)		66.0 (49 – 87)		55.3 (28 – 74)	70.4 (56 – 83)
Sex (F/M)	30/18	66/48	32/31	22/34		10/32		8/27	5/4
Disease duration at CSF (yr) *	-	2.9 (−1 – 16)	2.4 (−1 – 6)	2.8 (0 – 14)		6.6 (−1 – 22)		1.1 (0 – 10)	3.2 (0 – 7)
APOE ε4 presence (%)	29.2	50.0	38.1	28.6		23.8		20.0	11.1
CSF $A\beta_{42}$ (pg/mL) *	254.7 (94–423)	144.5 (31–405)	186.5 (78–355)	221.5 (82–386)		227.0 (78–338)		268.8 (8–464)	196.4 (110–347)
CSF t-tau (pg/mL) *	54.0 (20–161)	118.0 (21–405)	86.9 (18–254)	70.6 (7–203)		54.4 (20–127)		60.9 (21–271)	46.9 (18–80)
CSF p-tau (pg/mL) *	21.8 (2–101)	40.8 (7–135)	32.5 (5–116)	21.5 (4–62)		26.0 (10–69)		17.6 (6–196)	15.1 (7–23)
CSF t-tau/$A\beta_{42}$ *	0.25 (0.09–1.02)	1.01 (0.08–4.87)	0.59 (0.08–2.13)	0.38 (0.09–1.63)		0.26 (0.09–0.92)		0.33 (0.08–3.63)	0.25 (0.14–0.53)
CSF p-tau/t-tau *	0.41 (0.05–1.00)	0.38 (0.12–1.23)	0.40 (0.12–1.07)	0.35 (0.07–1.14)		0.54 (0.16–1.00)		0.41 (0.07–6.76)	0.37 (0.16–0.65)
CSF α-syn (ng/mL) *	0.464 (0.179–0.753)	0.487 (0.219–0.913)	0.519 (0.180–0.889)	0.401 (0.082–0.884)		0.421 (0.142–0.859)		0.400 (0.119–0.847)	0.358 (0.138–0.625)
CSF pS129 (ng/mL) *	0.085 (0.044–0.159)	0.084 (0.038–0.165)	0.082 (0.051–0.157)	0.087 (0.053–0.199)		0.084 (0.044–0.134)		0.088 (0.049–0.156)	0.091 (0.062–0.157)
CSF pS129/α-syn *	0.205 (0.088–0.888)	0.191 (0.062–0.529)	0.176 (0.057–0.548)	0.268 (0.073–1.139)		0.238 (0.086–0.653)		0.270 (0.073–1.147)	0.300 (0.129–0.465)

* Data shown is mean (range).

Table 2

Characteristics of the autopsy cohort (CSF hemoglobin <500 ng/mL).

	AD	FTLD		LRP				ALS
		FTLD	FTLD-AD	PD	PD-AD	LRP-AD	LRP-TDP	
N	29	12	4	3	4	12	2	5
Age at CSF sampling	70.7 (44–87)	66.4 (43–83)		70.0 (52–88)				57.4 (47–64)
Sex (F/M)	12/17	7/9		7/14				4/1
Disease duration at CSF (yr)	3.1 (0 – 12)	3.1 (0 – 9)		5.3 (1 – 20)				1.2 (1 – 2)
Age at Death	76.1 (47–92)	70.7 (44–86)		74.9 (62–90)				59.4 (51–67)
Survival after CSF (yr)	5.6 (1.5–13.9)	4.4 (1.2–10.4)		4.7 (1.1–10.7)				1.8 (0.2–4.0)
APOE ε4 presence (%)	55.2	12.5		52.4				20.0
CSF $A\beta_{42}$ (pg/mL)	134.3 (39–295)	195.4 (108–275)		170.9 (85–274)				279.2 (203–343)
CSF t-tau (pg/mL)	124.7 (20–304)	61.3 (22–143)		82.0 (22–196)				56.0 (25–102)
CSF p-tau (pg/mL)	34.4 (5–94)	12.0 (4–45)		24.1 (4–83)				8.4 (6–12)
CSF t-tau/$A\beta_{42}$	1.08 (0.21–3.40)	0.35 (0.15–1.15)		0.57 (0.13–1.72)				0.19 (0.12–0.30)
CSF p-tau/t-tau	0.45 (0.04–4.70)	0.21 (0.04–0.55)		0.32 (0.07–0.83)				0.19 (0.10–0.40)
CSF α-syn (ng/mL)	0.568 (0.248–1.120)	0.391 (0.216–0.698)		0.443 (0.227–0.725)				0.324 (0.197–0.579)
CSF pS129 (ng/mL)	0.077 (0.044–0.107)	0.069 (0.051–0.103)		0.081 (0.046–0.138)				0.089 (0.071–0.106)
CSF pS129/ α-syn	0.156 (0.051–0.348)	0.192 (0.103–0.373)		0.196 (0.075–0.392)				0.323 (0.138–0,499)

*Data shown is mean (range).

Table 3

Diagnostic and differential diagnostic values of CSF biomarkers in AD in the clinical cohort.

	α-syn*	pS129/α-syn*	$A\beta_{42}$*	t-tau*	p-tau*	t-tau/$A\beta_{42}$*	Combined model**
AD vs HC							
AUC	0.573	0.573	0.890	0.848	0.789	0.891	0.931†
Sens%	58.8	71.9	86.8	77.2	78.9	90.4	92.1
Spec%	60.4	45.8	83.3	83.3	72.9	81.3	85.4
p-value	0.142	0.141	5.13E-15	3.03E-12	6.25E-9	4.56E-15	5.03E-18
AD vs FTD							
AUC	0.760	0.767	0.860	0.831	0.857	0.873	0.893‡
Sens%	51.8	58.8	79.8	73.7	68.4	76.3	80.7
Spec%	89.3	82.1	80.4	78.6	89.3	87.5	85.7
p-value	3.94E-8	1.55E-8	2.69E-14	2.35E-12	4.43E-14	4.94E-16	8.42E-17
AD vs LBD							
AUC	0.751	0.746	0.854	0.849	0.768	0.886	0.900†
Sens%	78.1	79.8	88.6	75.4	77.2	78.9	89.5
Spec%	64.3	64.3	73.8	79.5	70.7	89.7	82.1
p-value	1.57E-6	2.57E-6	1.27E-11	8.17E-11	3.59E-7	1.73E-13	9.50E-14
AD vs ALS							
AUC	0.858	0.859	0.939	0.912	0.942	0.939	0.947‡
Sens%	87.7	83.3	94.7	80.7	95.6	86.8	96.5
Spec%	68.6	71.4	85.7	88.6	88.6	88.6	88.6
p-value	1.61E-10	1.35E-10	4.65E-15	1.88E-13	2.72E-15	1.72E-15	1.32E-15
AD vs PSP							
AUC	0.740	0.788	0.715	0.882	0.889	0.900	0.915†
Sens%	70.2	78.1	76.3	67.5	81.6	90.4	80.7
Spec%	77.8	77.8	66.7	100.0	88.9	88.9	100.0
p-value	0.017	0.004	0.032	1.41E-4	1.04E-4	4.34E-5	3.51E-5

* Logistic model contains the CSF marker, age and sex of participants;

** Logistic model contains CSF α-syn, Aβ42, t-tau† or p-tau‡, age and sex of participants.