ARTICLE IN PRESS

npj Parkinson's Disease

https://doi.org/10.1038/s41531-026-01341-8

Article in Press

# Plasma phosphorylated tau 217 detects amyloid-β in neuronal synuclein disease

Received: 17 January 2026

Accepted: 25 March 2026

Cite this article as: Smith, A.M., Lorkiewicz, S.A., Arslan, B. *et al.* Plasma phosphorylated tau 217 detects amyloid-β in neuronal synuclein disease. *npj Parkinsons Dis.* (2026). https://doi.org/10.1038/s41531-026-01341-8

**Alena M. Smith, Sara A. Lorkiewicz, Burak Arslan, Laia Montoliu-Gaya, Nicholas J. Ashton, Edward N. Wilson, Daniel Alcolea, Íñigo Rodríguez-Baz, Juan Fortea, Christina B. Young, Joseph R. Winer, Marian Shahid-Besanti, Hillary Vossler, Melanie J. Plastini, Tianyu Pan, Elena Vera-Campuzano, Isabel Sala, Justin H. Mendiola, Veronica Ramirez, Geoffrey A. Kerchner, Katrin I. Andreasson, Victor W. Henderson, Thomas J. Montine, Lu Tian, Elizabeth C. Mormino, Henrik Zetterberg, Kathleen L. Poston & Carla Abdelnour**

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

ARTICLE IN PRESS

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

ARTICLE IN PRESS

**Title: Plasma phosphorylated tau 217 detects amyloid-β in neuronal synuclein disease**

**Authors:** Alena M. Smith[1], Sara A. Lorkiewicz[1], Burak Arslan[2], Laia Montoliu-Gaya[2], Nicholas J. Ashton [2-4], Edward N. Wilson[1,5], Daniel Alcolea[6,7], Íñigo Rodríguez-Baz[6-8], Juan Fortea[6-8], Christina B. Young[1,9,10], Joseph R. Winer[1], Marian Shahid-Besanti[1], Hillary Vossler[1], Melanie J. Plastini[1], Tianyu Pan[11], Elena Vera-Campuzano[6,7], Isabel Sala[6,7], Justin H. Mendiola[1], Veronica Ramirez[1], Geoffrey A. Kerchner [12], Katrin I. Andreasson[1], Victor W. Henderson[1], Thomas J. Montine[13], Lu Tian[14], Elizabeth C. Mormino[1,5,15], Henrik Zetterberg[2,16-22], Kathleen L. Poston*[1,23], Carla Abdelnour*[1,6,7].

1. Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA.
2. Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, University of Gothenburg, Mölndal, Sweden.
3. Banner Alzheimer's Institute and University of Arizona, Phoenix, Arizona, USA.
4. Banner Sun Health Research Institute, Sun City, Arizona, USA.
5. Wu Tsai Neurosciences Institute, Stanford University School of Medicine, Stanford, CA, USA.
6. Sant Pau Memory Unit, IR SANT PAU, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.
7. Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain.
8. Barcelona Down Medical Center, Fundació Catalana de Síndrome de Down, Barcelona, Spain.
9. Department of Radiology, University of California Davis, Davis CA USA.
10. Department of Neurology, University of California Davis, Davis CA USA.
11. Department of Biomedical Data Science, School of Medicine. Department of Statistics, School of Humanities and Sciences. Stanford, CA, USA.
12. Pharma Product Development, F. Hoffmann-La Roche, Ltd., Roche, Basel, Switzerland.
13. Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA.
14. Department of Epidemology and Population Health, Stanford University School of Medicine, Stanford, CA, USA.
15. Molecular Imaging Program at Stanford (MIPS), Stanford University, Stanford, CA, USA.
16. Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.
17. Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK.
18. UK Dementia Research Institute at UCL, London, UK.
19. Hong Kong Center for Neurodegenerative Diseases, InnoHK, Hong Kong, China.
20. Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA.
21. Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA.
22. Centre for Brain Research, Indian Institute of Science, Bangalore, India.
23. Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA.

*equal contributors

**Corresponding author:** Carla Abdelnour
**Title:** MD, PhD
**Affiliation:** Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA. Sant Pau Memory Unit, IR SANT PAU, Hospital de la Santa Creu i Sant Pau, Barcelona,

ARTICLE IN PRESS

Spain. Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain.
**Department:** Neurology.
**Mailing address:** Sant Quintí, 77-79. 08041 Barcelona.
**E-mail address:** carla.abdelnour@gmail.com
**Telephone:** +34-662940955

**Title:** 77 characters with spaces
**Abstract:** 200 words
**Body of manuscript:** 4,628
**Number of figures:** 3
**Number of tables:** 2
**Supplementary information:** 3 supplementary figures and 10 supplementary tables.

## Abstract

Multiple proteinopathies commonly coexist in neurodegenerative diseases, making it essential to evaluate plasma biomarker performance in these complex diseases. While plasma biomarkers accurately detect amyloid-β pathology in Alzheimer's disease (AD), their performance is unknown in neuronal synuclein disease (NSD). We aimed to determine the accuracy of plasma pTau217, pTau181, Aβ42/40, GFAP, and NfL to detect amyloid-β in NSD, then establish and validate cut points for the most promising marker. We included 253 participants (180 discovery; 73 validation). In the discovery cohort, NSD status was defined by CSF α-synuclein seed amplification assay and amyloid-β status by CSF Aβ42/40. Participants included individuals with clinical Lewy body disease (LBD), AD, and cognitively unimpaired. Validation cohorts consisted of clinically diagnosed LBD participants. In the discovery cohort, plasma pTau217, pTau181, Aβ42/40, and GFAP significantly differed by amyloid-β status regardless of NSD status, while NfL was highest in NSD+/Aβ+ participants. Among all biomarkers, plasma pTau217 showed the best diagnostic performance (AUC=0.92, 95% CI=0.81-0.98). Applying plasma pTau217 cut points to pre-screen clinically diagnosed LBD participants reduced the need for confirmatory amyloid-β PET or CSF in 41-56%. These findings support plasma pTau217 as a minimally-invasive tool for identifying pathological amyloid-β in neuronal synucleinopathies with mixed Alzheimer's disease pathology.

## Introduction

In neurodegenerative diseases of aging, coexistence of pathological amyloid-β, tau, and α-synuclein is common and contributes to clinical heterogeneity, faster disease progression, and variable treatment response. Advances in biomarker technology now enable for accurate, scalable, and cost-effective detection of some of these proteinopathies. As these biomarkers move into clinical practice, it is essential to understand their performance in the context of mixed neurodegenerative diseases.

Lewy body disease (LBD), which includes Parkinson's disease (PD) and dementia with Lewy bodies (DLB), is characterized by the abnormal deposition of α-synuclein (αSyn). This diagnostic pathologic feature can be detected in cerebrospinal fluid (CSF) using the seed amplification assay (SAA), supporting the biologically-based definition of neuronal synuclein disease (NSD)(1). Concomitant amyloid-β pathology is frequently found in both NSD and clinically diagnosed PD and DLB(2–7), and is associated with worse clinical outcomes(2), earlier dementia onset(8), faster cognitive decline(9,10) and shorter survival(11). These findings underscore the

ARTICLE IN PRESS

importance of identifying NSD individuals with co-occuring amyloid-β pathology, and the potential value of testing anti-amyloid therapies in this population.

CSF and positron emission tomography (PET) imaging Aβ biomarkers are the gold standard for detecting amyloid-β pathology in Alzheimer's disease (AD). However blood-based Aβ biomarkers have emerged as promising tools with accuracy comparable to CSF and PET(12,13). Among them, plasma phosphorylated tau at threonine 217 (pTau217) has consistently outperformed other plasma biomarkers such as pTau181 and the Aβ42/40 ratio for detecting amyloid-β in AD(12,14,15). Combinations of these markers, either with each other or with markers of neuroinflammation (i.e. glial fibrillary acidic protein, GFAP) and neurodegeneration (i.e. neurofilament light chain, NfL), have shown lower performance than pTau217 alone(12). Based on this evidence, pTau217 is now recommended for clinical and research use(16).

Multiple studies have evaluated plasma pTau181(10,15,17–27), pTau217(10,21,28,29), Aβ42/40 ratio(17,19,20,25), GFAP(17,19,20,25,26,30,31), and NfL(17,19,20,22,23,25–28) in clinically diagnosed LBD participants. However, their diagnostic accuracy for detecting amyloid-β has not been evaluated in biologically defined NSD. This distinction is critical as Aβ plasma biomarkers are being considered as a routine component of NSD staging(1), particularly since prior studies show Aβ biomarker cut points can differ between "mixed" NSD plus AD pathology and "pure" AD pathology cases(32–34). Evaluating plasma biomarkers in NSD provides a closer approximation to underlying pathology than clinical diagnosis, which often does not predict the presence of Lewy bodies at autopsy(35–37).

As plasma biomarkers enter clinical use, their interpretation must align with the intended use population, as emphasized by the FDA-NIH Biomarker Working Group(38). In NSD, accurate interpretation of plasma biomarkers is crucial for their appropriate use as screening tools for patient selection and stratification in clinical trials, particularly as drug development begins to address mixed pathologies.

In this cross-sectional study, we assessed whether plasma pTau217, pTau181, Aβ42/40, GFAP, and NfL accurately detect amyloid-β in individuals with biologically defined NSD. We calculated plasma biomarker cut points using one- and two-reference standard approaches in a discovery cohort, then validated these cut points in two independent clinical LBD cohorts to assess their generalizability. By establishing and validating plasma biomarker cut points for detecting amyloid-β in NSD, this study addresses a critical gap in biomarker interpretation that currently limits their use in mixed pathology settings.

## Results

### Discovery cohort characteristics

This cross-sectional study included participants from two cohorts: Stanford University (SU) and the Sant Pau Initiative on Neurodegeneration (SPIN)(39). Participants were divided into one discovery cohort and two clinical validation cohorts. The discovery cohort consisted exclusively of SU participants, while SPIN participants contributed to one of the clinical validation cohorts. The discovery cohort included 180 SU participants with CSF αSyn SAA and Aβ42/40 data: 86 cognitively unimpaired (CU), 30 LBD cognitively unimpaired (LBD-CU), 30 LBD cognitively impaired (LBD-CI), and 34 AD. CSF biomarkers were used to determine αSyn status (NSD+/-) and Aβ status (Aβ+/-). Participants were classified into four biologically defined groups: NSD-/Aβ- (n=69), NSD-/Aβ+ (n=44), NSD+/Aβ- (n=39), and NSD+/Aβ+ (n=28). Median age was 69 years (min, max: 50-87), with 83 females (46.1%) and 97 males (53.9%). The NSD-/Aβ- group was significantly younger than the NSD-/Aβ+ group (*P*= 0.029). Otherwise, the groups were similar in age, sex, and years of education (Table 1).

ARTICLE IN PRESS

**Plasma biomarker levels stratified by CSF NSD and Aβ status**

In the SU discovery cohort, plasma levels of pTau217, pTau181, and GFAP were significantly higher, while Aβ42/40 was significantly lower in the Aβ+ compared to the Aβ- groups, regardless of NSD status (all $P<0.001$, Table 1 and Figure 1A-D). Plasma NfL levels were significantly higher in the NSD+/Aβ+ group than in all other groups (vs. NSD-/Aβ- $P<0.001$; vs. NSD-/Aβ+ $P=0.045$; vs. NSD+/Aβ- $P=0.012$), and significantly higher in the NSD-/Aβ+ than in the NSD-/Aβ- group ($P=0.014$) (Figure 1E).

Visual inspection identified one outlier in the NSD+/Aβ+ group with a pTau181 level of 10.43 pg/mL (7.9 SDs above the mean). Removing this outlier in a sensitivity analysis did not change the results. In another sensitivity analysis, we excluded symptomatic participants (those with cognitive and/or motor symptoms) from the NSD-/Aβ- group and asymptomatic participants from the NSD-/Aβ+, NSD+/Aβ+, and NSD+/Aβ- groups. Findings were consistent except for plasma NfL (Supplementary Figure 1). In this analysis, asymptomatic NSD-/Aβ- participants had lower plasma NfL levels than all other groups (vs. NSD-/Aβ+ $P=0.003$; vs. NSD+/Aβ- $P=0.042$; vs. NSD+/Aβ+ $P<0.001$), and symptomatic NSD+/Aβ+ participants had higher plasma NfL levels than symptomatic NSD+/Aβ- participants ($P=0.003$), suggesting that symptom severity might be associated with NfL levels.

To quantify plasma biomarker differences between Aβ+ and Aβ- participants, we calculated median fold-change, standard deviation, and 95% confidence intervals (Figure 1F). Plasma pTau217 showed the largest median fold-change between Aβ+ and Aβ- participants, with the highest value in the NSD+ group (2.79 [SD: 0.33], 95% CI = 2.3-3.61; Supplementary Table 1).

**Association of plasma biomarkers with age, sex and *APOE* ε4 carriership**

Results from linear regression models examining the effects of age, sex, *APOE* ε4 carriership, CSF Aβ and NSD status on plasma biomarker levels are shown in Supplementary Table 2. In both univariable and multivariable linear regression analyses, higher levels of pTau217 and pTau181 were associated with CSF Aβ positivity ($P<0.001$). These results remained consistent after excluding the pTau181 outlier. Lower Aβ42/40 was associated with both *APOE* ε4 carriership ($P=0.012$) and CSF Aβ positivity ($P<0.001$). Additionally, higher GFAP levels were associated with older age ($P=0.003$), CSF Aβ positivity ($P<0.001$), and NSD positivity ($P=0.044$), while male sex ($P=0.013$) was associated with lower GFAP levels. Finally, higher NfL levels were associated with older age, CSF Aβ positivity , and NSD positivity (all $P<0.001$).

**Diagnostic accuracy of plasma biomarkers for detecting amyloid-β**

To determine diagnostic accuracy of plasma biomarkers for detecting amyloid-β in NSD+ and NSD-, we used Receiver Operating Characteristic (ROC) curves. In NSD+ participants, plasma pTau217 and Aβ42/40 showed the highest accuracy for detecting Aβ positivity (pTau217 AUC: 0.92, 95% CI: 0.85-1.00; Aβ42/40 AUC: 0.90, 95% CI: 0.81-0.98, Figure 2A). Their diagnostic performances outperformed NfL (AUC: 0.72, 95% CI: 0.59-0.84; vs. pTau217 $P=0.003$, vs. Aβ42/40 $P=0.021$), but were comparable to pTau181 and GFAP (Supplementary Table 3). Including age, sex, and *APOE* ε4 carriership did not improve model accuracies. Sensitivity analyses confirmed that excluding the pTau181 outlier did not alter its diagnostic performance. However, pTau217 became significantly more accurate than pTau181 after this exclusion (pTau181 without outlier AUC: 0.79, 95% CI: 0.67-0.90; vs. pTau217 $P=0.046$).

ARTICLE IN PRESS

ARTICLE IN PRESS

In NSD- participants, plasma pTau217 and Aβ42/40 again demonstrated the highest accuracies for detecting Aβ positivity, with no significant difference between them (pTau217 AUC: 0.91, 95% CI: 0.86-0.97; Aβ42/40 AUC: 0.83, 95%CI: 0.75-0.92, Figure 2B). Plasma pTau217 outperformed pTau181 (AUC: 0.80, 95% CI: 0.72-0.88, $P$= 0.03), GFAP (AUC: 0.77, 95% CI: 0.69-0.86, $P$= 0.01), and NfL (AUC: 0.66, 95% CI: 0.56-0.76, $P$<0.001, Supplementary Table 4). Including age, sex, and *APOE* ε4 carriership significantly improved NfL's accuracy (AUC: 0.81, 95% CI: 0.72-0.89, vs. NfL alone $P$= 0.031), but was still less accurate than pTau217 alone ($P$=0.042).

In both NSD+ and NSD- groups, models incorporating all biomarkers achieved the highest accuracy (AUC: 0.96, 95% CI: 0.92-1), but their performance was not significantly better than models using pTau217 or Aβ42/40 alone.

The above analysis used Aβ PET positivity threshold of 36 centiloids (CL) as the gold standard to determine the CSF Aβ status (Aβ +/-, see Methods and Supplementary Figure 2). In an exploratory analysis using an Aβ PET positivity threshold of 24 CL (Figures 2C and 2D), key differences emerged. In NSD+ participants, pTau217 remained the most accurate biomarker (AUC: 0.92, 95% CI: 0.84-0.99), significantly outperforming pTau181, GFAP, and NfL (Supplementary Table 5). In NSD-, all biomarkers performed similarly, except for NfL, which showed the lowest accuracy (AUC: 0.57, 95% CI: 0.47-0.68, Supplementary Table 6). Adding age, sex, and *APOE* ε4 carriership significantly improved NfL's performance (AUC: 0.80, 95% CI: 0.71-0.88) compared to NfL alone ($P$= 0.002), while other biomarkers showed no significant improvement. In combined biomarker models, the only difference from analyses using the 36 CL Aβ PET positivity threshold was observed in NSD+ participants, in whom plasma pTau217 alone, but not Aβ42/40 alone ($P$= 0.015), performed comparably to all optimal biomarker combinations.

**Derivation and risk stratification of plasma pTau217 cut points for detecting amyloid-β**

Plasma pTau217 and Aβ42/40 demonstrated similar accuracy for detecting Aβ; however, pTau217 showed the best overall performance in both NSD+ and NSD- groups, with the largest median fold-change between Aβ+ and Aβ- participants. Consequently, we selected pTau217 to determine the accuracy of reference cut points for detecting Aβ in NSD.

In NSD+ participants from the SU discovery cohort, we established one-reference cut point for plasma pTau217 using the Youden Index (>0.54 pg/mL). We then derived two-reference cut points: one at 90% sensitivity (<0.66 pg/mL) and specificity (>0.69 pg/mL), and another at 95% sensitivity (<0.47 pg/mL) and specificity (>1.04 pg/mL). These cut points differed in NSD- participants (Supplementary Table 7).

Figure 3, panel A shows predicted probabilities of Aβ positivity under the two-reference model, stratifying participants into low, intermediate and high-risk categories. In the SU discovery cohort, NSD+ participants with a predicted probability <6% had a 95% chance of being Aβ-, and those with a predicted probability <36% had a 90% chance of being Aβ- (low probability). Conversely, NSD+ participants with predicted probabilities >94% had a 95% chance of being Aβ+ and individuals with predicted probabilities >53% had a 90% chance of being Aβ+ (high probability). Participants with predicted probabilities between 36% and 53% were classified as intermediate risk.

**Validation of plasma pTau217 cut points in two independent clinical LBD cohorts**

The two clinical validation cohorts included clinically-defined LBD participants without CSF αSyn data from the Stanford University (LBD-SU, n=29) and Sant Pau (LBD-SPIN, n= 44) cohorts. Amyloid-β status (Aβ +/-) was

ARTICLE IN PRESS

determined by Aβ PET in the LBD-SU cohort, and CSF Aβ42/40 in the LBD-SPIN cohort (Supplementary Figure 3). The LBD-SU clinical validation cohort (n=29) had a median age of 71 years (min, max: 57-81), with 6 females (20.7%), and 23 males (79.3%). The LBD-SPIN clinical validation cohort (n=44) had a median age of 78 years (min, max: 65-84), with 20 females (45.5%) and 24 males (54.5%) (Supplementary Table 8).

Application of reference cut points to the LBD-SU and LBD-SPIN clinical cohorts showed that the negative percentage agreement (NPA) was similar between the one- and two-reference cut point models at 90% sensitivity/specificity, ranging from 89.5% to 94.4% (Table 2). However, the two-reference cut point model significantly improved the positive percentage agreement (PPA) (two-reference cut point vs. one-reference cut point in LBD-SU: 100% vs. 72.7%, and in LBD-SPIN: 86.4% vs. 68.8%). At 95% sensitivity/specificity, the two-reference cut point model achieved perfect PPA (100%) in both cohorts while maintaining high NPA (LBD-SU: 93.8%, LBD-SPIN: 90.9%).

We then determined the number of people who would need confirmatory (CSF or PET) Aβ testing at different cut points. In the one-reference cut point model, the high NPA but relatively low PPA suggests that plasma pTau217-positive individuals would require additional testing to confirm Aβ positivity (Figure 3, Panel B). Using the two-reference cut point at 90% sensitivity/specificity, both intermediate- and high- risk individuals would require additional testing. In contrast, in the 95% sensitivity/specificity model, only the intermediate-risk group would require additional testing (Figure 3, Panel B).

In an exploratory analysis using a 24 CL threshold for Aβ PET positivity, we observed both differences and similarities. The two-reference cut point models significantly improved PPA, reaching 100% in the LBD-SU cohort, while in LBD-SPIN, PPA remained moderate (83.3% in the 90% sensitivity/specificity model, and 81.3% in the 95% sensitivity/specificity model). NPAs remained consistently high across cohorts for both one- and two-reference cut point models (87.5% to 100%; Supplementary Table 9).

To assess the potential impact of using plasma pTau217 on clinical trial recruitment, we calculated the number needed to screen to identify 100 Aβ+ and/or 100 Aβ– NSD participants under each model. The 95% sensitivity/specificity two-cut point model, while offering stringent accuracy, required screening the largest number of patients to identify 100 Aβ+ participants (see Figure 3, Panel B and C).

## Discussion

Our findings showed that plasma pTau217 accurately detects Aβ positivity in individuals with NSD. To our knowledge, this is the first study analyzing Aβ, neuroinflammation, and neurodegeneration plasma biomarkers in NSD. We identified plasma pTau217 as a reliable biomarker for Aβ positivity and applied, for the first time in this population, a two-reference cut point approach similar to that proposed in AD(13,16,40). Since mixed pathologic αSyn and amyloid-β accumulation cannot be diagnosed clinically, accessible biomarkers are critically needed for accurate diagnosis and patient management. Plasma pTau217 provides a cost-effective, minimally-invasive alternative to Aβ PET or CSF. This is particularly important as concomitant amyloid-β mixed pathology is common in NSD and is associated with faster functional and cognitive decline(2,3,9,10). Thus, detecting Aβ positivity in NSD could guide clinical decisions and inform clinical trial design.

In clinical trials targeting αSyn-related cognitive impairment or dementia, excluding participants with amyloid-β pathology may be necessary. Plasma pTau217 could serve as a prescreening tool reducing the need for additional tests. Furthermore, the high frequency of amyloid-β pathology in NSD suggests that anti-amyloid therapies may

ARTICLE IN PRESS

ARTICLE IN PRESS

be appropriate for some individuals. A recent NIH RFA calls for phase 2 clinical trials testing the efficacy of anti-amyloid monoclonal antibodies in DLB with concomitant Aβ positive biomarkers, expanding the application of targeting Aβ in neurodegenerative diseases beyond AD. Validated tools like plasma pTau217 could efficiently identify eligible participants for such trials(41,42).

In this context, both one- and two-reference cut points at 90% sensitivity/specificity effectively identified individuals without Aβ positivity, achieving high NPAs. The two-reference cut point method also demonstrated high PPA, allowing more individuals to avoid invasive or expensive testing compared to the one cut point approach. Additionally, the two-reference cut points were very close: 0.66 pg/mL at 90% sensitivity and 0.69 pg/mL at 90% specificity, reflecting a steep transition between Aβ negative and positive participants. This narrow range reduces the effect of small measurement variations and suggests that either threshold could be used depending on whether sensitivity or specificity is prioritized.

Plasma pTau217 may also help stratify trial participants, potentially improving the detection of differential treatment responses. For example, a phase 2A trial of neflamapimod in DLB found that participants with abnormal plasma pTau181 levels did not respond to treatment, leading to the exclusion of biomarker-positive participants in the subsequent phase 2B trial(43,44). These findings suggest that amyloid-β pathology may influence therapeutic responses in NSD and highlight the importance of accounting for mixed pathologies in drug development for neurodegenerative diseases.

In clinical practice, the goal may be ruling-in or ruling-out Aβ positivity to guide prognosis and management. More stringent cut points at 95% sensitivity/specificity may be appropriate in this setting, helping clinicians provide better guidance to patients and families regarding prognosis and monitoring. This approach may also reduce the need for additional Aβ testing. Potential false negative results, particularly in early or atypical cases, could be addressed by repeating plasma pTau217 when necessary, given that blood collection is minimally invasive and readily accesible.

ARTICLE IN PRESS

Additionally, this study showed that different Aβ PET positivity thresholds influence cut point calculations in NSD. Previous studies suggest that CSF AD biomarker levels in LBD are lower than in AD(32,33), indicating the need for disease-specific cut-points(45). Unlike AD, where amyloid-β pathology burden is consistently high, people with NSD can show a broader spectrum of amyloid-β accumulation, emphasizing a need for tailored biomarker thresholds(46).

Several studies have analyzed plasma pTau217, pTau181, Aβ42/40, GFAP and NfL in clinically diagnosed LBD patients (10,17–21,25,28). Using a biomarker-based definition, our findings demonstrated that pTau217, pTau181, and Aβ42/40 differed between Aβ+ and Aβ− individuals, regardless of NSD status or symptom presence. This is important, as studies have suggested different CSF Aβ and tau biomarker performance in those with and without LBD(41).

We also found elevated plasma GFAP levels in Aβ+ individuals regardless of NSD status, while NfL levels were highest in NSD participants with Aβ positivity. Since NfL reflects neuroaxonal injury rather than pathology-specific processes, higher levels in individuals with both NSD and AD likely reflect the cumulative influence of multiple pathological processes on neuronal integrity(47). Consistent with this finding, the associations of both GFAP and NfL with NSD positivity and Aβ positivity suggest that these markers reflect broader

ARTICLE IN PRESS

neuroinflammatory or neurodegenerative processes rather than being specific to αSyn or amyloid-β pathology(48).

A key strength of this study is the use of a biologically defined discovery cohort of NSD and Aβ status to assess biomarker performance, combined with external validation of plasma pTau217 in two clinical LBD cohorts. By establishing cut points in a cohort defined using the most validated biomarker for underlying αSyn pathology, we provide thresholds that might be applicable in clinical practice and trials. This design bridges the gap between biomarker-based research frameworks and clinical settings where αSyn status is often unknown, supporting the broader use of our findings. This study also has limitations. The clinical validation cohorts were relatively small. Additionally, all cohorts consisted of primarily non-Hispanic white individuals, which may limit the generalizability of our findings. Finally, future studies should evaluate whether longitudinal changes in plasma pTau217 reflect amyloid accumulation or disease progression, and whether comorbidities impact plasma biomarker levels in NSD.

In summary, this study supports the use of plasma pTau217 as a screening tool for amyloid-β in NSD and in clinically-defined LBD, highlighting a critical mixed-pathology dimension within the biomarkers landscape. Our findings suggest that plasma pTau217 could be used for screening and stratification in NSD clinical trials. These results are important because they integrate clinical and biomarker data in a multi-etiology framework for the diagnosis of mixed neurodegenerative diseases of aging.

## Methods

### Participants

This cross-sectional study included participants from two cohorts: SU and SPIN(39), recruited between 2011 and 2023. All participants or their legally authorized representatives provided written informed consent for participation according to the Declaration of Helsinki. The Institutional Review Boards of each academic institution approved the study protocols: the Institutional Review Board of Stanford University granted approval of the study protocols IRB-33727 and IRB-37604, the Institutional Review Board of Hospital Sant Pau granted approval of the study protocol IIBSP-DOW-2014-30. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

All participants underwent comprehensive clinical evaluation involving medical history, physical and neurological examination, and neuropsychological assessment. Diagnoses were based on international consensus criteria, similar to our prior studies(10,49) and included: LBD without cognitive impairment (LBD-CU; i.e., PD diagnosis according to UK Brain Biobank criteria(50) without objective impairment on neuropsychological testing), LBD with cognitive impairment (LBD-CI; i.e., diagnosis of PD with mild cognitive impairment(51), prodromal DLB(52), PD dementia(53) or DLB(54)), AD(55), and cognitively unimpaired (CU) individuals (i.e., older adults without parkinsonian symptoms who performed within normal age- and sex-adjusted ranges on neuropsychological testing). In the SU cohort, global cognition was measured with the Montreal Cognitive Assessment (MoCA)(56) in 183 participants and the Mini-Mental State Examination (MMSE)(57) in 26 participants. MMSE scores were converted to MoCA scores according to age, sex, and education based on Monsell et al.(58) In the LBD-SPIN cohort, global cognition was measured with the MMSE. MoCA and MMSE scores were obtained within a 6-month window of plasma collection.

Participants were divided into one discovery and two clinical validation cohorts. The discovery cohort included 180 SU participants with CSF αSyn SAA and Aβ42/40 data where we evaluated diagnostic accuracy of plasma biomarkers for detecting amyloid-β in NSD+ and NSD- groups. The two clinical validation cohorts comprised

ARTICLE IN PRESS

ARTICLE IN PRESS

clinically-defined LBD-CU and LBD-CI participants (CSF αSyn SAA data were not available) where we validated diagnostic accuracies of plasma biomarker cut points defined in the SU discovery cohort (Supplementary Figure 3).

**Biomarkers**

All biomarker processing for plasma, CSF, and Aβ PET imaging was performed blinded to clinical information. In the SU discovery cohort, CSF samples were obtained within six months of plasma collection. Most plasma and CSF samples were collected concurrently, with a median interval of 0.14 weeks (approximately 1 day; IQR: 0-4.9 weeks). Aβ PET was performed within one year of plasma collection, with a median of 11.7 weeks (IQR: 4-23.3 weeks). CSF and Aβ PET assessments occurred both before and after plasma collection, indicating no systematic ordering of biomarker acquisition relative to plasma collection. Given the slow longitudinal change of plasma, CSF, and Aβ PET measures, these time differences are unlikely to meaningfully affect cross-sectional biomarker correlations. In the SPIN clinical validation cohort, CSF samples were obtained at time of plasma collection.

CSF Aβ42/40 was used to determine Aβ status in all participants from the SU discovery cohort and the LBD-SPIN clinical validation cohort. Aβ PET was used to determine Aβ status in the LBD-SU clinical validation cohort and in an independent SU cohort, which was used to derive centiloid-based thresholds to calibrate CSF cut points.

ARTICLE IN PRESS

**Plasma**

SU research participants consented to donate blood samples during each annual research visit. Ethylenediaminetetraacetic acid (EDTA) plasma was collected by venipuncture, centrifuged for 10 minutes at 4$^{o}$C at 2000 x *g*, aliquoted in polypropylene tubes, and stored at -80$^{o}$C until biomarker measurement.

Plasma pTau181, Aβ42, and Aβ40 concentrations were measured by the Stanford Alzheimer's Disease Research Center (ADRC) Biomarker Core using the Lumipulse *G*1200 instrument. Detailed protocols have been published previously(10,59,60). Inter-batch variability was calculated by retesting six independent plasma aliquots one year after the initial analysis using different reagents batches and assay lot numbers. Plasma pTau181 was quantified using a modified version of the Lumipulse *G* CSF pTau181 assay (231654; Fujirebio Diagnostics)(60). The inter-batch coefficient of variation (CV) was 3.38%. All plasma samples were within the quantifiable range (0.16 to 10.43 pg/ml). Plasma Aβ42 and Aβ40 were measured using Lumipulse *G* assays(59). The inter-batch CV for the Aβ42/40 ratio was 2.93%, and values ranged from 0.0549 to 0.2031.

Plasma pTau217, GFAP, and NfL concentrations were measured at the University of Gothenburg Department of Psychiatry and Neurochemistry using the Single molecule array (Simoa®) HD-X platform (Quanterix, Billerica, MA), as detailed elsewhere(12). Plasma pTau217 for SU and SPIN participants was measured with the commercial ALZpath pTau217 assay (Quanterix, REF: 104371, LOT: 999024), in two independent batches. The batch-specific measurement range was 0.0072 to 30.0 pg/mL for SU plasma samples, and 0.0073-30.0 pg/mL for SPIN plasma samples. Plasma GFAP and NfL were measured with the commercial Neurology 4-plex E kit (Quanterix, REF: 103520, LOT: 503812). One SU participant's GFAP levels fell below the assay's lower limit of quantification and was excluded from statistical analysis.

**CSF αSyn, Aβ42, and Aβ40**

CSF sample collection procedures were similar across SU and SPIN cohorts. The complete protocol for CSF collection and biomarker analysis (CSF αSyn, Aβ42, Aβ40) in SU participants has been published

ARTICLE IN PRESS

previously(10,61,62). CSF αSyn status in the SU discovery cohort was determined at Amprion's CLIA Laboratory via αSyn seed amplification assay (SYNTap® Biomarker Test)(61). CSF samples were classified as aSyn "detected" (NSD+) or "not detected" (NSD-) based on a preestablished threshold for median maximum fluorescence of the triplicate wells within 150 hours. The complete protocol is published elsewhere(62). SU CSF Aβ42 and Aβ40 were quantified by the Stanford ADRC Biomarker Core using the Lumipulse G 1200 instrument, as previously described(63). SPIN cohort CSF Aβ42 and Aβ40 were quantified using the Lumipulse G600II at the Sant Pau Memory Unit laboratory, as previously described(64). Aβ status in LBD-SPIN cohort was determined using a CSF Aβ42/40 cut point of <0.06220.

**Aβ PET**

We acquired $^{18}$F-florbetaben imaging on a PET/MRI scanner (Signa 3T, GE Healthcare) at the Stanford University Richard M. Lucas Center for Imaging. Emission data were acquired 90-110 minutes post-injection and processed as previously described(65). Global cortical SUVRs were calculated using the whole cerebellum as reference and converted to centiloids (CL)(66).

CSF Aβ status was determined using Aβ PET as the references standard in the SU discovery cohort. Aβ PET positivity was defined using a threshold of 36 centiloids (CL) to maximize specificity (Supplementary Figure 2). This threshold was derived from a subset of SU participants (n=72) excluded from discovery and validation cohorts using a Gaussian Mixture Model. In an exploratory analysis, we used a 24 CL threshold(12) to maximaze sensitivity (Supplementary Table 10).

Both 36 and 24 CL Aβ PET thresholds were used to establish CSF Aβ42/40 cut points calculated with the Youden Index in ROC curve analyses, where abnormal CSF Aβ42/40 was defined as < 0.09 for 36 CL (AUC = 0.98, 95% CI: 0.95-1) and < 0.11 for 24 CL (AUC = 0.94, 95% CI: 0.84-1). With these CSF Aβ42/40 cut points, we classified 180 SU participants as either CSF Aβ+ or CSF Aβ- for analysis.

***APOE* Genotyping**

For SU participants, *APOE* genotype was obtained from National Cell Repository for Alzheimer's Disease (NCRAD) using a Fluidigm fingerprint panel, or determined using PCR restriction fragment length polymorphism analysis within the Stanford ADRC(67). For SPIN participants, DNA was extracted from whole blood samples using the DNeasy® Blood & Tissue kit (Qiagen) at the Sant Pau Memory Unit laboratory. *APOE* genotyping was performed by direct DNA sequencing of exon 4 to identify the rs429358 and rs7412 polymorphisms that determine the *APOE* E2, E3, and E4 alleles.

**Statistical analysis**

Plasma pTau217, pTau181, GFAP, and NfL data were log10-transformed due to skewed distributions. We summarized continuous variables using the mean (standard deviation) for normally distributed data, median (minimum, maximum) for non-normally distributed data, and proportions for categorical variables.

To assess group differences in continuous variables, we used either one-way ANOVAs or the Kruskal-Wallis tests, depending on data distribution. We performed post-hoc pairwise comparisons using *t*-tests or Mann-Whitney tests as appropriate. For categorical variables, we used either Pearson's $\chi^2$ or Fisher's Exact tests depending on observed proportions. We corrected for multiple comparisons using the Holm method.

ARTICLE IN PRESS

ARTICLE IN PRESS

To quantify the differences in plasma biomarker levels between Aβ+ and Aβ- participants in the SU discovery cohort, we calculated median fold-change, standard deviation, and 95% confidence intervals. With this cohort, we conducted univariable and multivariable linear regression analyses to evaluate associations between plasma biomarker levels and age, sex, *APOE* ε4 carriership (i.e., ε4 homozygotes or heterozygotes), CSF Aβ, and NSD status separately and jointly.

To determine diagnostic accuracy of plasma biomarkers for detecting Aβ positivity in NSD+ and NSD-, we used Receiver Operating Characteristic (ROC) curves. First, we analyzed each biomarker separately, incorporating age, sex, and *APOE* ε4 carriership in each model. Next, we combined multiple biomarkers into a single model. Model performance was compared with the DeLong test, and the best-performing biomarker was selected based on standard metrics. For this biomarker, we determined one-reference cut point using the Youden Index, as well as two-reference cut points based on 90% and 95% sensitivity and specificity. Then, we calculated the predicted probabilities for Aβ posititivy in the discovery and clinical validation cohorts using logistic regression under the two-reference models, and stratified participants into low, intermediate and high-risk. Finally, we calculated sensitivity, specificity, negative and positive percent agreement, as well as overall agreement.

Statistical analyses were performed using R version 4.3.0 (R Foundation for Statistical Computing). Two-sided *p*-values $\leq 0.05$ were statistically significant and 95% confidence intervals were reported when applicable.

**Data availability**

The datasets generated and analyzed during the current study are not publicly available due to participant privacy and confidentiality constraints. However, anonymized data from the current study are available from the corresponding author on reasonable request, contingent upon completion of Stanford University's data use agreement approval.

**Code availability**

The underlying code for this study's statistical analysis is not publicly available but may be made available to qualified researchers upon reasonable request from the corresponding author.

ARTICLE IN PRESS

**References**

1. Simuni T, Chahine LM, Poston K, Brumm M, Buracchio T, Campbell M, et al. A biological definition of neuronal α-synuclein disease: towards an integrated staging system for research. Lancet Neurol. 2024;23(2):178–90. doi:10.1016/s1474-4422(23)00405-2

2. Quadalti C, Palmqvist S, Hall S, Rossi M, Mammana A, Janelidze S, et al. Clinical effects of Lewy body pathology in cognitively impaired individuals. Nat Med. 2023;29(8):1964–70. doi:10.1038/s41591-023-02449-7

3. Shahid M, Kim J, Leaver K, Hendershott T, Zhu D, Cholerton B, et al. An increased rate of longitudinal cognitive decline is observed in Parkinson's disease patients with low CSF Aß42 and an APOE ε4 allele. Neurobiol Dis. 2019;127:278–86. doi:10.1016/j.nbd.2019.02.023

4. Ryman SG, Yutsis M, Tian L, Henderson VW, Montine TJ, Salmon DP, et al. Cognition at Each Stage of Lewy Body Disease with Co-occurring Alzheimer's Disease Pathology. Dugger B, editor. J Alzheimer's Dis. 2021;80(3):1243–56. doi:10.3233/JAD-201187

ARTICLE IN PRESS

5. Abdelnour C, Ferreira D, Oppedal K, Cavallin L, Bousiges O, Wahlund LO, et al. The combined effect of amyloid-beta and tau biomarkers on brain atrophy in dementia with Lewy bodies. Neuroimage Clin. 2020;27:102333. doi:10.1016/j.nicl.2020.102333.

6. Abdelnour C, Ferreira D, van de Beek M, Cedres N, Oppedal K, Cavallin L, et al. Parsing heterogeneity within dementia with Lewy bodies using clustering of biological, clinical, and demographic data. Alzheimers Res Ther. 2022;14(1):14. doi:10.1186/s13195-021-00946-w.

7. Esteller-Gauxax D, Pérez Millan A, Sarto J, Puey R, Guillén Soley N, Tort-Merino A, et al. α-Synuclein Seed Amplifications Assay in a Cohort With Cognitive Impairment: Performance and Interactions With CSF and Plasma Biomarkers. Neurology. 2025;105(7):e214040. doi:10.1212/WNL.0000000000214040

8. Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain. 2011;134(5):1493–505. doi:10.1093/brain/awr031

9. Abdelnour C, Van Steenoven I, Londos E, Blanc F, Auestad B, Kramberger MG, et al. Alzheimer's disease cerebrospinal fluid biomarkers predict cognitive decline in lewy body dementia. Mov Disord. 2016;31(8):1203–8. doi:10.1002/mds.26668

10. Abdelnour C, Young CB, Shahid-Besanti M, Smith A, Wilson EN, Ramos Benitez J, et al. Plasma pTau181 Reveals a Pathological Signature that Predicts Cognitive Outcomes in Lewy Body Disease. Ann Neurol. 2024;96(3):526–38. doi:10.1002/ana.27003

11. Jellinger KA, Seppi K, Wenning GK, Poewe W. Impact of coexistent Alzheimer pathology on the natural history of Parkinson's disease. J Neural Transm. 2002;109(3):329–39. doi:10.1007/s007020200027

12. Ashton NJ, Brum WS, Di Molfetta G, Benedet AL, Arslan B, Jonaitis E, et al. Diagnostic Accuracy of a Plasma Phosphorylated Tau 217 Immunoassay for Alzheimer Disease Pathology. JAMA Neurol. 2024;81(3):255–63. doi:10.1001/jamaneurol.2023.5319

13. Brum WS, Cullen NC, Janelidze S, Ashton NJ, Zimmer ER, Therriault J, et al. A two-step workflow based on plasma p-tau217 to screen for amyloid beta positivity with further confirmatory testing only in uncertain cases. Nat Aging. 2023;3(9):1079–90. doi:10.1038/s43587-023-00471-5

14. Figdore DJ, Griswold M, Bornhorst JA, Graff-Radford J, Ramanan VK, Vemuri P, et al. Optimizing cutpoints for clinical interpretation of brain amyloid status using plasma p-tau217 immunoassays. Alzheimers Dement. 2024;20(9):6506–16. doi:10.1002/alz.14140

15. Yu L, Boyle PA, Janelidze S, Petyuk VA, Wang T, Bennett DA, et al. Plasma p-tau181 and p-tau217 in discriminating PART, AD and other key neuropathologies in older adults. Acta Neuropathol. 2023;146(1):1–11. doi:10.1007/s00401-023-02570-4

16. Schindler SE, Galasko D, Pereira AC, Rabinovici GD, Salloway S, Suarez-Calvet M, et al. Acceptable performance of blood biomarker tests of amyloid pathology - recommendations from the Global CEO Initiative on Alzheimer's Disease. Nat Rev Neurol. 2024;20(7):426–39. doi:10.1038/s41582-024-00977-5

17. Bolsewig K, Van Unnik AAJM, Blujdea ER, Gonzalez MC, Ashton NJ, Aarsland D, et al. Association of Plasma Amyloid, P-Tau, GFAP, and NfL With CSF, Clinical, and Cognitive Features in Patients With Dementia With Lewy Bodies. Neurology. 2024;102(12):e209418. doi:10.1212/WNL.0000000000209418

ARTICLE IN PRESS

ARTICLE IN PRESS

18. Gonzalez MC, Ashton NJ, Gomes BF, Tovar-Rios DA, Blanc F, Karikari TK, et al. Association of Plasma p-tau181 and p-tau231 Concentrations With Cognitive Decline in Patients With Probable Dementia With Lewy Bodies. JAMA Neurol. 2022;79(1):32. doi:10.1001/jamaneurol.2021.4222

19. Donaghy PC, Firbank M, Petrides G, Lloyd J, Barnett N, Olsen K, et al. The relationship between plasma biomarkers and amyloid PET in dementia with Lewy bodies. Parkinsonism Relat Disord. 2022;101:111–6. doi:10.1016/j.parkreldis.2022.07.008

20. Vrillon A, Bousiges O, Gotze K, Demuynck C, Muller C, Ravier A, et al. Plasma biomarkers of amyloid, tau, axonal, and neuroinflammation pathologies in dementia with Lewy bodies. Alzheimers Res Ther. 2024;16(1):146. doi:10.1186/s13195-024-01502-y.

21. Hall S, Janelidze S, Londos E, Leuzy A, Stomrud E, Dage JL, et al. Plasma Phospho-Tau Identifies Alzheimer's Co-Pathology in Patients with Lewy Body Disease. Mov Disord. 2021;36(3):767–71. doi:10.1002/mds.28370

22. Batzu L, Rota S, Hye A, Heslegrave A, Trivedi D, Gibson LL, et al. Plasma p-tau181, neurofilament light chain and association with cognition in Parkinson's disease. NPJ Park Dis. 2022;8(1):154. doi:10.1038/s41531-022-00384-x

23. Pagonabarraga J, Perez-Gonzalez R, Bejr-Kasem H, Marin-Lahoz J, Horta-Barba A, Martinez-Horta S, et al. Dissociable contribution of plasma NfL and p-tau181 to cognitive impairment in Parkinson's disease. Park Relat Disord. 2022;105:132–8. doi:10.1016/j.parkreldis.2022.05.020

24. Thomas AJ, Hamilton CA, Heslegrave A, Barker S, Durcan R, Lawley S, et al. A Longitudinal Study of Plasma pTau181 in Mild Cognitive Impairment with Lewy Bodies and Alzheimer's Disease. Mov Disord. 2022;37(7):1495–504. doi:10.1002/mds.28994

25. Chouliaras L, Thomas A, Malpetti M, Donaghy P, Kane J, Mak E, et al. Differential levels of plasma biomarkers of neurodegeneration in Lewy body dementia, Alzheimer's disease, frontotemporal dementia and progressive supranuclear palsy. J Neurol Neurosurg Psychiatry. 2022;93(6):651–8. doi:10.1136/jnnp-2021-327788

26. Baiardi S, Quadalti C, Mammana A, Dellavalle S, Zenesini C, Sambati L, et al. Diagnostic value of plasma p-tau181, NfL, and GFAP in a clinical setting cohort of prevalent neurodegenerative dementias. Alzheimers Res Ther. 2022;14(1):153. doi:10.1186/s13195-022-01093-6

27. Alcolea D, Delaby C, Muñoz L, Torres S, Estellés T, Zhu N, et al. Use of plasma biomarkers for AT(N) classification of neurodegenerative dementias. J Neurol Neurosurg Psychiatry. 2021;92(11):1206–14. doi:10.1136/jnnp-2021-326603

28. Rousset RZ, Claessen T, Van Harten AC, Lemstra AW, Pijnenburg YAL, Van Der Flier WM, et al. Performance of plasma p-tau217 and NfL in an unselected memory clinic setting. Alzheimers Dement Diagn Assess Dis Monit. 2024;16(4):e70003. doi:10.1002/dad2.70003

29. Salvadó G, Ossenkoppele R, Ashton NJ, Beach TG, Serrano GE, Reiman EM, et al. Specific associations between plasma biomarkers and postmortem amyloid plaque and tau tangle loads. EMBO Mol Med. 2023;15(5):e17123. doi:10.15252/emmm.202217123

30. Tang Y, Han L, Li S, Hu T, Xu Z, Fan Y, et al. Plasma GFAP in Parkinson's disease with cognitive impairment and its potential to predict conversion to dementia. NPJ Park Dis. 2023;9(1):23. doi:10.1038/s41531-023-00447-7

ARTICLE IN PRESS

ARTICLE IN PRESS


31. Cousins KAQ, Irwin DJ, Chen-Plotkin A, Shaw LM, Arezoumandan S, Lee EB, et al. Plasma GFAP associates with secondary Alzheimer's pathology in Lewy body disease. Ann Clin Transl Neurol. 2023;10(5):802–13. doi:10.1002/acn3.51768

32. Cousins KAQ, Irwin DJ, Tropea TF, Rhodes E, Phillips J, Chen-Plotkin AS, et al. Evaluation of $ATN_{PD}$ Framework and Biofluid Markers to Predict Cognitive Decline in Early Parkinson Disease. Neurology. 2024;102(4):e208033. doi:10.1212/WNL.0000000000208033

33. Irwin DJ, Xie SX, Coughlin D, Nevler N, Akhtar RS, McMillan CT, et al. CSF tau and beta-amyloid predict cerebral synucleinopathy in autopsied Lewy body disorders. Neurology. 2018;90(12):e1038–46. doi:10.1212/WNL.0000000000005166

34. Jain L, Khrestian M, Formica S, Tuason ED, Pillai JA, Rao S, et al. ATN cerebrospinal fluid biomarkers in dementia with Lewy bodies: Initial results from the United States Dementia with Lewy Bodies Consortium. Alzheimers Dement. 2024;20(1):549–62. doi:10.1002/alz.13398

35. Samudra N, Fischer DL, Lenio S, Lario Lago A, Ljubenkov PA, Rojas JC, et al. Clinicopathological correlation of cerebrospinal fluid alpha-synuclein seed amplification assay in a behavioral neurology autopsy cohort. Alzheimers Dement. 2024;20(5):3334–41. doi:10.1002/alz.13799

36. Coughlin DG, MacLeod KR, Middleton JS, Bozoki AC, Galvin JE, Irwin DJ, et al. Association of CSF alpha-Synuclein Seeding Amplification Assay Results With Clinical Features of Possible and Probable Dementia With Lewy Bodies. Neurology. 2024;103(3):e209656. doi:10.1212/WNL.0000000000209656

37. Skogseth R, Hortobagyi T, Soennesyn H, Chwiszczuk L, Ffytche D, Rongve A, et al. Accuracy of Clinical Diagnosis of Dementia with Lewy Bodies versus Neuropathology. J Alzheimers Dis. 2017;59(4):1139–52. doi:10.3233/JAD-170274

38. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource [Internet]. Silver Spring (MD): Food and Drug Administration (US); 2016 [cited 2025 Jun 2]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK326791/

39. Alcolea D, Clarimón J, Carmona-Iragui M, Illán-Gala I, Morenas-Rodríguez E, Barroeta I, et al. The Sant Pau Initiative on Neurodegeneration (SPIN) cohort: A data set for biomarker discovery and validation in neurodegenerative disorders. Alzheimers Dement Transl Res Clin Interv. 2019;5(1):1. doi:10.1016/j.trci.2019.09.005

40. Hansson O, Edelmayer RM, Boxer AL, Carrillo MC, Mielke MM, Rabinovici GD, et al. The Alzheimer's Association appropriate use recommendations for blood biomarkers in Alzheimer's disease. Alzheimers Dement. 2022;18(12):2269-8686.

41. Gibson LL, Abdelnour C, Chong J, Ballard C, Aarsland D. Clinical trials in dementia with Lewy bodies: the evolving concept of co-pathologies, patient selection and biomarkers. Curr Opin Neurol. 2023;36(4):264–75. doi:10.1097/WCO.0000000000001173

42. Abdelnour C, Gibson LL, Batzu L, Aarsland D. How to advance the pharmacological management of cognitive impairment in Parkinson's disease. J Park Dis. 2025;0(0). doi:10.1177/1877718x251315645

43. Prins ND, de Haan W, Gardner A, Blackburn K, Chu HM, Galvin JE, et al. Phase 2A Learnings Incorporated into RewinD-LB, a Phase 2B Clinical Trial of Neflamapimod in Dementia with Lewy Bodies. J Prev Alzheimers Dis. 2024;11(3):549–57. doi:10.14283/jpad.2024.36


ARTICLE IN PRESS

ARTICLE IN PRESS

44. Alam JJ, Maruff P, Doctrow S, Chu HM, Conway J, Gomperts SN, et al. Association of Plasma Phosphorylated Tau With the Response to Neflamapimod Treatment in Patients With Dementia With Lewy Bodies. Neurology. 2023;101(17):e1708–17. doi:10.1212/WNL.0000000000207755

45. Weinshel S ID. Appropriateness of Applying Cerebrospinal Fluid Biomarker Cutoffs from Alzheimer's Disease to Parkinson's Disease. J Park Dis. 2022;12:1155–67.

46. Montoliu-Gaya L, Valeriano-Lorenzo E, Ashton NJ, López-González FJ, Lantero-Rodriguez J, Ruiz-González A, et al. Plasma tau biomarkers are distinctly associated with tau tangles and decreased with Lewy body pathology. Alzheimers Dement. 2025;21(8):e70562. doi:10.1002/alz.70562

47. Delaby C, Alcolea D, Carmona-Iragui M, Illán-Gala I, Morenas-Rodríguez E, Barroeta I, et al. Differential levels of Neurofilament Light protein in cerebrospinal fluid in patients with a wide range of neurodegenerative disorders. Sci Rep. 2020;10(1):9161. doi:10.1038/s41598-020-66090-x

48. Teunissen CE, Verberk IMW, Thijssen EH, Vermunt L, Hansson O, Zetterberg H, et al. Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. Lancet Neurol. 2022;21(1):66–77. doi:10.1016/s1474-4422(21)00361-6

49. Shahid M, Rawls A, Ramirez V, Ryman S, Santini VE, Yang L, et al. Illusory Responses across the Lewy Body Disease Spectrum. Ann Neurol. 2023;93(4):702–14. doi:10.1002/ana.26574

50. Gibb WR LA. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry. 1988;51:745–52. doi:10.1136/jnnp.51.6.745

51. Litvan I, Goldman JG, Troster AI, Schmand BA, Weintraub D, Petersen RC, et al. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. Mov Disord. 2012;27(3):349–56. doi:10.1002/mds.24893

52. McKeith IG, Ferman TJ, Thomas AJ, Blanc F, Boeve BF, Fujishiro H, et al. Research criteria for the diagnosis of prodromal dementia with Lewy bodies. Neurology. 2020;94(17):743–55. doi:10.1212/WNL.0000000000009323 PubMed Central PMCID: PMC7274845.

53. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord. 2007;22(12):1689–707. doi:10.1002/mds.21507

54. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and managament of dementia with Lewy bodies. Neurology. 2017;89(1):88–100. doi:10.1212/WNL.0000000000004058

55. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263–9. doi:10.1016/j.jalz.2011.03.005

56. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment. J Am Geriatr Soc. 2005;53(4):695–9. doi:10.1111/j.1532-5415.2005.53221.x

57. Folstein MF, Folstein SE, McHugh PR. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189–98. doi:10.1016/0022-3956(75)90026-6

ARTICLE IN PRESS

ARTICLE IN PRESS

58. Monsell SE, Dodge HH, Zhou XH, Bu Y, Besser LM, Mock C, et al. Results From the NACC Uniform Data Set Neuropsychological Battery Crosswalk Study. Alzheimer Assoc Disord. 2016;30(2):134–9. doi:10.1097/WAD.0000000000000111

59. Trelle AN, Young CB, Vossler H, Ramos Benitez J, Cody KA, Mendiola JH, et al. Plasma Abeta(42)/Abeta(40) is sensitive to early cerebral amyloid accumulation and predicts risk of cognitive decline across the Alzheimer's disease spectrum. Alzheimers Dement. 2025;21(2):e14442. doi:10.1002/alz.14442

60. Wilson EN, Young CB, Ramos Benitez J, Swarovski MS, Feinstein I, Vandijck M, et al. Performance of a fully-automated Lumipulse plasma phospho-tau181 assay for Alzheimer's disease. Alzheimers Res Ther. 2022;14(1):172. doi:10.1186/s13195-022-01116-2

61. Middleton JS, Hovren HL, Kha N, Medina MJ, MacLeod KR, Concha-Marambio L, et al. Seed amplification assay results illustrate discrepancy in Parkinson's disease clinical diagnostic accuracy and error rates. J Neurol. 2023;270(12):5813–8. doi:10.1007/s00415-023-11810-2

62. Plastini MJ, Abdelnour C, Young CB, Wilson EN, Shahid-Besanti M, Lamoureux J, et al. Multiple biomarkers improve diagnostic accuracy across Lewy body and Alzheimer's disease spectra. Ann Clin Transl Neurol. 2024; 11(5):1197-1210. doi:10.1002/acn3.52034

63. Wilson EN, Swarovski MS, Linortner P, Shahid M, Zuckerman AJ, Wang Q, et al. Soluble TREM2 is elevated in Parkinson's disease subgroups with increased CSF tau. Brain. 2020;143(3):932–43. doi:10.1093/brain/awaa021

64. Alcolea D, Pegueroles J, Muñoz L, Camacho V, López-Mora D, Fernández-León A, et al. Agreement of amyloid PET and CSF biomarkers for Alzheimer's disease on Lumipulse. Ann Clin Transl Neurol. 2019;6(9):1815–24. doi:10.1002/acn3.50873

65. Winer JR, Vossler H, Young CB, Smith V, Romero A, Shahid-Besanti M, et al. 18F-PI-2620 Tau PET is associated with cognitive and motor impairment in Lewy body disease. Brain Commun. 2024;7(1):fcae458. doi:10.1093/braincomms/fcae458

66. Royse SK, Minhas DS, Lopresti BJ, Murphy A, Ward T, Koeppe RA, et al. Validation of amyloid PET positivity thresholds in centiloids: a multisite PET study approach. Alzheimers Res Ther. 2021;13(1):99. doi:10.1186/s13195-021-00836-1

67. Kerchner G, Berdnik D, Shen J, Bernstein J, Fenesy M, Deutsch G, et al. APOE ε4 worsens hippocampal CA1 apical neuropil atrophy and episodic memory. Neurology. 2014;82(8):691–7. doi:10.1212/WNL.0000000000000154

**Acknowledgements**

ENW received support from the Knight Inititiative for Brain Resilience. DA received support from the Institute of Health Carlos III (ISCIII) grants PI18/00435, PI22/00611, INT19/00016, INT23/00048 jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, "Una manera de hacer Europa". VWH received suppor from NCRAD grant P50 AG047366, and NACC grant P30 AG066515.

ECM received support from NIH grant R21AG058859. HZ is a Wallenberg Scholar and a Distinguished Professor at the Swedish Research Council supported by grants from the Swedish Research Council (#2023-00356, #2022-01018 and #2019-02397), the European Union's Horizon Europe research and innovation

ARTICLE IN PRESS

programme under grant agreement No 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C, and #ADSF-24-1284328-C), the European Partnership on Metrology, co-financed from the European Union's Horizon Europe Research and Innovation Programme and by the Participating States (NEuroBioStand, #22HLT07), the Bluefield Project, Cure Alzheimer's Fund, the Olav Thon Foundation, the Erling-Persson Family Foundation, Familjen Rönströms Stiftelse, Familjen Beiglers Stiftelse, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre, the UK Dementia Research Institute at UCL (UKDRI-1003), and an anonymous donor. KLP received support from NIH grants K23 NS075097, R01NS115114. CA received support from the Susan and Charles Berghoff Foundation and the ARISTOS program that has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101081334.

These funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Author contributions**

CA and AMS had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: CA, KLP.
Acquisition, analysis or interpretation of data: CA, AMS, SAL, KLP, BA, LMG, NJA, ENW, DA, IRB, CBY, JRW, MSB, HV, MJP, TP, EVC, IS, JHM, KIA, VWH, TJM, LT, ECM, HZ.
Drafting of the manuscript: CA, AMS, SAL, KLP.
Critical review of the manuscript for important intellectual content: CA, KLP, AMS, SAL, BA, LMG, NJA, ENW, DA, IRB, JF, CBY, JRW, MSB, HV, MJP, TP, EVC, IS, JHM, VR, GAK, KIA, VWH, TJM, LT, ECM, HZ.
Statistical analysis: CA, AMS, SAL.
Obtained funding: CA, KLP.
Administrative, technical, or material support: CA, KLP, AMS, SAL, BA, LMG, NJA, ENW, DA, IRB, JF, HV, TP, JHM, VR, GAK, VWH, TJM, LT, ECM, HZ.
Supervision: CA, KLP.

**Competing interests**

These authors declare competing interests: LMG has received speaker fees from Quanterix and Esteve, and served as consultant for Quanterix. MJP is currently a full-time employee at Amprion Inc. DA has participated in advisory boards for Fujirebio-Europe, Roche Diagnostics, Grifols S.A. and Lilly, and received speaker honoraria from Fujirebio-Europe, Roche Diagnostics, Nutricia, Krka Farmacéutica S.L., Zambon S.A.U., Neuraxpharm, Alter Medica, Lilly and Esteve Pharmaceuticals S.A. DA holds a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx NeuroSciences N.V., WO2019175379 Markers of synaptopathy in neurodegenerative diseases). JF has served on advisory boards, adjudication committees, or speaker honoraria from AC Immune, Adamed, Alzheon, Biogen, Eisai, Esteve, Fujirebio, Ionis, Laboratorios Carnot, Life

ARTICLE IN PRESS

ARTICLE IN PRESS

Molecular Imaging, Lilly, Lundbeck, Novo Nordisk, Perha, Roche, Zambón, Spanish Neurological Society, T21 Research Society, Lumind foundation, Jérôme-Lejeune Foundation, Alzheimer's Association, National Institutes of Health USA, and Instituto de Salud Carlos III. JF also holds a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx NeuroSciences N.V., WO2019175379 Markers of synaptopathy in neurodegenerative diseases). HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZpath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Enigma, LabCorp, Merck Sharp & Dohme, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Quanterix, Red Abbey Labs, reMYND, Roche, Samumed, ScandiBio Therapeutics AB, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures sponsored by Alzecure, BioArctic, Biogen, Cellectricon, Fujirebio, LabCorp, Lilly, Novo Nordisk, Oy Medix Biochemica AB, Roche, and WebMD, is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, and is a shareholder of MicThera (outside submitted work). KLP has been on the Scientific Advisory Board for Amprion and has been a consultant for Novartis, Lilly, BioArctic, Biohaven, Curasen and Neuron23. CA has received honoraria as a speaker from Hoffman-La Roche LTD, Nutricia, Schwabe Farma Ibérica SAU and Zambon; and is member of the Board of Directors of the Lewy Body Dementia Association, the Scientific Committee of Lewy Body España, and the Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART) Advisory Council.

All other authors (AMS, SAL, BA, NJA, ENW, IRB, CBY, JRW, MSB, HV, TP, EVC, IS, JHM, VR, GAK, KIA, VWH, TJM, LT, and ECM) declare no financial or non-financial competing interests. No authors have pending patent applications related to the work described in this manuscript.

**Additional information**

The authors would like to express their gratitude to the research participants. We would like to thank the Stanford ADRC Clinical and Imaging Core, and specifically Dr. Michael Zeineh, Dr. Greg Zaharchuk, and Dr. Guido Davidzon for the amyloid-β PET scan reviews.

**Correspondence** and requests for material should be addressed to Carla Abdelnour.

**Figure Legends**

ARTICLE IN PRESS

**Figure 1. Plasma biomarker levels (pg/mL) across NSD/Aβ groups.** Panel 1A-1E: Bars with asterisks represent significant differences between log-transformed plasma biomarker levels. Plasma pTau217, pTau181, Aβ42/40, and GFAP levels were abnormal in Aβ+ groups, regardless of NSD status. Plasma NfL levels were higher in the NSD+/Aβ+ group compared to the other groups; and in the NSD-/Aβ+ group compared to the NSD/Aβ- group. Plasma pTau217 showed the largest median fold-change regardless of NSD status, with the highest median concentration observed in the NSD+ group (2.79 [SD: 0.33], 95% CI = 2.30-3.61; see Supplementary Table 1).

**Figure 2. ROC models for detecting amyloid-β using individual plasma biomarkers in NSD+ and NSD- participants.** Figure 2 compares diagnostic accuracies of individual plasma biomarker receiver operating characteristic (ROC) models for detecting amyloid-β. **Panels 2A** and **2C** show ROC area under the curve (AUC) results for NSD+ participants whereas **panels 2B** and **2D** show ROC AUC results for NSD- participants. **Panels 2A** and **2B** show models using a CSF Aβ42/40 cut point calculated based on Aβ PET $\geq$ 36 centiloids (CL). **Panels 2C and 2D** show models using a CSF Aβ42/40 cut point calculated based on Aβ PET $\geq$ 24 CL. Plasma pTau217 outperforms other biomarkers for the detection of amyloid-β in NSD+ participants. Individual

ARTICLE IN PRESS

biomarker models performed similarly when detecting amyloid-β in NSD- participants, with no biomarker significantly outperforming the other.

**Figure 3. Validation of plasma pTau217 in clinical LBD cohorts. Panel 3A** shows the predicted probability of amyloid-β positivity based on a logistic regression model including z-transformed plasma pTau217 levels across cohorts. Each dot represents an individual participant, with blue dots indicating amyloid-β negative and red dots indicating amyloid-β positive. Predicted probabilities are shown for the NSD+ SU discovery cohort (left). Dashed lines indicate the probability thresholds corresponding to 90% (thick line) and 95% (thin line) sensitivity or specificity, demonstrating low- and high-risk cut points on the probability distribution, applying a similar approach as Brum et al. (doi: 10.1038/s43587-023-00471-5). **Panel 3B** shows a workflow of amyloid-β detection with plasma pTau217 in the clinical validation cohorts. On the left, the flowchart presents the categorization of patients based on their plasma pTau217 levels: positive, intermediate, or negative results. The right panel presents the workflow using different reference cut points. The top section presents the number and percentage of patients that will require confirmatory testing (with PET or CSF), while the bottom section presents the number and percentage of patients that will not require additional testing. The color-coded boxes indicate a potential context of use (COU) for the different reference cut points: clinical trial (green) and clinical practice (yellow), assuming in clinical trials would bias towards more confirmatory testing for those suspected of being amyloid-β positive. **Panel 3C** shows sample size estimates for hypothetical clinical trials, which are calculated using the NSD+ SU discovery cohort. In Scenario 1 the goal was to find 100 NSD who were amyloid-β positive, versus Scenario 2 where the goal was to find 100 NSD who were amyloid-β negative. The number of plasma pTau217 positives (or negatives) needed was calculated as 100 divided by the PPV (or NPV). The number to screen was calculated as plasma pTau217 positives (or negatives) needed divided by the percentage of plasma pTau217 positivity (or negativity.

**Table 1. Characteristics of the Stanford University discovery cohort**

| | NSD-/Aβ- (n=69) | NSD-/Aβ+ (n=44) | NSD+/Aβ- (n=39) | NSD+/Aβ+ (n=28) | Total Cohort (n=180) | *p* value* |
|---|---|---|---|---|---|---|
| **Age, years** median (min, max) | 66 (51, 87) | 71 (54, 86) | 69 (50, 82) | 70 (57, 83) | 69 (50, 87) | 0.031 |
| **Sex, male** No. (%) | 38 (55.1) | 20 (45.5) | 22 (56.4) | 17 (60.7) | 97 (53.9) | 0.589 |
| **Years of education** median (min, max) | 16 (5, 20) | 16 (12, 20) | 17.0 (12, 20) | 16.0 (12, 20) | 16.0 (5, 20) | 0.935 |
| **APOE ε4 carrier** No. (%) | 14 (20.3)[b,d] | 29 (65.9)[a,d] | 13 (33.3) | 16 (57.1)[a,b] | 72 (40.0) | < 0.001 |
| **Clinical diagnosis**, No. (%) | | | | | | |
| Cognitively Unimpaired | 56 (81.2)[b,c,d] | 20 (45.5)[a,c,d] | 5 (12.8)[a,b,d] | 5 (17.9)[a,b,c] | 86 (47.8) | < 0.001 |
| AD | 8 (11.6) | 22 (50.0) | 0 (0.0) | 4 (14.3) | 34 (18.9) | |
| LBD Cognitively Impaired | 2 (2.9) | 1 (2.3) | 10 (25.6) | 17 (60.7) | 30 (16.7) | |
| LBD Cognitively Unimpaired | 3 (4.3) | 1 (2.3) | 24 (61.5) | 2 (7.1) | 30 (16.7) | |
| **Clinical status**, No. (%) | | | | | | |
| Asymptomatic | 56 (81.2)[b,c,d] | 20 (45.5)[a,c,d] | 5 (12.8)[a,b] | 5 (17.9)[a,b] | 86 (47.8) | < 0.001 |
| Symptomatic | 13 (18.8) | 24 (54.5) | 34 (87.2) | 23 (82.1) | 94 (52.2) | |
| **MoCA score** median (min, max) | 27 (6, 30)[b,d] | 25 (1, 30)[a] | 26 (8, 30)[d] | 22 (3, 30)[a,c] | 26 (1, 30) | < 0.001 |
| **CSF Aβ42/40** median (min, max) | 0.13 (0.09, 0.15)[b,d] | 0.06 (0.04, 0.09)[a,c] | 0.12 (0.09, 0.15)[b,d] | 0.07 (0.04, 0.09)[a,c] | 0.11 (0.04, 0.15) | < 0.001 |

ARTICLE IN PRESS

| | | | | | | |
|---|---|---|---|---|---|---|
| **Plasma pTau217, pg/mL** median (min, max) | 0.34 (0.02, 1.88)[b,d] | 0.84 (0.31, 3.87)[a,c] | 0.35 (0.06, 2.41)[b,d] | 0.96 (0.30, 3.29)[a,c] | 0.47 (0.02, 3.87) | < 0.001 |
| **Plasma pTau181, pg/mL** median (min, max) | 1.43 (0.83, 3.52)[b,d] | 1.99 (1.26, 5.41)[a,c] | 1.47 (0.93, 4.39)[b,d] | 1.92 (1.24, 10.43)[a,c] | 1.60 (0.83, 10.43) | < 0.001 |
| **Plasma GFAP, pg/mL** mean (SD) | 128.45 (64.56)[b,d] | 207.13 (92.14)[a,c] | 140.89 (57.06)[b,d] | 247.07 (121.46)[a,c] | 168.84 (93.09) | < 0.001 |
| **Plasma NfL, pg/mL** mean (SD) | 20.85 (9.51)[b,d] | 25.01 (9.12)[a,d] | 23.67 (9.03)[d] | 31.87 (12.85)[a,b,c] | 24.18 (10.51) | 0.001 |
| **Plasma Aβ42/40** mean (SD) | 0.10 (0.01)[b,d] | 0.08 (0.01)[a,c] | 0.10 (0.01)[b,d] | 0.08 (0.01)[a,c] | 0.09 (0.01) | <0.001 |

Note. NSD status was determined by CSF αSyn SAA (not detected/detected); Aβ status was determined by CSF Aβ42/40 (normal/abnormal). Clinical status = presence (i.e., symptomatic) or absence (i.e., asymptomatic) of cognitive and motor symptoms; *$p$ values for descriptive comparisons are uncorrected; [a]$p<0.05$ compared with NSD-/Aβ-; [b]$p<0.05$ compared with NSD-/Aβ+; [c]$p<0.05$ compared with NSD+/Aβ-; [d]$p<0.05$ compared with NSD+/Aβ+. Abbreviations: NSD, Neuronal Synuclein Disease; Aβ, amyloid-β; AD, Alzheimer's disease; LBD, Lewy body disease; MoCA, Montreal Cognitive Assessment; CSF, cerebrospinal fluid; pTau217, phosphorylated tau 217; pTau181, phosphorylated tau 181; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain. Missing data: APOE ε4 carriership: 15 (8.3%); MoCA: 2 (1.1%); Plasma pTau217: 3 (1.7%); Plasma pTau181: 7 (3.9%); Plasma GFAP and NfL: 1 (0.6%); Plasma Aβ42/40: 32 (17.8%).

**Table 2. Plasma pTau217 one- and two-reference cut points for amyloid-β positivity in discovery and clinical validation cohorts**

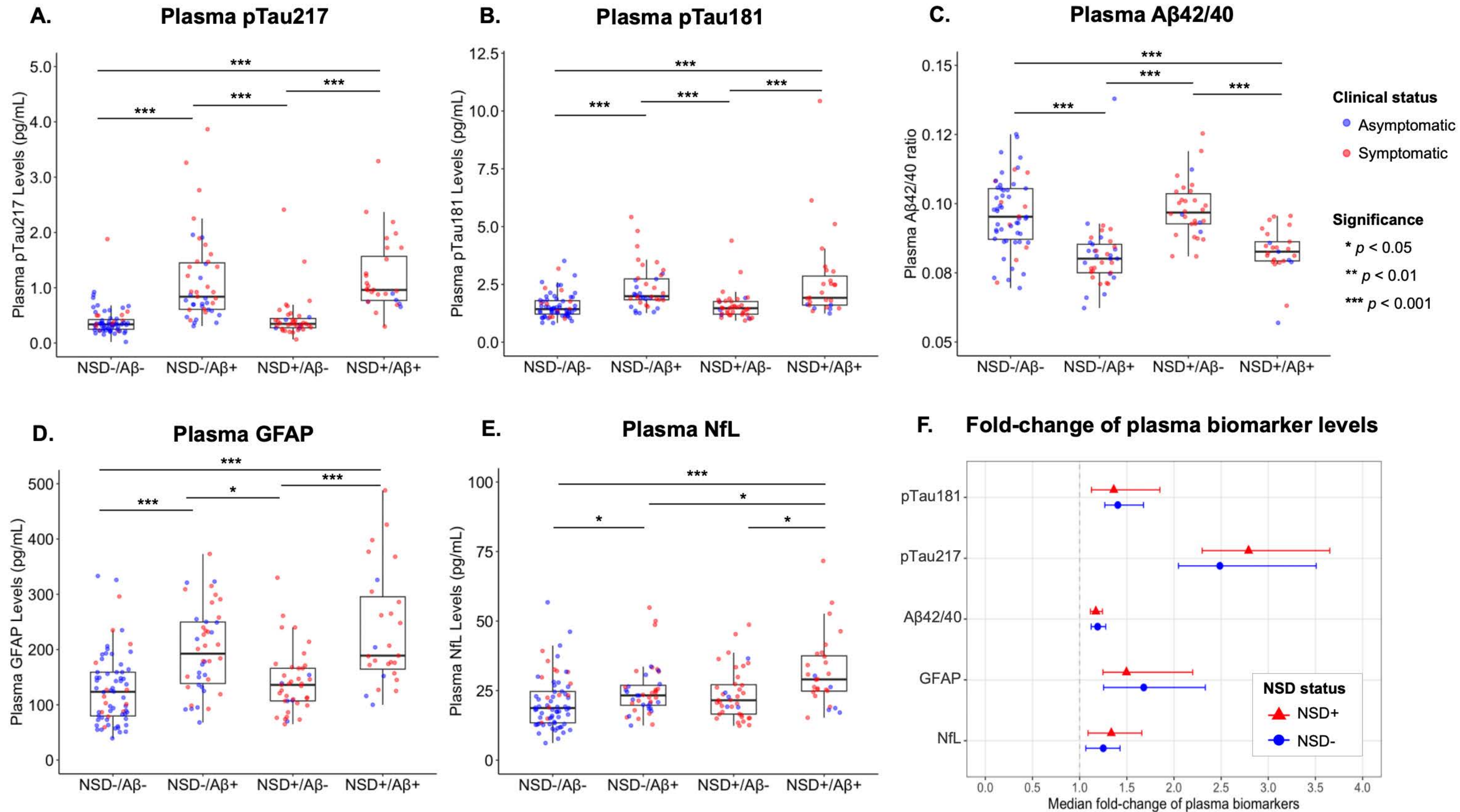
| | **One cut point (Youden Index)** | | | | **Two cut points 90% Se/Sp** | | | **Two cut points 95% Se/Sp** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pTau217 positive > 0.54 pg/mL | | | | pTau217 positive >0.69 pg/mL<br>pTau217 negative <0.66 pg/mL | | | pTau217 positive >1.04 pg/mL<br>pTau217 negative <0.47 pg/mL) | | |
| **Cohort** | **SU Discovery (NSD+)** | **LBD-SU** | **LBD-SPIN** | **Cohort** | **SU Discovery (NSD+)** | **LBD-SU** | **LBD-SPIN** | **SU Discovery (NSD+)** | **LBD-SU** | **LBD-SPIN** |
| **Plasma Aβ positive**, No. (%) | 33 (49.3) | 11 (37.9) | 32 (72.7) | **Plasma Aβ positive**, No. (%) | 29 (43.4) | 6 (20.7) | 22 (50.0) | 13 (19.4) | 2 (6.9) | 8 (18.2) |
| | | | | **Plasma Aβ intermediate**, No. (%) | 1 (1.5) | 1 (3.4) | 3 (6.8) | 23 (34.3) | 11 (37.9) | 25 (56.8) |
| **Plasma Aβ negative**, No. (%) | 34 (50.7) | 18 (62.1) | 12 (27.3) | **Plasma Aβ negative**, No. (%) | 37 (55.2) | 22 (75.9) | 19 (43.2) | 31 (46.3) | 16 (55.2) | 11 (25.0) |
| **Sensitivity**, % | 96.4 | 88.9 | 95.7 | **Sensitivity, lower cut point**, % | 89.3 | 75.0 | 90.5 | 91.7 | 66.7 | 88.9 |
| **Specificity**, % | 84.6 | 85.0 | 52.4 | **Specificity, upper cut point**, % | 89.5 | 100.0 | 85.0 | 93.8 | 100.0 | 100.0 |

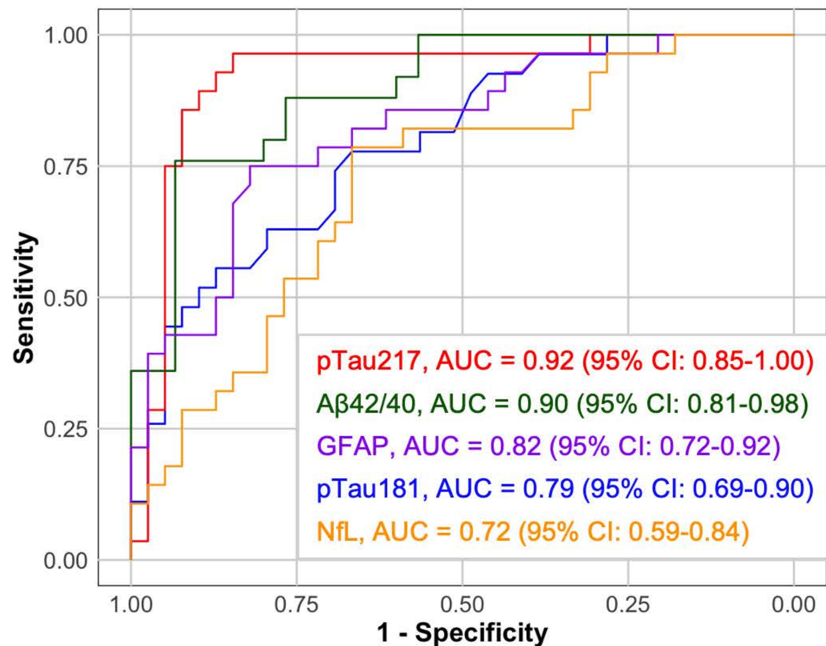
ARTICLE IN PRESS

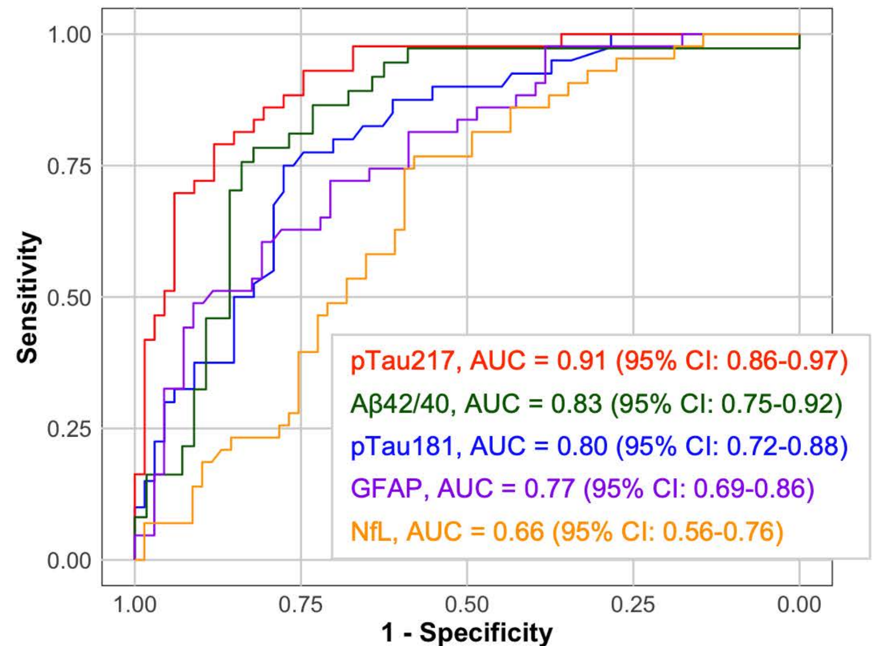
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PPA**, % | 81.8 | 72.7 | 68.8 | **PPA, upper cut point**, % | 86.2 | 100.0 | 86.4 | 84.6 | 100.0 | 100.0 |
| **NPA**, % | 97.1 | 94.4 | 91.7 | **NPA, lower cut point**, % | 91.9 | 90.9 | 89.5 | 96.8 | 93.8 | 90.9 |
| **OPA**, % | 89.6 | 86.2 | 75.0 | **OPA for plasma pTau217 positive and negative**, % | 89.4 | 92.9 | 87.8 | 93.2 | 94.4 | 94.7 |

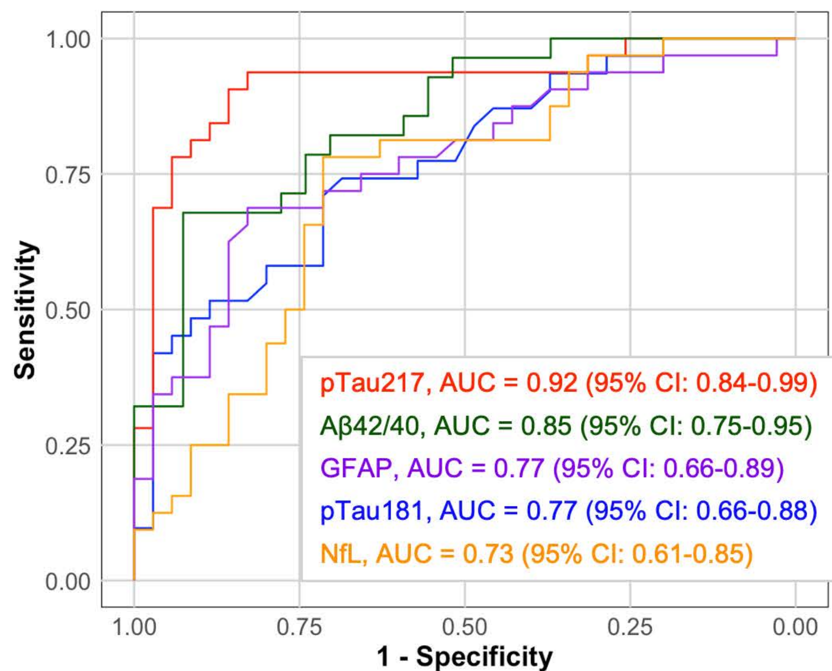
Note. The one-reference cut point for plasma pTau217 was calculated using the Youden Index. Two-reference cut points were calculated at 90% sensitivity/90% specificity, and at 95% sensitivity/95% specificity. Cut points were established in the biologically defined NSD+ SU Discovery cohort and applied to the clinically defined LBD-SU and LBD-SPIN cohorts. Amyloid-β positivity was determined by CSF Aβ42/40 status (normal/abnormal) using a 36 centiloid Aβ PET reference threshold. Abbreviations: Se, sensitivity; Sp, specificity; Aβ, amyloid-β; SU, Stanford University; LBD, Lewy body disease; SPIN, Sant Pau Initiative on Neurodegeneration; phosphorylated tau 217; PPA, positive percentage agreement; NPA, negative percentage agreement; OPA, overall percentage agreement; NSD+, individuals with Neuronal Synuclein Disease.

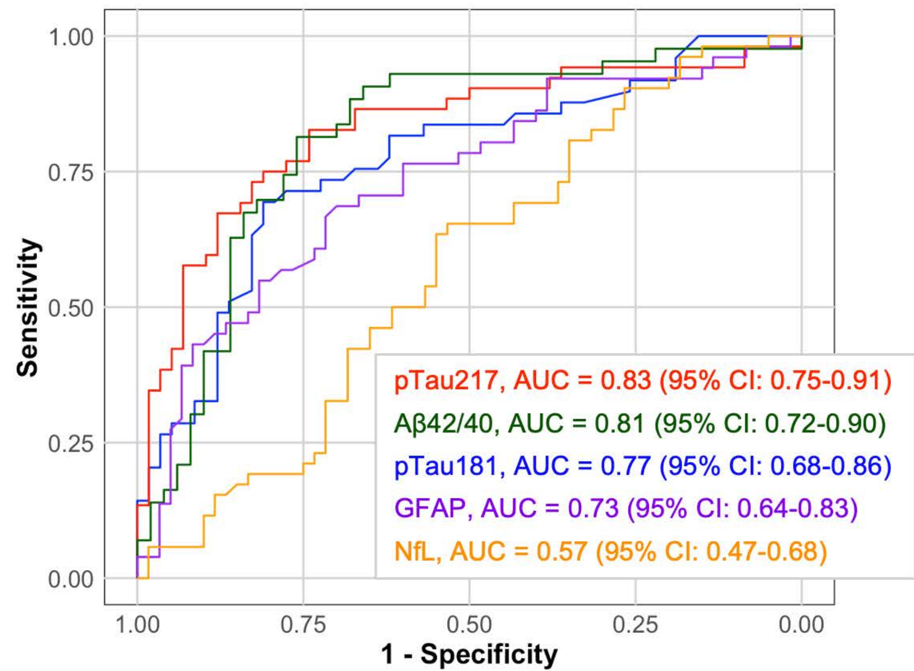
ARTICLE IN PRESS

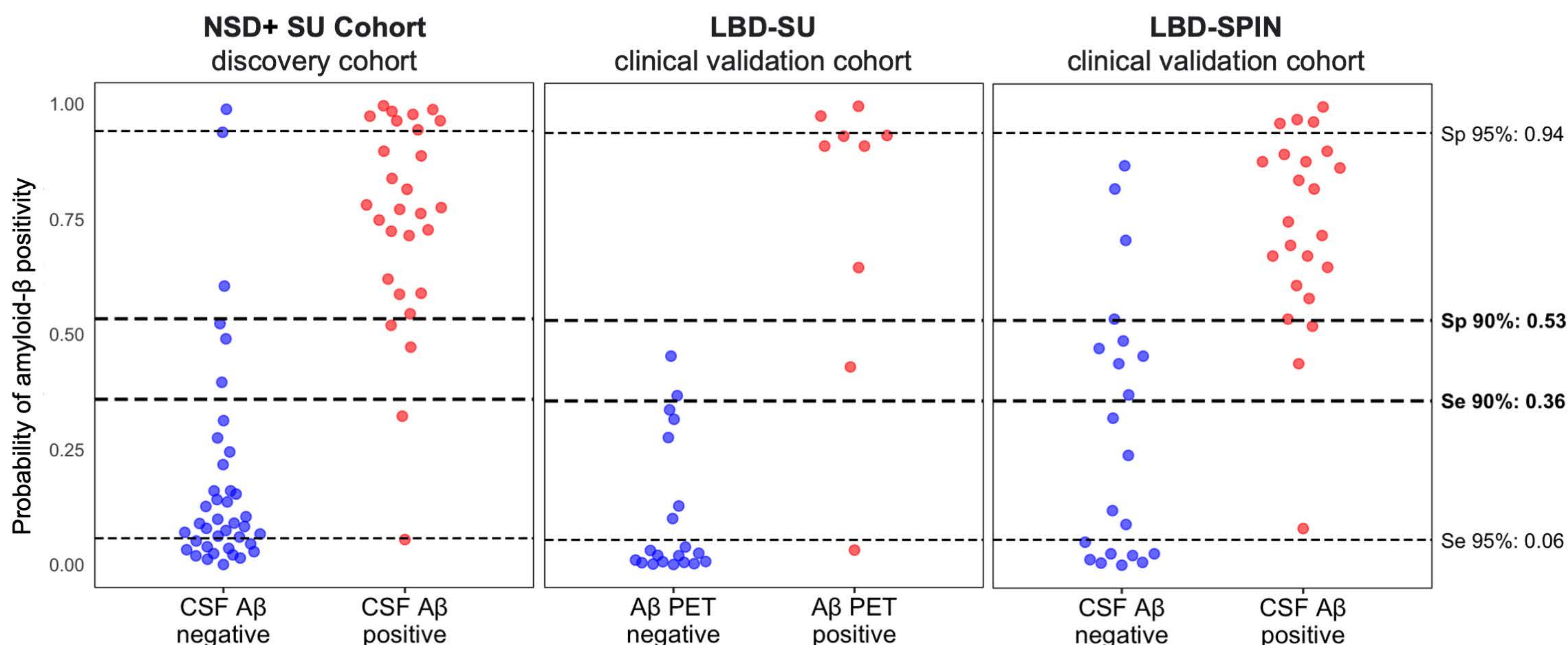

A.
Plasma pTau217
Plasma pTau217 Levels (pg/mL)
5.0
4.0
3.0
2.0
1.0
0.0
***
***
***
***
NSD-/Aβ-
NSD-/Aβ+
NSD+/Aβ-
NSD+/Aβ+
B.
Plasma pTau181
Plasma pTau181 Levels (pg/mL)
12.5
10.0
7.5
5.0
2.5
0.0
C.
Plasma Aβ42/40
Plasma Aβ42/40 ratio
0.15
0.12
0.10
0.08
0.05
Clinical status
Asymptomatic
Symptomatic
Significance
* p < 0.05
** p < 0.01
*** p < 0.001
D.
Plasma GFAP
Plasma GFAP Levels (pg/mL)
500
400
300
200
100
0
*
E.
Plasma NfL
Plasma NfL Levels (pg/mL)
100
75
50
25
0
F.
Fold-change of plasma biomarker levels
pTau181
pTau217
Aβ42/40
GFAP
NfL
NSD status
NSD+
NSD-
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Median fold-change of plasma biomarkers

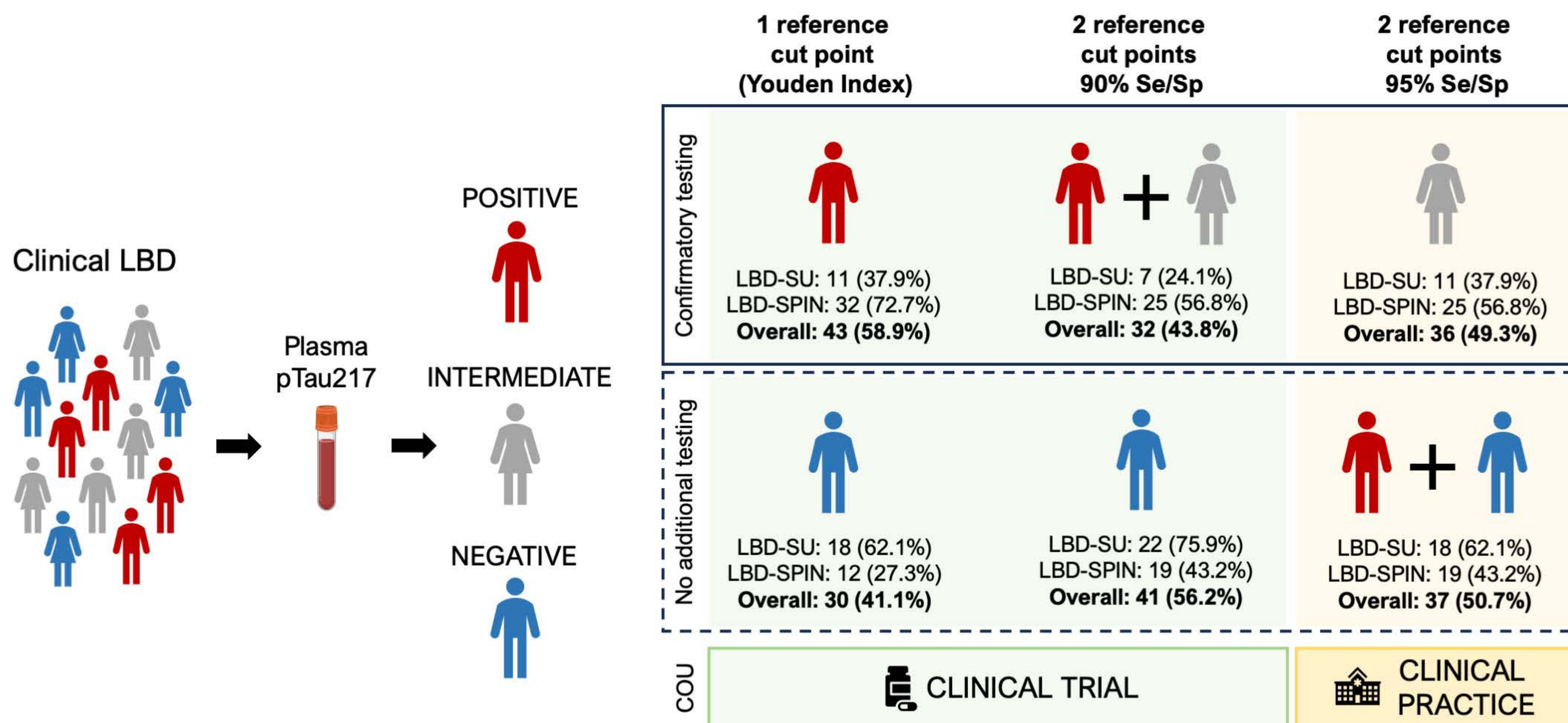

A.
NSD+ 36CL
Sensitivity
1 - Specificity
1.00
0.75
0.50
0.25
0.00
pTau217, AUC = 0.92 (95% CI: 0.85-1.00)
Aβ42/40, AUC = 0.90 (95% CI: 0.81-0.98)
GFAP, AUC = 0.82 (95% CI: 0.72-0.92)
pTau181, AUC = 0.79 (95% CI: 0.69-0.90)
NfL, AUC = 0.72 (95% CI: 0.59-0.84)


B.
NSD- 36CL
Sensitivity
1 - Specificity
1.00
0.75
0.50
0.25
0.00
pTau217, AUC = 0.91 (95% CI: 0.86-0.97)
Aβ42/40, AUC = 0.83 (95% CI: 0.75-0.92)
pTau181, AUC = 0.80 (95% CI: 0.72-0.88)
GFAP, AUC = 0.77 (95% CI: 0.69-0.86)
NfL, AUC = 0.66 (95% CI: 0.56-0.76)


C.
NSD+ 24CL
Sensitivity
1 - Specificity
1.00
0.75
0.50
0.25
0.00
pTau217, AUC = 0.92 (95% CI: 0.84-0.99)
Aβ42/40, AUC = 0.85 (95% CI: 0.75-0.95)
GFAP, AUC = 0.77 (95% CI: 0.66-0.89)
pTau181, AUC = 0.77 (95% CI: 0.66-0.88)
NfL, AUC = 0.73 (95% CI: 0.61-0.85)


D.
NSD- 24CL
Sensitivity
1 - Specificity
1.00
0.75
0.50
0.25
0.00
pTau217, AUC = 0.83 (95% CI: 0.75-0.91)
Aβ42/40, AUC = 0.81 (95% CI: 0.72-0.90)
pTau181, AUC = 0.77 (95% CI: 0.68-0.86)
GFAP, AUC = 0.73 (95% CI: 0.64-0.83)
NfL, AUC = 0.57 (95% CI: 0.47-0.68)




**C. Sample size estimates for hypothetical clinical trials in NSD**

| SU discovery cohort | Scenario 1: To include 100 amyloid-β positive LBD participants | | |
|---|---|---|---|
| | 1 reference cut point (Youden Index) | 2 reference cut points 90% Se/Sp | 2 reference cut points 95% Se/Sp |
| Plasma pTau217 positivity, % | 49.3% | 43.3% | 19.4% |
| PPV, % | 81.8% | 86.2% | 84.6% |
| Number of plasma pTau217 positives needed | ~122 | ~116 | ~118 |
| Number to screen | ~248 | ~248 | ~610 |

| SU discovery cohort | Scenario 2: To include 100 amyloid-β negative LBD participants | | |
|---|---|---|---|
| | 1 reference cut point (Youden Index) | 2 reference cut points 90% Se/Sp | 2 reference cut points 95% Se/Sp |
| Plasma pTau217 negativity, % | 50.7% | 55.2% | 46.3% |
| NPV, % | 97.1% | 91.9% | 96.8% |
| Number of plasma pTau217 negatives needed | ~103 | ~109 | ~103 |
| Number to screen | ~203 | ~197 | ~123 |