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RESEARCH ARTICLE

Plasma p-tau181/A β ₁₋₄₂ ratio predicts A β -PET status and correlates with CSF-p-tau181/A β ₁₋₄₂ and future cognitive decline

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Abstract

Background: In Alzheimer's disease (AD), plasma amyloid beta (A β)₁₋₄₂ and phosphorylated tau (p-tau) predict high amyloid status from A β positron emission tomography (PET); however, the extent to which combination of these plasma assays can predict remains unknown.

Methods: Prototype Simoa assays were used to measure plasma samples from participants who were either cognitively normal (CN) or had mild cognitive impairment (MCI)/AD in the Australian Imaging, Biomarkers and Lifestyle (AIBL) study.

Results: The p-tau181/A β ₁₋₄₂ ratio showed the best prediction of A β -PET across all participants (area under the curve [AUC] = 0.905, 95% confidence interval [CI]: 0.86–0.95) and in CN (AUC = 0.873; 0.80–0.94), and symptomatic (AUC = 0.908; 0.82–1.00) adults. Plasma p-tau181/A β ₁₋₄₂ ratio correlated with cerebrospinal fluid (CSF) p-tau181 (Elecsys, Spearman's ρ = 0.74, P < 0.0001) and predicted abnormal CSF A β (AUC = 0.816; 0.74–0.89). The p-tau181/A β ₁₋₄₂ ratio also predicted future rates of cognitive decline assessed by AIBL Preclinical Alzheimer Cognitive Composite or Clinical Dementia Rating Sum of Boxes (P < 0.0001).

Discussion: Plasma p-tau181/A β ₁₋₄₂ ratio predicted both A β -PET status and cognitive decline, demonstrating potential as both a diagnostic aid and as a screening and prognostic assay for preclinical AD trials.

KEYWORDS

Alzheimer's disease, amyloid beta amyloid imaging, blood biomarkers, blood diagnostic for Alzheimer's disease, cerebrospinal fluid, phosphorylated tau, plasma phosphorylated tau181, plasma amyloid beta, positron emission tomography

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1 | INTRODUCTION

Alzheimer's disease (AD), the most common neurodegenerative dementia, is characterized by a preclinical and prodromal phase of >20 years, during which amyloid beta ($A\beta$) accumulates as plaques in the brain extracellular environment, and with aggregation of tau in neurofibrillary tangles, drives neurodegeneration that gives rise to cognitive decline and ultimately dementia.¹ $A\beta$ fragments exist in a number of distinct bioavailable pools, with the more insoluble load measurable by positron emission topography ($A\beta$ -PET) and soluble $A\beta$ detectable in the cerebrospinal fluid (CSF).²⁻⁵ Soluble phosphorylated tau (p-tau) can be detected in CSF, and PET can image insoluble aggregates of tau, all of which can aid AD diagnosis in research settings.⁶⁻⁸ However, the use of PET and CSF to detect $A\beta$ and tau in clinical or community settings is limited by the cost, requirements for specific expertise and equipment, and risk of adverse events. This restricts their use in programs seeking to identify individuals at risk for AD.

The advent of ultra-high-sensitivity detection methods, including immunoprecipitation-mass spectrometry and single molecule array detection (including Simoa and other platforms) has enabled $A\beta$ and p-tau species to be measured in blood plasma with increased dynamic range. When analyzed with specific detector and capture antibodies, plasma $A\beta$ and p-tau levels are associated strongly with levels of the same biomarkers measured from CSF and with PET. For example plasma $A\beta_{1-42}$ and the $A\beta_{1-42/1-40}$ ratio predict brain $A\beta$ burden, and tau measured at different phosphorylation sites (threonine₁₈₁ [p-tau181], threonine₂₁₇ [p-tau217], threonine₂₃₁ [p-tau231]) shows concordance with CSF p-tau levels (concordance ≈ 0.8) and can predict $A\beta$ -PET burden (area under the curve [AUC] values ranging from 0.8 through 0.9).⁹⁻¹⁴ Clinical-pathological models show that understanding about the presence and severity of AD is improved when markers of amyloid and tau levels are considered simultaneously,¹⁵⁻¹⁸ with one study demonstrating how combinations of plasma levels of $A\beta$ and p-tau relate to $A\beta$ -PET burden.¹⁹ The first aim of this study was to investigate whether two different plasma p-tau markers (p-tau181 and p-tau231), incorporated into a ratio with $A\beta_{42}$, can improve predictions of $A\beta$ burden over single analytes compared to PET or CSF sampling. Additionally, these analytes were investigated for an association with future cognitive decline. The second aim was to explore how ratios of plasma tau and amyloid were associated with disease progression. Both aims are tested in the complete cohort, as well as cognitively normal (CN) and cognitively impaired (CI: mild cognitive impairment [MCI] and AD) groups to gain a better understanding of the results.

2 | METHODS

2.1 | Study participants

The Australian Imaging, Biomarkers and Lifestyle (AIBL) study is a prospective longitudinal cohort of adults over the age of 60 designed to understand the natural history of AD, with recruitment and testing procedures described in detail previously.²⁰ Participants undergo 18

RESEARCH IN CONTEXT

- 1. Systematic review:** The authors reviewed the literature, found via PubMed, for analyses on plasma and cerebrospinal fluid (CSF) levels of phosphorylated tau (p-tau) and amyloid beta ($A\beta$), which are highly specific markers of Alzheimer's disease. Recent studies in CSF using fully automated immunoassays have demonstrated that the p-tau/ $A\beta_{1-42}$ ratio shows better concordance with $A\beta$ positron emission tomography (PET) compared to p-tau alone. To the best of our knowledge, only one small study has explored the p-tau/ $A\beta_{1-42}$ ratio in plasma showing an increased performance of discriminating $A\beta^+$ (positive) from $A\beta^-$ (negative) individuals.
- 2. Interpretation:** Our research has demonstrated the predictive capability of the p-tau181/ $A\beta_{1-42}$ ratio to predict both $A\beta$ -PET status at multiple Centiloid thresholds and time points, as well as to predict change in both early and late cognition. The ratio may serve as an excellent tool for clinical trial prescreening.
- 3. Future directions:** Future work could focus on relationships between plasma biomarkers and changes in cognition, accounting for features such as apolipoprotein E $\epsilon 4$ allele status.

monthly neuropsychological and clinical assessments and blood donations, with AIBL clinical classification confirmed by an expert clinical panel consisting of a neurologist, geriatrician, and neuropsychologist (all blinded to biomarker status). All participants from the AIBL study, who had been classified clinically as being CN, with mild cognitive impairment (MCI) or dementia, and who had both CSF samples and $A\beta$ -PET scans, were used in this study. Participants with either MCI or AD were classed in a CI subgroup for statistical comparisons separate from the CN group. Ethical approval was provided through St. Vincent's Health and Hollywood Private Hospital, and all participants provided written informed consent.

2.2 | Biospecimen collection

Blood collection was conducted on overnight fasting participants between 9:00 am and 10:30 am in K3-ethylenediaminetetraacetic acid tubes (7.5 ml S-monovette 01.1605.008) containing pre-added prostaglandin E1 (33 ng/ml of whole blood, Sapphire Biosciences) to prevent platelet activation, a potential source of peripheral $A\beta$. To generate plasma, blood was centrifuged at room temperature at $200 \times g$ for 10 minutes to collect platelet-rich plasma, which was then spun at $800 \times g$ for 10 minutes, aliquoted into 0.5 ml aliquots (2D cryobankIT, NUN374088), snap frozen within 2 hours of collection and then stored in vapor phase liquid nitrogen (LN₂).

CSF was collected in the morning after overnight fasting via lumbar puncture using a Temena (Polymedic) spinal needle micro-tip (22/27G × 103 mm; CAT 21922-27). CSF was collected by aspiration or gravity drip into 15 ml polypropylene tubes (Greiner Bio-One188271) on wet ice, centrifuged within 1 hour at 2000 × *g* for 10 minutes at 4°C, transferred to a new 15 ml tube to remove any gradient effect, and aliquoted into 2D NUNC cryovials and stored in vapor phase LN₂.

Within AIBL, CSF collection is non-compulsory and can have intermittent follow-up collections. In this article Assessment 1 refers to the plasma matching the first CSF collection, and Assessment 2 refers to plasma collected matching a second CSF collection, either at 18-month or longer follow-up intervals.

2.3 | Clinical cognitive measures

Calculation of the Alzheimer's Disease Cooperative Study Preclinical Alzheimer Cognitive Composite in AIBL (AIBL-PACC) has been described previously.²¹ For each assessment, an individual's AIBL-PACC is computed by averaging the baseline-standardized scores of the Mini-Mental State Examination (MMSE), California Verbal Learning Test-II (CVLT-II), Logical Memory II, and Digit Symbol Coding.²² For analyses using both plasma biomarker and longitudinal cognition, the baseline clinical classification and cognitive status of individuals was aligned with the assessment at which the plasma and CSF collection occurred first (Assessment 1). Longitudinal analyses then used all available cognitive measurements with respect to the plasma biomarker baseline (up to nine 18-month assessments). The number of assessments available with cognitive data for participants in CN/CI groups are shown in Table S1 in supporting information.

2.4 | Aβ-PET imaging

Aβ-PET imaging was performed with four different radiotracers: ¹¹C-Pittsburgh compound B (PiB), ¹⁸F-NAV4694 (NAV), ¹⁸F-flutemetamol (FLUTE) or ¹⁸F-florbetapir (FBP). Aβ-PET scans were spatially normalized using CapAIBL²³ and the Centiloid (CL) method was applied.^{24,25} Aβ-PET (positive/negative) groups were derived using three CL thresholds: CL < 15, 20, and 25 as negative; ≥ 15, 20, and 25 as positive. Reporting on three thresholds has been used here, as variable CL thresholds have been used in the literature, with < 15 CL selected as a threshold for no neuritic plaques (CL threshold when calculated as +2 standard deviations from young normal ranging between 8 and 16 CL depending upon the tracer, and < 12 compared to neuropathology), and ≥ 20 and ≥ 25 CL frequently used as the threshold for amyloid positivity corresponding to moderate plaque density.^{26–28} Increasing the positivity threshold improves the correlation between neuropathological and clinicopathological diagnosis, but also allows for Consortium to Establish a Registry for Alzheimer's Disease (CERAD) sparse neuritic plaques in a negative scan. CL data were not available for all participants at each of the assessments where plasma was collected. For Assessments 1 and 2, there were 169 and 65 participants from a total of 233 and 100,

respectively, with Aβ-PET measured at the same assessment as the plasma. For the remaining participants, the Aβ-PET status was imputed by binning the CL value (into bins derived via the 15, 20, and 25 CL thresholds) if a positive scan occurred prior to plasma collection and a negative scan post-plasma collection.

2.5 | CSF analysis

CSF analysis in AIBL has been described previously¹⁵ using the Roche Elecsys electrochemiluminescence immunoassays for Aβ_{1–42}, Elecsys Total (t-)tau and Elecsys p-tau181, run on cobas e 601, cobas e 602, and MODULAR ANALYTICS E170 analyzers. A total of 155 participants had Elecsys measurements at the same time as the plasma (CN: N = 106, MCI: N = 28, AD: N = 21). AIBL participant CSF and plasma samples were selected given biospecimen availability.

2.6 | Assays and analytics

The set-up of the Amyblood (Simoa) assay was essentially as described in Thijssen et al.²⁹ In short, C-terminal monoclonal antibodies (mAb), ADx102 and ADx103, are coupled to the paramagnetic carboxylated beads. mAb ADx101 was used as detection antibody in biotinylated form, with an antibody/biotin ratio of 32. Samples were diluted respectively 4 and 20 times for Aβ_{1–42} and Aβ_{1–40}. Both assays were performed on the automated Quanterix Simoa HD-X platform using a two-step protocol (80-7 cadences).

2.7 | Plasma p-tau assay

In the plasma p-tau protocol the phospho-specific mAbs ADx252 and ADx253 were conjugated to paramagnetic beads.³⁰ ADx252 is specific to p-tau181 and non-reactive toward p-threonine₁₇₅ (p-tau175) or p-tau231/p-serine₂₃₅ (p-S235). ADx253 has a specificity toward p-tau231 and absence of reactivity toward p-S235 or p-tau181/175. In addition, the phosphorylation of additional phospho-sites did not affect the reactivity (Figures S1 and S2 in supporting information). Detection of p-tau was done using a N-terminal-specific tau mAb ADx204 in biotinylated form, that recognizes all tau forms except those phosphorylated at tyrosine₁₈³⁰ (ADx204 biotin/ratio of 32 for p-tau181 and ADx204/biotin ratio of 128 for p-tau231). After 8 minutes of centrifugation at 10,000 × *g* plasma samples were diluted five-fold and were run on the Quanterix Simoa HD-X platform using a two-step protocol of 80-14 cadences for the p-tau181 assay and 80-7 cadences for p-tau231. Calibration of both p-tau assays was done with a single synthetic peptide covering the relevant antibody epitopes. The seven calibrator points ranged between 50 pg/ml and 0.78 pg/ml, and 50 pg/ml and 0.39 pg/ml respectively, for p-tau181 and p-tau231. A five-parameter curve-fit algorithm with 1/Y² weighting was used to convert average enzymes per bead into p-tau concentrations. Details of the assay specifications are described in Table S2 in supporting

TABLE 1 Study demographic characteristics

	Total sample	A β ⁻	A β ⁺	P-value
N (%)	233	142 (61%)	91 (39%)	
Sex male, N (%)	124 (53%)	72 (51%)	52 (57%)	0.34
Mean age, years (SD)	72.9 (6.1)	72.4 (6.3)	73.5 (5.8)	0.18
APOE ϵ 4 carriage, N (%)	74 (32%)	26 (18%)	48 (53%)	<0.0001
Tracer florbetapir N %	60 (26%)	44 (26%)	16 (26%)	
Tracer flutemetamol N %	73 (31%)	42 (31%)	31 (31%)	
Tracer NAV/PiB N %	100 (43%)	56 (43%)	44 (43%)	0.069
Mean CL (SD)	34.2 (45.2)	1.8 (9.6)	80 (34.9)	<0.0001
Diagnosis CN N %	168 (72%)	124 (53%)	44 (19%)	
Diagnosis MCI N %	34 (15%)	16 (7%)	18 (8%)	
Diagnosis AD N %	31 (13%)	2 (1%)	29 (12%)	<0.0001
Median MMSE, (MAD)	28 (1.5)	29 (1.5)	28 (3)	<0.0001
Median CDR-SB, (MAD)	0 (0)	0 (0)	0.5 (0.7)	<0.0001
Mean AIBL PACC (SD)	-0.62 (1.25)	-0.19 (0.73)	-1.31 (1.58)	<0.0001
Plasma p-tau181/A β ₁₋₄₂ matching A β -PET N (%)		92 (93%)	57 (81%)	
Plasma p-tau181/A β ₁₋₄₂ discordant to A β -PET N (%)		7 (7%)	13 (19%)	

Characteristics measured at Assessment 1 and compared between A β -PET groups using a CL threshold at 20CL.

Abbreviations: A β , amyloid beta; AD, Alzheimer's disease; AIBL, Australian Imaging, Biomarkers and Lifestyle study; APOE, apolipoprotein E; CDR-SB, Clinical Dementia Rating Sum of Boxes; CL, Centiloid; CN, cognitively normal; MAD, maximum absolute deviation; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NAV, ¹⁸F-NAV4694; PACC, Preclinical Alzheimer Cognitive Composite; PET, positron emission tomography; PiB, Pittsburgh compound B; p-tau, phosphorylated tau; SD, standard deviation.

information. Further analytical performance of the p-tau assays has been described previously.³⁰

p-tau assay phospho-specificity was assessed by checking sandwich assay reactivity using synthetic peptides containing the (non-)phosphorylated epitopes for each respective phospho-site threonine₁₈₁ and/or threonine₂₃₁ up to a concentration of 1000 pg/ml. Reactivity dependency of phosphorylation at other phospho-sites was also checked by using peptides with non-phosphorylation on threonine₁₇₅ and serine₂₃₅ up to a concentration of 1000 pg/ml (Figures S1 and 2).

Further information on sample analysis, quality control panel testing, run-to-run variability, accuracy, and intra-run precision can be found in the supporting information.

2.8 | Statistical analyses

Statistical analyses were performed to test four main hypotheses: (1) determine which of the biomarkers and their ratios have the largest mean difference between A β -PET groups; (2) investigate the capability of each of the biomarkers and their ratios to predict A β -PET groups; (3) investigate how well plasma biomarkers correlate with their corresponding CSF biomarkers, and compare how well plasma biomarkers predict CSF A β ₁₋₄₂ compared to predicting A β -PET; and (4) using the

best performing plasma biomarker, assess its ability to predict cognitive decline using both a late and early parameter of cognitive change (linear mixed effects models). Full details for the statistical analyses are in the supporting information. Only tables/plots for Assessment 1 are shown in the main text, while results from Assessment 2 are shown in supporting information.

3 | RESULTS

3.1 | Study demographic characteristics

Of the 233 participants with plasma collected at their first assessment, and the 100 participants with plasma taken at their second, 39% in Assessment 1 (Table 1), and 38% at Assessment 2 (Table S3 in supporting information) were A β -PET⁺. There was no difference in age or sex between the A β -PET groups ($P>0.05$); however, A β -PET⁺ participants were more likely to carry at least one apolipoprotein E (APOE) ϵ 4 allele ($P<0.0001$); have higher CL values ($P<0.0001$); and perform worse on MMSE, the AIBL PACC score, and Clinical Dementia Rating Sum of Boxes (CDR-SB; $P<0.0001$). Comparisons of sample demographics for Assessment 1 are shown in Table 1, and for Assessment 2 in Table S3. Comparison of group mean biomarker levels showed large differences between A β -PET groups for the p-tau181 (Cohen's D: 1.19) and

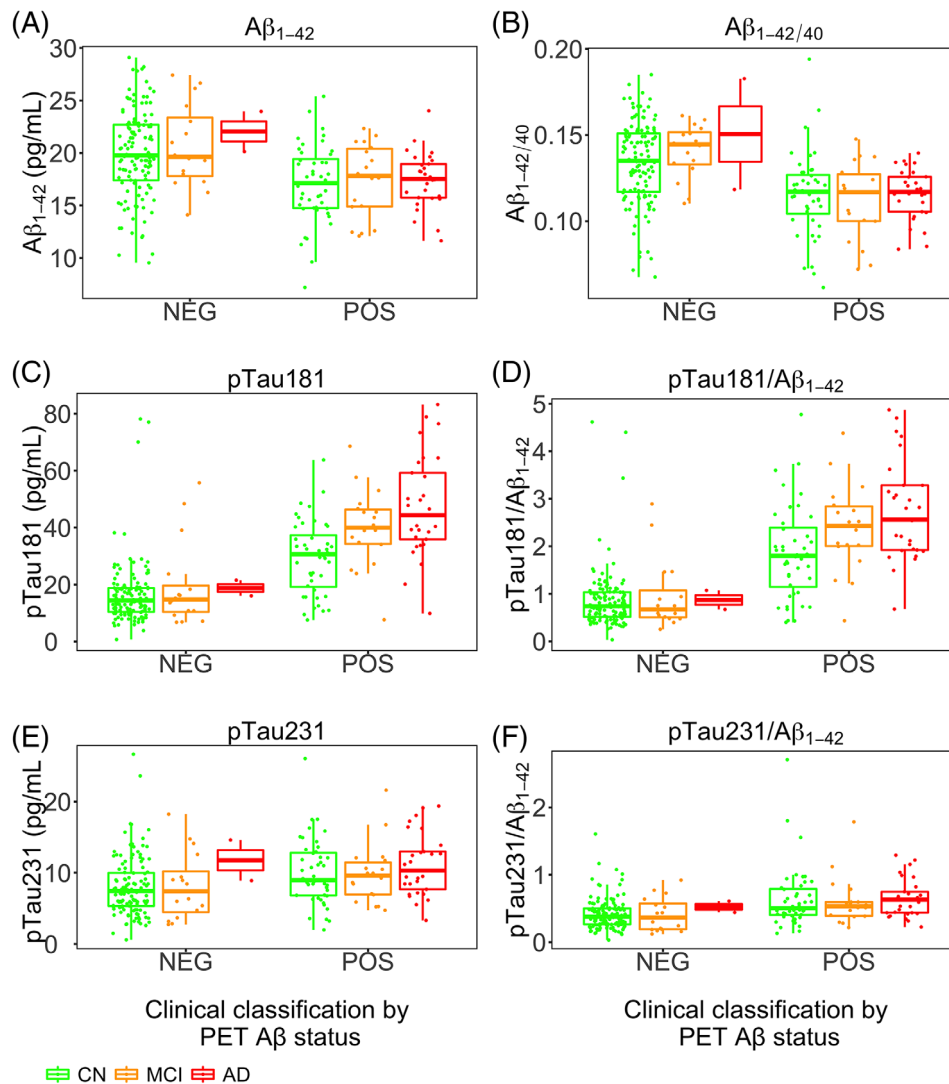


FIGURE 1 Box and whisker plot for $A\beta_{1-42}$, p-tau181 and p-tau231, $A\beta_{1-42}/1-40$ ratio, p-tau181/ $A\beta_{1-42}$ ratio, and p-tau231/ $A\beta_{1-42}$ ratio at Assessment 1. Individual markers ($A\beta_{1-40}$, $A\beta_{1-42}$ p-tau181, and p-tau231) are measured in pg/mL. A, $A\beta_{1-42}$. B, $A\beta_{1-42}/40$. C, pTau181. D, pTau181/ $A\beta_{1-42}$. E, pTau231. F, pTau231/ $A\beta_{1-42}$. Plots represent the five-number summary, with the median, first, and third quartiles shown in each box. $A\beta$, amyloid beta; NEG, negative; PET, positron emission tomography; POS, positive; p-tau, phosphorylated tau

p-tau181/ $A\beta_{1-42}$ ratio (Cohen's D: 1.29) at both assessments ($P < 0.0001$; Table S4 in supporting information; Figure 1). Other markers such as body mass index and education were assessed; however, they were not significantly associated with $A\beta$ -PET and as such were not considered further.

3.2 | Plasma biomarkers to predict $A\beta$ -PET

Receiver operating characteristic (ROC) analyses of plasma data to predict $A\beta$ -PET groups at both assessments and across three CL thresholds demonstrated strong predictive capability for both the p-tau181 marker alone and its ratio with $A\beta_{1-42}$ (Table 2; Tables S5, 6, and 7 in supporting information; Figure S1). Using the ratio to predict $A\beta$ -PET groups (CL threshold at 20 CL) performed significantly better than both the p-tau181 marker alone and any combination

of markers as tested via multivariate modelling. p-tau231 alone did not significantly differ from the base model and further information on the predictive capability comparisons is presented in Table S9 in supporting information. Concentrating on the p-tau181/ $A\beta_{1-42}$ ratio and restricting the sample set to the CN adults only (Assessment 1), predictive performance was reduced slightly (AUC complete group 0.88, CN 0.83), while in the CI subgroup it increased to 0.91. Predictive performance was similar at Assessment 2, with higher AUC values despite the smaller sample size (Table S5). Assessing the predictive capability in models using confounders age, sex, APOE $\epsilon 4$ allele status, and tracer showed only small improvements in AUC values (AUC [95% confidence interval] unadjusted: 0.883 [0.83–0.93], adjusted: 0.889 [0.84–0.94]). All models including plasma p-tau181 out-performed the base model ($P < 0.0001$, Table S7, Figure S2 and Figure S3 in supporting information). Positive and negative predictive values for the p-tau181/ $A\beta_{1-42}$ ratio remained similar whether

TABLE 2 AUC values across complete and stratified groups using CL threshold at 20

	Complete sample	CN	CI
Biomarker	AUC (95% CI)	AUC (95% CI)	AUC (95% CI)
N ($A\beta^-/A\beta^+$)	142/91	124/44	18/47
$A\beta_{1-42}$	0.705 (0.64–0.77)	0.705 (0.62–0.79)	0.741 (0.6–0.88)
$A\beta_{1-42/1-40}$	0.731 (0.69–0.81)	0.728 (0.64–0.81)	0.852 (0.74–0.96)
p-tau181	0.862 (0.81–0.92)	0.808 (0.72–0.89)	0.868 (0.75–0.98)
p-tau231	0.66 (0.59–0.73)	0.646 (0.55–0.74)	0.636 (0.47–0.8)
p-tau181/ $A\beta_{1-42}$	0.883 (0.83–0.93)	0.832 (0.75–0.91)	0.908 (0.82–1)
p-tau231/ $A\beta_{1-42}$	0.713 (0.65–0.78)	0.692 (0.6–0.78)	0.696 (0.54–0.85)

Abbreviations: 95% CI: 95% confidence interval; $A\beta$, amyloid beta; AUC, area under the curve; CI, cognitively impaired (contains participants with either mild cognitive impairment or Alzheimer's disease); CL, Centiloid; CN, cognitively normal; p-tau, phosphorylated tau.

using ROC models from the individual biomarkers, or from models including both biomarker and confounders age, sex, tracer, and APOE $\epsilon 4$ allele status at both assessments (Table S8 in supporting information).

3.3 | Biomarker correlation and agreement among plasma, CSF, and PET

Associations between markers of tau and amyloid measured from plasma and CSF (Figure 2) were strongest for the p-tau181/ $A\beta_{1-42}$ ratio, with a Spearman's rho value of 0.75 ($P < 0.0001$). p-tau181 and the $A\beta_{1-42/1-40}$ ratio were the next strongest with rho values of 0.53 and 0.45 ($P < 0.0001$), while $A\beta_{1-42}$ showed more variation between plasma and CSF rho = 0.32 ($P < 0.0001$). Assessing agreement between the plotted CL values and p-tau181/ $A\beta_{1-42}$ ratio values binned into quadrants (Figure 3 and Figure S2) demonstrated strong agreement between the two markers. Of the 99 participants at Assessment 1 with a CL value less than 20, 92 participants (93%) were both $A\beta$ -PET⁻ and p-tau181/ $A\beta_{1-42}$ negative, while only 7 participants (7%) were $A\beta$ -PET⁻ and p-tau181/ $A\beta_{1-42}$ positive. Of the 70 participants who were $A\beta$ -PET⁺, 57 participants (81%) were also p-tau181/ $A\beta_{1-42}$ positive, while 13 participants (19%) were $A\beta$ -PET⁺ and p-tau181/ $A\beta_{1-42}$ negative.

3.4 | Plasma versus CSF biomarkers to predict $A\beta$ -PET status

Comparing the performance of $A\beta_{1-42}$, p-tau181, and p-tau181/ $A\beta_{1-42}$ to predict the $A\beta$ -PET status at each of the three thresholds (Figure S3) in plasma (measured using Simoa assays) and CSF (measured using Elecsys assays), it was clear that the $A\beta_{1-42}$ assay for CSF outperformed the plasma assay (@CL15 $P = 0.012$, @CL20 $P = 0.0003$, @CL25 $P = 0.0003$), while the CSF p-tau181 assay performed no differently than the plasma assay (@CL15 $P = 0.887$, @CL20 $P = 0.822$, @CL25 $P = 0.856$). For the ratio, however, there was no difference in AUC values

between the plasma and CSF assay at 15 CL ($P = 0.409$), with a weak but significant difference using the CL threshold at 20 ($P = 0.033$) but not at 25 ($P = 0.051$). As for $A\beta$ -PET, ROC analyses were performed using CSF $A\beta_{1-42}$ as the outcome variable (Table S9; Figure S1E and 1F). AUC values were lower for plasma biomarkers to predict CSF- $A\beta$ compared to predicting $A\beta$ -PET status; however, the p-tau181/ $A\beta_{1-42}$ ratio still had the highest AUC (0.82 [95% confidence interval: 0.71–0.89]). Differences in p-tau181 values may be attributed to the different antibodies used in the pT181 tau assays. While the Simoa assay detects an N terminal fragment the Elecsys assay detects a fragment within the aa 170-205 of the tau441 protein when phosphorylated at threonine 181.³¹

3.5 | Plasma p-tau181/ $A\beta_{1-42}$ predicts change in cognition at both early and late stages of AD

Analyses of associations between levels of the plasma p-tau181/ $A\beta_{1-42}$ ratio with change in cognition over time and adjusting for age, sex, tracer, and APOE $\epsilon 4$ allele status showed a significant increase in CDR-SB (Figure 4D, $P < 0.0001$) in the CI group, a significant increase in CDR-SB (Figure 4C, $P = 0.015$) in the CN group and a significant decrease in the AIBL PACC score in the CN group over time (Figure 4A, $P = 0.0002$). While there was a significant change for the PACC score over time in the CI group, there was no difference between the slopes for low/high p-tau181/ $A\beta_{1-42}$ ratio groups (Figure 4B). Repeating these analyses using the CSF p-tau181/ $A\beta_{1-42}$ ratio showed only the CDR-SB to have a significant difference in the change in cognition for those with a higher p-tau181/ $A\beta_{1-42}$ ratio compared to a low p-tau181/ $A\beta_{1-42}$ ratio in the CI group (Figure S4D, $P = 0.003$).

4 | DISCUSSION

In the current study we have used the Amyblood assay on the ultra-sensitive Simoa platform to measure, in the AIBL cohort, plasma $A\beta_{1-40}$ and $A\beta_{1-42}$, and a prototype assay for measuring p-tau181 and p-tau231, to determine that p-tau181/ $A\beta_{1-42}$ ratio is the highest

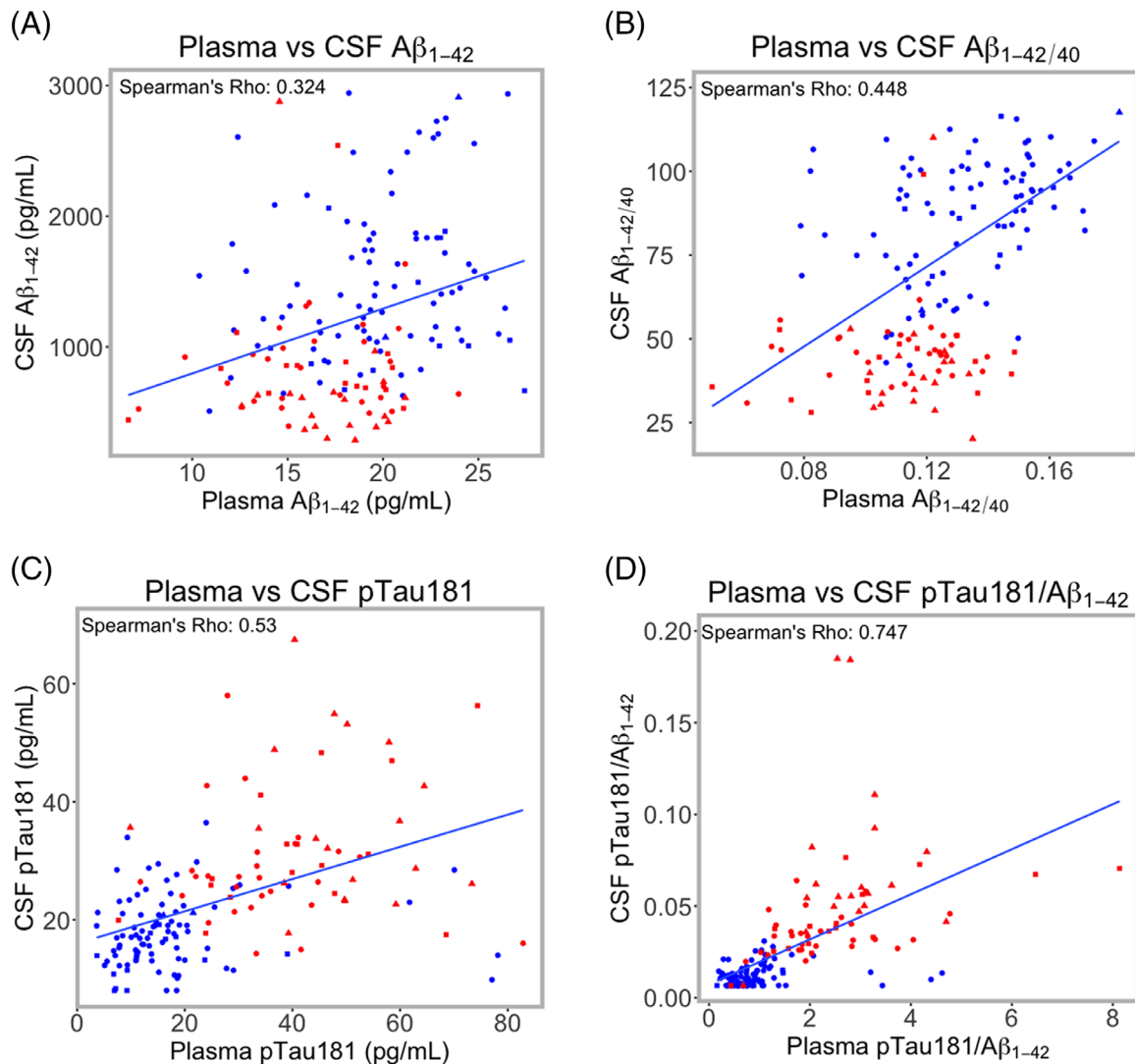


FIGURE 2 Correlation between plasma and CSF biomarkers. Sample size for correlation plots at Assessment 1 was $N = 155$. Linear fit lines are drawn irrespective of $A\beta$ -PET status. Red points represent participants who were $A\beta$ -PET⁺; blue points represent participants who were $A\beta$ -PET⁻. Circle points represent those participants who were CN, square points represent those participants with MCI, triangle points represent those participants with AD. A, Plasma versus CSF $A\beta_{1-42}$. B, Plasma versus CSF $A\beta_{1-42}/1-40$. C, Plasma versus CSF pTau181. D, Plasma versus CSF pTau181/ $A\beta_{1-42}$. $A\beta$, amyloid beta; AD, Alzheimer's disease; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; PET, positron emission tomography; p-tau, phosphorylated tau

performing test compared to individual measurements in predicting amyloid-positive status. Moreover, we observed that only the plasma p-tau181/ $A\beta_{1-42}$ ratio was correlated strongly with the CSF p-tau181/ $A\beta_{1-42}$ ratio, and that this plasma ratio predicted future cognitive decline within the CN subgroup for PACC, and the CI subgroup for CDR-SB.

In a cohort of 233 participants, comprising CN, MCI, and AD participants, the p-tau181/ $A\beta_{1-42}$ ratio yielded an AUC of 0.91 for determining amyloid positivity when analyzed against a PET amyloid threshold of 25 CL. When separated into CN and CI cohorts, the AUCs remained very high (CN = 0.87, CI = 0.91). The AUCs improved as the CL threshold was increased. This compares favorably with other Simoa $A\beta$ and p-tau analyses whose AUCs range between 0.66 to 0.88,^{11,32-34} and is similar to AUCs for the only current US Food and Drug Adminis-

tration (FDA)-approved plasma test for determining amyloid positivity, which in a cohort of predominately CDR-SB 0 had an AUC of 0.88 and 0.94 when run without and with other covariates of age and $APOE \epsilon 4$ on a mass spectrometry platform.³⁵ In the AIBL study cohort, addition of $APOE \epsilon 4$, sex, age, and PET tracer only marginally improved the performance of the plasma test, potentially reducing the need for measuring other parameters when used as a trial-screening tool. Analyses incorporating p-tau181 improved significantly, although mildly, to the addition of covariates, possibly related to the age-dependent accumulation that CSF tau is known to display.³⁶ To our knowledge, only one other study investigated the ratio of p-tau181/ $A\beta_{1-42}$ in plasma in relation to $A\beta$ -PET, which produced an AUC of 0.89 in both CN and MCI participants.¹⁹ The current work, conducted in a larger cohort and in a different ethnic group, supports the utility of this measurement

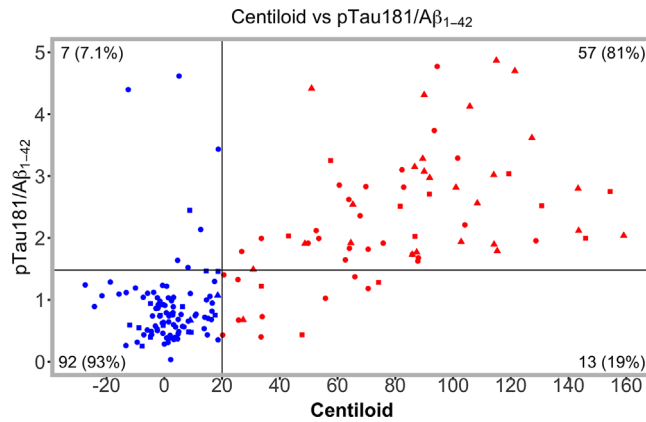


FIGURE 3 Agreement between $A\beta$ -PET and plasma p-tau181/ $A\beta_{1-42}$ ratio at Assessment 1. Threshold line for $A\beta$ -PET was set at 20 CL. Threshold for the p-tau181/ $A\beta_{1-42}$ ratio was set using the Youden's Index (1.48) from the ROC model for $A\beta$ -PET status with the CL threshold set at 20 CL at Assessment 1. Red points represent participants who were $A\beta$ -PET⁺; blue points represent participants who were $A\beta$ -PET⁻. Circle points represent those participants who were CN, square points represent those participants with MCI, triangle points represent those participants with AD. $A\beta$, amyloid beta; AD, Alzheimer's disease; CL, Centiloid; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; PET, positron emission tomography; p-tau, phosphorylated tau; ROC, receiver operating characteristic

approach. Other works, while not using a ratio, have also identified that combination analyses on p-tau, $A\beta$, and clinical features can better distinguish AD from unimpaired populations³⁷ than analyses of analytes alone.

Compared to CSF measures of p-tau181, $A\beta_{1-40}$ and $A\beta_{1-42}$, measured in a subset of this cohort on the Elecsys immunoassay, the ratio of p-tau181/ $A\beta_{1-42}$ in both the CSF and plasma was the only measure that was highly correlated, although measured on different platforms. The p-tau181/ $A\beta_{1-42}$ ratio has long been considered to have the best statistical power of the CSF markers for representing AD, as it incorporates both hallmarks of the AD disease pathway and represents both early and late stages of the disease pathway. Indeed this ratio in CSF has helped identify late-stage changes in synaptic and neuronal degradation markers and inflammatory markers associated with AD,³⁸ as well as high concordance with $A\beta$ -PET.^{15-17,20} Indeed, the CSF p-tau181/ $A\beta_{1-42}$ ratio was previously shown as well to be the best predictor of cognitive decline in a combined Alzheimer's Disease Neuroimaging Initiative/BioFinder cohort.³⁹ Here analyses of the plasma markers at a second time point in this study showed increased performance of all markers, including the p-tau181/ $A\beta_{1-42}$ ratio, at predicting amyloid status at Assessment 2, which may reflect further accumulation of positive biomarker signals compared to a static baseline in the amyloid-negative cohort. More analyses in other cohorts are needed to test if this plasma ratio can act as a proxy for the CSF measurements to suitably correlate with synaptic degradation biomarkers.

Determination of amyloid chronicity is an estimation of where an individual's current level of amyloid sits temporally on an amyloid accu-

mulation pathway. This accumulation pathway, which is non-linear in accumulation over the initial 20-year preclinical phase, and is independent of the individual's starting age, requires biomarkers that can detect concurrent pathological hallmarks, including accumulation of p-tau, to aid understanding of the near-term risk of phenotypic cognitive decline. Analyses here into longitudinal cognitive outcomes with up to 10 years follow-up, showed that the ratio was able to identify those with early cognitive change as demonstrated via the AIBL PACC, and showed a clear separation in the CDR-SB in both CN and CI groups. The ability to detect cognitive change in those classed as CN further demonstrates the ratio's capacity to detect the minority of this population with amyloid, thus acting as an alternative to PET imaging. For CDR-SB, those with the high ratio performed significantly worse compared to those with a low ratio, suggesting that even once impaired, participants' cognitive performance can be staged as per their ratio level; a very useful trait for clinical trial recruitment. These data suggest that the ratio may assist in the determination of disease chronicity and should be investigated across more longitudinal plasma collection points.

The p-tau181 analyte alone was the next best performing measurement, while the p-tau231 analyte did not perform better than the $A\beta$ measurements, nor add anything when in ratio with the $A\beta$ for correlating with $A\beta$ -PET or CSF- $A\beta$ levels. Further longitudinal analysis, and incorporation of tau-PET measurements into the analysis may reveal temporal specificities for changes in p-tau231 more subtle than what can be detected in this work.

A limitation of the current study is that some of the subgroup analyses were performed on small groups of individuals. At Assessment 1, there were very few participants in the CI group that were $A\beta$ -PET negative, reflecting the AIBL study design. Furthermore, subgroup analyses for participants with samples at Assessment 2 were small in size and results should be taken with caution.

Presently the FDA has provisionally approved the first immunogenic anti-amyloid therapy for mild AD and there is a need for cheap, non-invasive blood tests to not only predict amyloid burden to assist in identifying participants at risk for developing AD, and to confirm AD diagnosis, but to aid in determining the temporal location along the biomarker accumulation pathway. The plasma p-tau181/ $A\beta_{1-42}$ ratio predicts here $A\beta$ -PET status but also hints at predicting near-term future cognitive decline more accurately than $A\beta$ -PET status alone.

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CONFLICTS OF INTEREST

Eugeen Vanmechelen is cofounder and shareholder of ADx NeuroSciences. Erik Stoops is employee and shareholder of ADx NeuroSciences. Jeroen Vanbrabant, Nele Dewit, and Kimberley Mauroo are employees of ADx NeuroSciences. Paul Maruff is a full-time employee of Cogstate Ltd. Christopher C. Rowe has received research grants from NHMRC, Enigma Australia, Biogen, Eisai, and Abbvie; he is on the scientific advisory board for Cerveau Technologies; and

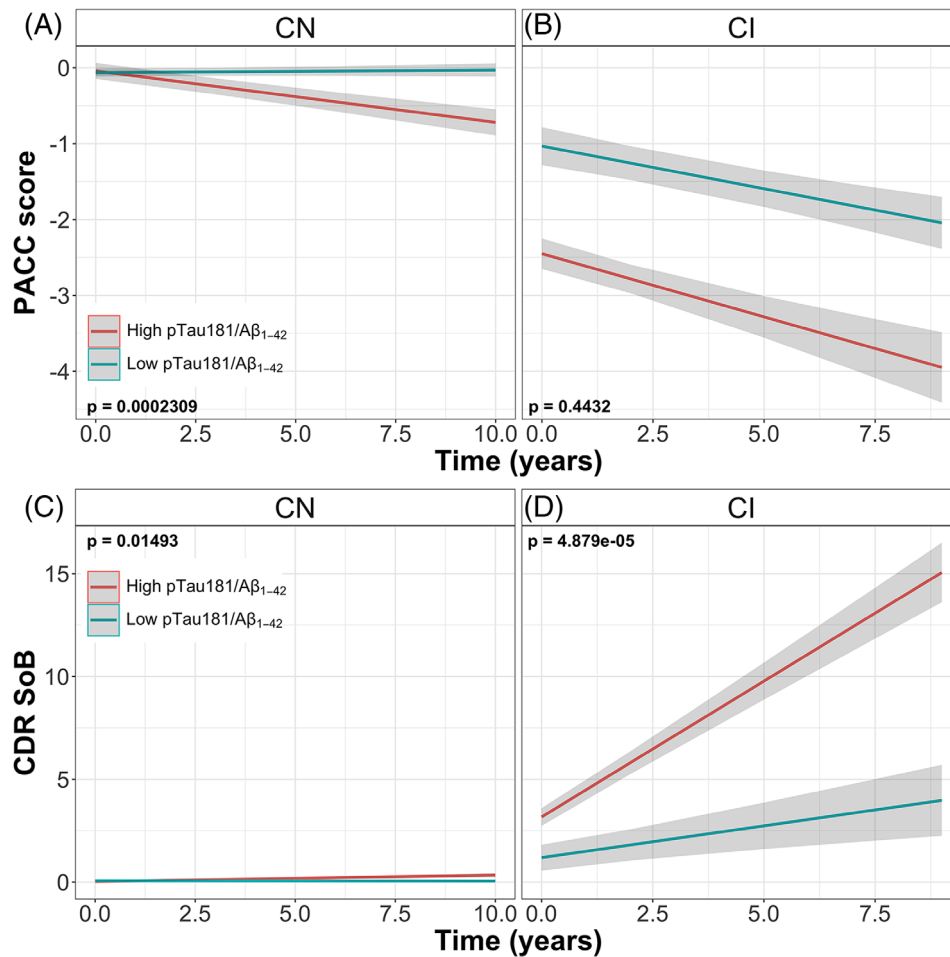


FIGURE 4 Cognitive decline in CDR-SoB and the AIBL PACC score using the plasma between CN/CI groups and the p-tau181/A β_{1-42} ratio as measured by linear mixed effects models. Time on the x-axis refers to the first AIBL assessment whereby the plasma was collected. Sample sizes for cognitive collection points for the plasma sample set are shown in Table S1. The binary p-tau181/A β_{1-42} ratio was created using the Youden's Index (1.48) created from the ROC model using plasma p-tau181/A β_{1-42} versus A β -PET using a CL threshold at 20 CL. Threshold for the plasma p-tau181/A β_{1-42} ratio was 1.483. A β , amyloid beta; AD, Alzheimer's disease; AIBL, Australian Imaging, Biomarkers and Lifestyle study; CDR-SoB, Clinical Dementia Rating Sum of Boxes; CL, Centiloid; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; PACC, Preclinical Alzheimer Cognitive Composite; PET, positron emission tomography; p-tau, phosphorylated tau; ROC, receiver operating characteristic

consulted for Prothena, Eisai, Roche, and Biogen Australia. The other authors did not report any conflict of interest. Author disclosures are available in the [supporting information](#)

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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