Plasma $A\beta_{42/40}$ ratio, p-tau$181$, GFAP, and NfL across the Alzheimer's disease continuum: A cross-sectional and longitudinal study in the AIBL cohort

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Plasma Aβ42/40 ratio, p-tau181, GFAP, and NfL across the Alzheimer’s disease continuum: A cross-sectional and longitudinal study in the AIBL cohort

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Abstract

Introduction: Plasma amyloid beta (Aβ)1-42/Aβ1-40 ratio, phosphorylated-tau181 (p-tau181), glial fibrillary acidic protein (GFAP), and neurofilament light (NfL) are putative blood biomarkers for Alzheimer’s disease (AD). However, head-to-head
cross-sectional and longitudinal comparisons of the aforementioned biomarkers across the AD continuum are lacking.

**Methods:** Plasma Aβ1-42, Aβ1-40, p-tau181, GFAP, and NfL were measured utilizing the Single Molecule Array (Simoa) platform and compared cross-sectionally across the AD continuum, wherein Aβ-PET (positron emission tomography)–negative cognitively unimpaired (CU Aβ−, n = 81) and mild cognitive impairment (MCI Aβ−, n = 26) participants were compared with Aβ-PET–positive participants across the AD continuum (CU Aβ+, n = 39; MCI Aβ+, n = 33; AD Aβ+, n = 46) from the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) cohort. Longitudinal plasma biomarker changes were also assessed in MCI (n = 27) and AD (n = 29) participants compared with CU (n = 120) participants. In addition, associations between baseline plasma biomarker levels and prospective cognitive decline and Aβ-PET load were assessed over a 7 to 10-year duration.

**Results:** Lower plasma Aβ1-42/Aβ1-40 ratio and elevated p-tau181 and GFAP were observed in CU Aβ+, MCI Aβ+, and AD Aβ+, whereas elevated plasma NfL was observed in MCI Aβ+ and AD Aβ+, compared with CU Aβ− and MCI Aβ−. Among the aforementioned plasma biomarkers, for models with and without AD risk factors (age, sex, and apolipoprotein E (APOE) ε4 carrier status), p-tau181 performed equivalent to or better than other biomarkers in predicting a brain Aβ−/+ status across the AD continuum. However, for models with and without the AD risk factors, a biomarker panel of Aβ1-42/Aβ1-40, p-tau181, and GFAP performed equivalent to or better than any of the biomarkers alone in predicting brain Aβ−/+ status across the AD continuum. Longitudinally, plasma Aβ1-42/Aβ1-40, p-tau181, and GFAP were altered in MCI compared with CU, and plasma GFAP and NfL were altered in AD compared with CU. In addition, lower plasma Aβ1-42/Aβ1-40 and higher p-tau181, GFAP, and NfL were associated with prospective cognitive decline and lower plasma Aβ1-42/Aβ1-40, and higher p-tau181 and GFAP were associated with increased Aβ-PET load prospectively.

**Discussion:** These findings suggest that plasma biomarkers are altered cross-sectionally and longitudinally, along the AD continuum, and are prospectively associated with cognitive decline and brain Aβ-PET load. In addition, although p-tau181 performed equivalent to or better than other biomarkers in predicting an Aβ−/+ status across the AD continuum, a panel of biomarkers may have superior Aβ−/+ status predictive capability across the AD continuum.

**KEYWORDS**
Alzheimer’s disease, amyloid beta, blood biomarkers, brain amyloid beta, diagnosis, glial fibrillary acidic protein, longitudinal monitoring, neurofilament light, p-tau181, single molecule array

**HIGHLIGHTS**
- Area under the curve (AUC) of p-tau181 ≥ AUC of Aβ42/40, GFAP, NfL in predicting PET Aβ−/+ status (Aβ−/+).
- AUC of Aβ42/40+p-tau181+GFAP panel ≥ AUC of Aβ42/40/p-tau181/GFAP/NfL for Aβ−/+.
- Longitudinally, Aβ42/40, p-tau181, and GFAP were altered in MCI versus CU.
- Longitudinally, GFAP and NfL were altered in AD versus CU.
INTRODUCTION

Abnormal amyloid beta (Aβ) and tau buildup in the brain measured with positron emission tomography (PET), and Aβ42 and phosphorylated-tau181 (p-tau181) levels in the cerebrospinal fluid (CSF) are the current core biomarkers of Alzheimer’s disease (AD). These biomarkers reflect AD neuropathology and begin to manifest two decades before the appearance of clinical symptoms.1,2 However, the high cost, low throughput, and exposure to radiation associated with PET and the perceived invasiveness and expertise associated with lumbar puncture have all highlighted the need for surrogate markers in the blood.

Plasma Aβ (Aβ1-42/Aβ1-40 ratio), p-tau181, glial fibrillary acidic protein (GFAP) and neurofilament light (NfL) are some of the putative blood-based biomarkers for AD.3,4 Circulating levels of these biomarkers have been reported to reflect AD-related neuropathological processes such as impaired clearance of brain Aβ, disruption of the axonal cytoskeletal structure, and reactive astrogliosis.3,5-9 Previous studies have reported lower plasma Aβ1-42 and Aβ1-42/Aβ1-40 ratio5,10-14 and higher plasma p-tau181 and GFAP in preclinical AD, prodromal AD, and AD dementia.5,6,12,15-17 In addition, blood-based NFL levels have been observed to be higher in both prodromal AD and AD dementia.18-20

However, head-to-head studies of the aforementioned plasma biomarkers across the AD continuum are lacking. Therefore, in the current study, we carried out a head-to-head comparison of plasma Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NFL alterations between Aβ-PET-negative (Aβ−) and Aβ-PET-positive (Aβ+) individuals across the AD continuum and evaluated the Aβ−/+ status predictive performance of these biomarkers against each other before and after the addition of AD risk factors, as well as evaluated their Aβ−/+ predictive performance as a biomarker panel before and after the addition of AD risk factors. In addition, we investigated the longitudinal changes in plasma biomarkers between the diagnostic groups over 36 months and investigated the association of plasma biomarkers at baseline with prospective cognitive decline and brain Aβ-PET load over a duration of 7 to 10 years.

METHODS

Participants

Participants were from the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) cohort. Participant exclusion criteria are described in detail elsewhere.21 Briefly, exclusion criteria comprised a history of non-AD dementia, schizophrenia, bipolar disorder, significant current (but not past) depression, Parkinson disease, cancer (other than basal cell skin carcinoma) within the last 2 years, symptomatic stroke, uncontrolled diabetes, or current regular alcohol use exceeding two standard drinks per day for women or four per day for men. Participants were classified as individuals with AD based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRSA) criteria22 and mild cognitive impairment (MCI) based on reduced cognitive performance often involving memory, representing a high-risk state for the development of AD.23,24 Participants were defined as preclinical AD (cognitively unimpaired [CU] Aβ+), prodromal AD (MCI Aβ+), or AD (AD Aβ+) for cross-sectional analyses based on clinical criteria and Aβ+ status. Plasma Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NFL data were available for 225 participants (81 CU Aβ−, 39 CU Aβ+, 26 MCI Aβ−, 33 MCI Aβ+, and 46 AD Aβ+) at timepoint 1. Follow-up samples were not available for 49 of the 225 participants at timepoint 1. Therefore, plasma biomarker data at the 18- and 36-month follow-up timepoints were available for 80 CU Aβ− (79 CU Aβ− for p-tau181), 40 CU Aβ+, 13 MCI Aβ−, 14 MCI Aβ+, and 29 AD Aβ+ (28 AD Aβ+ for p-tau181) participants. Aβ−/+ status for participants who did not undergo an Aβ-PET scan at any given timepoint was determined from the previous/next immediate timepoint. Participants were defined as CU (n = 120), MCI (n = 27), or AD (n = 29) based on clinical criteria only, for longitudinal analyses, albeit all AD were Aβ+. All participants provided written informed consent before participation. This study was approved by the Human Research Ethics Committees of St. Vincent’s Health (HREC/028/06) and Austin Health (HREC/18/Austin/201) in Melbourne and Hollywood Private Hospital (HPH215) and Edith Cowan University (ECU1878 Martins) in Perth, and Macquarie University (520221061636006) in Sydney.

Measurement of plasma p-tau181, Aβ1-40, Aβ1-42, GFAP, and NFL

Ethylenediaminetetraacetic acid (EDTA) plasma p-tau181, Aβ1-40, Aβ1-42, GFAP, and NFL concentrations were measured utilizing the ultra-sensitive single molecule array (Simoa) platform. Level of p-tau181 was measured using the P-Tau 181 V2 Simoa Advantage Assay (QTX-103714, Quanterix, Billerica, MA), with calibrators and samples run in duplicates. Average Coefficient of Variation CV% for p-tau181 Aβ42/40, p-tau181, GFAP, and NFL are associated with prospective cognitive decline.

Aβ42/40, p-tau181, GFAP, and NFL are associated with increased PET Aβ load prospectively.
RESEARCH IN CONTEXT

1. Systematic Review: The authors reviewed the literature using PubMed. Several studies have been conducted on the diagnostic performance of individual plasma biomarkers; however, head-to-head comparisons of the putative Alzheimer’s disease (AD) plasma biomarkers cross-sectionally and longitudinally across the AD continuum are lacking.

2. Interpretation: Our findings suggest that among the plasma biomarkers included in this study, phosphorylated tau181 (p-tau181) performed ≥ the other biomarkers in predicting brain amyloid beta (Aβ)−/+ status across the AD continuum. However, a biomarker panel of Aβ1-42/Aβ1-40, p-tau181, and glial fibrillary acidic protein (GFAP) performed ≥ any of the biomarkers alone in predicting brain Aβ−/+ positron emission tomography (PET) status across the AD continuum. Longitudinally, Aβ1-42/Aβ1-40, p-tau181, and GFAP were altered in prodromal AD, and GFAP and neurofilament light (NFL) were altered in AD. Aβ1-42/Aβ1-40, p-tau181, GFAP, and NFL were associated with prospective cognitive decline and Aβ1-42/Aβ1-40, p-tau181, and GFAP were associated with increased Aβ PET load prospectively.

3. Future Directions: Further studies need to validate the current observations in independent cohorts including establishment of clinical cutoffs for implementation in clinical settings.

was 5.58%. Aβ1-40, Aβ1-42, GFAP, and NFL were measured using the Neurology 4-Plex E kit (QTX-103670, Quanterix, Billerica, MA), where calibrators were run in duplicates and samples in singlicates. Average CV% of previous batches run in duplicate in our laboratory for Aβ1-40, Aβ1-42, GFAP, and NFL were 1.56%, 2.91%, 3.26%, and 3.20%, respectively. Quality control (QC) was attained by assessing the levels of the positive controls provided in the Simoa kits. The analytical lowest limit of quantification was 0.338 pg/mL for p-tau181, 4.08 pg/mL for the positive controls provided in the Simoa kits. The analytical lowest CV% of previous batches run in duplicate in our laboratory for Aβ1-40, Aβ1-42, GFAP, and NFL were 1.56%, 2.91%, 3.26%, and 3.20%, respectively.

2.3 | Neuroimaging

All participants underwent Aβ PET imaging with either 11C-Pittsburgh Compound B (PiB), 18F-NAV4694 (NAV), 18F-Flutemetamol (FLUTE), or 18F-Florbetapir (FBP) to determine neocortical Aβ load. PiB, NAV, and FBP PET scan acquisition consisted of 20 min (4 x 5 min) dynamic scans acquired at 50 min after an intravenous bolus injection of 370 MBq (± 10%) for PiB or 185 MBq (± 10%) for NAV or FBP (± 10%). Similarly, the participants who received FLUTE also underwent a 20 min (4 x 5 min) PET acquisition starting at 90 min after injection of 185 MBq (± 10%) of FLUTE. All Aβ imaging results were expressed in Cen tiloids (CL). Aβ−PET scans were spatially normalized using CapAIBL. The standard CL method was applied to determine Aβ burden. A CL value >20 was selected to determine a high Aβ (Aβ+) scan.

2.4 | Neuropsychological testing

Participants underwent a comprehensive battery of neuropsychological tests as described previously. For this study, the primary measures used to examine global cognitive abilities were the Mini-Mental State Examination (MMSE; scores range from 0 to 30, indicating severe impairment to no impairment), Clinical Dementia Rating scale (CDR; scores range from 0 to 3, indicating no impairment to severe impairment), CDR-Sum of Boxes (CDR-SOB; scores range from 0 to 18, indicating no impairment to severe impairment), and the Preclinical Alzheimer Cognitive Composite (PACC) constructed using episodic memory, executive function, and orientation as described previously.

2.5 | Statistical analyses

Descriptive statistics including means and standard deviations were calculated for each group with comparisons employing Kruskal-Wallis tests for continuous variables with non-parametric distributions, general linear models for continuous variables with parametric distributions, and chi-square tests for categorical variables. Linear models employed to compare plasma biomarkers between groups cross-sectionally were adjusted for covariates age, sex, apolipoprotein E (APOE) ε4 carrier status, Aβ−PET tracer, and site. Logistic regression with Aβ−/+ as response was used to evaluate predictive models and receiver-operating characteristic (ROC) curves were constructed from the logistic scores. To determine the diagnostic performance of each protein in distinguishing between groups, the R package cut point was used. The areas under the curves (AUCs) for different plasma proteins were compared using DeLong test. Linear mixed-effects models were used to compare plasma biomarkers longitudinally between diagnostic groups and were adjusted for the covariates age, sex, APOE ε4 carrier status, Aβ−/+ status, and PET tracer. Associations between plasma biomarker levels at timepoint 1 with prospective longitudinal cognitive decline were investigated using linear mixed-effects models adjusting for age, sex, APOE ε4 carrier status, years of education, and Aβ−/+ status in all participants and in the cognitively unimpaired and cognitively impaired subsets. Associations between plasma biomarker levels at timepoint 1 with subsequent longitudinal Aβ−PET load were investigated using linear mixed-effects models adjusting for age, sex, APOE ε4 carrier status, and Aβ−/+ status in all participants and in the cognitively unimpaired and cognitively impaired subsets. The models utilized for the whole sample (all participants) also included cognitive status as an additional covariate. Cognitive data were available for an average period of 6.5 years and Aβ−PET data were available for an average period of 6.5 years and Aβ−PET data were available for an average
period of 4.5 years for participants whose plasma samples were available at timepoint 1. Plasma biomarkers were natural log transformed to better approximate normality and variance homogeneity as required for analyses. All analyses and data visualization were carried out using IBM SPSS (v27) or R (v4.0.4). p < 0.05 was considered as statistically significant and all statistical tests were two-tailed.

3 | RESULTS

3.1 | Cohort characteristics

Participant cohort characteristics are presented in Table 1. There was no significant difference in the frequency of males and females, mean age, or mean body mass index (BMI) between CU Aβ−, CU Aβ+, MCI Aβ−, MCI Aβ+, and AD Aβ+ groups; however, the frequency of the APOE ε4 carriers was significantly higher in the Aβ+ groups (CU Aβ+, MCI Aβ+, and AD Aβ+) compared with Aβ− groups (CU Aβ− and MCI Aβ−) as expected. Significant differences in cognitive performance between groups were observed, wherein lower MMSE and PACC scores and higher CDR-SOB scores were observed in MCI (Aβ− and Aβ+) and AD Aβ+ compared with CU (Aβ− and Aβ+) as expected. Timepoints 2 (Table S1A) and 3 (Table S1B) had similar cohort characteristics.

3.2 | Association of AD risk factors, age, sex, and APOE ε4 carrier status, and BMI with plasma biomarkers

Although plasma Aβ1-42/Aβ1-40 ratio was not observed to correlate with age, plasma p-tau181, GFAP, and NfL correlated with age in all participants, and after stratifying participants based on diagnosis, except in the AD group, where only plasma NfL was observed to correlate with age (Table S2A). Plasma GFAP was observed to be significantly higher in females compared with males in all participants and after stratification by diagnosis, following correction for potential confounding variables, except in the AD group (Table S2B). No significant differences in plasma biomarker levels were observed between APOE ε4 non-carriers and carriers in all participants and after stratification by diagnosis, following correction for potential confounding variables (Table S2C). Lower BMI, likely to be a consequence of the disease rather than a risk factor, correlated inversely with p-tau181, GFAP, and NfL (Table S2D).

3.3 | Cross-sectional comparison of plasma biomarkers between groups

3.3.1 | Aβ1-42/Aβ1-40 ratio

Plasma Aβ1-42/Aβ1-40 ratio was significantly lower in CU Aβ+, MCI Aβ+, and AD Aβ+ compared with CU Aβ− (p < 0.0001) and MCI Aβ− (p < 0.0001), whereas no significant difference was observed between CU Aβ+ and MCI Aβ− and MCI Aβ− and AD Aβ− and between CU Aβ− and MCI Aβ− (Figure 1). Similar observations were found after bias correction and bootstrapping with 1000 random samples (Table S3). Absolute value data of Aβ1-42 and Aβ1-40 at timepoint 1 are presented in Table S4.

3.3.2 | p-tau181

Plasma p-tau181 was significantly higher in CU Aβ+, MCI Aβ+, and AD Aβ− compared with CU Aβ− (p < 0.0001) and MCI Aβ− (p < 0.0001), whereas no significant difference was observed between CU Aβ+, MCI Aβ+, and AD Aβ+ compared with MCI Aβ− and AD Aβ+ (Figure 1). Similar observations were found after bias correction and bootstrapping with 1000 random samples, except that higher p-tau181 was also observed in AD Aβ+ compared with MCI Aβ+ (Table S3).

3.3.3 | GFAP

Plasma GFAP was significantly higher in CU Aβ+, MCI Aβ+, and AD Aβ+ compared with CU Aβ− (p < 0.0001) and MCI Aβ− (p < 0.0005), whereas no significant difference was observed between CU Aβ+ and MCI Aβ+ and between CU Aβ− and MCI Aβ−; however, plasma GFAP was observed to be higher in AD Aβ+ compared with CU Aβ+ (p < 0.01) and MCI Aβ+ (p < 0.001) (Figure 1). Similar observations were found after bias correction and bootstrapping with 1000 random samples (Table S3).

3.3.4 | NfL

Plasma NfL was significantly higher in MCI Aβ+ compared with CU Aβ− (p = 0.014) and MCI Aβ− (p = 0.031) and higher in AD Aβ+ compared with CU Aβ− (p < 0.0001), CU Aβ+ (p < 0.005), MCI Aβ+ (p < 0.001), and MCI Aβ− (p = 0.049) (Figure 1). Similar observations were found after bias correction and bootstrapping with 1000 random samples, except that no significant difference was observed in NfL levels between AD Aβ+ and MCI Aβ+ (p = 0.071, Table S3).

Mean differences and confidence intervals of Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NfL between CU Aβ−/MCI Aβ− and CU Aβ+/MCI Aβ+/AD Aβ+ are presented in Table S4. These observations were consistent before and after adjusting for covariates age, sex, APOE ε4 carrier status, Aβ-PET tracer, and site. Figure S1 shows similar findings at timepoints 2 and 3. Similar observations were noted on adding BMI as a covariate along with other covariates (data not shown).

3.4 | Diagnostic performance of plasma Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NfL

The diagnostic performance parameters of plasma biomarkers including AUCs, specificity, sensitivity, accuracy, negative predictive value,
### Table 1: Participant characteristics at timepoint 1

<table>
<thead>
<tr>
<th>Timepoint 1</th>
<th>Total Sample</th>
<th>CU Aβ−</th>
<th>CU Aβ+</th>
<th>MCI Aβ−</th>
<th>MCI Aβ+</th>
<th>AD Aβ+</th>
<th>P</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>225</td>
<td>81</td>
<td>39</td>
<td>26</td>
<td>33</td>
<td>46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex, Female (%)</td>
<td>50.67</td>
<td>53.09</td>
<td>51.28</td>
<td>46.15</td>
<td>39.39</td>
<td>56.52</td>
<td>0.606</td>
<td>-</td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
<td>74.23 (7.22)</td>
<td>73.74 (5.96)</td>
<td>74.9 (6.96)</td>
<td>71.31 (11.46)</td>
<td>75.61 (5.66)</td>
<td>75.17 (7.20)</td>
<td>0.234</td>
<td>-</td>
</tr>
<tr>
<td>Mean body mass index (SD)</td>
<td>26.19 (4.54)</td>
<td>26.71 (4.32)</td>
<td>25.29 (4.65)</td>
<td>27.28 (5.05)</td>
<td>25.84 (4.76)</td>
<td>25.69 (4.32)</td>
<td>0.339</td>
<td>-</td>
</tr>
<tr>
<td>APOE ε4 carriage, N (%)</td>
<td>104 (46.22)</td>
<td>21 (25.93)</td>
<td>21 (53.85)</td>
<td>2 (7.69)</td>
<td>24 (72.73)</td>
<td>36 (78.26)</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Mean MMSE (SD)</td>
<td>26.84 (4.15)</td>
<td>29.04 (1.03)</td>
<td>28.92 (1.24)</td>
<td>27.27 (1.89)</td>
<td>27.58 (1.48)</td>
<td>20.41 (4.87)</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Mean CDR-SOB (SD)</td>
<td>1.43 (2.66)</td>
<td>0.025 (0.11)</td>
<td>0.026 (0.11)</td>
<td>0.519 (0.264)</td>
<td>0.606 (0.325)</td>
<td>6.21 (2.36)</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Mean PACC score (SD)</td>
<td>−0.844 (1.53)</td>
<td>0.175 (0.65)</td>
<td>0.177 (0.74)</td>
<td>−1.105 (0.80)</td>
<td>−1.446 (0.53)</td>
<td>−3.55 (0.77)</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Aβ PET tracer</td>
<td>148/4/65/8</td>
<td>51/1/28/1</td>
<td>22/0/17/0</td>
<td>20/1/5/0</td>
<td>23/0/8/2</td>
<td>32/2/7/5</td>
<td>0.021</td>
<td>-</td>
</tr>
<tr>
<td>Mean Aβ PET Centiloid (SD)</td>
<td>41.65 (46.65)</td>
<td>1.31 (6.70)</td>
<td>61 (26.85)</td>
<td>0.30 (7.01)</td>
<td>77.63 (30.01)</td>
<td>102.31 (28.55)</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Mean hippocampal volume, right, cm³ (SD)</td>
<td>2.79 (0.43)</td>
<td>2.97 (0.31)</td>
<td>2.98 (0.27)</td>
<td>2.91 (0.30)</td>
<td>2.7 (0.33)</td>
<td>2.15 (0.31)</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Mean hippocampal volume, left, cm³ (SD)</td>
<td>2.72 (0.44)</td>
<td>2.89 (0.31)</td>
<td>2.89 (0.28)</td>
<td>2.84 (0.36)</td>
<td>2.74 (0.30)</td>
<td>2.04 (0.31)</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Mean Aβ1-42/ Aβ1-40 ratio (SD)</td>
<td>0.054 (0.011)</td>
<td>0.058 (0.010)</td>
<td>0.047 (0.008)</td>
<td>0.062 (0.011)</td>
<td>0.050 (0.008)</td>
<td>0.049 (0.007)</td>
<td>&lt;0.0001^a</td>
<td>&lt;0.0001^a</td>
</tr>
<tr>
<td>Mean p-tau181 pg/mL (SD)</td>
<td>3.01 (1.64)</td>
<td>2.16 (1.14)</td>
<td>3.67 (2.02)</td>
<td>1.87 (0.74)</td>
<td>3.65 (1.39)</td>
<td>4.12 (1.42)</td>
<td>&lt;0.0001^a</td>
<td>&lt;0.0001^a</td>
</tr>
<tr>
<td>Mean GFAP pg/mL (SD)</td>
<td>179.60 (85.09)</td>
<td>135.06 (54.67)</td>
<td>205.26 (84.76)</td>
<td>205.26 (84.76)</td>
<td>205.26 (84.76)</td>
<td>205.26 (84.76)</td>
<td>&lt;0.0001^a</td>
<td>&lt;0.0001^a</td>
</tr>
<tr>
<td>Mean NFL pg/mL (SD)</td>
<td>25.66 (14.05)</td>
<td>22.46 (11.62)</td>
<td>25.15 (10.56)</td>
<td>20.49 (10.00)</td>
<td>28.56 (17.80)</td>
<td>32.58 (16.66)</td>
<td>&lt;0.0001^a</td>
<td>&lt;0.0001^a</td>
</tr>
</tbody>
</table>

Kruskal-Wallis tests were used for continuous variables with non-parametric distributions and general linear models were used for continuous variables with parametric distributions, whereas chi-square tests were used for categorical variables. Data for composite AIBL PACC scores are presented for 79 CU Aβ−, 39 CU Aβ+, 25 MCI Aβ−, 32 MCI Aβ+, and 35 AD individuals, data for hippocampal volume are presented for 73 CU Aβ−, 35 CU Aβ+, 17 MCI Aβ−, 21 MCI Aβ+, and 31 AD individuals and Centiloid data are presented for 81 CU Aβ−, 39 CU Aβ+, 24 MCI Aβ−, 30 MCI Aβ+, and 40 AD individuals based on data availability. Aβ−/+ status for participants who did not undergo an Aβ PET scan at timepoint 1 was determined from the next immediate timepoint. CU individuals comprised 55 non-subjective memory complainers (non-SMC: Aβ− = 39, Aβ+ = 16) and 65 SMC (Aβ− = 42, Aβ+ = 23). P^a are adjusted for age, sex, site, APOE ε4 carriage, and Aβ PET tracer, p < 0.05 was considered significant. †Represents plasma biomarkers natural log transformed to better approximate normality and variance heterogeneity. CU: cognitively unimpaired, MCI: mild cognitively impaired, AD: Alzheimer’s disease, MMSE: Mini-Mental State Examination, CDR-SOB: Clinical Dementia Rating Sum of Boxes, PACC score: Preclinical Alzheimer Cognitive Composite score, Aβ: amyloid beta, PIB: C Pittsburgh Compound B, NAV: F-NAV4694, FLUTE: F-Flutemetamol, FBP: F-Florbetapir, PET: positron emission tomography, p-tau181: phosphorylated-tau 181, GFAP: glial fibrillary acidic protein, NFL: neurofilament light chain.

Positive predictive value, and Youden’s optimal cut point are presented in Table S5.

#### 3.4.1 CU Aβ− versus CU Aβ+

The AUCs of Aβ1-42/Aβ1-40 ratio (AUC = 0.836), p-tau181 (AUC = 0.805), and GFAP (AUC = 0.749) were significantly different, but all had significantly higher AUCs than NFL (AUC = 0.609, p < 0.01) in distinguishing between the groups (Table S6A, Figure 2).

#### 3.4.2 CU Aβ− versus MCI Aβ+

P-tau181 had a significantly higher AUC (AUC = 0.858) than GFAP (AUC = 0.716, p = 0.019) and NFL (AUC = 0.641, p < 0.001), but not
FIGURE 1  Boxplots comparing plasma Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NfL between CU Aβ-, CU Aβ+, MCI Aβ-, MCI Aβ+, and AD Aβ+ groups at timepoint 1. Plasma measures were compared between groups using linear models with age, sex, APOE ε4 carrier status, PET tracer, and site as covariates. Data from 81 CU Aβ-, 39 CU Aβ+, 26 MCI Aβ-, 33 MCI Aβ+, and 46 AD Aβ+ participants were utilized for analyses. The line segments within each boxplot represent the median of the data. p-values were obtained from natural log-transformed plasma biomarker data to better approximate normality and variance homogeneity. p < 0.05 was considered statistically significant.

compared with Aβ1-42/Aβ1-40 ratio (AUC = 0.772) in distinguishing between the groups (Table S6B, Figure 2).

3.4.3 | CU Aβ− versus AD Aβ+

p-tau181 (AUC = 0.920) and GFAP (AUC = 0.904) had significantly higher AUCs than Aβ1-42/Aβ1-40 ratio (AUC = 0.784, p < 0.01) and NfL (AUC = 0.717, p < 0.0001) in distinguishing between the groups (Table S6C, Figure 2).

3.4.4 | MCI Aβ− versus MCI Aβ+

p-tau181 (AUC = 0.902) had a significantly higher AUC compared with GFAP (AUC = 0.730, p < 0.01) and NfL (AUC = 0.646, p < 0.0001), but not compared with Aβ1-42/Aβ1-40 ratio (AUC = 0.825) in distinguishing between the groups (Table S6D, Figure 2).

3.4.5 | MCI Aβ− versus AD Aβ+

p-tau181 (AUC = 0.957) had a significantly higher AUC compared with Aβ1-42/Aβ1-40 ratio (AUC = 0.839, p = 0.036) and NfL (AUC = 0.741, p < 0.0001), but not compared with GFAP (AUC = 0.868) in distinguishing between the groups (Table S6E, Figure 2).

3.5 | Diagnostic performance of plasma Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NfL along with AD risk factors

3.5.1 | CU Aβ− versus CU Aβ+

On adding the plasma biomarkers to a base model (BM) incorporating the AD risk factors age, sex, and APOE ε4 allele carrier status, Aβ1-42/Aβ1-40 ratio+BM (AUC = 0.859), p-tau181+BM (AUC = 0.812), and GFAP+BM (AUC = 0.826) had no significant differences between their AUCs but had significantly higher AUCs compared with the BM (AUC = 0.694, p < 0.01) and NfL+BM (AUC = 0.708, p < 0.01) in distinguishing between the groups (Table S7A, Figure 2).

3.5.2 | CU Aβ− versus MCI Aβ+

Aβ1-42/Aβ1-40 ratio+BM (AUC = 0.884) and p-tau181+BM (AUC = 0.874) had significantly higher AUCs than BM (AUC = 0.809,
FIGURE 2  Receiver-operating characteristic (ROC) curves for distinguishing between (A) CU Aβ− and CU Aβ+, (B) CU Aβ− and MCI Aβ+, (C) CU Aβ− and AD Aβ+, (D) MCI Aβ− and MCI Aβ+, and (E) MCI Aβ− and AD Aβ+ participants at timepoint 1. ROC curves are presented for (i) Aβ1-42/Aβ1-40, p-tau181, GFAP, and NFL, Aβ1-42/Aβ1-40+p-tau181+GFAP, and Aβ1-42/Aβ1-40+p-tau181+GFAP+NFL and (ii) base model comprising AD risk factors, age, sex, APOE ε4 allele status (BM), BM+Aβ1-42/Aβ1-40, BM+p-tau181, BM+GFAP, BM+NFL, BM+Aβ1-42/Aβ1-40+p-tau181+GFAP, and BM+Aβ1-42/Aβ1-40+p-tau181+GFAP+NFL. Data from 81 CU Aβ−, 39 CU Aβ+, 26 MCI Aβ−, 33 MCI Aβ+, and 46 AD Aβ+ participants were utilized for analyses. AUC: area under the curve; CI: confidence interval.
FIGURE 2 Continued

3.5.3 CU Aβ− versus AD Aβ+

Aβ1-42/Aβ1-40 ratio+BM (AUC = 0.884), p-tau181+BM (AUC = 0.910), GFAP+BM (AUC = 0.959), and NfL+BM (AUC = 0.866)
had significantly higher AUCs than BM (AUC = 0.803, \( p = 0.018 \)), and GFAP+BM had a significantly higher AUC than \( A_\beta^{1-42}/A_\beta^{1-40} \) ratio+BM (\( p < 0.01 \)) and NfL+BM (\( p < 0.01 \)) in distinguishing between the groups (Table S7C, Figure 2).

### 3.5.4 MCI A\( \beta^- \) versus MCI A\( \beta^+ \)

\( A_\beta^{1-42}/A_\beta^{1-40} \) ratio+BM (AUC = 0.952) had a significantly higher AUC compared with BM (AUC = 0.900, \( p = 0.048 \)), and p-tau181+BM (AUC = 0.958) had significantly higher AUCs compared with BM (\( p = 0.018 \)), GFAP+BM (AUC = 0.911, \( p = 0.028 \)), and NfL+BM (AUC = 0.904, \( p = 0.015 \)) in distinguishing between the groups (Table S7D, Figure 2).

### 3.5.5 MCI A\( \beta^- \) versus AD A\( \beta^+ \)

\( A_\beta^{1-42}/A_\beta^{1-40} \) ratio+BM (AUC = 0.947), p-tau181+BM (AUC = 0.969), and GFAP+BM (AUC = 0.965) had significantly higher AUCs compared with BM (AUC = 0.895, \( A_\beta^{1-42}/A_\beta^{1-40} \) ratio+BM: \( p = 0.032 \), p-tau181+BM: \( p < 0.01 \), GFAP+BM; \( p = 0.013 \)), but not compared with NfL+BM (AUC = 0.926) in distinguishing between the groups (Table S7E, Figure 2).

In addition, we assessed whether combining the BM with the plasma biomarkers significantly improved plasma biomarker diagnostic performance. In distinguishing between CU A\( \beta^- \) and CU A\( \beta^+ \), we noted a significantly higher AUC when combining BM with GFAP in a model compared with GFAP alone (\( p = 0.049 \)). In distinguishing between MCI A\( \beta^- \) groups, CU A\( \beta^- \) and AD A\( \beta^+ \) groups, MCI A\( \beta^- \) and AD A\( \beta^+ \) groups, and MCI A\( \beta^- \) and AD A\( \beta^+ \) groups, we noted significantly higher AUCs when combining BM with GFAP, BM with p-tau181, and BM with NfL compared with BM alone (\( p = 0.019 \), CU A\( \beta^- \) vs. AD A\( \beta^+ \); \( p = 0.011 \), MCI A\( \beta^- \) vs. MCI A\( \beta^+ \); \( p = 0.014 \), MCI A\( \beta^- \) vs. AD A\( \beta^+ \); \( p = 0.017 \), and BM with GFAP compared with GFAP alone (\( p < 0.01 \), MCI A\( \beta^- \) vs. MCI A\( \beta^+ \); \( p < 0.01 \), CU A\( \beta^- \) vs. AD A\( \beta^+ \); \( p < 0.01 \), MCI A\( \beta^- \) vs. MCI A\( \beta^+ \); \( p = 0.028 \)). No significant difference in diagnostic performance of p-tau181 across the AD continuum was observed before and after the addition of the BM (Table S8).

### 3.6 Diagnostic performance of a panel of plasma biomarkers comprising \( A_\beta^{1-42}/A_\beta^{1-40} \) ratio, p-tau181, GFAP, and NfL

#### 3.6.1 CU A\( \beta^- \) versus CU A\( \beta^+ \)

A model incorporating \( A_\beta^{1-42}/A_\beta^{1-40} \) ratio, p-tau181, and GFAP (with and without NfL) had a significantly higher AUC (AUC = 0.898, \( A_\beta^{1-42}/A_\beta^{1-40} \) ratio: \( p = 0.016 \), p-tau181: \( p < 0.01 \), GFAP: \( p < 0.001 \), NfL: \( p < 0.001 \)).
p < 0.0001) than any of these proteins alone in distinguishing between the groups (Table S6A, Figure 2).

3.6.2  |  CU Aβ− versus MCI Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) had a significantly higher AUC (AUC = 0.886) compared with the AUC of Aβ1-42/Aβ1-40 ratio (p < 0.01), GFAP (p < 0.001), and NfL (p < 0.0001), but not p-tau181 in distinguishing between the groups (Table S6B, Figure 2).

3.6.3  |  CU Aβ− versus AD Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) had a significantly higher AUC (AUC = 0.958) compared with the AUC of Aβ1-42/Aβ1-40 ratio (p < 0.0001), GFAP (p < 0.01), and NfL (p < 0.0001), but not p-tau181 in distinguishing between the groups (Table S6C, Figure 2).

3.6.4  |  MCI Aβ− versus MCI Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) had a significantly higher AUC (AUC = 0.941) compared with the AUC of Aβ1-42/Aβ1-40 ratio (p = 0.011), GFAP (p < 0.01), and NfL (p < 0.0001), but not p-tau181 in distinguishing between the groups (Table S6D, Figure 2).

MCI Aβ− versus AD Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) had a significantly higher AUC (AUC = 0.967) compared with the AUC of Aβ1-42/Aβ1-40 ratio (p < 0.01), GFAP (p = 0.012), and NfL (p < 0.001), but not p-tau181 in distinguishing between the groups (Table S6E, Figure 2).

3.7  |  Diagnostic performance of a panel of plasma biomarkers comprising plasma Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NfL along with AD risk factors

3.7.1  |  CU Aβ− versus CU Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) along with BM was observed to have a significantly higher AUC (AUC = 0.924) than Aβ1-42/Aβ1-40 ratio+BM (p = 0.014), p-tau181+BM (p < 0.01), GFAP+BM (p < 0.01), and NfL+BM (p < 0.0001) in distinguishing between the groups (Table S7A, Figure 2).

3.7.2  |  CU Aβ− versus MCI Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) along with BM was observed to have a significantly higher AUC (AUC = 0.938) than Aβ1-42/Aβ1-40 ratio+BM (p = 0.026), p-tau181+BM (p < 0.01), GFAP+BM (p < 0.01), and NfL+BM (p < 0.001) in distinguishing between the groups (Table S7B, Figure 2).

3.7.3  |  CU Aβ− versus AD Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) along with BM was observed to have a significantly higher AUC (AUC = 0.978) than Aβ1-42/Aβ1-40 ratio+BM (p < 0.001), p-tau181+BM (p < 0.01), GFAP+BM (p = 0.016), and NfL+BM (p < 0.001) in distinguishing between the groups (Table S7C, Figure 2).

3.7.4  |  MCI Aβ− versus MCI Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) along with BM was observed to have a significantly higher AUC (AUC = 0.976) than BM (p = 0.018), GFAP+BM (p = 0.027), and NfL+BM (p = 0.016), but not p-tau181 or Aβ1-42/Aβ1-40 ratio, in distinguishing between the groups (Table S7D, Figure 2).

3.7.5  |  MCI Aβ− versus AD Aβ+

A model incorporating Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP (with and without NfL) along with BM was observed to have a significantly higher AUC (AUC = 0.988) than BM (p < 0.01), Aβ1-42/Aβ1-40 ratio+BM (p = 0.025), NfL+BM (p = 0.013), but not GFAP+BM and p-tau181+BM, in distinguishing between the groups (Table S7E, Figure 2).

In addition, whether combining the BM with the plasma biomarker panel significantly improved the diagnostic performance of the plasma biomarker panel was assessed. No significant improvement was observed after combining the BM with the plasma biomarker panel when compared with the plasma biomarker panel in distinguishing CU Aβ− versus CU Aβ+, MCI Aβ− versus MCI Aβ+, and MCI Aβ− versus AD Aβ+ groups. In distinguishing between CU Aβ− and MCI Aβ+, significantly higher AUCs were noted on combining the BM with the plasma biomarker panel compared with the plasma biomarker panel alone (p = 0.043) (Table S9).

3.8  | Longitudinal changes in plasma biomarkers in MCI and AD compared with CU

Plasma Aβ1-42/Aβ1-40 ratio decreased significantly (p = 0.024), and plasma p-tau181 (p ≤ 0.01) and GFAP (p < 0.01) increased
Figure 3 Longitudinal changes in plasma biomarkers over 36 months between CU, MCI, and AD groups. Estimated marginal means of plasma biomarkers Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NfL for CU (blue), MCI (yellow), and AD (red) participants are presented at three timepoints, 18 months apart. Data for Aβ1-42/Aβ1-40 ratio, GFAP, and NfL are presented in 120 CU, 27 MCI, and 29 AD participants and for p-tau181 are presented in 119 CU, 27 MCI, and 28 AD. Error bars represent ±1SE.

Table 2 Longitudinal changes in plasma biomarkers over 36 months in MCI and AD individuals compared to CU individuals

<table>
<thead>
<tr>
<th></th>
<th>CU versus MCI</th>
<th></th>
<th>CU versus AD</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>B (SE)</td>
<td>p</td>
<td>B (SE) a</td>
<td>p a</td>
</tr>
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<td>0.008</td>
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<td>GFAP</td>
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<td>0.059 (0.023)</td>
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<td></td>
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<tr>
<td>NFL</td>
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<td>0.630</td>
<td>−0.009 (0.020)</td>
<td>0.653</td>
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<tr>
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<td>2e-04</td>
<td>0.071 (0.019)</td>
<td>2e-04</td>
</tr>
</tbody>
</table>

Longitudinal changes in plasma proteins were compared between CU and MCI participants and, CU and AD participants, using linear mixed models, before and after (P) adjustment for the covariates age, sex, APOE ε4 carrier status, Aβ−/+ PET status, and Aβ PET tracer. Data from 120 CU, 27 MCI, and 29 AD participants were utilized for Aβ1-42/Aβ1-40 ratio, GFAP, and NfL and from 119 CU, 27 MCI, and 28 AD participants for p-tau181. CU: cognitively unimpaired, MCI: mild cognitively impaired, AD: Alzheimer’s disease. Plasma biomarkers were natural log transformed to better approximate normality and variance homogeneity. p < 0.05 was considered significant.

Significantly in MCI compared with CU over 36 months before and after correcting for covariates age, sex, APOE ε4 carrier status, Aβ−/+ status, and tracer (Table 2). In addition, plasma GFAP (p = 0.049) and NfL (p < 0.001) increased significantly in AD compared with CU over 36 months before and after correcting for covariates (Figure 3, Figure S2, Table 2).
3.9 | Association of baseline plasma biomarker levels with prospective cognitive decline and Aβ-PET load

Analyses were performed to investigate whether plasma biomarker levels from a single timepoint were associated with prospective cognitive decline and cerebral Aβ accumulation. In all participants, lower baseline plasma Aβ1-42/Aβ1-40 ratio was associated with increased future cognitive decline (MMSE: 0.041; CDR-SOB: 0.049) and higher baseline p-tau181 (MMSE: p < 0.0001; CDR-SOB: p < 0.0001; PACC: p < 0.0001), GFAP (MMSE: p < 0.0001; CDR-SOB: p < 0.0001; PACC: p < 0.0001), and NfL (MMSE: p < 0.0001; CDR-SOB: p < 0.0001; PACC: p < 0.0001) measures were observed to be associated with increased future cognitive decline (Table 3). On stratifying participants based on cognitive status, in cognitively unimpaired participants, baseline plasma Aβ1-42/Aβ1-40 ratio was not observed to be associated with future cognitive decline; however, higher baseline plasma p-tau181 (PACC: p < 0.001), GFAP (PACC: p = 0.020) and NfL (MMSE: p = 0.019; CDR-SOB: p = 0.046) measures were observed to be associated with increased future cognitive decline (Table 3). In cognitively impaired participants (MCI and AD), lower baseline plasma Aβ1-42/Aβ1-40 ratio was associated significantly with prospective decline in CDR-SOB (p = 0.020). Furthermore, higher baseline plasma p-tau181 (MMSE: p < 0.0001; CDR-SOB: p < 0.0001; PACC: p < 0.0001), GFAP (MMSE: p < 0.0001; CDR-SOB: p < 0.0001; PACC: p < 0.01), and NfL (MMSE: p < 0.01; CDR-SOB: p < 0.01; PACC: p < 0.01) measures were observed to be associated with increased future cognitive decline (Table 3). In addition, lower baseline plasma Aβ1-42/Aβ1-40 ratio (p < 0.001) and higher p-tau181 (p < 0.0001) and GFAP (p < 0.01) were observed to be associated with increased future Aβ-PET load in all participants; however, upon stratification by cognitive impairment status, the preceding observations remained significant only in cognitively unimpaired participants. Relationships between low and high plasma biomarker levels at baseline (based on the optimal cut point at Youden’s index for comparisons between CU Aβ– and AD Aβ+) and the rate of change in cognition and brain Aβ–PET load are presented in Figure S3.

4 | DISCUSSION

In the current study we showed that plasma Aβ1-42/Aβ1-40 ratio was lower, and p-tau181 and GFAP levels were higher in Aβ+ individuals across the AD continuum, and that plasmaNFL levels were higher in cognitively impaired Aβ+ individuals compared with controls. p-tau181 followed by GFAP showed the highest change in magnitude in Aβ+ compared with Aβ– individuals along the AD continuum. To our knowledge this is the first head-to-head study cross-sectionally investigating plasma Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NFL along the AD continuum employing Aβ+ defined preclinical AD, prodromal AD, and AD participants in a highly characterized Australian cohort utilizing an ultrasensitive platform. We also showed that Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP had non-significant differences in their discriminative capabilities for preclinical AD based on AUCs, and outperformed NFL. In the cognitively impaired stages, we showed that p-tau181 outperformed NFL and Aβ1-42/Aβ1-40 ratio or GFAP. Furthermore we showed that combining plasma biomarkers (particularly Aβ1-42/Aβ1-40 ratio, p-tau181, or GFAP) with the known AD risk factors, age, sex, and APOE ε4 carrier status, most often significantly improved the discriminative performance of the known AD risk factors between CU Aβ+/MCI Aβ+/AD Aβ+ and Aβ– CU individuals. On the other hand, we also showed that although the discriminative performance of Aβ1-42/Aβ1-40 ratio, GFAP, and NFL improved when the AD risk factors were combined with the plasma biomarkers, this was not the case for p-tau181. In our longitudinal analyses, we showed that the plasma Aβ1-42/Aβ1-40 ratio decreased and p-tau181 increased in MCI participants, GFAP increased in MCI and AD participants, and NFL increased in AD participants over 36 months compared with controls. We also showed that baseline plasma Aβ1-42/Aβ1-40 ratio, p-tau181, GFAP, and NFL levels are associated with prospective cognitive decline and baseline plasma Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP are associated with prospective Aβ-PET load.

Our observations of lower plasma Aβ1-42/Aβ1-40 ratio,10,12,29 and elevated plasma p-tau18115,16,29,30 and GFAP12,17,31 in preclinical AD, prodromal AD, and AD, corroborate findings from earlier studies; however, in the current study we did not always observe a consistent progressive magnitude decrease in plasma Aβ1-42/Aβ1-40 ratio or increase in plasma p-tau181 levels and GFAP levels across the AD continuum. Further validation studies are required to confirm whether these observations could be attributed to the differences in sample size between groups. Our observations of elevated NFL in prodromal AD and AD but not in Aβ+ defined preclinical AD are also in line with previous studies.32–34 In addition, abnormal NFL levels have been reported in other neurological diseases, such as multiple sclerosis,35 Parkinson disease36,37 and other diseases affecting the central nervous system,38 thus serving as a putative marker of neurological insults or ongoing neuroaxonal damage but unspecific to AD.

Although head-to-head studies for plasma biomarkers across the AD continuum are largely missing, one study reported that p-tau181 outperformed Aβ1-42/Aβ1-40 ratio, GFAP, and NFL in differentiating between AD and CU; however, unlike the current study, these findings are not from Aβ–/+ status confirmed participants.3 A study demonstrated that diagnosis of AD based on clinical criteria has limited sensitivity and specificity,29 whereas Aβ-PET and CSF biomarkers have over 90% sensitivity and specificity.40,41 In the current study, we observed that there was no significant difference in the discriminative performance of p-tau181 and GFAP between AD Aβ+ and CU Aβ–, and that both outperformed Aβ1-42/Aβ1-40 ratio and NFL. Our observations of non-significant differences between the AUCs of p-tau181 and GFAP in CU Aβ– versus CU Aβ+ are in line with our previous observations in an independent cohort, wherein plasma p-tau181 and GFAP had non-significant differences in their discriminative capabilities for preclinical AD and both significantly outperformed plasma NFL.16 Strikingly, in the current study at timepoint 1, plasma Aβ1-42/Aβ1-40 ratio showed unexpectedly high AUCs in differentiating between CU Aβ– and CU Aβ+ (AUC = 0.84, 95% CI: 0.77–0.91),


### TABLE 3  Association of baseline plasma biomarkers with longitudinal cognitive decline and brain Aβ-PET load

<table>
<thead>
<tr>
<th></th>
<th>Aβ42/40 ratio</th>
<th>P-tau181</th>
<th>GFAP</th>
<th>NfL</th>
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<tr>
<td><strong>MMSE</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>All participants</strong></td>
<td>0.911 (0.442)</td>
<td>-0.927 (0.177)</td>
<td>-0.870 (0.180)</td>
<td>-0.884 (0.199)</td>
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<td><strong>B (SE)</strong></td>
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<td>5.52E-07</td>
<td>3.29E-06</td>
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<td><strong>P</strong></td>
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<td>3.29E-06</td>
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<td><strong>CU participants</strong></td>
<td>0.094 (0.090)</td>
<td>-0.029 (0.042)</td>
<td>-0.074 (0.041)</td>
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<tr>
<td><strong>B (SE)</strong></td>
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<td>0.499</td>
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</tr>
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<td><strong>P</strong></td>
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<td><strong>CDR-SOB</strong></td>
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<td><strong>All participants</strong></td>
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<td>-0.027 (0.035)</td>
<td>0.012 (0.016)</td>
<td>0.011 (0.017)</td>
<td>0.028 (0.019)</td>
</tr>
<tr>
<td><strong>B (SE)</strong></td>
<td>0.441</td>
<td>0.460</td>
<td>0.507</td>
<td>0.131</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.441</td>
<td>0.460</td>
<td>0.507</td>
<td>0.131</td>
</tr>
<tr>
<td><strong>CI participants</strong></td>
<td>-1.209 (0.509)</td>
<td>0.932 (0.172)</td>
<td>0.765 (0.173)</td>
<td>0.608 (0.186)</td>
</tr>
<tr>
<td><strong>B (SE)</strong></td>
<td>0.020</td>
<td>7.63E-07</td>
<td>3.37E-05</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.020</td>
<td>7.63E-07</td>
<td>3.37E-05</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>PACC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All participants</strong></td>
<td>0.069 (0.042)</td>
<td>-0.100 (0.018)</td>
<td>-0.070 (0.018)</td>
<td>-0.090 (0.020)</td>
</tr>
<tr>
<td><strong>B (SE)</strong></td>
<td>0.102</td>
<td>9.76E-08</td>
<td>2.05E-04</td>
<td>1.35E-05</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.102</td>
<td>9.76E-08</td>
<td>2.05E-04</td>
<td>1.35E-05</td>
</tr>
<tr>
<td><strong>CU participants</strong></td>
<td>0.034 (0.038)</td>
<td>-0.064 (0.017)</td>
<td>-0.042 (0.018)</td>
<td>-0.041 (0.020)</td>
</tr>
<tr>
<td><strong>B (SE)</strong></td>
<td>0.374</td>
<td>3.37E-04</td>
<td>0.020</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.374</td>
<td>3.37E-04</td>
<td>0.020</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>CI participants</strong></td>
<td>0.213 (0.141)</td>
<td>-0.214 (0.048)</td>
<td>-0.166 (0.048)</td>
<td>-0.156 (0.049)</td>
</tr>
<tr>
<td><strong>B (SE)</strong></td>
<td>0.139</td>
<td>6.66E-05</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.139</td>
<td>6.66E-05</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Aβ-PET</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All participants</strong></td>
<td>-6.035 (1.555)</td>
<td>2.823 (0.675)</td>
<td>2.075 (0.708)</td>
<td>1.473 (0.786)</td>
</tr>
<tr>
<td><strong>B (SE)</strong></td>
<td>1.56E-04</td>
<td>4.72E-05</td>
<td>0.003</td>
<td>0.063</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>1.56E-04</td>
<td>4.72E-05</td>
<td>0.003</td>
<td>0.063</td>
</tr>
<tr>
<td><strong>CU participants</strong></td>
<td>-6.014 (1.521)</td>
<td>2.844 (0.706)</td>
<td>2.215 (0.767)</td>
<td>1.212 (0.866)</td>
</tr>
<tr>
<td><strong>B (SE)</strong></td>
<td>1.28E-04</td>
<td>9.71E-05</td>
<td>0.005</td>
<td>0.165</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>1.28E-04</td>
<td>9.71E-05</td>
<td>0.005</td>
<td>0.165</td>
</tr>
<tr>
<td><strong>CI participants</strong></td>
<td>-5.664 (4.302)</td>
<td>2.711 (1.656)</td>
<td>1.619 (1.569)</td>
<td>1.467 (1.670)</td>
</tr>
<tr>
<td><strong>B (SE)</strong></td>
<td>0.196</td>
<td>0.107</td>
<td>0.307</td>
<td>0.384</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.196</td>
<td>0.107</td>
<td>0.307</td>
<td>0.384</td>
</tr>
</tbody>
</table>

Relationships between plasma biomarkers and change in cognition (represented by MMSE, CDR-SOB, and PACC scores) were assessed using linear mixed effects models adjusting for age, sex, APOE ε4 carrier status, and years of education. Models for all participants were also adjusted for cognitive status. p < 0.05 was considered as statistically significant. Plasma biomarkers were natural log transformed to better approximate normality and variance homogeneity.
not seen previously using the Simoa platform. Similar analyses between the same CU Aβ− and CU Aβ+ participants at follow-up visit timepoint 2 generated an AUC of 0.78 (95% CI: 0.70-0.87) and timepoint 3 generated AUC = 0.79 (95% CI: 0.70-0.87). It could be posited that this superior performance of plasma Aβ1-42/Aβ1-40 ratio in preclinical AD at timepoint 1 compared to the later timepoints may be reflective of the nature of the early changes of this biomarker in the AD pathogenesis trajectory; however, further confirmatory studies are required.

Combining plasma biomarkers (particularly Aβ1-42/Aβ1-40 ratio, p-tau181, or GFAP) with the known AD risk factors most often significantly improved the discriminative performance of the AD risk factors between CU Aβ+ /MCI Aβ+ /AD Aβ+ and Aβ−/ CU individuals. However, combining the AD risk factors with the plasma biomarkers improved the discriminative performance of Aβ1-42/Aβ1-40 ratio, GFAP, and NfL but not p-tau181. Similar to our findings, previous studies have reported improved plasma Aβ1-42/Aβ1-40 ratio or GFAP performance when combined with AD risk factors in differentiating between Aβ−/− individuals, whereas plasma p-tau181 combined with AD risk factors did not significantly perform better than p-tau181 alone. This may suggest that p-tau181 levels are largely independent of age, sex, and APOE ε4 carrier status in distinguishing CU Aβ+, MCI Aβ+, and AD Aβ+ from Aβ−/ CU individuals.

Furthermore, our observations within the current study suggest that employing a panel of plasma biomarkers comprising Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP may provide better discriminative performance than individual plasma biomarkers, particularly when combined with the AD risk factors. In line with our observations, Janelidze and colleagues reported a significantly higher AUC when combining p-tau181 with Aβ42/Aβ40 ratio compared with Aβ42/Aβ40 ratio alone in differentiating between Aβ− and Aβ+ individuals. In addition, Verberk and colleagues showed that a panel comprising Aβ1-42/Aβ1-40 ratio, GFAP, age, and APOE ε4 carrier status optimally identified Aβ+ individuals, and also reported no significant improvements with the addition of NfL similar to our findings with regard to NfL. However, further studies investigating an optimal panel of biomarkers along with AD risk factors are required.

To date only a handful of studies have investigated longitudinal changes in the aforementioned plasma biomarkers in clinically classified MCI and AD. In the current study, we observed a longitudinal decrease in plasma Aβ1-42/Aβ1-40 ratio and a longitudinal increase in plasma p-tau181 in MCI participants compared with controls; however, no significant longitudinal changes were observed in plasma Aβ1-42/Aβ1-40 ratio and p-tau181 levels in AD participants compared with controls. These findings are consistent with previous CSF and plasma familial AD studies reporting that alterations in Aβ1-42/Aβ1-40 ratios and p-tau181 levels along the disease trajectory ultimately begin to plateau following the first progressive symptom (e.g., memory, motor, or behavior) onset. Furthermore, Rodriguez and colleagues show that the trajectory of p-tau181 is associated with the duration of AD status, wherein increases in plasma p-tau181 in AD patients were observed up to 8 to 4 years prior to death, which later plateaued. Given that we do not have data on the duration of AD status for participants in the current study, further studies are required to investigate the trajectory of p-tau181 levels in AD participants from disease onset to death. A previous study reported significant longitudinal increases in GFAP in MCI Aβ+ and MCI who progressed to dementia compared with MCI Aβ− and stable MCI, respectively. Within the current study, we show that GFAP longitudinally increased in MCI and AD compared with controls, and although NfL did not significantly increase longitudinally in MCI, a significant longitudinal increase was observed in AD compared with controls. These findings suggest a sequence in the progression of biomarkers reflecting the underlying pathological process.

In the current study we showed that plasma biomarker levels are associated with prospective cognitive decline. Our observations of the association of baseline plasma biomarker levels with prospective cognitive decline are in line with previous studies, wherein lower baseline plasma Aβ42/Aβ40 ratio or Aβ42 levels have been reported to be associated with faster cognitive decline and higher baseline plasma p-tau181, GFAP, and NfL levels have been reported to be associated with faster cognitive decline. Furthermore, observations from the current study extend results from previous findings, wherein the majority of the aforementioned studies report associations in sample sets comprising a mix of CU and CI individuals, and not independently.

Baseline plasma Aβ1-42/Aβ1-40 ratio, p-tau181, and GFAP were also observed to be associated with future brain Aβ accumulation, in line with previous reports. Schindler and colleagues reported a 15-fold greater risk of conversion to Aβ+ in Aβ− cognitively normal individuals with plasma Aβ42/Aβ40 ratio < 0.1218 compared with individuals with plasma Aβ42/Aβ40 ratio > 0.1218. In addition, Shen and colleagues reported that individuals with abnormal baseline plasma p-tau181 levels had a higher risk of progression to pathological brain amyloid load. Furthermore, Pareira and colleagues have reported that plasma GFAP levels predicted Aβ accumulation before and after adjusting for age, sex, baseline Aβ status, presence of cognitive impairment, and tau PET load.

The strengths of the current study include Aβ+ defined classification, the availability of serial plasma measurements to assess longitudinal changes in plasma biomarkers, and the availability of longitudinal data on cognition and brain Aβ-PET load. It is acknowledged that this study also has its limitations. Aβ+ defined classification was not used to assess longitudinal changes in plasma biomarkers as only a modest Aβ-PET sample size with follow-up timepoints was available; however, analyses were adjusted for Aβ−/+ status at baseline. Preliminary, longitudinal changes in plasma biomarkers in groups classified using both clinical and Aβ−/+ status are; however, presented in Table 510, albeit further validation studies are required. In addition, analyses could not include tau-PET−/+ status to assess early or late preclinical AD stages, given that these data were not available for the analyzed sample set. Furthermore, the measurement of Aβ42/Aβ40 using the Simoa platform has been reported to perform inferiorly to immuno precipitation followed by mass-spectrometry methods or the Elecsys immunoassay with respect to its predictive performance for Aβ−/+ status.

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To conclude, results from the current study suggest that plasma biomarkers are altered cross-sectionally and longitudinally, sequentially along the AD continuum, and are associated with prospective cognitive decline and increase in brain Aβ-PET load. These findings provide further evidence of the diagnostic and prognostic potential of plasma biomarkers. Findings from the current study have significance and potential implications for (1) clinical trials (e.g., identifying preclinical and prodromal AD participants for clinical trials) and demonstrating superiority of some biomarkers/combinations for this distinction earlier in the AD continuum, compared to NfL and (2) clinical translation (e.g., earlier, and simpler precision diagnosis of AD). Studies comparing differences in the putative plasma biomarkers between AD and other non-AD neurodegenerative diseases and non-neurodegenerative psychiatric disorders in clinical settings are required. Further in-depth head-to-head comparisons between the putative plasma biomarkers between AD and other non-AD neurodegenerative diseases and non-neurodegenerative psychiatric disorders in clinical settings are required. Further in-depth head-to-head comparisons between the putative plasma and CSF AD biomarkers are required; however, Tables S11-S12 and Figure S4 show comparisons and associations of plasma versus CSF Aβ42 and p-tau181 pilot data. Future validation studies are required with an emphasis on more ethnically diverse populations.

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AUTHOR CONTRIBUTIONS
Pratishtha Chatterjee and Ralph N. Martins conceptualised the study. Steve Pedrini measured plasma protein concentrations using the Simoa platform. Pratishtha Chatterjee carried out the statistical analyses, data visualization and interpretation, and James D. Doecke, Abhay K. Singh, and Penghao Wang validated the statistical analyses. Victor L. Villemagne, Vincent Doré, and Christopher C. Rowe provided input on neuroimaging data. Pratishtha Chatterjee wrote the manuscript. All authors critically reviewed the manuscript.

CONFLICT OF INTERESTS
VV is and has been a consultant or paid speaker at sponsored conference sessions for Eli Lilly, Life Molecular Imaging, ACE Barcelona, and IXICO. S.R.S has received grant support from the National Health and Medical Research Council, Alzheimer’s Association (USA) Research Grant, Alzheimer’s Drug Discovery Foundation, and the BrightFocus Foundation and honorarium for lectures from the Mature Adults Learning Association Inc. K.T., H.R.S., and R.N.M. are Directors of SMarT Minds Western Australia. H.R.S. has been partially supported by the Australian Alzheimer’s Research Foundation, Western Australia. H.R.S. has received reimbursements from Alector and Alnylam Pharmaceuticals. PM is a full-time employee of Cogstate Ltd. C.C.R. has received research grants from NHMRC, Enigma Australia, Biogen, Eisai, and Abbvie. He is on the scientific advisory board for Cereveau Technologies and has 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.

REFERENCES


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


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