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Plasma Amyloid-β as a Biomarker in Alzheimer's Disease: The AIBL Study of Aging

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Abstract. Amyloid-β (Aβ) plays a central role in the pathogenesis of Alzheimer's disease (AD) and has been postulated as a potential biomarker for AD. However, there is a lack of consensus as to its suitability as an AD biomarker. The objective of this study was to determine the significance of plasma Aβ as an AD biomarker and its relationship with Aβ load and to determine the effect of different assay methods on the interpretation of Aβ levels. Plasma Aβ_{1-40}, Aβ_{1-42}, and N-terminal cleaved fragments were measured using both a commercial multiplex assay and a well-documented ELISA in 1032 individuals drawn from the well-characterized Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Further, Aβ levels were compared to Aβ load derived from positron-emission tomography (PET) with the Pittsburgh compound B (PiB). Lower Aβ_{1-42} and A$\beta_{1-42/1-40}$ ratio were observed in patients with AD and inversely correlated with PiB-PET derived Aβ load. However,

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assay methodology significantly impacted the interpretation of data. The cross-sectional analysis of plasma Aβ isoforms suggests that they may not be sufficient *per se* to diagnose AD. The value of their measurement in prognosis and monitoring of AD interventions needs further study, in addition to future longitudinal comparisons together with other predictors, which will determine whether plasma Aβ has diagnostic value in a panel of biomarkers.

Keyword: Alzheimer's disease, amyloid-β, biomarkers, diagnosis, Pittsburgh Compound B, positron-emission topography

Supplementary data available online: http://www.j-alz.com/issues/20/vol20-4.html#supplementarydata02

INTRODUCTION

Epidemiological studies have estimated that approximately 24 million people suffered from dementia worldwide in 2001, with this figure projected to double by 2020 and increase to 81 million by 2040 [1]. While there is no cure for the most common form of dementia (Alzheimer's disease, AD), several clinical trials are showing promise. Further, there is a growing body of evidence that lifestyle interventions may also delay the onset of AD [2]. The development of an effective and early diagnostic test for AD is essential, as it may allow detection of disease before the onset of symptoms, when damage to the brain can be reasonably expected to be minimal. Neuropsychological and clinical assessments currently distinguish between cognitively normal individuals and patients with AD with an overall accuracy of ∼80% [3]. However, accuracy of diagnosis declines at the early stages of AD due to overlap in neuropsychological presentation between prodromal AD and normal aging. Amyloid-β (Aβ), intimately involvement in the pathogenesis of AD, is speculated to be a plausible biomarker for AD. A decline in Aβ levels in the cerebrospinal fluid (CSF) has been reported to help distinguish between patients with AD and normal elderly individuals [4–8]. Further, several studies using Enzyme-Linked Immuno-Sorbent Assays (ELISA) report a relationship between ratiometric levels of Aβ ($A\beta_{1-42/1-40}$) in CSF, compared with healthy aged controls [9,10], although with overlapping data ranges. Further, recent advances in Aβ imaging through the use of Pittsburgh compound B (PiB) coupled with positron-emission tomography (PET) [11, 12] have yielded promising data. Significant relationships have been described between PiB-PET derived Aβ load and memory and cognitive performance [13], apolipoprotein E (*APOE*) genotype [14,15], and CSF biomarkers [16,17]. Whilst these results are promising, different sampling sources, such as blood, may be more accessible to the wider community.

Studies of plasma Aβ have been inconclusive with some studies reporting an increase in $A\beta_{1-42}$ and a decrease in $A\beta_{1-40}$ in plasma of patients with AD, whereas others have presented contradictory results or equivocal changes [6,18–30] or initially elevated Aβ species followed by significant decreases in $A\beta_{42}$ and $A\beta_{1-42/1-40}$ ratio at disease onset [31]. Further, studies have found no clear correlation between the levels of Aβ in plasma and CSF [6,9,30,32]. Many factors may contribute to variations between studies including assay methodologies and sample sizes. As Aβ concentration in plasma is by some estimates a hundredth of that in CSF [6], its accurate and reliable measurement requires, first, an assay that is not only very specific but also has the sensitivity to detect low amounts of Aβ peptides, and second, standardized procedures which will minimize degradation, maximize solubility and be reproducible across laboratories. Adequate sample size is a further potential confounder with a larger sample size providing greater confidence in the results by minimizing potential skewing and bias. Currently, only the Rotterdam study [23] and the more recent German [33] and French [34] multicentre research studies present data from cohorts well in excess of hundreds, with most studies reporting on cohorts of around 200 participants [6,7,21,24]. Further, the cohort used, no matter the size, needs to be comprehensively clinically characterized, such that any grouping based on the diagnosis of AD is as accurate as possible.

This study, as part of the longitudinal Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging, aimed to address these issues through utilizing a large, well-characterized cohort [35]. Here we report plasma Aβ data from baseline using two separate methodologies, a commercial multiplex assay and a well-documented ELISA technique. Additionally, in a subset of the cohort, correlations with PiB-PET derived Aβ plaque load are also reported.

METHODS AND MATERIALS

The AIBL Cohort

The cohort recruitment process and neuropsychological, cognitive, and mood assessments have been de-

scribed previously [35]. A clinical review panel met to discuss baseline classifications to ensure that diagnoses were made in a consistent manner according to internationally agreed criteria [36–38]. The final clinical classification of individuals, that presented with a diagnosis of AD or mild cognitive impairment (MCI) at baseline or were healthy controls who required further investigation, was based on the panels consensus diagnosis and was made prior to obtaining variables such as Aβ levels, PiB-PET imaging, or *APOE* genotype, determined as previously described [39]. The institutional ethics committees of Austin Health, St Vincent's Health, Hollywood Private Hospital and Edith Cowan University granted ethics approval for the AIBL study. All volunteers gave written informed consent before participating in the study.

Sample collection and Aβ assays

Plasma Aβ was measured using a commercial kit (INNO-BIA plasma Aβ assay, Innogenetics, Inc.; Perth and Melbourne sites) and a well-documented double sandwich ELISA (Mehta ELISA) technique [6,9, 24] (Perth site only). Plasma was isolated from 15 mL of whole blood collected in standard EDTA tubes with added prostaglandin E1 (Sapphire Biosciences, 33.3 ng/ml) and stored in liquid nitrogen prior to the analysis of all samples [35]. The INNO-BIA multiplex assay, based on the Luminex xMAP technique, allows for the simultaneous measurement of $A\beta_{1-40}$ and $A\beta_{1-42}$ (module A) or Aβ fragments ($A\beta_{n-40}$ and $A\beta_{n-42}$; module B). Both modules were run according to manufacturer's instructions with the addition of an inter-plate probe wash step (Perth site). Assays were read on a Luminex xMAP reader system (Bio-Plex 200 System, Bio-Rad). The Mehta ELISA utilizes, as the capture assembly, the monoclonal antibody 6E10 and two different biotinylated polyclonal antibodies for the detection of $A\beta_{1-40}$ and $A\beta_{1-42}$. The assay was carried out as previously described [6,24] with absorbance measurements collected at 450nm (FluoroStar, BMG).

Brain imaging

A subset of 286 participants was randomly selected for PiB-PET imaging using the methodology described previously [13,40]. Briefly, 3D T1 MPRAGE and a T2 turbospin echo and FLAIR sequence MRI was acquired for screening and co-registration with the PET images. PET standardized uptake value (SUV) data acquired 40-70 min post ^{11}C-PiB injection were summed and normalized to the cerebellar cortex SUV, resulting in a region to cerebellar ratio termed the SUV ratio (SUVR). This subset of samples was assayed at both sites using the INNO-BIA assay (batch numbers were identical across sites) with a total of 255 individuals presenting with a complete set of data.

Statistical analysis

Statistical analyses were conducted using PASW Statistics (version 17.0.2; SPSS Inc., USA). Variables were assessed for conformation to a normal distribution with departure corrected using Box-Cox transformations [41]; in most cases where transformation was required the calculated lambda equated to a natural logarithm (ln) or ln(X+1) transformation, in the case of Aβ ratios. Continuous variables were analyzed by one-way Analysis of Variance (ANOVA) with Tukey's HSD post-hoc analysis, whilst categorical variables were analyzed using the χ^2-test. Differences in Aβ levels were further assessed in an analysis of covariance (ANCOVA) that adopted a general linear model (GLM) procedure, adjusting for age, gender, and *APOE* genotype. Correlations between Aβ isoforms, between assays and between Aβ levels and SUVR were assessed using Pearson's bivariate correlations. Estimated risk ratios for the conversion to MCI (from healthy control) or AD (from healthy control or MCI) were determined by multinomial logistic regression for standard deviation change and tertile increases in Aβ isoforms or ratios.

RESULTS

Baseline descriptive statistics are presented in Table 1 with variation from Ellis and colleagues [35] attributed to withdrawals and incomplete datasets. Cognitive performance was significantly different across groups, as was *APOE*-ε4 allele frequency (AD>MCI>HC (healthy control)). Likewise, age was significantly different across groups; however, no statistical differences in gender were observed. Of the potential confounding cardiovascular factors, body mass index and high density lipoprotein, only the former showed significant differences (AD<HC: Tukey's HSD, $P < 0.001$). There were no overall statistical differences between genders within clinical classifications with respect to age, performance on the Mini-Mental Status Examination and *APOE*-ε4 genotype frequency.

Table 1
Baseline sociodemographic variables. Break down of major variables including potential confounding variables in the entire AIBL cohort and the PiB-PET subset; MMSE, Mini-Mental State Examination; HDL, High Density Lipoprotein; BMI, Body Mass Index. BMI data was available on 903 individuals (194 in PiB-PET subset). [1]ANOVA without covariates (except gender and *APOE* genotype where the χ^2-test was performed)

	Entire cohort ($n = 1032$)				PiB-PET subset ($n = 255$)			
	HC ($n = 724$)	MCI ($n = 122$)	AD ($n = 186$)	P^1	HC ($n = 167$)	MCI ($n = 51$)	AD ($n = 38$)	P^1
Age (years)	70.0 ± 7.0	75.9 ± 7.5	78.6 ± 8.5	< 0.001	71.7 ± 7.4	75.2 ± 7.5	73.1 ± 8.9	0.022
Gender (% Female)	57.7	55.7	62.9	0.361	52.1	47.1	60.5	0.450
MMSE Score	28.9 ± 1.2	26.2 ± 2.6	19.2 ± 5.0	< 0.001	28.7 ± 1.2	27.1 ± 2.2	20.8 ± 5.5	< 0.001
APOE-ε4 pos. (%)	26.9	49.6	61.3	< 0.001	43.7	54.0	60.5	0.115
HDL (mmol/L)	1.68 ± 0.46	1.64 ± 0.42	1.68 ± 0.46	0.703	1.67 ± 0.45	1.61 ± 0.43	1.75 ± 0.50	0.421
BMI	26.6 ± 4.7	25.7 ± 3.8	24.8 ± 4.5	< 0.001	26.4 ± 4.1	26.0 ± 4.8	25.7 ± 5.2	0.597

Table 2
Baseline plasma Aβ levels in the AIBL cohort. Plasma Aβ levels measured via the [1]INNO-BIA plasma Aβ assay kit (Innogenetics Inc) and the [2]Mehta ELISA. Plasma $A\beta_{n-40}$ and $A\beta_{n-42}$ levels measured using the INNO-BIA plasma Aβ assay (Innogenetics Inc). Aβ ratios were calculated from measured plasma Aβ isoform/fragment levels. Values represent mean ± S.D. [3]ANOVA without covariates, [4]ANCOVA controlling for age, gender, and *APOE* genotype

	Entire cohort ($n = 1032$)				
	HC ($n = 724$)	MCI ($n = 122$)	AD ($n = 186$)	*Crude P*[3]	*P (Model 2)*[4]
$A\beta_{1-40}$(pg/mL)[1]	153.4 ± 40.2	152.9 ± 51.5	155.1 ± 44.2	0.877	0.176
$A\beta_{1-42}$(pg/mL)[1]	32.4 ± 9.7	30.2 ± 11.9	30.0 ± 10.2	< 0.001	0.003
$A\beta_{1-42/1-40}$ Ratio[1]	0.221 ± 0.097	0.216 ± 0.120	0.199 ± 0.056	0.001	0.385
$A\beta_{n-40}$ (pg/mL)[1]	141.4 ± 48.0	152.0 ± 56.3	158.9 ± 48.0	< 0.001	0.079
$A\beta_{n-42}$ (pg/mL)[1]	35.4 ± 31.1	31.1 ± 15.2	33.4 ± 21.7	0.166	0.273
$A\beta_{n-42/n-40}$ Ratio[1]	0.294 ± 0.277	0.265 ± 0.262	0.241 ± 0.191	0.012	0.225
$A\beta_{1-40}$ (pg/mL)[2]	124.7 ± 64.3	141.5 ± 73.8	128.3 ± 71.0	0.066	0.071
$A\beta_{1-42}$ (pg/mL)[2]	48.1 ± 33.7	50.1 ± 23.5	44.8 ± 20.0	0.065	0.092
$A\beta_{1-42/1-40}$ Ratio[2]	0.414 ± 0.152	0.410 ± 0.203	0.424 ± 0.259	0.830	0.824

Aβ characterization using a multiplex Aβ assay and an Aβ ELISA

Plasma $A\beta_{1-40}$, $A\beta_{1-42}$ and plasma Aβ fragment ($A\beta_{n-40}$, $A\beta_{n-42}$) levels, measured in both assays, are presented in Table 2. Plasma $A\beta_{1-40}$ was strongly correlated with $A\beta_{1-42}$ (INNO-BIA assay, $R = 0.470$, $P < 0.001$, Supplemental Fig. 1 (supplement available online: http://www.j-alz.com/issues/20/ vol20-4.html#supplementarydata02); ELISA, $R = 0.573$, $P < 0.001$; Supplemental Fig. 2). Inverse correlations for both Aβ isoforms were observed between assays ($A\beta_{1-40}$, $R = -0.185$, $P = 0.003$; $A\beta_{1-42}$, $R = -0.172$, $P = 0.006$; Fig. 1A, B), while $A\beta_{1-42/1-40}$ ratio did not show any correlation ($R = -0.095$, $P = 0.129$; Fig. 1C).

ELISA measured plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels and $A\beta_{1-42/1-40}$ ratio were not significantly altered across groups, although $A\beta_{1-40}$ and $A\beta_{1-42}$ levels tended to be higher for MCI than other groups. However, significant differences were observed for Aβ levels as measured via the INNO-BIA plasma Aβ assay. $A\beta_{1-42}$ levels were decreased in both AD and MCI groups compared to HC (Tukey's HSD: MCI>HC, $P = 0.037$; AD>HC, $P = 0.01$); however, there was no statistical difference between MCI and AD groups (Tukey's HSD, $P = 0.981$). $A\beta_{1-42/1-40}$ ratio was significantly lower in the AD group compared to the HC group (Tukey's HSD, $P = 0.008$). There were no significant differences in $A\beta_{1-40}$. Only $A\beta_{1-42}$ levels remained significant after controlling for age, gender, and *APOE* genotype. High $A\beta_{1-42}$ levels were found to be associated with a decreased risk for dementia compared to controls (Table 3); individuals in the upper tertile of both AD and MCI groups had a 2-fold decreased risk but no significant risk was attributed to comparisons between MCI and AD. Neither $A\beta_{1-40}$ levels nor $A\beta_{1-42/1-40}$ ratio were associated with altered risk. While controlling for age in a GLM ANCOVA, a strong main effect of age was consistently observed. When plasma Aβ isoforms and ratios were plotted against age for each group (Supplemental Fig. 3), it revealed that differences between groups are more apparent in the earlier age groups; however, these differences did not reach statistical significance.

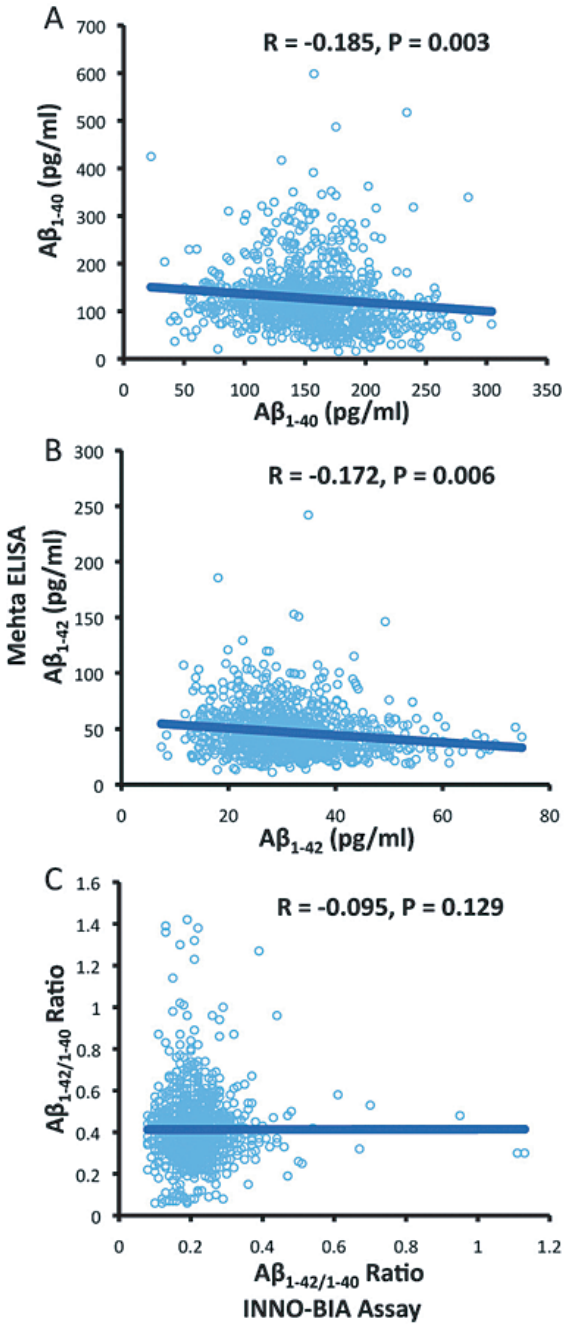

Fig. 1. Comparison of the INNO-BIA plasma Aβ assay to the Mehta double sandwich ELISA. Correlation of Plasma Aβ_{1-40} (A), Aβ_{1-42} (B), and A$\beta_{1-42/1-40}$ ratio (C) as measured using the INNO-BIA plasma Aβ assay against that measured using the Mehta double sandwich ELISA.

Plasma Aβ_{n-40} was significantly elevated in both AD and MCI when compared to HC (Tukey's HSD: AD, $P < 0.001$; MCI, $P = 0.044$) with no difference observed between MCI and AD (Tukey's HSD, $P = 0.504$). The significance of this finding is tempered by levels of Aβ_{n-40} being significantly lower than the levels of Aβ_{1-40} (T-Test, $P < 0.001$). In contrast, the observed decrease in Aβ_{1-42} in AD was not accompanied by either a concomitant change in Aβ_{n-42} levels or any statistical differences between Aβ_{n-42} and Aβ_{1-42} within groups. However, the decreasing A$\beta_{1-42/1-40}$ ratio was reflected by a parallel significant decrease in A$\beta_{n-42/n-40}$ ratio, although like full-length ratios this failed to remain significant after controlling for age, gender, and *APOE* genotype. Levels of Aβ_{n-40} were associated with significantly altered risk for dementia (Table 3); individuals in the upper tertile had a 1.8-fold increased risk for AD. While a higher A$\beta_{n-42/n-40}$ ratio was associated with a decreased risk for dementia compared to controls; individuals in the upper tertile had a 2-fold decreased risk.

Correlation of plasma Aβ and PiB-PET imaging (SUVR)

The PiB-PET subset was generally representative of the entire cohort with respect to age, cognition, frequency of the *APOE*-ε4 allele, and gender (Table 1), with the exception that the PiB-PET control subset had a higher frequency of the *APOE*-ε4 allele and the PiB-PET AD subset were slightly younger. As previously reported [42], PiB-PET provided good distinction between different groups, with significant differences between all clinical classifications (Tukey's HSD: HC<MCI ($P < 0.001$) < AD ($P < 0.001$); Table 4). This association held after controlling for age, gender, and *APOE* genotype, for which age and *APOE* genotype had strong main effects (ANCOVA, $P < 0.001$). The PiB-PET subset revealed contrasting results to the full cohort in regards to plasma Aβ_{1-40}, which was decreased in AD individuals compared to HC individuals (Tukey's HSD, $P = 0.049$). Plasma Aβ_{1-42} showed a similar trend to the entire cohort, with significantly decreased levels in MCI and AD groups compared to HC (Tukey's HSD: MCI, $P < 0.001$; AD, $P = 0.007$). A significant difference in A$\beta_{1-42/1-40}$ ratios was apparent across groups; however, this association was driven by differences between MCI and HC groups (Tukey's HSD, $P < 0.001$).

Comparison of plasma Aβ levels between sites revealed absolute levels of both isoforms were higher at the Melbourne site but a strong correlation was observed between sites (Aβ_{1-40}, $R = 0.511$, $P < 0.001$; Aβ_{1-42}, $R = 0.566$, $P < 0.001$; A$\beta_{1-42/1-40}$, $R =$

Table 3
Risk ratios for conversion to MCI or AD. Risk ratios per standard deviation (SD) increase and per tertile increase in Aβ isoforms and ratios were estimated from baseline data. Odds ratios (OR) and 95% Confidence Intervals (CI) are presented. T_{LOW}, *lowest tertile;* T_{MID}, *middle tertile;* T_{HIGH}, *highest tertile.* $^*P$-trend for tertile increasess

	Per SD increase		Tertiles			
	OR (95% CI)	P	T_{LOW}	T_{MID}	T_{HIGH}	P*
HC → AD						
Aβ_{1-40}	0.88 (0.71–1.04)	0.113	1.0 (Ref)	0.73 (0.46–1.16)	0.72 (0.45–1.15)	0.288
Aβ_{1-42}	0.78 (0.65–0.93)	0.007	1.0 (Ref)	0.69 (0.44–1.09)	0.45 (0.28–0.73)	0.005
A$\beta_{1-42/1-40}$ Ratio	0.86 (0.68–1.08)	0.207	1.0 (Ref)	1.08 (0.69–1.71)	0.80 (0.49–1.30)	0.461
Aβ_{n-40}	1.14 (1.00–1.48)	0.048	1.0 (Ref)	1.47 (0.89–2.42)	1.88 (1.15–3.05)	0.040
Aβ_{n-42}	0.94 (0.78–1.11)	0.504	1.0 (Ref)	0.71 (0.45–1.13)	0.78 (0.49–1.24)	0.325
A$\beta_{n-42/n-40}$ Ratio	0.83 (0.67–1.03)	0.088	1.0 (Ref)	0.69 (0.44–1.08)	0.61 (0.37–0.98)	0.093
MCI → AD						
Aβ_{1-40}	1.00 (0.81–1.24)	0.962	1.0 (Ref)	1.31 (0.73–2.38)	0.89 (0.51–1.57)	0.437
Aβ_{1-42}	1.01 (0.81–1.26)	0.916	1.0 (Ref)	1.66 (0.94–2.93)	0.97 (0.54–1.72)	0.156
A$\beta_{1-42/1-40}$ Ratio	0.83 (0.63–1.09)	0.182	1.0 (Ref)	1.24 (0.71–2.14)	1.25 (0.69–s2.28)	0.675
Aβ_{n-40}	1.14 (0.88–1.30)	0.569	1.0 (Ref)	1.20 (0.64–2.25)	1.26 (0.69–2.29)	0.746
Aβ_{n-42}	1.10 (0.87–1.38)	0.443	1.0 (Ref)	0.80 (0.46–1.38)	1.55 (0.85–2.84)	0.099
A$\beta_{n-42/n-40}$ Ratio	0.91 (0.69–1.18)	0.470	1.0 (Ref)	1.37 (0.79–2.36)	1.28 (0.70–2.35)	0.486
HC → MCI						
Aβ_{1-40}	0.88 (0.71–1.04)	0.167	1.0 (Ref)	0.56 (0.33–0.94)	0.76 (0.46–1.24)	0.087
Aβ_{1-42}	0.77 (0.63–0.94)	0.009	1.0 (Ref)	0.41 (0.25–0.69)	0.48 (0.29–0.79)	0.001
A$\beta_{1-42/1-40}$ Ratio	1.04 (0.84–1.28)	0.739	1.0 (Ref)	0.86 (0.52–1.42)	0.68 (0.41–1.14)	0.342
Aβ_{n-40}	1.14 (0.88–1.30)	0.245	1.0 (Ref)	1.37 (0.81–2.31)	1.66 (0.99–2.79)	0.151
Aβ_{n-42}	0.86 (0.70–1.05)	0.133	1.0 (Ref)	0.99 (0.62–1.60)	0.60 (0.35–1.03)	0.124
A$\beta_{n-42/n-40}$ Ratio	0.92 (0.74–1.13)	0.418	1.0 (Ref)	0.52 (0.32–0.85)	0.45 (0.27–0.76)	0.003

Table 4
Plasma Aβ levels in the PiB-PET subset. Plasma Aβ levels measured via the INNO-BIA plasma Aβ assay kit (Innogenetics Inc) at the [1]Perth and [2]Melbourne sites. A$\beta_{1-42/1-40}$ ratios were calculated from measured plasma Aβ isoform levels. Values represent mean $\pm$ S.D.[3]ANOVA without covariates, [4]ANCOVA controlling for age, gender, and *APOE* genotype

	PiB-PET subset ($n = 255$)				
	HC ($n = 167$)	MCI ($n = 51$)	AD ($n = 38$)	*Crude P*[3]	*P (Model 2)*[4]
PiB-PET (SUVR)	1.44 ± 0.43	1.93 ± 0.63	2.30 ± 0.40	< 0.001	< 0.001
Aβ_{1-40} (pg/mL)[1]	152.9 ± 35.4	143.1 ± 42.3	137.5 ± 34.0	0.033	0.010
Aβ_{1-42} (pg/mL)[1]	33.6 ± 10.2	27.2 ± 10.3	28.6 ± 10.3	< 0.001	< 0.001
A$\beta_{1-42/1-40}$ Ratio[1]	0.231 ± 0.109	0.206 ± 0.099	0.210 ± 0.057	0.022	0.114
Aβ_{1-40} (pg/mL)[2]	222.8 ± 44.8	227.7 ± 43.0	228.4 ± 51.7	0.682	0.902
Aβ_{1-42} (pg/mL)[2]	39.3 ± 15.0	34.8 ± 8.7	35.5 ± 9.9	0.010	0.016
A$\beta_{1-42/1-40}$ Ratio[2]	0.183 ± 0.076	0.155 ± 0.034	0.157 ± 0.032	0.004	0.019

0.475, $P < 0.001$; Supplemental Fig. 4) and trends across clinical classification were similar for Aβ_{1-42} and A$\beta_{1-42/1-40}$ ratio. After correction for age, gender, and *APOE* genotype all the associations held, except for that of A$\beta_{1-42/1-40}$ ratio, at the Perth site. Correlations of plasma Aβ isoforms and ratios with SUVR derived from PiB-PET imaging were consistent between sites. Negligible correlations were apparent between Aβ_{1-40} and SUVR (Fig. 2A), while Aβ_{1-42} and A$\beta_{1-42/1-40}$ ratio showed significant inverse correlations with SUVR (Fig. 2B and 2C). When analyzed within groups, these correlations were no longer statistically significant (Supplementary Table 1).

DISCUSSION

Numerous studies have investigated Aβ levels in plasma and their value as a biomarker for AD with generally inconclusive results. A range of confounding factors may account for the disparate findings reported in the existing literature [6,7,18–30,33]. One factor is the cohort under study, which needs to be both sufficient in size and thoroughly characterized to pro-

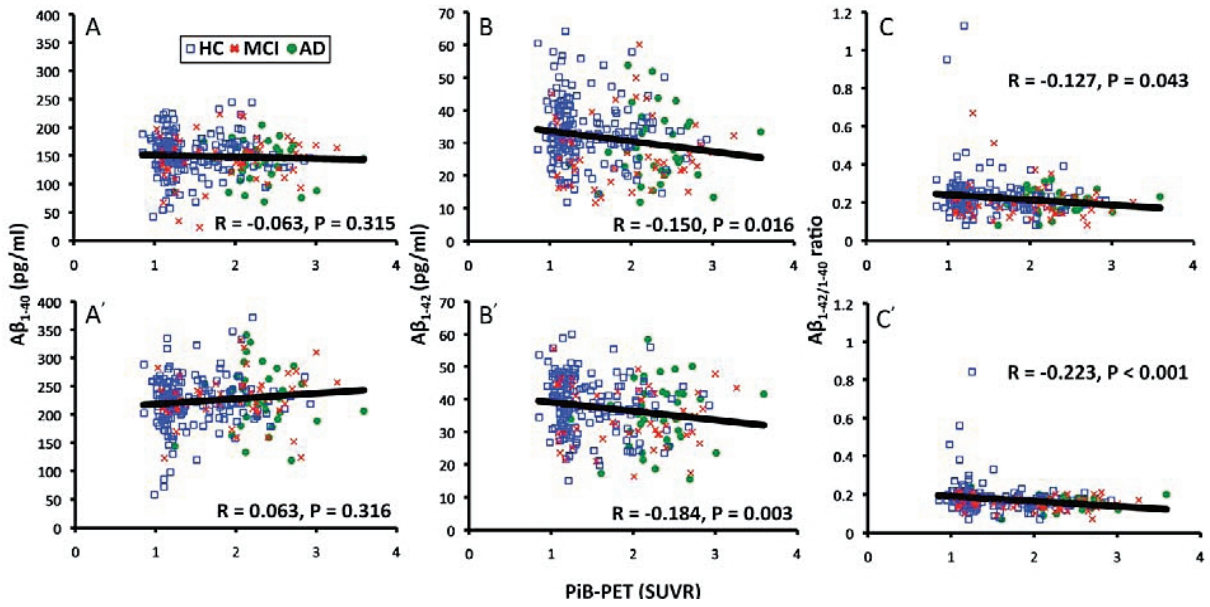

Fig. 2. Correlation of PiB PET imaging with plasma Aβ measurements. Aβ_{1-40} (A), Aβ_{1-42} (B), and the A$\beta_{1-42/1-40}$ ratio (C) as measured by the INNO-BIA plasma Aβ assay were plotted against the corresponding SUVR. Pearson's correlations were consistent between Perth (A, B, C) and Melbourne (A', B', C') sites.

vide the most confidence for interpreting data. The current study addressed these issues by drawing participants from the AIBL cohort, which represents one of the largest and most thoroughly clinically characterized cohorts. A further related factor is age; this and a previous study [20] suggest that different age groups may have differences in plasma Aβ regulation patterns, which implies that there may be a distinctive pattern of Aβ levels depending on the age of onset of AD.

Perhaps the greatest mitigating factor for meaningful interpretation of the literature is the lack of a standardized method for measuring Aβ levels in plasma, which raises numerous issues that may impact upon the reproducibility of results. This was evident in the current study through comparisons of the INNO-BIA plasma Aβ assay with the ELISA [6]. ELISA obtained plasma Aβ levels tended to be lower, in the case of Aβ_{1-40}, and higher, in the case of Aβ_{1-42}, than levels determined by the INNO-BIA plasma Aβ assay. It was interesting to note that both plasma Aβ_{1-40} and Aβ_{1-42} concentrations measured using the ELISA showed an increasing trend from HC to MCI but a drop with AD, a similar observation to that of previous studies [18, 24]. Generally, results were not comparable between assays as evidenced by an inverse, or a complete lack of, correlation. Clearly, different assaying methodologies and detection techniques can result in very different outcomes. Different assays invariably make use of antibodies (polyclonal or monoclonal) produced under different conditions and/or from different sources which may have different affinities for monomeric or oligomeric forms, thus potentially explaining differences in the final concentration of Aβ detected from the same sample. It is also possible that different diluents and/or dilution factors used in assaying procedures may contribute to the variation in detectable levels of Aβ as described in a recent study [30]. In the current study samples were not diluted when assayed using the ELISA, whereas samples were diluted for the INNO-BIA plasma Aβ assay. Overall, the combination of antibody specific binding differences with differences in sample preparation and methodology between assays may contribute to the current lack of consensus in the literature. The development of a commercially available multiplex assay has gone some way to addressing these issues.

To our knowledge there are only two comparably sized studies, a German multi-center study [33] and the French Three-City Study [34], and one smaller study [30], that utilized the INNO-BIA plasma Aβ assay. While direct comparisons with the German study are difficult due to the lack of a healthy control group, the general trend for decreasing Aβ levels observed in these two European studies was also evident in the current study. The Aβ levels detected in these two studies are similar to that observed in measurements from the

Melbourne site and thus generally higher than the Perth site. However, $A\beta_{1-40}$ and $A\beta_{1-42}$ levels determined at the Perth site are comparable to the third study [30], suggesting that site-to-site variability exists and further standardization of methodology is required. For example, these differences may be attributed to the addition of an inter-plate wash step at the Perth site that may prevent potential carry-over, especially if numerous plates are measured in a single batch.

This study is one of the first to investigate the value of plasma Aβ in context with PiB-PET derived Aβ load. Previous significant relationships described between PiB-PET derived Aβ load and memory and cognitive performance [13] and *APOE* genotype [14,15] were confirmed in this study. Further, significant correlations between PiB-PET derived Aβ load and plasma Aβ were observed in this study, similar to that reported between CSF biomarkers and Aβ load [16,17] whereby increased Aβ load was reflected by decreased plasma Aβ levels. As Aβ is known to circulate through the periphery, these findings raise the possibility that equilibrium exists between the different pools, which may be the mechanism involved in the circulation of Aβ through the blood brain barrier and thus explain the findings of this study. Initial stages of the disease would see a concomitant increase in Aβ in the brain/CSF and the periphery, however, with disease progression and increased amyloid plaque burden, there is a resultant decline in peripheral circulating Aβ, in particularly $A\beta_{1-42}$ [9,24]. A second plausible hypothesis is that decreases in peripheral circulating Aβ may be a result of increases in cell-associated membrane bound Aβ, a mechanism akin to that in the CSF. However, these results when placed in context with prior literature that failed to observe correlations between CSF and plasma Aβ [6,9,30,32], suggests that relationships between the two circulating pools of Aβ and with PiB-PET derived Aβ load warrant further investigation.

To summarize, numerous mitigating factors may influence Aβ measurements and, when combined with the lack of a comprehensive understanding of the plasma Aβ kinetics, has resulted in a lack of consensus as to the suitability of plasma Aβ as an AD biomarker. The development of a standardized methodology for the measurement of plasma Aβ would go some way to addressing some of these mitigating factors. Under the conditions employed in this cross-sectional analysis of the baseline phase of the AIBL study, plasma Aβ isoforms and ratios are not markedly different across different clinical classifications, with statistically significant differences unlikely to provide sufficient power to serve independently as an AD biomarker. However, Aβ may be of optimal value when interpreted in the context of other clinical features or biomarkers, whereby multiple potential predictors are considered simultaneously. To address this and the limitations discussed above, a longitudinal study involving the measurement of other biomarkers is underway within the AIBL study in order to determine conclusively whether plasma Aβ may contribute towards the development of an early diagnostic test for AD.

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