

Blood-Based Biomarkers

Altered levels of blood proteins in Alzheimer's disease longitudinal study: Results from Australian Imaging Biomarkers Lifestyle Study of Ageing cohort

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Abstract

Introduction: A blood-based biomarker panel to identify individuals with preclinical Alzheimer's disease (AD) would be an inexpensive and accessible first step for routine testing.

Methods: We analyzed 14 biomarkers that have previously been linked to AD in the Australian Imaging Biomarkers lifestyle longitudinal study of aging cohort.

Results: Levels of apolipoprotein J (apoJ) were higher in AD individuals compared with healthy controls at baseline and 18 months ($P = .0003$) and chemokine-309 (I-309) were increased in AD patients compared to mild cognitive impaired individuals over 36 months ($P = .0008$).

Discussion: These data suggest that apoJ may have potential in the context of use (COU) of AD diagnostics, I-309 may be specifically useful in the COU of identifying individuals at greatest risk for progressing toward AD. This work takes an initial step toward identifying blood biomarkers with potential use in the diagnosis and prognosis of AD and should be validated across other prospective cohorts.

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by extracellular deposition of amyloid β (A β) in senile plaques and intracellular formation of neurofibrillary

tangles of hyperphosphorylated tau protein. Although a small percentage of AD patients carry specific mutations that cause the disease at an earlier age, most patients are considered sporadic with a later age of onset. For this subgroup, the major risk factor is the $\epsilon 4$ allele of the *APOE* gene. Currently, the definitive diagnosis of AD can be performed only postmortem with the analysis of senile plaques in the brain parenchyma, although positron emission tomography (PET) scan modalities have received regulatory approval that can detect the

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presence of A β among living individuals. Furthermore, the search for biomarker panels has also been extended to cerebrospinal fluid (CSF) in AD and other neurological diseases [1–3]. It is to note that the examination of CSF, which reflects more closely what happens in the brain, has the disadvantage that CSF collection requires trained people, it is associated with a higher degree of risks, and it is not suitable for repeated draws. Although PET and CSF biomarkers are currently the gold standard to detect amyloid living patients, these biomarkers are expensive and cannot be used in routine clinical care. Blood-based biomarkers provide cost- and time-effective methods that can be used as the first step in a multistage neurodiagnostic process that would significantly streamline and cost contain this novel strategy. Recent years have witnessed an exponential increase in the investigation of blood-based biomarkers that have diagnostic and prognostic potential in AD. The identification of blood-based biomarkers that can identify groups that are at a higher risk for AD within routine clinical care (e.g., within primary care clinics) before the clinical manifestation would make possible targeted early treatments aimed at postponing the onset of the clinical disease itself. Such a paradigm would be advantageous not only for the individuals at risk, but also for the whole community, as delaying the onset of the disease would lighten the economic burden that is associated with the care of these patients.

In the recent years, several groups have published reports that associate AD to a specific group of biomarkers that largely focus on (1) discriminating between AD cases and controls (i.e., putative “context of use” [COU] related to AD diagnostics) or (2) predict the onset of the disease (i.e., putative COU related to AD prognostics). With regards to diagnostics, Ray et al. [4] defined a group of 18 plasma biomarkers out of a larger group of 120 analyzed that discriminated AD from controls with high accuracy. Later in 2008, research based on this 18 biomarker panel found that using 5 of these 18 was sufficient to differentiate between AD and controls with the same accuracy [5]. However, two subsequent analyses of these 18 proteins in different cohorts [6,7] failed to validate the biomarker panel finding significant differences between AD and controls only in five and three biomarkers, respectively. In 2012, the current group [8] identified a panel of 18 blood-based biomarkers that distinguished between AD and controls with high accuracy in the AIBL cohort and replicated the findings in the ADNI cohort. At the same time, an independent group analyzed two cohorts (TARC and ADNI) and found a panel of 11 biomarkers that accurately discriminated AD from controls across both serum and plasma [9], which was a continuation of their previous work [10] in which a panel of 30 biomarkers was identified. Another research group [11] reported that a panel of three blood-based biomarkers was able to discriminate AD from controls with a correct classification of more than 80%. In 2012, Johnstone et al. [12] reported a panel of 11 biomarkers in ADNI cohort identified preclinical AD. Other groups have performed similar analysis and identified a plasma biomarker panel in AD [13]. Some other studies have also associated

blood-based biomarker panels with microvascular pathology, brain atrophy, and cognitive decline in AD [14,15]. The common problem in all these studies is the variability of the biomarkers evaluated and the statistical analysis used to determine the diagnostic and prognostic value of the panel for AD with sufficient sensitivity and specificity. In this study, a panel of 14 biomarkers that have been identified as significant in 1 or more of the previous analyses was selected for analysis using the well-characterized AIBL cohort, baseline and longitudinal samples. These analyses were undertaken with the goal of determining if biomarker levels were different in AD compared with healthy controls (HCs) and mild cognitive impairment across time points, hence demonstrating their diagnostic value. In addition, if these biomarkers were associated with brain A β burden as assessed by PET results suggesting that different levels may be able to predict which individuals will convert to MCI/AD. The longitudinal analysis in AD patients was also carried out to study whether these biomarkers were associated with the progression of the disease.

2. Materials and methods

2.1. Population sample

2.1.1. The AIBL cohort

The cohort recruitment process including the neuropsychological, lifestyle, and mood assessments have been previously described in detail [16]. In brief, the AIBL study recruited a total of 1166 participants over the age of 60 years at baseline, of whom 54 were excluded because of comorbid disorders or consent withdrawal. Using the NINCDS-ARDA international criteria for AD diagnosis [17] and symptomatic predementia phase criteria for MCI diagnosis [18], a clinical review panel determined disease classifications at each assessment time point to ensure accurate and consistent diagnoses among the participants. According to these diagnostic criteria, participants were classified into one of three groups; AD, MCI, or HCs. At baseline, there were a total of 768 HCs, 133 MCI, and 211 AD subjects.

The AIBL study is a prospective, longitudinal study, following participants at 18-month intervals. This particular study reports on 711 individuals who completed the full study assessment and corresponding blood sample collection at baseline, 18 months and 36 months follow-up time points.

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.

2.1.2. Sample collection and APOE genotyping

Plasma was isolated from whole blood and collected in standard EDTA tubes with prostaglandin E1 (33.3 ng/mL, Sapphire Biosciences, NSW, Australia) added. On

completion of blood fractionation, samples were aliquoted and immediately stored in liquid nitrogen until required for analysis. DNA was isolated from whole blood using a QIAamp DNA Blood Midi Kit (Qiagen, VIC, Australia) according to the manufacturer's protocol, and *APOE* genotype was determined through either polymerase chain reaction (PCR) amplification and restriction enzyme digestions, as previously described [19], or through TaqMan genotyping assays (Life Technologies, Mulgrave, VIC, Australia) for rs7412 (assay ID: C____904973_10) and rs429358 (assay ID: C____3084793_20). For TaqMan assays, PCRs and real-time fluorescence measurements were carried out on a ViiA™ 7 real-time PCR system (Applied Biosystems, VIC, Australia) using the TaqMan GTXpress Master Mix (Life Technologies) methodology per manufacturer's instructions.

2.1.3. Plasma biomarker assay

Aliquots were prepared according to the volume required for each set of assays and stored at -80°C . All samples were assayed in duplicate via a multiplex biomarker assay platform using ECL on the SECTOR Imager 2400A from Meso Scale Discovery (MSD; <http://www.mesoscale.com>). The analytes requiring similar dilution were grouped together in the multiplexing plate by the manufacturer. The MSD platform has been used extensively to assay biomarkers associated with a range of human diseases including AD. ECL measures have well-established properties of being more sensitive and requiring less sample volume than conventional enzyme-linked immunosorbent assay, the gold standard for most assays. The biomarkers assayed were chosen from previously generated and cross-validated AD algorithms from various biomarker studies [8–10,20] and included the following: thrombopoietin (TPO), interleukin-18 (IL-18), fatty acid binding protein (FABP3), pancreatic polypeptide Y (PPY), chemokine I309 (I309), serum amyloid A (SAA), C-reactive protein (CRP), soluble vascular adhesion molecule 1 (sVCAM1), soluble intercellular adhesion molecule 1 (sICAM1), alpha-2-macroglobulin (A2M), beta-2 microglobulin (B2M), Factor VII (FVII), adiponectin (adipo), apolipoprotein J (apoJ).

Plates were washed and blocked as per manufacturer instructions using the supplied buffers. Samples were diluted according to each respective assay group and applied to the plate, along with standards. Plates were then sealed and incubated at room temperature for 2 hours. Plates were washed three times using phosphate buffered saline Tween 20 (PBST), then secondary detection antibodies were added and plates were sealed and incubated for a further hour. Plates were washed three times with PBST and read solution was added according to the assay instructions. Plates were immediately read using an MSD plate reader. The supplied software was used to determine standard curve and sample concentration, according to 5-PL curve-fitting techniques. The final protein biomarker concentration was reported in $\mu\text{g/mL}$ units.

2.1.4. Assessment of neocortical $A\beta$ via PiB-PET

A subset of the AIBL cohort ($n = 287$) underwent carbon 11-labeled Pittsburgh compound B-positron emission tomography (^{11}C -PiB-PET) imaging at baseline to measure brain $A\beta$ burden, as previously described [21]. PET standardized uptake value (SUV) data were summed and normalized to the cerebellar cortex SUV values to obtain the region to cerebellar ratio (standardized uptake value ratio [SUVR]). A threshold of 1.5 SUVR was used to discriminate between high (PiB+) and low (PiB-) brain $A\beta$ burden [22]. Of the total 711 participants reported on here, 180 individuals underwent PiB-PET imaging at baseline, 158 at 18-month follow-up, and 120 underwent at 36-month follow-up.

2.1.5. Statistical methodology

Descriptive statistics including means, standard deviations, and frequencies were calculated across clinical classifications. Gender and *APOE* $\epsilon 4$ allele comparisons were assessed using chi squared (χ^2) test and Fisher's exact test where necessary. Analysis of mean biomarker levels between clinical classifications adjusted for age, gender, and *APOE* $\epsilon 4$ allele status to assess the potential of these proteins as biomarkers for AD was performed using proportional odds logistic regression (for three group analyses, HC/MCI/AD as the outcome) and generalized linear modeling (GLM, for two groups, HC vs. AD as the outcome, binomial family). These statistical models were used as they are able to assess the differences in mean biomarker levels, adjusted for confounders under the assumption that there is a certain level of uncertainty in the outcome (clinical classification is not 100% correct). *P*-values were compared a Bonferroni adjusted alpha (α), with the number of biomarkers tested as the adjustment factor ($\alpha = 0.05/14, 0.00036$).

For the longitudinal analyses, linear mixed-effects modeling (LMM, using the biomarker as the [Gaussian] outcome at each time point) was used to assess mean biomarker levels over time for stable HC and AD groups individually, adjusted for age, gender, site, and *APOE* $\epsilon 4$ allele status. Assessment of biomarkers longitudinally between HC/MCI and AD groups was performed using cumulative link mixed models (CLMMs) for the three group clinical classification comparison, and the generalized linear mixed models (GLMMs, binomial family) for the individual group comparisons. GLM combined with receiver operating characteristic (ROC) analyses were combined to perform 100-fold repeated random subsampling validation for disease predictions.

Correlations between quantitative SUVR and all the biomarkers were carried out using Spearman's rank correlation analysis (ρ). A cutoff value of 1.5 for SUVR was used as the most appropriate criterion for biomarker evaluation. The R statistical software environment, version 2.15 was used for all statistical analyses (Team, R Development Core. 2009. R: A Language and Environment for Statistical Computing Manual).

3. Results

3.1. Population demographics

Baseline, 18-month, and 36-month follow-up time points demographic data, *APOE* ϵ 4 allele status, and Mini-Mental State Examination (MMSE) for the AIBL cohort are presented in Table 1. PiB-SUVRs for the AIBL imaging subcohort are also presented in Table 1. Plasma levels of biomarkers (pg/mL) TPO, IL-18, FABP3, PPY, I309, SAA, CRP, sVCAM1, sICAM1, A2M, B2M, FVII, adipo, apoJ were assayed in 554 healthy controls, 65 participants with MCI, and 92 participants with AD (total $N = 711$ at baseline). Age, *APOE* ϵ 4 allele status, and MMSE were significantly different between clinical classifications at baseline, 18 months, and 36 months ($P < .0001$). There was no difference in the proportion of females to males at either time point ($P > .05$). Total number of participants from the AIBL imaging subcohort was lower compared with the total group. PiB-SUVR was significantly higher in the MCI and AD groups compared with the HC group ($P < .0001$).

3.2. Association of biomarkers between clinical classification at the time of collection

Comparing biomarker levels between HC and MCI groups before adjustment for cofounders, FABP3 was significantly higher at both baseline ($P = .00002$) and 18-month ($P = .0001$) time points, but not at 36 months ($P = .0096$) (Table 2). This significance however was abrogated after adjusting for age, gender, and *APOE* ϵ 4 allele status. For the MCI versus AD group comparison, PPY stood out at 18 months, with significantly higher levels in AD compared with MCI participants, even after adjustment for both cofounders and multiple testing ($P = .0001$). Comparing biomarker levels between HC and AD groups across the three time points, 7 of 14 biomarkers measured were significantly higher in AD compared to HC at baseline and 18 months, and six biomarkers were significantly higher at 36 months. After adjustment for cofounders, apoJ at baseline and at 18 months was marginally significant after adjustment for multiple comparisons ($P = .0004$). Those associations that were not replicated at multiple time points

still showed differences between comparative groups; however, these were only significant at the nominal significance level (without Bonferroni correction, $\alpha = 0.05$, Table 2).

3.3. Comparison of mean biomarker levels between HC and AD groups over 36 months

Assessing mean biomarker levels over time between clinical classifications (three groups, CLMM), adjusted for age, gender, site, and *APOE* ϵ 4 allele status, strongest associations were seen for I309 ($P = .01$), sVCAM ($P = .04$), B2M ($P = .02$), and ApoJ ($P = .01$), although none of these reached the Bonferroni corrected threshold. Conducting pairwise comparisons over time (GLMM), levels of I309 were significantly increased in AD participants compared with MCI participants over time ($P = .00076$). Before adjustment for covariates, levels of PPY were also higher in AD participants compared with MCI participants over time ($P = .009$).

3.4. Biomarker trends over time for HC and AD groups

To assess biomarker levels over time, stratified data (two groups; only those that remained either HC ($N = 590$) or AD ($N = 109$) over 36 months [stable groups]) were assessed, adjusted for covariates age, gender, site, and *APOE* ϵ 4 allele status using LMM. TPO levels were significantly decreased for the HC group ($B = -0.03 \pm 0.005$, $P < .0001$), but not for the AD ($B = -0.02 \pm 0.01$, $P = .332$); however, the slope was similar. CRP levels for HC decreased ($B = -0.05 \pm 0.02$, $P = .05$), whereas for the AD group, they increased, albeit this was not significant ($B = 0.14 \pm 0.08$, $P = .09$). sVCAM1 levels in the AD group increased over time ($B = 0.03 \pm 0.01$, $P = .02$); however, this was not seen for the HC group ($B = 0.001 \pm 0.004$, $P = .73$). For IL-18, the decrease in biomarker levels over time was slightly stronger for the HC group ($B = -0.03 \pm 0.006$, $P = .0002$) compared with the AD group ($B = -0.02 \pm 0.02$, $P = .18$). Both A2M and B2M increased over time in HC and AD groups; however, rates were slightly different, with HC mean levels for A2M increasing more than that for the AD group (HC:

Table 1
Demographic characteristics

	Baseline			18 months			36 months			P-values
	HC	MCI	AD	HC	MCI	AD	HC	MCI	AD	
N	554	65	92	543	51	116	526	50	129	
Age (mean SD)	69.79 (6.51)	74.84 (7.54)	77.01 (7.43)	71.12 (6.34)	76.05 (7.28)	78.68 (7.63)	72.65 (6.26)	77.56 (7.47)	80.41 (7.39)	<.0001
Gender (F/M)	330/224	36/29	52/40	328/215	23/28	67/49	318/208	25/25	73/56	>.05
<i>APOE</i> ϵ 4 (-ve/+ve)	401/153	32/33	28/64	395/148	32/19	34/82	387/139	29/21	41/88	<.0001
MMSE (median IQR)	29 (2)	27 (3)	21 (4.25)	29 (2)	27 (3)	19 (8)	29 (2)	26.5 (3)	16 (10)	<.0001
SUVR (N)	127	32	21	116	19	23	95	18	24	
SUVR (mean SD)	1.36 (0.38)	2.02 (0.57)	2.29 (0.5)	1.35 (0.37)	2.03 (0.63)	2.32 (1.35)	1.38 (0.37)	1.8 (0.65)	2.43 (0.53)	<.0001

Abbreviations: HC, healthy control; MCI, mild cognitive impairment; AD, Alzheimer's disease; SD, standard deviation; MMSE, Mini-Mental State Examination; IQR, interquartile range; SUVR, standardized uptake value ratio.

Table 2
Cross-sectional comparisons across three clinical classifications namely HC, MCI, and AD

Time point	Biomarker	Mean (SD)			HC/MCI/AD		HC versus MCI		HC versus AD		MCI versus AD		
		HC	MCI	AD	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	
Baseline	TPO	453.72 (128.25)	486.4 (133.06)	498.25 (151.94)	0.0012	0.0356	0.0563	0.2509	0.0039	0.0502	0.5974	0.5387	
	IL-18	201.62 (78.55)	202.11 (99.01)	205.86 (102.95)	0.9283	0.8336	0.8567	0.7213	0.9954	0.8138	0.8969	0.5814	
	FABP3	6417.9 (2498.74)	7664.95 (2577.08)	7707.77 (2993.31)	2.02E-08	0.3356	2.18E-05	0.1285	3.41E-06	0.8375	0.9936	0.4419	
	PPY	151.51 (123.25)	195.11 (190.74)	226.44 (153.94)	3.16E-05	0.1075	0.1249	0.9325	7.56E-06	0.037	0.0775	0.1454	
	I309	1.76 (0.89)	1.66 (0.66)	2.25 (1.43)	0.0015	0.2862	0.5181	0.117	1.13E-05	0.0167	0.001	0.0066	
	SAA	5,014,328.58 (12,441,294.73)	4,556,617.17 (5,400,336.74)	10,066,848.34 (25,001,707.84)	0.019	0.0582	0.2092	0.2693	0.0319	0.1127	0.6153	0.6228	
	CRP	2,623,075.41 (5,483,161.45)	2,105,933.51 (1,992,259.53)	2,764,309.64 (5,501,614.76)	0.1497	0.9508	0.905	0.5195	0.059	0.4626	0.1728	0.4252	
	sVCAM	412,578.76 (93,418.88)	443,844.82 (107,111.58)	464,594.39 (118,439.3)	7.59E-06	0.1634	0.012	0.3654	4.09E-05	0.0855	0.3422	0.7705	
	sICAM	258,715.61 (57,091.32)	274,167.46 (70,100.74)	275,757.24 (57,768.89)	0.0046	0.1765	0.0524	0.3084	0.0165	0.6791	0.8869	0.9136	
	A2M	1,747,511,738.03 (513,469,652)	1,855,714,846.34 (602,037,583.95)	1,893,399,396.14 (581,856,370.49)	0.0062	0.4693	0.1635	0.8476	0.0101	0.4797	0.5236	0.4959	
	B2M	2,302,005.26 (854,220.32)	2,564,836.72 (962,444.25)	2,600,875.68 (868,669.09)	6.45E-05	0.6796	0.0223	0.7112	0.0002	0.283	0.5081	0.975	
	FVII	855,774.28 (204,030.04)	879,676.06 (200,143.09)	870,519.29 (211,777.27)	0.33	0.4349	0.3435	0.3465	0.5341	0.8441	0.7428	0.6244	
	Adipo	72,058,918.21 (37,972,553.53)	85,795,000.94 (47,271,598.65)	91,367,246.13 (52,484,686.66)	9.37E-06	0.0461	0.0067	0.0898	9.60E-05	0.0402	0.5956	0.9282	
	ApoJ	34,840,490.7 (6,569,730.63)	36,905,488.48 (7,081,682.5)	38,901,971.01 (8,064,135.24)	2.70E-07	0.0003	0.0183	0.0875	4.31E-07	0.0004	0.1232	0.1435	
	18 months	TPO	439.01 (124.56)	484.25 (130.48)	489.65 (168.28)	0.0005	0.0172	0.0176	0.0276	0.0034	0.0836	0.8974	0.4575
		IL-18	197.31 (78.42)	202.49 (92.26)	201.59 (101.17)	0.9834	0.9396	0.8507	0.5852	0.9475	0.8877	0.86	0.4857
FABP3		6560.26 (2371.26)	7983.14 (2970.32)	7921.63 (3075.55)	3.03E-09	0.2022	0.0001	0.1219	5.15E-08	0.4821	0.8588	0.8237	
PPY		160.98 (124.66)	164.33 (162.1)	231.27 (157.33)	3.65E-05	0.0443	0.4921	0.0557	1.44E-07	0.01	0.0002	0.0001	
I309		1.82 (1.04)	1.72 (0.91)	2.25 (1.28)	0.0021	0.9447	0.369	0.0669	0.0001	0.1752	0.0028	0.0202	
SAA		4,508,476.82 (10,011,818.68)	6,010,980.54 (13,928,739.6)	11,051,022.73 (26,601,726.7)	0.0049	0.0331	0.8312	0.8133	0.0007	0.007	0.0519	0.2179	
CRP		2,324,026.89 (3,678,293.91)	3,244,249.76 (7,168,549.06)	3,750,314.02 (9,648,464.51)	0.2513	0.5808	0.8488	0.9327	0.2133	0.5945	0.5862	0.9336	
sVCAM		413,866.28 (94,396.07)	451,372.28 (128,980.99)	461,761.39 (123,431.77)	3.82E-05	0.5612	0.0336	0.6051	0.0001	0.5823	0.5645	0.8394	
sICAM		256,940.34 (63,741.88)	276,181.68 (81,976.23)	273,854.69 (65,996.05)	0.0086	0.629	0.07	0.3302	0.022	0.8845	0.8916	0.5686	
A2M		1,799,156,641.14 (533,559,105.75)	1,818,415,510.22 (540,494,569.01)	1,966,783,241.78 (593,726,925.78)	0.0051	0.6885	0.7756	0.5794	0.002	0.4514	0.097	0.1402	
B2M		2,380,739.82 (906,482.97)	2,586,551.41 (1,058,022.97)	2,792,433.32 (1,002,092.54)	2.00E-06	0.4936	0.1107	0.754	1.18E-06	0.1792	0.1148	0.3201	
FVII		859,839.14 (217,735.63)	843,890.76 (211,518.29)	904,916.05 (221,993.04)	0.1079	0.094	0.636	0.9626	0.0399	0.275	0.0974	0.3038	
Adipo		73,899,317.71 (40,137,703.56)	87,609,586.8 (51,060,964.01)	89,456,380.55 (47,630,520.81)	2.76E-05	0.0681	0.0197	0.0158	0.0001	0.0711	0.7607	0.399	
ApoJ		35,076,852.22 (6,760,610.92)	36,935,240.02 (7,250,908.7)	39,828,311.84 (8,650,920.67)	3.59E-09	0.0003	0.0635	0.1112	7.05E-10	0.0004	0.0451	0.523	
36 months		TPO	437.79 (119.73)	463.2 (105.38)	478.3 (153.32)	0.0012	0.1789	0.1478	0.6842	0.0023	0.4755	0.5599	0.3682
		IL-18	194.68 (77.96)	199.61 (97.69)	194.57 (94.75)	0.7527	0.876	0.8155	0.5699	0.6417	0.7694	0.6611	0.4462
	FABP3	6953.36 (2904.37)	7988.9 (3178.54)	8026.43 (3242.37)	1.14E-05	0.6319	0.0096	0.7746	0.0001	0.4757	0.8671	0.5791	
	PPY	162.11 (118.95)	172.88 (151.87)	228.83 (165.22)	0.0004	0.2124	0.7835	0.1079	2.56E-05	0.0651	0.0111	0.0119	
	I309	1.91 (1.12)	1.72 (0.89)	2.29 (1.41)	0.0054	0.5469	0.1233	0.0118	0.0001	0.5706	0.0007	0.0266	
	SAA	6,591,850.97 (20,102,101.96)	6,152,281.83 (9,696,054.8)	11,689,850.22 (28,348,629.61)	0.0022	0.0106	0.3237	0.1837	0.0018	0.016	0.3668	0.8677	
	CRP	2,815,106.56 (6,320,376.91)	2,580,568.27 (3,838,300.23)	4,135,642.49 (10,667,549.77)	0.2797	0.9952	0.6139	0.9748	0.3071	0.9831	0.8702	0.8581	
	sVCAM	427,124.68 (103,231.67)	458,365.34 (108,775.08)	493,257.79 (138,934.86)	1.67E-07	0.0134	0.0351	0.2046	1.60E-07	0.0136	0.1919	0.6776	
	sICAM	264,728.18 (65,030.13)	273,235.93 (73,269.22)	290,037.45 (80,897.55)	0.0017	0.1356	0.406	0.624	0.001	0.3142	0.2503	0.5674	
	A2M	1,827,578,796.9 (545,505,679.48)	1,778,523,439.16 (433,650,399.82)	1,981,649,902.93 (624,365,511.97)	0.1339	0.6535	0.4509	0.2519	0.0359	0.6007	0.0396	0.1643	
	B2M	2,449,111.13 (900,460.4)	2,776,486.26 (1,441,411.42)	2,869,544.1 (1,083,739.71)	5.85E-05	0.1774	0.1267	0.4804	4.51E-05	0.1172	0.25	0.5588	
	FVII	859,473.47 (211,784.3)	828,173.72 (239,319.27)	920,802.51 (236,389.85)	0.4943	0.5917	0.3422	0.3441	0.2094	0.4345	0.1037	0.0791	
	Adipo	76,652,983.41 (42,470,109.59)	95,692,502.42 (128,009,842.11)	91,388,867.36 (48,296,710.64)	0.0017	0.2182	0.4015	0.358	4.92E-05	0.0149	0.0003	0.0246	
	ApoJ	35,855,560.96 (6,862,794.16)	36,925,546.32 (7,663,254.78)	39,890,373.94 (8,623,166.76)	0.0027	0.0448	0.6585	0.752	0.001	0.0456	0.0799	0.0771	

Abbreviations: HC, healthy control; MCI, mild cognitive impairment; AD, Alzheimer's disease; SD, standard deviation; TPO, thrombopoietin; IL-18, interleukin-18; FABP3, fatty acid binding protein; PPY, pancreatic polypeptide Y; I309, chemokine I309; SAA, serum amyloid A; CRP, C-reactive protein; sVCAM, soluble vascular adhesion molecule; sICAM, soluble intercellular adhesion molecule; A2M, alpha-2-macroglobulin; B2M, beta-2 microglobulin; FVII, Factor VII; adipo, adiponectin; apoJ, apolipoprotein J.

B = 0.008 ± 0.004 , $P = .03$; AD: B = 0.006 ± 0.01 , $P = .57$), while the mean AD levels for B2M increased more than that for the HC group (HC: B = 0.001 ± 0.001 , $P = .81$; AD: B = 0.03 ± 0.01 , $P = .05$).

3.5. Capability of a combined biomarker model to diagnose AD

To assess the diagnostic capability of the strongest biomarkers for AD at each time point (found via stepwise modeling), an ROC analysis was performed after building a generalized linear model using *APOE* $\epsilon 4$ allele status, age, sVCAM1, PPY, I309, apoJ, SAA, and CRP (main effects model only) with dependent variable HC (set as 0) and AD (set as 1). Training the model using a random 70% of the data, and testing the model on the remaining 30%, and iterating through this 100 times enabled cross-validated prediction estimates of approximately 78%–79% sensitivity and specificity, and AUC values at approximately 85% for all three time points. Repeating this with age, gender, and *APOE* $\epsilon 4$ allele status only, sensitivity, specificity, and AUC values were approximately 77%, 77%, and 83%, respectively.

3.6. Cross-sectional differences between transitional, nontransitional, and stable AD

We further grouped AIBL study participants based on the change in clinical classification over 36 months [23]. Participants were classified into three groups: (1) those HC that did not transition (nontransition), (2) those who transitioned from either HC or MCI to either MCI or AD, including MCI participants (transition), and (3) stable AD participants (stable AD) over the 36-month period (Table 3). Because transitions were classed as change from baseline, statistics from baseline are not shown. Table 4 summarizes the cross-sectional differences in the levels of biomarkers between different categories.

Before adjustment for confounders, biomarkers such as PPY, I309, and B2M were able to significantly differentiate ($P < .00036$) between nontransitional and stable AD categories at both 18- and 36-month time points. At the 18-month but not at the 36-month time point, FABP3 and apoJ were able to differentiate between nontransitional and

stable AD ($P < .00036$). At the 36-month but not at the 18-month time point, SAA and sVCAM were able to differentiate between nontransitional and stable AD ($P < .00036$). None of these associations were statistically significant after adjustment for age, gender, and *APOE* $\epsilon 4$ allele status. Finally, apoJ was significantly increased in the stable AD group compared with the nontransition group at 18 months, both before and after adjustment for confounders ($P < .00036$).

Comparing biomarker levels between nontransition and transition groups at both 18- and 36-month time points, FABP3 and sVCAM were significantly increased in the transition groups over the nontransition groups ($P < .00036$), but only before adjustment for confounders. Adipo and PPY were also significantly increased at the 18-month time point in the transition group ($P < .00036$) compared with the nontransition group, but only before adjustment for confounders. Those associations that were not replicated at multiple time points still showed differences between comparative groups; however, these were only significant at the nominal significance level (without Bonferroni correction, $\alpha = 0.05$, Table 4).

3.7. Correlation with SUVR

Associations of biomarker levels with quantitative SUVR were assessed among the participants who underwent PiB-PET at all three collection time points showing weak-to-moderate associations. Table 5 shows the correlation coefficients (ρ values) from correlation analyses between SUVR and each protein, at baseline, 18 and 36 months. There were correlations for TPO (BL, 18M), FABP3 (BL, 18M), PPY (BL, 18M), I309 (BL), B2M (BL), adipo (18M, 36M), A2M (36M), and apoJ (36M) with SUVR within the MCI subgroup. Within AD group, correlation with SUVR was observed for FABP3 (36M) and apoJ (18M).

4. Discussion

AD is characterized by a series of pathological events. These include amyloid β ($A\beta$) and tau protein deposition, oxidative damage, and inflammation, resulting in neuronal cell death and symptoms of cognitive dysfunction. For

Table 3

Demographics table showing participants in three classifications namely nontransition, transition, and stable AD groups

	18 months			36 months			<i>P</i> -values	
	Nontransition	Stable AD	Transition	Nontransition	Stable AD	Transition	18 months	36 months
<i>N</i>	520	80	79	504	78	91		
Age (mean SD)	70.93 (6.23)	78.64 (7.63)	76.6 (7.62)	72.48 (6.14)	80.75 (7.24)	78.09 (7.67)	<.0001	<.0001
Gender (F/M)	314/206	46/34	41/38	305/199	45/33	49/42	.344	.469
<i>APOE</i> $\epsilon 4$ (–ve/+ve)	375/145	23/57	40/39	366/138	23/55	45/46	<.0001	<.0001
MMSE (median SD)	29 (1.33)	19 (5.73)	26 (3.21)	29 (1.18)	14 (7.16)	25 (4.79)	<.0001	<.0001
SUVR (<i>N</i>)	110	14	29	93	9	33		
SUVR (mean SD)	1.35 (0.36)	2.26 (0.52)	2.1 (1.35)	1.39 (0.37)	2.29 (0.55)	2.07 (0.68)	1.18E–18	8.64E–13

Abbreviations: AD, Alzheimer's disease; SD, standard deviation; MMSE, Mini-Mental State Examination; SUVR, standardized uptake value ratio.

Table 4
Cross-sectional differences between transitional, nontransitional, and stable AD group

Biomarker	Time point	Mean (SD)			All groups		Nontransitional versus stable AD		Nontransition versus transition		Stable AD versus transition	
		Nontransition	Stable AD	Transition	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
TPO	18 months	734,927.17 (915,001.31)	1,213,129.15 (1,769,353.4)	892,786.19 (910,036.55)	0.001	0.008	0.003	0.031	0.001	0.011	0.608	0.649
IL-18	18 months	785,441.3 (882,205.56)	941,067.44 (1,499,137.36)	864,354.28 (1,182,220.84)	0.851	0.780	0.848	0.703	0.843	0.596	0.955	0.647
FABP3	18 months	656,531.23 (650,406.72)	1,216,359.82 (1,600,944.1)	1,210,246.46 (1,189,060.66)	2.54E-09	0.089	4.66E-06	0.609	5.04E-10	0.108	0.732	0.407
PPY	18 months	754,884.28 (942,461.94)	1,100,224.7 (863,039.37)	933,822.1 (1,500,440.09)	0.001	0.645	1.59E-06	0.012	1.89E-04	0.244	0.005	0.005
I309	18 months	745,631.85 (889,056.6)	1,177,579.61 (1,258,715.97)	742,981.38 (861,920.76)	0.037	0.307	1.16E-04	0.132	0.011	0.785	0.006	0.026
SAA	18 months	725,269.34 (856,747.47)	1,220,882.21 (1,766,987.01)	892,634.17 (1,079,246.36)	0.024	0.132	0.005	0.012	0.012	0.038	0.230	0.531
CRP	18 months	802,298.22 (1,013,351.45)	756,708.33 (1,093,736.16)	931,625.38 (1,460,346.78)	0.259	0.715	0.078	0.383	0.196	0.491	0.302	0.433
sVCAM	18 months	692,349.99 (783,842.15)	1,089,117.41 (1,411,766.29)	970,385 (1,438,177.48)	1.39E-04	0.695	0.001	0.580	5.73E-05	0.528	0.552	0.973
sICAM	18 months	715,202.24 (925,269.56)	954,110.77 (1,174,231.67)	955,533.97 (1,243,870.25)	0.013	0.720	0.040	0.758	0.011	0.732	0.880	0.732
A2M	18 months	1,799,650,345.22 (534,498,021.57)	1,976,590,545.15 (633,002,758.16)	1,847,123,591.56 (500,192,450.05)	0.006	0.611	0.006	0.669	0.310	0.798	0.196	0.312
B2M	18 months	2,381,850.43 (907,635.57)	2,765,830.14 (965,757.82)	2,658,577.56 (1,076,595.34)	5.79E-06	0.598	1.83E-05	0.267	0.012	0.989	0.246	0.500
fVII	18 months	860,000.98 (217,912.04)	896,958.07 (219,752.27)	871,258.44 (219,811.81)	0.150	0.197	0.127	0.553	0.639	0.431	0.452	0.932
Adipo	18 months	73,569,851.3 (39,896,168.21)	88,122,933.79 (49,723,473.27)	91,055,858.46 (47,752,718.81)	1.36E-05	0.046	0.002	0.148	1.61E-04	0.002	0.505	0.124
ApoJ	18 months	35,079,670.75 (6,775,600.88)	40,217,838.62 (8,576,304.99)	37,220,101.86 (7,678,193.84)	4.58E-09	2.54E-04	1.11E-09	2.20E-04	0.012	0.072	0.020	0.151
TPO	36 months	703,881.17 (822,936.35)	1,077,448.34 (1,296,890.2)	788,618.72 (667,868.9)	0.009	0.264	0.015	0.646	0.005	0.499	0.583	0.728
IL-18	36 months	746,645.04 (858,530.23)	810,618.65 (1,376,366.07)	838,000.74 (1,111,612.79)	0.792	0.951	0.453	0.691	0.714	0.638	0.526	0.807
FABP3	36 months	822,703.27 (1,049,183.88)	981,094.25 (933,201.02)	1,201,488.19 (1,363,089.1)	5.49E-05	0.855	0.006	0.292	5.38E-05	0.495	0.695	0.338
PPY	36 months	781,974.64 (989,104.79)	1,151,973.57 (1,092,561.08)	954,990.77 (1,174,520.34)	0.006	0.603	1.10E-04	0.059	0.002	0.445	0.046	0.054
I309	36 months	838,533.81 (1,078,746.36)	1,353,072.01 (1,746,175.95)	804,245.28 (931,700.18)	0.145	0.071	1.10E-04	0.235	0.047	0.255	0.001	0.011
SAA	36 months	767,652.82 (800,384.12)	1,345,618.34 (1,628,667.49)	981,199.98 (1,443,722.11)	0.007	0.032	1.80E-04	0.004	0.002	0.007	0.063	0.327
CRP	36 months	814,023.99 (954,192.13)	1,026,815.36 (1,628,202.09)	743,721.04 (864,164.17)	0.326	0.893	0.979	0.328	0.382	0.887	0.416	0.354
sVCAM	36 months	792,271.17 (881,653.84)	1,384,448.99 (1,597,444.07)	1,030,504.74 (1,333,138.23)	5.53E-06	0.118	5.85E-07	0.005	4.81E-07	0.020	0.051	0.107
sICAM	36 months	810,655.53 (993,834.31)	1,252,178.56 (1,731,630.77)	1,025,007.5 (1,135,948.28)	0.004	0.431	0.003	0.269	0.002	0.206	0.353	0.473
A2M	36 months	1,829,309,760.37 (546,555,071.17)	1,994,538,938.13 (671,081,010.33)	1,846,566,354.11 (471,139,174.28)	0.133	0.671	0.030	0.503	0.929	0.366	0.059	0.236
B2M	36 months	2,449,468.3 (902,701.5)	2,906,874.61 (1,081,026.87)	2,732,263.3 (1,245,439.21)	9.75E-05	0.181	1.34E-04	0.125	0.038	0.580	0.133	0.315
fVII	36 months	858,762.71 (211,954.37)	908,875.33 (231,706.93)	888,053.93 (244,355.77)	0.524	0.574	0.242	0.613	0.760	0.678	0.238	0.182
Adipo	36 months	76,456,574.56 (42,427,744.89)	89,827,129.94 (50,476,152.8)	96,095,398.58 (98,293,155.58)	0.001	0.096	1.76E-04	0.025	0.212	0.443	0.023	0.286
ApoJ	36 months	35,837,220.52 (6,875,230.87)	40,429,122.93 (8,704,482.98)	37,761,901.99 (7,900,428.96)	0.003	0.061	0.015	0.205	0.048	0.152	0.556	0.552

Abbreviations: AD, Alzheimer's disease; SD, standard deviation; TPO, thrombopoietin; IL-18, interleukin-18; FABP3, fatty acid binding protein; PPY, pancreatic polypeptide Y; I309, chemokine I309; SAA, serum amyloid A; CRP, C-reactive protein; sVCAM, soluble vascular adhesion molecule; sICAM, soluble intercellular adhesion molecule; A2M, alpha-2-macroglobulin; B2M, beta-2 microglobulin; fVII, Factor VII; adipo, adiponectin; apoJ, apolipoprotein J.

Table 5
Correlation between SUVR and protein biomarkers

Biomarker	All groups	HC	MCI	AD
Baseline				
TPO	0.085	0.039	0.315	-0.284
IL-18	0.097	0.095	0.086	0.147
FABP3	0.186	0.068	0.522	0.242
PPY	0.21	0.116	0.345	-0.011
I309	0.047	-0.051	0.354	-0.189
SAA	0.065	0.073	0.056	-0.021
CRP	-0.009	0.019	-0.069	0.168
sVCAM	0.194	0.111	0.272	-0.305
sICAM	0.159	0.101	0.181	0.053
A2M	0.064	0.063	0.103	-0.11
B2M	0.147	0.128	0.237	-0.005
fVII	0.068	0.027	0.164	0.167
Adipo	0.069	0.016	0.099	-0.062
ApoJ	0.03	0.013	0.01	-0.129
18 months				
TPO	0.103	0.033	0.563	-0.264
IL-18	0.036	-0.02	0.094	0.158
FABP3	0.168	0.029	0.434	0.187
PPY	0.179	0.109	0.328	-0.11
I309	0.03	-0.025	0.246	-0.197
SAA	-0.009	-0.04	0.07	0.005
CRP	-0.087	-0.052	-0.176	0.091
sVCAM	0.141	0.041	0.012	0.024
sICAM	0.067	0.039	0.094	-0.11
A2M	0.011	-0.022	0.035	-0.191
B2M	0.077	-0.005	0.176	-0.239
fVII	-0.019	-0.087	0.211	-0.008
Adipo	0.082	0.01	0.258	-0.167
ApoJ	-0.006	-0.026	-0.023	-0.294
36 months				
TPO	0.082	0.072	0.066	0
IL-18	0.067	0.074	-0.105	0.191
FABP3	0.113	-0.012	0.21	0.399
PPY	0.116	-0.008	0.197	-0.139
I309	-0.004	-0.045	0.026	0.052
SAA	0.006	0.039	-0.013	0.069
CRP	-0.079	0.045	-0.079	0.104
sVCAM	0.158	0.063	0.052	0.13
sICAM	0.101	0.132	-0.013	0.095
A2M	-0.043	-0.024	0.235	-0.098
B2M	0.004	0.005	0	0.02
fVII	-0.115	-0.097	-0.279	-0.163
Adipo	0.081	0.004	0.382	-0.098
ApoJ	-0.005	-0.007	-0.265	-0.085

Abbreviations: SUVR, standardized uptake value ratio; HC, healthy control; MCI, mild cognitive impairment; AD, Alzheimer's disease; TPO, thrombopoietin; IL-18, interleukin-18; FABP3, fatty acid binding protein; PPY, pancreatic polypeptide Y; I309, chemokine I309; SAA, serum amyloid A; CRP, C-reactive protein; sVCAM, soluble vascular adhesion molecule; sICAM, soluble intercellular adhesion molecule; A2M, alpha-2-macroglobulin; B2M, beta-2 microglobulin; fVII, Factor VII; adipo, adiponectin; apoJ, apolipoprotein J.

effective treatment, early diagnosis of AD is essential. However, apart from amyloid imaging, there are no established blood biomarkers for AD. Many biomarkers reported in the literature were limited by cohorts that were not characterized specifically for AD or were the result of cross-sectional studies. Blood-based biomarkers are considered

by many as a significant step forward to improve diagnostic specificity and as a bridge between potential interventions and monitoring of the disease progression.

The current selection of biomarkers has been highlighted by previous work and warranted validation as to their usefulness. In this regard, we have measured their changes in the participants of the well characterized AIBL cohort where cognitive and lifestyle characteristics of each participant are well documented and may be compared. This work was undertaken to set the stage for a programmatic line of work seeking to identify biomarkers of potential relevance to predicting risk for future AD and current clinical diagnosis. Although the results from the current are internally and not externally validated, the biomarkers investigated were chosen on the basis that they had previously shown some association with AD, thus by assessing each of the markers in a large and well-characterized cohort, we have been able to contribute to the evidence base defining their potential use in AD prediction and diagnosis. The current work clearly identifies signals across multiple plasma-based biomarkers that warrant further investigation and shows that many markers are not validated after adjustment for disease-modifying confounders.

Initial cross-sectional analysis of our data with proportional odds logistic regression revealed that 11 of the 14 tested analyte levels changed over the 36 months between HC, MCI, and AD before adjustment for covariates and multiple comparisons. The analytes that demonstrated no change were IL-18, CRP, and fVII. After accounting for the influences of age, sex, *APOE* genotype, and site with the generalized linear model statistical method, we found that the apoJ and I309 levels are raised in AD category compared with HC and MCI participants, respectively, at each of the baseline, 18- and 36-month sample times. This suggests that these molecules might be affected or even have direct roles in the brain as it undergoes pathological changes.

ApoJ, also known as clusterin, was initially discovered over 20 years ago as a molecule which causes some testicular cells and erythrocytes to cluster and aggregate [24]. It appears to be associated with apoptosis in tissues that have been injured or undergoing regression or involution [25–29]; however, its pathological significance remains unclear. Determining the primary function of apoJ is complicated by its propensity to interact with a range of molecules, including itself [30]. It has been thought of as an extracellular heat shock protein due to the presence of a heat shock DNA element in its promoter region [31]. It behaves much like an extracellular version of small heat shock proteins that chaperone misfolded proteins to remain soluble [32]. Its expression is upregulated in many processes including development [33], response to injury and stress, apoptosis [34,35], and neurodegeneration [36]. Therefore, it is no surprise that we find apoJ to be significantly increased in our AD participants compared with their HC counterparts in all of the assessment periods. On examination of apoJ, when the status of participants transits

from HC to MCI or AD, it appears that this analyte does not change markedly. These results suggest that apoJ, as measured in the current protocols, may be most relevant to the specific COU of detecting AD and perhaps monitoring progression within AD rather than the COU of predicting future risk of AD although more work is needed.

In human plasma, this molecule is carried as a component of high density lipoprotein (HDL) [37,38]. As a molecule in the brain, it exists as a lower molecular weight form [39] that is bound to HDL particles [40]. It has been identified in noncovalent reversible complexes with soluble A β and some evidence suggests that it might cross the blood-brain barrier [41,42]. The molecular weight differences between liver-derived plasma apoJ and the astrocyte-derived form in the brain are likely due to variation in glycosylation between these two compartments.

This may be of functional significance for the AD brain because intracellular forms of apoJ lack glycosylation [43] before secretion and also appear to lack chaperone activity [44]. These characteristics coupled with its ability to bind a variety of molecules has led to the theory that secreted apoJ is a molecular chaperone for extracellular misfolded proteins [45–47]. There has been speculation that apoJ might be a physiological carrier of A β , and indeed, there is evidence showing a reduction of A β toxicity through its sequestering action [48]. Because of this, interest in post-translational modification of apoJ has expanded. Although it is known that differentially modified forms of apoJ are made by different tissues [39,49], the significance of these modifications are slowly being discovered [50].

Altered levels of apoJ are not only important for AD [51–53], but also for other conditions [54,55], some of which are linked to metabolic syndrome [56–60], another risk factor for AD. It would be interesting to investigate the altered apoJ levels observed between the AD and healthy participants over time in light of post-translational modifications. Further monitoring of our cohort is still ongoing to gather more conclusive data. ApoJ may yet be a useful early monitoring tool in combination with other biomarkers for cases of suspected AD.

PPY also demonstrated differences when examining our data in those participants whose clinical status was AD. This applied for all three periodic assessments, where the AD group exhibited higher levels of PPY compared with HC. This molecule has been identified in previous blood-based biomarker investigations [3,8,9,61,62] as being associated with MCI and AD but its role is currently unclear. PPY is a small signaling molecule secreted by PP cells at the periphery of Islets of Langerhans within the pancreas and released into the circulation after a meal [63]. The most widely accepted function for PPY is the regulation of postprandial appetite suppression via actions on the gastrointestinal tract and brain [64] via the Y4 and Y5 neuropeptide receptors which initiate vagal signaling [65,66]. However, it has also been shown to control other pancreatic secretions [67–71]. An impaired feeding

induced response from the gut that produces low levels of PPY is associated with obesity and hyperphagia [72,73], whereas excess PPY results in weight loss [74]. In the AIBL cohort, the AD participants have lower average weight [16], suggesting that an overproduction of PPY might be a factor. This could arise from an over stimulation of the vagal nerve [75]. Interestingly, vagal nerve stimulation was once considered as a treatment for AD [76] because of previously observed improvements in cognition and depression from this treatment [77,78]. This suggests that increased PPY may have relationship with dietary physiology and might be a response to AD brain pathology hence is most likely relevant for COU of AD diagnosis.

Adipo is a protein hormone which has come to light due to its connection with antiobesity [79] and neuroprotective effects. It regulates many functions including inflammatory response [80], food intake, glucose regulation [81], and fatty acid catabolism in the periphery. It is secreted into the circulation by adipose tissue as a collection of full-length species ranging up to trimeric and larger species and even globular form. Indeed, low levels of adipo are considered a risk factor for metabolic syndrome [82], which itself is a risk factor for AD. In our cohort, increased adipo levels were seen in the AD participants over their HC counterparts at baseline and 36-month sampling periods. This increase is consistent with previous work [83,84] which also shows that there is some correlation in CSF. Although the 18-month period showed a similar trend, this might suggest that the alteration is a gradual process in response to changing brain pathology. This may also be a response to neuroinflammation because it is a relatively abundant anti-inflammatory adipokine which concomitantly reduces expression of proinflammatory cytokines [85] while increasing anti-inflammatory cytokines [86]. This work suggests that adipo may be of most use in the specific COU of AD diagnostics.

Among the biomarkers tested in the transition from MCI to AD, chemokine I-309 (CCL1) was found to show the most significant changes. This is consistent with previous work in CSF where it was associated with cognitive decline [1] and in other biomarker panels [9,87]. This molecule is a glycoprotein that is secreted by activated T-lymphocytes [88,89] and is related to a class of inflammatory cytokines that carry the C-C motif. Its primary function appears to be to attract monocytes, natural killer cells, immature B cells, and dendritic cells, which all carry the necessary CCR8 receptor [90]. It also elicits activation of monocyte intracellular calcium mobilization as part of the immune response [89].

Generally, chemokines are produced locally at sites of inflammation or infection to regulate recruitment of other immune cells such as leukocytes and lymphocytes. The consistent increase of I-309 in the MCI-AD transition participants could indicate increasing brain injury or pathology. Chemokines are typically released by endothelial cells on stimulation by inflammatory cytokines [91–93], but I-309 is unusual because it is released by the more mobile

T-lymphocytes, which could suggest a wider immune response from the body. One of the possible effects may be to attract immune cells to injured areas, such as to help clear away amyloid as seen in studies of the similar CCL2 receptor [94]. The current data suggest that plasma I-309 levels may be most useful in the COU of detecting AD and distinguishing AD from MCI. Interestingly, we also find it to be changed significantly in those who underwent transition from MCI to AD over all three test periods. It is possible that specific alterations of I-309 at specific time points may also be indicative of imminent likelihood of transition from MCI to AD.

sVCAM1 belongs to a class of cell adhesion molecules (CAMs), binding proteins that appear to be important in inflammatory or injury processes involving the endothelium and blood cells, such as platelets. These soluble molecules are generally present in the circulation near sites of injury to the endothelium, such as an atherosclerotic plaque [95–99]. After cytokine activation, CAMs are released into the circulation, which may then be detected. It has been reported that measurement of these molecules could be useful in diagnosis of cardiovascular injury [100–103]. When comparing the HC with AD participants, sVCAM did not change markedly until 36 months in our study. This delay might indicate that sVCAM might be a reparatory physiological response to injury and inflammation, rather than an initiator that directly influences the pathology because it is upregulated in endothelial cells that have been stimulated by cytokines [104]. It mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to the vasculature [105,106] and is implicated in the early stages of atherosclerosis [107]. This suggests that peripheral vascular injury contributes to the pathology of the AD-affected brain. Further follow-up examinations of the cohort would help to shed more light on the physiological processes involved.

There are significant advantages of the present study over much prior work. First, the current work examined longitudinal change in multiple previously identified potential AD blood-based biomarkers. In addition, the sample size is significantly larger than much prior work and the deep phenotypic characterization of the AIBL cohort is an additional advantage. In the current work, multiple biomarkers were examined longitudinally to identify a set of markers for further examination in the COU of diagnosing and predicting future risk of AD. In this work, multiple markers were supported as potential diagnostic AD biomarkers while others were only likely useful for future risk prediction. As was pointed out recently by an international working group (including the current team), the first step in moving biomarker discovery to potential clinical use is the identification and initial support of the biomarkers within the specific COU. The current work addresses that first step and sets the stage for movement toward (1) additional replication of these biomarkers (individually and in combination) as well as (2) discovery

of additional biomarkers of relevance to this specific COU. The AIBL cohort provides a unique cohort for these next steps, which will then be replicated across independent cohorts.

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RESEARCH IN CONTEXT

1. Systematic review: Blood biomarkers have attracted a great deal of attention in recent times in regards to early diagnosis and monitoring of Alzheimer's disease (AD); however, there is a huge inconsistency in the field.
2. Interpretation: We have used plasma samples from the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study of Ageing cohort to specifically answer the question if plasma protein biomarkers can be used for diagnostic accuracy. Our results show that levels of I309 and PPY were significantly increased in AD participants compared with MCI participants over time. We also showed apolipoprotein J to be increased in AD patients compared with healthy participants longitudinally.
3. Future directions: The current work addresses that first step and sets the stage for movement towards (1) additional replication of these biomarkers (individually and in combination) as well as (2) discovery of additional biomarkers of relevance to this specific COU. The AIBL cohort provides a unique cohort for these next steps, which will then be replicated across independent cohorts.

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