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Original Article

A BLOOD BASED PREDICTOR FOR NEOCORTICAL Aβ BURDEN IN ALZHEIMER'S DISEASE: RESULTS FROM THE AIBL STUDY

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Running head: Neocortical Aβ Burden Prediction

ABSTRACT

Dementia is a global epidemic with Alzheimer's disease (AD) being the leading cause. Early identification of patients at risk of developing AD is now becoming an international priority. Neocortical Aβ Burden (NAB), as assessed by positron emission tomography (PET) represents one such marker for early identification. These scans are expensive and are not widely available, thus there is a need for cheaper and more widely accessible alternatives. Addressing this need, a blood biomarker based signature having efficacy for the prediction of NAB and that can be easily adapted for population screening, is described. Blood data (176 analytes measured in plasma) and PiB-PET measurements from 273 participants from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study were utilised. Univariate analysis was conducted to assess the difference of plasma measures between high and low NAB groups and cross-validated machine-learning models were generated for predicting NAB. These models were applied to 817 non-imaged AIBL subjects and 82 subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) for validation. Five analytes showed significant difference between subjects with high compared to low NAB. A machine-learning model (based on nine markers) achieved sensitivity and specificity of 80% and 82%, respectively for predicting NAB. Validation using the ADNI cohort yielded similar results (sensitivity 79%, specificity 76%). These results show that a panel of blood-based biomarkers is able to accurately predict NAB, supporting the hypothesis for a relationship between a blood-based signature and Aβ accumulation, therefore providing a platform for developing a population based screen.

INTRODUCTION

The only definitive diagnosis for Alzheimer's disease (AD) is by histological examination at autopsy, with clinical diagnosis based on medical history, clinical examination and neuropsychological testing[1]. However, clinical symptoms only present after significant irreversible neurological degeneration has occurred[2]. Thus, there is a need for a simple population based screening test that is able to identify subjects at risk of developing AD at the pre-symptomatic stages[3].

The hallmark histological markers of AD are the presence of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles (NFT) principally comprised of hyperphosphorylated Tau (pTau)[4]. Currently two leading sets of biomarkers are available which show strong correlations with these histological markers of AD. Firstly, imaging markers which bind Aβ, (e.g. Pittsburgh Compound B; PiB) coupled with Positron Emission Tomography (PET), that provide semi-quantitative assessments of neocortical Aβ burden (NAB)[5]. The other leading set of biomarkers is derived from cerebral spinal fluid (CSF) and includes measures of Aβ (in particular the $Aβ_{1-42}$ species) that correlate with Aβ in the brain and total Tau and pTau that correlate with NFTs[6]. However, both of these sets of biomarkers have limited use as general population-screening tools for AD. PET imaging is relatively expensive and its availability is limited, whilst the collection of CSF requires a lumbar puncture which is invasive. Widespread population screening requires alternative approaches to estimate NAB derived from more accessible tissues, such as blood.

Given the strong association between NAB and AD pathology and the need of population blood-based screening tests to identify those individuals at risk of developing AD, this study presents a blood biomarker panel whose composite score correlates with NAB, as assessed by PiB-PET. The results are validated using samples taken from an independent study.

METHODS

a)   Cohorts:

(i)   The Australian Imaging, Biomarkers and Lifestyle (AIBL) Study.

Detailed information on the study design and enrolment procedures has been discussed elsewhere[7]. The AIBL study is a prospective longitudinal study of aging, integrating data from neuroimaging, biomarkers, lifestyle, clinical and neuropsychological analysis. Eligible volunteers, aged over 60 years and fluent in English, were classified into three groups: 1) individuals meeting NINCDS-ADRDA criteria for AD[1], 2) individuals meeting criteria for Mild Cognitive Impairment (MCI)[8, 9] and 3) cognitively healthy individuals (HC). The institutional ethics committees of Austin Health, St Vincent's Health, Hollywood Private Hospital and Edith Cowan University approved the AIBL study, and all volunteers gave written informed consent before participating.

  Blood Collection, Processing and Analytes Assays.

Whole blood (80 mL) was collected in the morning (after overnight fasting) by venipuncture (blood processing procedure, is detailed in the Supplementary Methods Section 1 a) of Supplementary Material). EDTA plasma samples were shipped to Myriad Rules-Based Medicine (Myriad RBM; Austin, Texas, www.rulesbasedmedicine.com), on dry ice, for analysis using commercially available multiplexed Luminex human discovery 151MAP panel. All assays were validated according to CLIA standards and no samples were older than 18 months at the time of analysis. The assays used for EDTA plasma Aβ analysis were both the well-documented double sandwich ELISA technique[10-12] and the commercial luminex based kit (INNO-BIA plasma Aβ forms, Innogenetics, NV, Belgium), as previously described[13]. EDTA Plasma APOE protein levels were measured using a commercial assay (Pan APOE ELISA, MBL co., Ltd.)[14]. Apolipoprotein E (*APOE*) genotyping using polymerase chain reaction amplification and restriction enzyme digestions[15]. Plasma was collected in Sarstedt (01.1608.100 S-Monovette 7.5 mL) lithium heparin tubes for the measurement of seven metal ion levels using induction coupled plasma mass spectrometry (ICP-MS).

(ii)   Alzheimer's Disease Neuroimaging Initiative (ADNI):

For validation purposes, data from 82 participants were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. Details on the study design, enrolment procedures and sample collection are given in Supplementary Methods (Section 1 b) of Supplementary Material).

b)   Dataset Quality Control

1090 of the AIBL participants underwent full blood analyses comprised of 57 pathology blood analytes and 169 plasma analytes (151 from the Myriad RBM xMap discovery panel version one, 13 Metals, Apolipoprotein E (APOE) levels, Innogenetics and Mehta based ELISAs for Aβ1-40 and Aβ1-42). Data points outside 5 standard deviations of the median were treated as missing along with any failed assays (the measurement were below the detectable limit, for the analytes measured on the Myriad RBM multiplex platform) from the blood sample analyses. Analytes with over five percent missing data (see Supplementary Material; Supplementary Table 1, for list), or any subjects with over 50 percent missing data (not applicable in this instance as no AIBL participant who had undergone both imaging and full blood analyses had more than 50% missing data) were removed. This resulted in a working dataset of 176 blood analytes and two ratios (53 Pathology, 111 MyriadRBM, 7 Metals, APOE levels, Innogenetics and Mehta Aβ1-40, Aβ1-42 and their ratios; listed in Supplementary Table 1) for 1090 AIBL subjects, 273 of whom had undergone PiB-PET imaging. The pattern of missingness in the resulting dataset was random (i.e. the missing data was not biased to a particular clinical diagnostic group) and missing data points were imputed using multiple imputations by chain equation16 (all data in the working data set was used). Median values of the hundredth iteration of 100 multiple imputations were used. To adopt normality and to ensure uniformity of residuals in the imputation (and the ensuing) analyses all variables were log transformed prior to imputation. The same quality control procedures were applied independently to the validation (ADNI) blood analyte data, resulting in 176 blood analytes and one ratio for 82 subjects, 113 of the analytes and one ratio overlapping with those in the AIBL dataset

c)   Image Analysis

273 AIBL subjects and 82 ADNI subjects, for who relevant blood samples had been analysed, underwent PiB-PET imaging. The PiB imaging methodology of the AIBL and ADNI studies is detailed elsewhere[5, 17]. The AIBL and ADNI PiB-PET scans for each participant were spatially normalised to a customized PiB-PET template in the Montreal Neurological Institute reference space using Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Centre for Neuroimaging, London, United-Kingdom). The spatially normalised PiB-PET images were then scaled using the cerebellar grey matter as reference region for the generation of Standardized Uptake Value ratios (SUVR). Regional analysis was obtained using the Automated Anatomical Labeling (AAL)[18] template restricted to the grey matter (GM) segmentation. The GM masking of the AAL ensured that the PiB measurement did not contain any CSF or white matter voxels. Regional measurements were averaged across both hemispheres. Neocortical Aβ burden (NAB) was expressed as the average SUVR of the mean of frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions. A SUVR cut-off of 1.3, determined through a cluster analysis of HC individuals, was used to classify participants as belonging to a high or a low NAB group.

d)   Statistical Analysis

Specific details of the statistical software and settings used throughout this section are also given in Supplementary Methods (Section 1 d) of Supplementary Material).

(i)   Demographic Evaluation

The demographic make-up of both the AIBL and ADNI imaged cohorts, split by high and low NAB, for Clinical Classification (HC, MCI or AD), age, gender, *APOE ε4* status, years of education and Clinical Dementia Rate Scale (CDR) sum of boxes are given by Table 1. Demographic differences between the high and low NAB were assessed using a χ^2 test for the categorical variables, analysis of variance (ANOVA) for the continuous variables was applied, due to the non-normality of the CDR sum of boxes the Mann-Whitney U test was applied. Also the demographic make-up of both the AIBL imaged and non-imaged cohorts as well as the ADNI imaged cohort, split by Clinical Classification, are given in Supplementary Table 2.

(ii)   Exploratory Analyses

The 176 blood analytes were assessed, using Analysis of Covariance (ANCOVA), to see if their concentrations differed between participants with high versus low NAB. The results were corrected for age, site, gender and *APOE ε4* carrier status , in addition to these main effects, the pairwise interactions between NAB status and age, ApoE ε4 carries status, and gender were also included in the models. To minimize any false positive results the p-values were adjusted for false discovery rate (FDR)[19].

(iii)   Variable Selection and Model Generation

Data for the 273 imaged AIBL participants was initially used for variable selection and model generation; data from the ADNI imaged and AIBL non-imaged cohorts was set aside at this stage. Measures of the 176 blood analytes (along with age, gender, *APOE* genotype and years of education) were considered in variable selection and model generation to predict the continuous SUVR values, through cross-validated Random Forest (RF) analysis. The smallest panel of analytes that gave the least squared error in the cross validation predictions of SUVR was chosen for in-depth validation analysis in attempt to assess potential for use as a biomarker panel for the prediction of NAB status.

To ensure that the identified analytes were robust to the analysis protocol employed, the identified panel was assessed by a second analysis protocol which was based on the binary response of NAB status: namely, cross-validated Support Vector Machine (SVM) analysis with a RBF kernel coupled.

In an attempt to specifically quantify the value added by the blood biomarkers, six separate cross-validated RF sub-models were constructed. Three of the sub-models also included a clinical variable (CDR sum of boxes), incorporated to improve model performance and to provide a comparative assessment of the added value of the blood biomarkers. The six sub-models assessed were model 1 (M1) blood-based markers, age, *APOE* genotype and CDR sum of boxes; model 2 (M2) blood-based markers, age and *APOE* genotype; model 3 (M3) blood-based markers and; model 4 (M4) age, *APOE* genotype and CDR sum of boxes; model 5 (M5) age and CDR sum of boxes; model 6 (M6) age and *APOE* genotype. For validation purposes the resultant sub-models were applied to the imaged ADNI cohort to predict high or low NAB status.

To ensure that the signal was specifically associated with NAB and not just with clinical or cognitive status, the best performing of these sub-models was assessed for efficacy in individual clinical diagnosis groups[a]. Finally, the best performing of these sub-models was also applied to the non-imaged AIBL cohort. As no actual NAB information was available for this cohort, the percentages of those predicted to have high NAB for each clinical diagnosis group was compared to those of the imaged AIBL cohort and the literature.

Three-fold cross validation with 100 repeats was adopted and sensitivities, specificities and area under the curve (AUC) performance indicators with standard deviations (SD) were calculated. The predictions of SUVR were split into predicted high or low NAB, based on cut offs providing the most equal sensitivities and specificities, for the purpose of reporting performance statistics.

RESULTS

a)   Demographics

Table 1 describes the demographic characteristics of the AIBL and ADNI imaged cohorts. There was a large prevalence of MCI and AD subjects, as well as an enrichment of *APOE ε4* carriers, in the high NAB groups when compared with the low NAB groups for both AIBL and ADNI. There was also an enrichment of elderly subjects in the AIBL high NAB group.

b)   Exploratory Analysis: Blood-based analyte differences between high and low NAB

Supplementary Table 1 details the age, gender, site and *APOE ε4* carrier status adjusted means of the blood-based analytes for the two NAB groups. After adjusting for false discovery rates, five analytes showed a significant difference between the NAB groups: Immunoglobulin M-1 (IgM-1) and free thyroxine (FT4) were found to be lower in the high

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[a] Only one imaged AIBL AD subject had low NAB status, therefore accurate performance statistics could not be calculated for the AIBL AD group; for the ADNI cohort there were only 3 HC subjects (all low NAB status) and no AD subjects with low NAB status so performance statistics were not calculated for the individual clinical diagnosis groups in ADNI.

NAB group (p = 0.019 and 0.009 respectively, Figure 1, A and B); whilst macrophage inflammatory protein 1α (MIP1α), pancreatic polypeptide (PPY) and vascular cell adhesion protein (VCAM-1) were all found to be elevated in the high NAB group (p = 0.027, 0.01 and 0.01 respectively, Figure 1 C-E).

c)   Variable Selection and Model Generation

(i)   Biomarker Identification

The RF variable selection analysis identified a panel of six biomarkers ($A\beta_{1-42}$, chemokine ligand 13 (CXCL-13), IgM-1, interleukin 17 (IL-17), PPY and VCAM-1) as well as Age and *APOE* genotype. Together, these analytes form the model M2. Three of these analytes (IgM-1, PPY and VCAM-1) were also identified as being significantly different between the NAB groups using ANCOVA, Figure 1 B, D and E.

(ii)   Performance Statistics

The cross-validated RF model (M2) achieved a sensitivity, specificity and AUC of 79.6% (SD=1.6%), 79.4% (SD=1.4%) and 83.9% (SD=1.0%), respectively; the cross-validated SVM model achieved a similar sensitivity, specificity and AUC of 74.2% (SD=0.8%), 75.7% (SD=1.4%) and 83.7% (SD=1.6%), respectively. Full performance statistics for the six cross-validated RF sub-models are given by Table 2 and Figure 2A. Based on the AUC, it can be seen that addition of the blood-based markers to the models, resulted in increases in performance of 9% above the demographic and CDR sum of boxes alone (M1 *cf.* M4) and nearly 14% above the demographic variables alone (M2 *cf.* M6). The inclusion of CDR sum of boxes improved the models by 4% (M1 *cf.* M2) and 8% (M4 *cf.* M6). The addition of the blood-based markers increased performance by approximately 5% (M2 *cf.* M4 and M3 *cf.* M5) in contrast to the addition of CDR sum of boxes.

M1 achieved a sensitivity, specificity and AUC of 80.0% (SD=1.1%), 74.2% (SD=1.2%) and 78.4% (SD=0.7%), respectively, in the HC group and a sensitivity, specificity and AUC of 80.1% (SD=1.0%), 81.2% (SD=0.9%) and 81.6% (SD=0.5%), respectively, in the MCI group. Thus, relatively consistent performance is displayed across the overall dataset and both the HC and MCI groups.

(iii)   Application to the ADNI validation samples

Unfortunately there were no measurements of IL-17 in the validation cohort. Instead, the median IL-17 measurement from the AIBL cohort was substituted for each of the 82 ADNI samples. Then, the six RF sub-models (M1: M6) generated using AIBL samples were applied to the ADNI validation dataset, performance statistics are given by Table 2 and Figure 2B. It can be seen that M1 achieves 84.7% AUC when applied to the ADNI cohort. It should be noted that dropping IL-17 from the AIBL model results in an AUC of 83.9% (SD=1.1%).

(iv)   Application to the non-imaged AIBL samples

The RF model M1 was applied to the 817 AIBL participants who had not undergone the imaging protocol, to predict their expected NAB. All AD, 87% of MCI and 35% of HC participants were predicted to have high NAB, compared with 98% AD, 69% MCI and 34% HC deemed to have high NAB by imaging protocol in the AIBL imaged cohort (Figure 3).

DISCUSSION

This study describes how blood-based molecular signatures can be implemented to predict a person's neocortical amyloid burden (NAB). The signature presented here achieves sensitivity, specificity and AUC of 79.6%, 82.4% and 87.6%, respectively, in the AIBL cohort. Validation in a comparable and independent second cohort, ADNI, achieved similar levels of accuracy with sensitivity, specificity and AUC of 78.3%, 76.3% and 84.7%, respectively. The signature is represented by a RF model of a short list of markers, namely: age, *APOE* genotype, $A\beta_{1-42}$, CXCL-13, IgM-1, IL-17, PPY, VCAM-1 and includes a clinical variable (CDR sum of boxes) which slightly improves model performance (increase in AUC of 4%). However, the blood based panel is shown to have efficacy above that of CDR sum of boxes with a 9% additional increase in AUC when the blood panel is added to a model of age, *APOE* genotype and CDR sum of boxes and a 5% comparative increase in AUC if the blood panel is added to age and *APOE* genotype instead of CDR sum of boxes. It should be noted that IL-17 was not included in the validation signature due to assay failure in the ADNI cohort, regardless the performance statistics of the validation signature were comparable with those of the AIBL signature. Further, removal of IL-17 from the AIBL signature (AUC of 83.9%) was still comparable to that of the validation signature. It should also be noted that an SVM model showed similar performance statistics, using the same set of blood-based markers indicating that the set of markers are robust to the choice of algorithm.

Frank and colleagues[20] describe an ideal biomarker for AD as: able to detect a fundamental pathological feature, be validated in neuropathologically confirmed cases, have sensitivity and specificity each above 80%, be reliable, reproducible, noninvasive, simple to perform and inexpensive. Here it has been demonstrated that an additional 10% has been added to performance statistics by inclusion of a small number of blood measurements, over a more traditional signature based on demographic and clinical variables. Thus, inclusion of blood based measurements means that a signature for the prediction of NAB in line with the desirable features of a biomarker as described above has been achieved. In translation to a clinical setting it means that only the measurement of a small number of blood analytes along with standard demographic and clinical variables are necessary to obtain an indication of the NAB in a patient. Thus, such a signature, after further validation, would provide a much needed first pass community level screening option which in turn could provide justification for more invasive or costly confirmation tests such as CSF

analysis or PET, which currently do not lend themselves to widespread population screening due to their invasive or costly nature.

The ADNI cohort provided little data with which to validate our signature in the HC class (N=3), however we observed that performance statistics in the imaged AIBL cohort were consistent across clinical diagnosis groups. This indicates that the identified signal is appropriately tracking with NAB status, opposed to solely tracking with clinical or cognitive status. Percentages of individuals predicted to have high NAB were estimated in the non-imaged AIBL cohort for the specific clinical diagnostic groups (Figure 3). In line with the literature[21-24], the model predicted all of AD, 87% of MCI and around a 35% of HC participants to have high NAB. Given that 35% of HC participants were identified to be at risk of having high NAB over 13% of the MCI group, where arguably the HC participants have better clinical and cognitive representations than their MCI counterparts, further indicates that the signal identified is associated with NAB status and not that of clinical or cognitive status.

The inclusion of age and *APOE* genotype markers, commonly corrected for in models associated with AD as they are the two leading risk factors for AD[25], is not an unexpected result. Among the other analytes, pancreatic polypeptide has been identified by several other AD proteomic studies[26-28]. Pancreatic polypeptide is produced by the endocrine F cells in the periphery of the pancreatic islets, and levels in plasma dramatically increase in response to food ingestion[29, 30] playing a role in regulating satiety[31] as well as insulin release and gut emptying[30]. While PPY levels are positively correlated with age[29], we observed a significant increase in the high NAB group, after adjusting for age. The significance of the increase observed in subjects with high NAB and in AD subjects[26] is unclear. However, as elevated PPY has been associated with inflammation[32], the elevation of PPY in the high NAB group maybe part an immune signature as previously reported[33].

A number of the other biomarkers, CXCL-13, IgM-1, IL-17 and VCAM-1 also have reported involvement in (acute) immune response and/or immune signaling (see OMIM[34] records 605149, 147020, 603149, 192225, 109535, 123260). For the classification of AD and HC subjects in other proteomic studies[26, 28, 33] similar immune based signatures have been reported.

Alzheimer's pathological Aβ processing is evident in the brain; however, there is ongoing debate if this is also represented in the plasma[13, 35-39] (Rembach *et al.*)[b]. A number of studies have shown that Aβ species in plasma and blood cells including the plasma ratio of $A\beta_{1-42}/A\beta_{1-40}$ have a mild correlation with NAB, with the direction of the relationship being Aβ species dependent [13, 40, 41] (Rembach *et al.*)[a].

The inclusion of biomarkers other than those assessed in this contribution (Supplementary Table 1a) may improve the efficacy of the model presented: in an attempt to explore this, additional blood analytes are being measured within AIBL. As longitudinal monitoring of the AIBL and other cohorts progress, indicators of the efficacy of such models in predicting progression to disease will be able to be examined. Additionally, further validation of the findings presented here for disease specificity in other disease cohorts is essential.

Estimates of NAB using CSF Aβ measurements have been reported[42], but these assessments rely on obtaining CSF, a procedure considered invasive by many. Also, there are studies, reporting the efficacy of blood-based signatures in discriminating between AD and aged matched controls[26-28, 33, 43]. To our knowledge, this contribution is the first study to report efficacy, validated in an independent separate cohort, for a (non-invasive) blood-based predictor of NAB. The clinical implications of fulfilling AD biomarker criteria[20] are crucial and especially relevant now that a more sensible paradigm has been proposed as the new diagnostic criteria for AD[3, 44, 45] MCI[46], and preclinical AD[3] in a model that integrates cognitive, biochemical and imaging biomarkers to provide a better predictive framework. With some further improvement in biomarker selection, the method described here will allow wide and non-invasive population screening, as well as participant selection for therapeutic trials that, after confirmation of high NAB by imaging or CSF, might also be used to follow up these individuals over time or monitor the efficacy of anti-Aβ therapy.

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NAB has been demonstrated by many to be a good predictor for progression to AD[3, 5, 22, 47-49]. Also biomarker signatures similar to the one presented here can easily be adapted to a low-cost routine test or rolled out as a high throughput population screen. Thus, this work may represent the first step in developing an economical screening tool for early detection of individuals at risk of developing AD, thus allowing earlier disease-specific therapeutic interventions aimed at halting or slowing down this devastating disease.

Conflict of Interest Statement:

We declare that Colin L. Masters and Ashley I. Bush are consultants with Prana Biotechnology. Further, a patent has been filed covering the biomarker algorithm from this work and the author's institutions may benefit from commercialisation of this patent.

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TABLES

Table 1. The Demographic and Clinical make-up of the AIBL and ADNI imaged sub-cohorts. [*]Interquartile Range (IQR).

		High Neocortical SUVR (>1.3)	Low Neocortical SUVR (<1.3)	p-value
AIBL Imaged Cohort	Number of Participants: [N]	160	113	
	Clinical Diagnosis: [N (%)]	HC: 72 (45) MCI: 40 (25) AD: 48 (30)	HC: 97 (86) MCI: 15 (13) AD: 1 (1)	<0.001
	Age: [mean (SD)]	74.5 (7.7)	69.7 (7.2)	<0.001
	Gender; Males: [N (%)]	79 (49)	53 (47)	0.78
	Years of Education: [N (%)]	<9: 20 (13) 9-12: 63 (39) 13-15: 34 (21) >15: 43 (27)	<9: 8 (7) 9-12: 43 (38) 13-15: 19 (17) >15: 43 (38)	0.05
	CDR Sum of Boxes: [median (IQR[*])]	0.5 (0.0 - 3.0)	0.0 (0.0 - 0.0)	<0.001
	APOE ε4 carrier: [N (%)]	106 (66)	32 (28)	<0.001
ADNI Imaged Cohort	Number of Participants: [N]	59	23	
	Clinical Diagnosis: [N (%)]	HC: 0 (0) MCI: 42 (71) AD: 17 (29)	HC: 3 (13) MCI: 18 (78) AD: 2 (9)	<0.01
	Age: [mean (SD)]	78.9 (8.3)	78.4 (7.7)	0.78
	Gender; Males: [N (%)]	20 (34)	7 (30)	0.97
	Years of Education: [N (%)]	<9: 0 (0) 9-12: 10 (17) 13-15: 11 (19) >15: 38 (64)	<9: 0 (0) 9-12: 7 (30) 13-15: 1 (4) >15: 15 (65)	0.15
	CDR Sum of Boxes: [median (IQR[*])]	2.0 (1.0 - 3.0)	1.0 (0.5 – 2.0)	0.03
	APOE ε4 carrier: [N (%)]	37 (63)	6 (26)	<0.01

Table 2. The receiver operating characteristics for the efficacy of the cross-validated RF sub-models applied to both the

AIBL and the ADNI imaged sub-cohorts

Model	Markers	AIBL Imaged Cohort			ADNI Imaged Cohort		
		Sens% (SD%)	Spec% (SD%)	AUC% (SD%)	Sens %	Spec %	AUC %
M1	Age, *APOE* Genotype, CXCL-13, IgM-1, IL-17, PPY, VCAM-1, $A\beta_{1-42}$, CDR sum of boxes	79.6 (1.3)	82.4 (1.2)	87.6 (0.7)	78.3	76.3	84.7
M2	Age, *APOE* Genotype, CXCL-13, IgM-1, IL-17, PPY, VCAM-1, $A\beta_{1-42}$	79.6 (1.6)	79.4 (1.4)	83.9 (1.0)	73.9	74.6	81.7
M3	Age, CXCL-13, IgM-1, IL-17, PPY, VCAM-1, $A\beta_{1-42}$	73.2 (1.8)	73.3 (1.8)	80.8 (1.7)	68.1	73.6	68.8
M4	Age, *APOE* Genotype, CDR sum of boxes	71.4 (1.1)	71.5 (1.1)	78.3 (0.8)	72.7	67.9	73.7
M5	Age, CDR sum of boxes	69.0 (2.1)	68.9 (2.2)	76.0 (2.0)	59.1	62.3	64.5
M6	Age, *APOE* Genotype	66.7 (1.6)	66.7 (1.6)	70.2 (1.3)	77.3	62.3	67.8

FIGURE LEGENDS

Figure 1. Univalent differences of blood analytes between the high and low NAB groups of the imaged AIBL sub-cohort. A: FT4; B: IgM; C: MIP 1α; D: PPY; E: VCAM 1. The horizontal line within the box represents the median, the lower and upper boundaries of the box represent the lower and upper quartiles, which define the interquartile range (IQR) and the whiskers are 1.5 times the IQR.

Figure 2. A: ROC curves for the cross-validated RF sub-models applied to the imaged AIBL sub-cohort; B: ROC curves for RF sub-models applied to the imaged ADNI sub-cohort. [Black = M1, Orange = M2, Blue = M3, Green = M4, Pink = M5, Grey = M6].

Figure 3. A: Actual SUVR values for the imaged AIBL sub-cohort split by Clinical Diagnosis; B: Predicted SUVR values for the non-imaged AIBL sub-cohort split by Clinical Diagnosis.