A blood-based signature of cerebral spinal fluid Aβ_{1-42} status

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Abstract

It is increasingly recognized that Alzheimer's disease (AD) exists before dementia is present and that shifts in amyloid beta occur long before clinical symptoms can be detected. Early detection of these molecular changes is a key aspect for the success of interventions aimed at slowing down rates of cognitive decline. Recent evidence indicates that of the two established methods for measuring amyloid, a decrease in cerebral spinal fluid (CSF) amyloid β_{1-42} (Aβ_{1-42}) may be an earlier indicator of Alzheimer's disease risk than measures of amyloid obtained from Positron Emission Topography (PET). However, CSF collection is highly invasive and expensive. In contrast, blood collection is routinely performed, minimally invasive and cheap.

In this work, we develop a blood-based signature that can provide a cheap and minimally invasive estimation of an individual's CSF amyloid status. We show that a Random Forest model derived from plasma analytes can accurately predict subjects as having abnormal (low) CSF Aβ_{1-42} levels indicative of AD risk (0.84 AUC, 0.73 sensitivity, and 0.76 specificity). Post-hoc analysis indicates that only six analytes are required to achieve similar performance. Furthermore, we show across an independent validation cohort that individuals with predicted abnormal CSF Aβ_{1-42} levels transitioned to an AD diagnosis over 120 months significantly faster than those predicted with normal CSF Aβ_{1-42} levels.

This is the first study to show that a plasma protein signature, together with age and *APOEϵ4* genotype, is able to predict CSF Aβ_{1-42} levels with high accuracy. Biomarkers in plasma have previously been shown to be predictive of PET amyloid levels. This work further highlights the potential for developing a blood-based signature for improved AD screening, critical for drug and intervention trials.

Keywords: Alzheimer's Disease, Amyloid β_{1-42}, Biomarkers, Cerebral Spinal Fluid, risk prediction, Blood-based biomarkers, Random Forest

1. Introduction

Alzheimer's disease (AD) is a terminal neurodegenerative disease that has historically been diagnosed based on "clinically significant" cognitive decline of an individual and exclusion of other conditions. However, it is increasingly recognized that AD is a decades-long neurodegenerative process, with shifts in amyloid beta (Aβ_{1-42}) providing the first indicators of disease development, long before "Alzheimer's dementia" (significant cognitive decline) is clinically apparent[1–5].

There is currently no cure or disease-modifying therapy for this terminal illness despite hundreds of clinical trials being conducted since 2002 [6, 7]. It is hypothesized that the high failure rate of AD trials is in part due to the inclusion of clinically diagnosed AD patients, who are therefore in the late stages of the disease, as well as the discovery that many patients, up to 20% in one study [8], were retrospectively found to have normal levels of amyloid and hence did not have AD [9]. Given these findings, there is a great interest in

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amyloid screening for clinical trial enrichment in order to recruit individuals at the earliest stages of AD, where intervention are thought to have the greatest chance of success [10], and ensure that included individuals are amyloid positive (i.e have abnormal levels of amyloid), a necessary precondition for the development of AD. This selective screening is a precursor for the longer-term goal of population screening for AD [11].

There are currently two established methods to measure an individual's amyloid burden: either in vivo in the form of reduced levels of Aβ_{1-42} in the cerebral spinal fluid (CSF) or increased uptake of radioactive tracers that bind selectively to the Aβ fibrillary aggregates by PET imaging.

Unfortunately, existing methodologies for measuring an individual's amyloid levels suffer drawbacks that limit their utility for either clinical trial recruitment or population screening. Lumbar punctures are highly invasive, with this factor alone likely to be sufficient to limit applicability for screening. While PET scans are less invasive, they are far more expensive and access to PET scanning facilities is limited in some regions. Despite this, nearly all current trials related to amyloid now require positive amyloid imaging at baseline to ensure accurate diagnosis, a cost-intensive process[7].

Despite their invasiveness, recent studies have found evidence that changes in CSF Aβ_{1-42} may indicate AD risk long before these same changes are reflected in PET Aβ imaging[12–14]. Palmqvist et al [13] have shown shown compelling evidence that changes in CSF Aβ_{1-42} occur up to a decade before the same signal is found by PET Aβ imaging. These results indicate that CSF may be a more suitable measure for early detection, whereas Aβ PET contributes independent information that is more related to disease progression and downstream pathology.

To bypass the invasiveness of CSF collection, there is a strong interest in finding blood-based markers that yield the same information about amyloid status as would be obtained from CSF. There have been a number of studies which have shown that a blood protein signature reflects AD brain pathology as measured by PET [15–24]. But as of yet, no study has looked at the association between blood proteins and CSF amyloid levels.

Here, we evaluated the ability of proteomic and metabolomic data to predict the levels of CSF Aβ_{1-42} using a Random Forest (RF) approach and explore which types of measurements lead to the strongest predictive performance. We then find the minimal set of features required to achieve comparable levels of predictive performance. Finally, we demonstrate the robustness and utility of these predictive models demonstrating across a held-out validation cohort of individuals with mild cognitive impairment (MCI) that subjects with predicted abnormal CSF Aβ_{1-42} levels showed a faster rate of cognitive decline (measured by transition to AD) than those with predicted normal CSF Aβ_{1-42} levels.

2. Methods

2.1. Overview of cohort and measurements

The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a large, multicenter, longitudinal neuroimaging study, launched in 2004 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and non-profit organizations.

ADNI is a longitudinal study of older adults, designed to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD.

After quality control, a total of 566 individuals were present in the ADNI cohort (Table 1).

This cohort was split into a training and a validation cohort with 356 and 210 individuals with and without measures of CSF Aβ_{1-42}, respectively. The training set was used to build predictive models and evaluate their performance directly using the measured Aβ_{1-42} levels while the validation cohort was not used to build the predictive model, but was instead used to evaluate the generalizability and utility of the predicted CSF status. Further demographic information for these cohorts can be found in Supplementary tables 2 to 4.

All individuals in this study had 297 measures at baseline including age, $APOE\epsilon4$ carrier status, 149 protein levels (including 146 RBM proteins, homocysteine, as well as $A\beta_{1-40}$ and $A\beta_{1-42}$) and 146 LC-MS/MS metabolites. For the training cohort, levels of neurofilament light chain (NFL) in plasma were also available. All missing data points were imputed using an unsupervised RF approach. Details of these measures and their association quality control procedures are provided in Supplementary material.

2.2. Binary and regression modelling tasks

The core challenge in this work was to produce a model that predicts if an individual's CSF Aβ_{1-42} levels are below the recognized clinical threshold of 192pg/ml, indicating an abnormal CSF Aβ_{1-42} level and hence AD risk. Given the continuous CSF Aβ_{1-42} measures in the ADNI cohort, two approaches were considered for predicting this binary status: a 'regression' task, learning the continuous CSF Aβ_{1-42} levels and thresholding these post-prediction and a 'binary' task, learning the dichotomized CSF Aβ_{1-42} status based on clinical thresholds directly. While both tasks result in a binary classifier, they face different trade-offs. The regression task makes use of the full information in the CSF levels but needs to learn a suitable threshold to convert its continuous predictions into suitable binary labels whereas the binary task only learns from the dichotomized CSF

Diagnosis	CN		MCI		AD	
With CSF Aβ_{1-42}	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
Number of participants (n)	0	58	198	198	10	102
Age (mean, (sd))	-	75.11 (5.77)	75.13 (7.32)	74.35 (7.48)	73.73 (10.04)	74.90 (7.91)
Gender; female (n, (%))	-	28 (48.28)	75 (37.88)	65 (32.82)	4 (40.00)	43 (42.16)
Years of education (mean, (sd))	-	15.67 (2.78)	15.48 (3.09)	15.81 (2.99)	14.70 (2.06)	15.16 (3.29)
***APOEϵ4* carriers (n, (%))**	-	5 (8.62)	105 (53.03)	106 (53.54)	5 (50.00)	71 (69.61)
CSF $A\beta_{1-42}$ (mean, (sd))	-	250.84 (21.08)	-	163.44 (52.76)	-	142.69 (39.15)

Table 1: Demographic characteristics of the ADNI data set used.

levels. Given these trade-offs, we have investigated both modelling approaches throughout this work.

2.3. Statistical modeling using RF

Random Forests were selected as the modeling approach to predict CSF Aβ_{1-42} levels for both the binary and regression tasks. Random Forests are a widely used ensemble method that have a number of advantages for the small sample size and disparate types of features observed in the ADNI dataset. Due to their use of bagging, RFs can be evaluated without the need for an independent validation set by evaluating the *'out-of-bag'* (OOB) performance. Details of the RF motivation, implementation and parameters used can be found in the Supplementary Methods. For the regression models, a threshold on the continuous predictions was necessary to generate binary labels. Here, we selected the cutoff along the Receiver Operating Characteristic (ROC) curve that maximizes Cohens kappa, a function of both the sensitivity and specificity [25]. We also compared the performance of RFs against other machine learning algorithms (Supplementary Methods, Supplementary Figure 1 demonstrating that RFs were comparable or better than all other approaches.

2.4. Evaluating importance of different modalities

The input variables were separated into four classes: a commonly used baseline model (B) including age and *APOE*ϵ4 carrier status; Proteomics (P), including 149 protein levels; Metabolomics (M), including 146 metabolites and lipids; and plasma Neurofilament Light (NFL). NFL was analyzed separately as it has shown a lot of promise as a marker of neuronal injury and was only available for the training cohort.

Six separate random forests were created using different subsets of these features to determine which type of features were most useful for modeling CSF Aβ_{1-42}. We denoted these models by the combination of features they included; for example 'BPM' refers to a model built using only the Base, Proteomics and Metabolomics features. The best performing model was selected for all subsequent analysis.

2.5. Measures of model performance

Model performance based on the out-of-bag predictions was summarized by area under the ROC curve (AUC), accuracy, sensitivity and specificity. R^2 values were also calculated for the regression task. Increases in AUC between models were tested for significance using a one-tailed DeLong test [26].

2.5.1. Discovery of smallest feature subset required for strong predictive performance

For the best-performing model in the training cohort, we determined the minimum set of individual analytes necessary to achieve high predictive performance. We determined the impact of each feature by evaluating their *Variable Importance* defined as the difference in the prediction error on the out-of-bag data when a given feature was permuted and unpermuted [27]. A forward stepwise procedure was then used: RFs were independently trained over increasing numbers of features, starting with the feature with the highest Variable Importance and consecutively adding in the next ranked feature. Each RF was then evaluated in terms of its AUC. Finally, we selected the smallest subset of features that achieved within 1% of the maximal observed AUC.

2.6. Survival Analysis

A survival analysis was performed to determine if the rate of conversion from MCI to AD was different between those with predicted low and normal CSF Aβ_{1-42} levels,

	Regression					Binary			
Feature set	AUC	R^2	Acc	Sens	Spec	AUC	Acc	Sens	Spec
BPM+NFL	**0.83**	0.31	0.75	0.80	0.65	0.81	0.73	0.98	0.22
BP+NFL	**0.85**	0.33	0.76	0.76	0.77	**0.84**	0.76	0.97	0.34
BPM	0.82	0.31	0.76	0.77	0.74	**0.82**	0.73	0.98	0.21
BP	**0.84**	0.32	0.74	0.73	0.76	**0.84**	0.75	0.95	0.34
BM	0.78	0.26	0.72	0.71	0.74	0.77	0.71	0.94	0.24
B	0.77	0.29	0.72	0.75	0.66	0.73	0.69	0.77	0.53

Table 2: Out of bag performances (area under the receiver operator curve, AUC; accuracy, Acc; sensitivity, Sens; specificity, Spec and R^2 for the regression models) for the different Random Forest models using different feature sets. Left and right halves are for the Regression and Binary tasks respectively. Bold faced text on AUCs indicates the best performing model or those that are statistically equivalent (via Delong's test, with a Bonferroni-corrected significance threshold of 0.05/5=0.01). Features sets describe combinations of (B) baseline model (age and $APOE\epsilon4$ genotype), (P) Proteomics, (M) Metabolomics and (NFL) plasma neurofilament light.

enabling us to determine if our predictions lead to useful clinical outcomes in the validation cohort.

Three separate analyses were performed: using the measured CSFS status on the training set (n=198), and using the predicted CSF status from either the BP and BP6 (model with the smallest set of features) models in the validation set (n=198). For each analysis, we have examined the hazard ratios using Cox regression and used log-rank tests to compare the survival distribution of low/normal CSF Aβ_{1-42} stratifications in the three analyses, as well to compare equivalence between the actual and predicted stratifications.

3. Results

3.1. Models utilizing protein levels accurately predicts CSF positivity

We evaluated the ability of blood-based biomarkers to predict CSF $A\beta_{1-42}$ normal/abnormal status using RFs trained using six different subsets of input variables, treating the prediction as a regression or binary task. The performance metrics from these 12 RFs are shown in Table 2 with their corresponding ROC curves shown in Figure 1.

We observed strong overall predictive performance (OOB AUC $\geq$ 0.84) for both the regression and binary tasks. All sets of features outperformed the base model of age and $APOE\epsilon4$ carrier status with BP+NFL based models leading to the highest AUC in both tasks.

Using the BP+NFL features resulted in an R^2 of 0.33 for the regression task. The automatically derived threshold for this regression RF was 188.6 pg/mL, close to the clinically validated cutoff of 192 pg/mL. Using this threshold to dichotomize the continuous predictions yielded an out-of-bag accuracy of 0.77, with a sensitivity of 0.76 and a specificity of 0.77. Investigation of the continuous predictions prior to thresholding showed that the range of the predicted $A\beta_{1-42}$ values were reduced compared to the actual levels but maintained a strong correlation (Supplementary Figure 4).

Similar results could be observed for learning the dichotomized CSF labels directly (AUC 0.84, accuracy 0.76).For the binary task, a slight drop in both AUC and accuracy, as well as an altered trade-off between sensitivity and specificity was observed across all different feature sets compared to the regression task.

While all sets of features outperformed the base model, models that made use of the protein level measurements consistently achieved the strongest predictive performance. While the BP+NFL RF had the highest AUC in both the regression and binary tasks, the drop in performance was not significant when NFL was removed, i.e. BP feature set (p = 0.024 and 0.33 for regression and binary tasks, respectively)Given that NFL is only available for the training set and may be conflated with neuronal injury (see Discussion), we have used the BP feature set as the basis for analysis for the rest of our analysis.

To ensure that our imputation procedure did not bias our results, we also built similar models using only complete cases (n = 130), obtaining similar AUCs of 0.80 and 0.79 for the regression and binary RFs despite a substantial drop in sample size (Supplementary Figure 2).

3.2. Model performance is maintained using only six proteins

The models described so far used all ($>$ 140) available features. In practice, measuring hundreds of analytes is costly, negating a key advantage of using blood biomarkers for screening. Given this, we have conducted a post-hoc analysis of the BP regression RF to identify the smallest number of features that still achieved high accuracy. Using a stepwise feature selection procedure (Methods), we identified a subset of six plasma analytes (Figure 3) critical for model performance: Chromogranin-A (CGA), Eotaxin 3, Aβ_{1-42} (AB42), C-reactive protein (CRP), Apolipoprotein E (APOE) and Insulin. This combination of protein levels, together with age and $APOE\epsilon4$ is denoted as BP6 and achieved an OOB AUC, sensitivity and specificity of 0.85, 0.8 and 0.8 respectively on the training cohort. As these performance measures are based on data that was also used to select the six proteins, these measures provide an overly

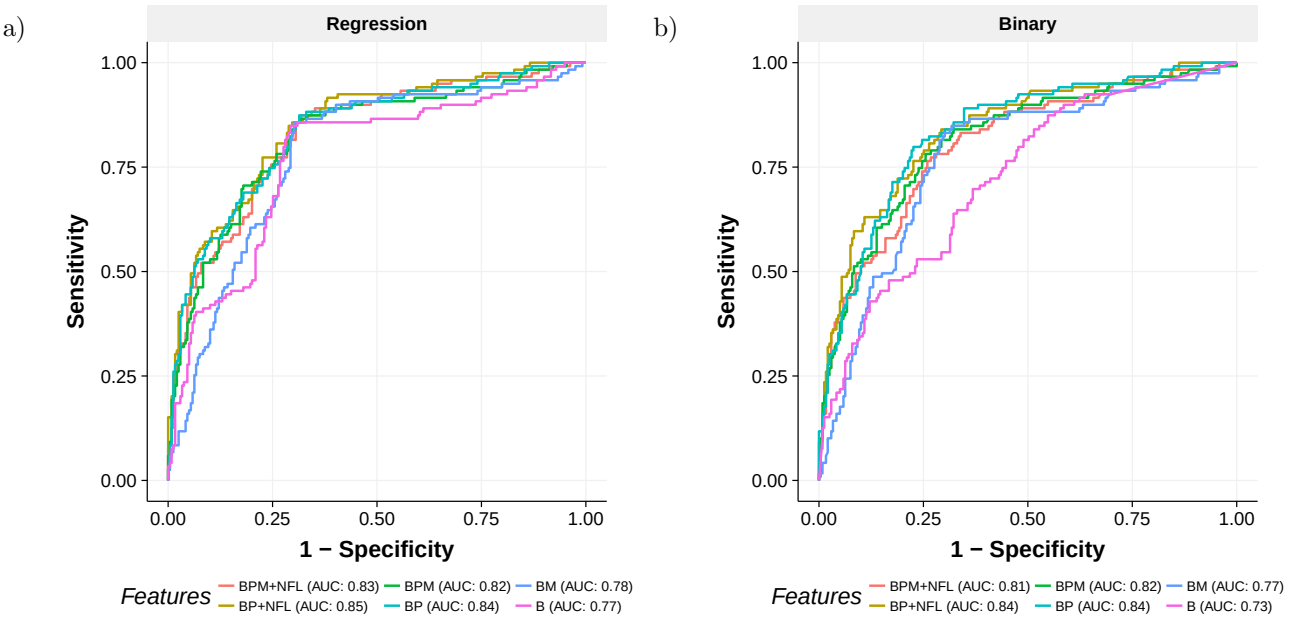

Figure 1: ROC curves comparing different sets of features to determine predictive value for the a) regression, and B) binary tasks. Different colours of lines correspond to different feature sets with corresponding AUCs indicating in the legend under each plot.

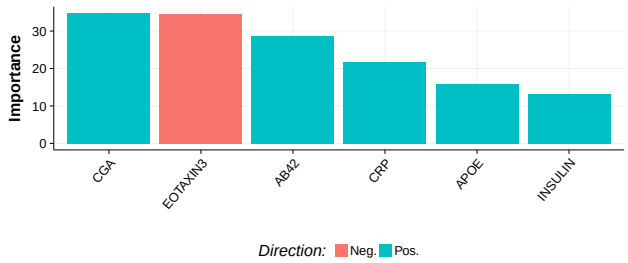

Figure 2: Variable importance of the smallest set of proteins that achieve an OOB AUC within 1% of the maximum OOB observed from including base features (age and $APOE\epsilon4$ carrier status) and increasing numbers of proteins. Colour indicates the direction of association with CSF $A\beta_{1-42}$, as determined by examining the sign of the linear regression coefficient.

optimistic estimation as to the model's ability to predict CSF $A\beta_{1-42}$ status on unseen data [28].

3.3. Validation of clinical utility

To demonstrate the utility of our modeling on unseen data, we conducted a survival analysis over the validation cohort (n=198), evaluating the probability of baseline MCI individuals transitioning to AD diagnosis over 120 months, stratified by predicted CSF $A\beta_{1-42}$ status from either the BP or reduced BP6 model. These survival distributions could then be compared to those of the real $A\beta$ status observed over the training cohort. Given the demographic similarity of the two cohorts, we would expect to see strong similarities in rates of conversion.

From Figure 3, we observed that in all cases, the predicted low CSF $A\beta_{1-42}$ group transitioned to AD significantly faster than the $A\beta_{1-42}$ normal group (log rank test $p = 8.72\times10^{-7}, 2.43\times10^{-4}, 5.78\times10^{-4}$ for the three plots left to right, respectively). Comparing the predictions from the BP and BP6 models on the validation cohort to the actual CSF $A\beta_{1-42}$ status on the training cohort, we find that there is no significant difference between the survival distributions for either the normal (log-rank test $p = 0.26, 0.17$) or abnormal (log-rank test $p = 0.49, 0.48$) survival distributions, respectively, reflecting the overlapping confidence intervals of the hazard ratios.

The highly significant differences in conversion rates between the predicted normal/low strata, especially from the more parsimonious BP6 model, together with their similarity to the survival distributions of the actual CSF measures, provide strong evidence that our blood-based biomarkers have similar utility to actual CSF $A\beta_{1_42}$ levels, given the similar properties of the two cohorts (Table 1).

As a further validation, we compared the distribution of continuous predictions (i.e. before thresholding) in the training and validation cohorts, finding similar distributions with no significant difference (via Mann-Whitney U test) across the different diagnosis groups (Supplementary Figure 3), providing further evidence of the robustness of our predictions in the held-out validation set.

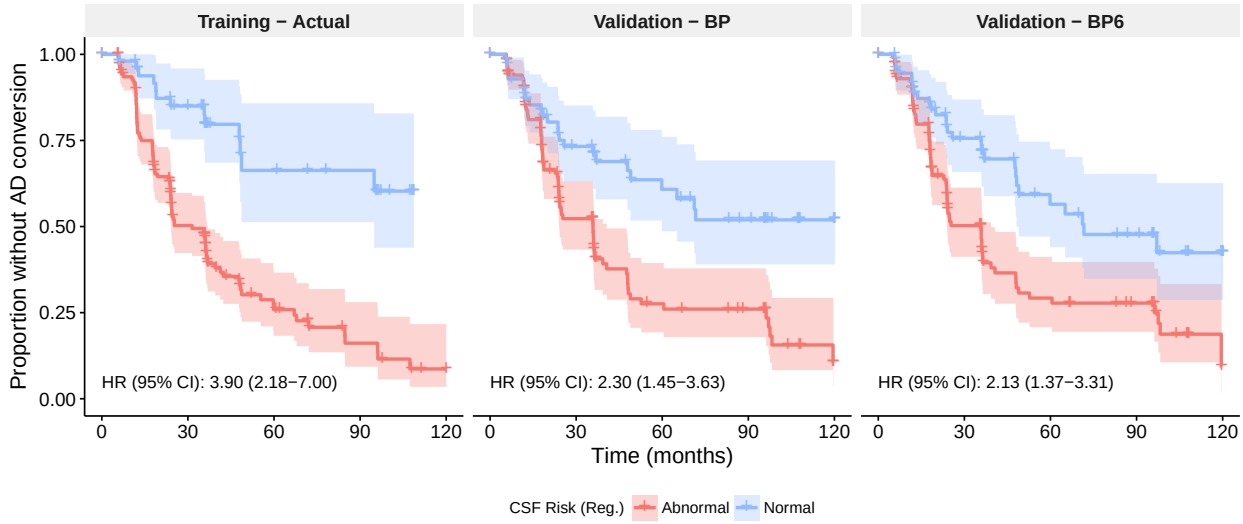

Figure 3: Kaplan-Meier curves for a) the training cohort stratified by actual CSF A142 status. b) the validation cohort stratified by predicted CSF Aβ_{1-42} status from the BP model and c) the validation cohort stratified by predicted CSF Aβ_{1-42} status from the BP6 model. The transparent ribbons along the curves represent the 95% confidence intervals. Hazards ratios and 95% confidence intervals for the abnormal group compared to the normal are shown in the bottom left of each subplot. In all cases, the low CSF Aβ_{1-42} group transitioned to AD diagnosis significantly faster then the normal group ($p = 8.72\times10^{-7}, 2.43\times10^{-4}, 5.78\times10^{-4}$ for the three plots left-right). CN individuals were not included in this analysis given that there were no CN individuals present in the validation cohort.

4. Discussion

The most positive results from AD trials to date have been found in patients with early forms of the disease, leading to an increasing awareness that treatments are likely to be most successful if applied at the earliest stages of AD [10]. While some AD clinical trials are enriching pre-symptomatic AD individuals with PET screening, recent findings that shifts in CSF amyloid can be observed up to a decade before those from PET may indicate that CSF positive individuals are more suitable for clinical trial enrichment [29]. Direct measurement of CSF biomarkers is too invasive to be used in such a screening test [30]. However, the development of a minimally-invasive, low-cost solution that provides the same type of information would overcome these limitations.

This current study provides the first steps towards a minimally invasive test for CSF Aβ_{1-42} status by examining whether plasma biomarkers could be used to predict CSF Aβ_{1-42} levels cross-sectionally. We demonstrated that a RF predicting CSF Aβ_{1-42} normal/abnormal status using age, *APOEϵ4* carrier status and protein levels achieved a high AUC, sensitivity and specificity of 0.84, 0.73 and 0.76 respectively. Compared to the base model (age and *APOEϵ*4 genotype) the inclusion of the plasma analytes improved the performance (AUC) by 6-9%. To make the model more suitable for clinical application, we identified a RF (BP6) consisting of only six plasma analytes that still achieved a high AUC, sensitivity and specificity of 0.85, 0.80 and 0.80 respectively These predictive models were then validated on a separate cohort of individuals to demonstrate that MCI subjects with predicted abnormal CSF Aβ_{1-42} (low) levels transitioned to an AD diagnosis over 120 months at a significantly higher rate than those predicted with high CSF Aβ_{1-42} levels, and that these rates were similar to those observed in a demographically similar cohort of MCIs using actual CSF Aβ_{1-42} levels. This is a strong validation of our modelling as the blood-based biomarkers for CSF Aβ_{1-42} status is only useful if they can replicate the behavior of the actual Aβ_{1-42} status for clinically relevant endpoints for individuals that were not used to build the predictive model.

A number of studies have previously investigated the use of blood analytes to predict the burden of amyloid in the neocortex, as measured by PET [15, 16, 18–20, 22, 23]. Some of these studies showed similar performance metrics to those reported in this work ($>$ 0.80 AUCs [15, 23, 24] or $>$ 0.78 Accuracy[17]), indicating that prediction of PET and CSF *A*β_{1-42} status are of similar difficulty. PET Aβ is directly related to brain fibrillar amyloid, whereas CSF amyloid is a marker of soluble Aβ_{1-42} and they may, therefore, give different insights into AD progression and mechanisms. For example, CSF Aβ_{1-42} has been shown to be associated with *APOEϵ4* whereas PET has been shown to have a greater association with tau [31]. Thus, the development of a blood-based screening test for CSF Aβ_{1-42} levels is a complementary approach to existing blood-based biomarkers of PET amyloid status.

The use of metabolites appeared to be of limited

utility for predicting CSF Aβ_{1-42}. In both the regression and binary tasks, models containing metabolites and proteomic data (BPM+NFL, BPM) achieved equivalent or worse AUCs than models containing only the proteomic data (BP+NFL, BP). Furthermore, we observed that the use of the base features and metabolites alone (BM) lead to only small improvements above the baseline model. These findings can be contrast with the utility of metabolites in predicting PET Aβ_{1-42} positivity [22] and their association with AD more broadly[32]. It may be that more sophisticated pre-processing of metabolite levels, such as adjusting for lifestyle and medication usage, or alternative methods for integrating this source of data [33], may be required in order to find robust associations with CSF Aβ_{1-42} status.

The subset of features found to be critical for our reduced-feature random forest model included age, *APOEϵ4* genotype, Chromogranin-A (CGA), Eotaxin 3, Aβ_{1-42} (AB42), C-reactive protein (CRP), Apolipoprotein E (APOE) and Insulin. Several of these identified proteins have known associations with Alzheimer's disease and two of the proteins, APOE and Aβ_{1-42} (AB42), have been previously associated PET amyloid levels [15, 19].

Unsurprisingly, the levels of plasma APOE are associated with CSF amyloid levels. *APOEϵ4* is the strongest genetic risk factor for AD. APOE is involved in the clearance of Aβ_{1-42} [34–36] and there is a strong relationship between *APOE4* genotype and APOE plasma levels, where *APOEϵ4* carriers have lower plasma levels [37, 38].

Plasma Aβ_{1-42} showed a positive relationship in our model for CSF Aβ_{1-42}, in line with a prior observation [39]. This is interesting, as the link between alterations of Aβ_{1-42} levels in the blood and the progression of the disease is still controversial and studies assessing the Aβ_{1-42} concentration in blood of AD patients have produced conflicting results [39–45].

Chromogranin A (CGA) is associated with synaptic function, and has traditionally been used as an indicator of neuroendocrine tumors [46]. However, more recent work has shown that CGA has a degree of co-localisation with amyloid plaques in the brain [47, 48]. However, levels of CGA in the CSF and blood serum do not appear to be correlated [49], and serum CGA has not previously been linked to AD.

Eotaxin 3, also known as C-C chemokine ligand 26 (CCL26), plays an important role in the innate immune system and has been found to be dysregulated in AD patients[50]. CSF Eotaxin-3 has been shown to be significantly elevated in patients with prodromal AD, however, Eotaxin-3 levels in plasma or the CSF do not correlate with rates of disease progression [50, 51]

A number of previous studies have demonstrated an association between C-reactive protein (CRP) and AD, with the general consensus that individuals with AD have decreased serum levels of CRP [52–54]. Notably, there is also a strong association between the *APOEϵ4* polymorphism and low levels of CRP [55, 56].

CSF Aβ_{1-42} has been associated with plasma insulin levels [57], and insulin resistance (IR), measured as a function of plasma insulin and glucose levels [58]. Insulin resistance has also previously been associated with an increased risk of developing AD across multiple studies [59]. However, determining the direction of association between IR and AD, including CSF biomarkers, remains an active area of research [60].

This study has several limitations. The training and validation cohorts are both part of the ADNI study and thus all measures were conducted on the same platforms. Hence further cross-cohort and cross-platform replication is required. This remains an ongoing issue within the development of all AD biomarkers relating to early screening and requires significant future investment [61]. Furthermore, the current cohort is neuropathology biased, i.e. 84% of the cohort have MCI or AD, and thus likely to have neuronal damage, potentially confounding the analysis of CSF Aβ_{1-42} status. While this motivated us to exclude plasma neurofilament light (NFL), which has been shown to be strongly associated with disease progression in AD rather than an early biomarker of AD [62], untangling the relationship between disease status, disease progression and CSF Aβ_{1-42} remains challenging. Finally, it needs to be noted that there are other medical conditions that are known to affect CSF Aβ_{1-42} levels and it is unclear whether these affect any of the patients in our cohort.

The early identification of AD disease is paramount and a major global focus as the success of disease-modifying or preventative therapies in AD may depend on detecting the earliest signs of abnormal amyloid-beta load. The differences between CSF Aβ_{1-42} and PET Aβ_{1-42} in preclinical stages of AD could also have implications in clinical trial settings. Blood-based biomarkers of amyloid can serve as the first step in a multistage screening procedure, similar to those that have been clinically-implemented in cancer, cardiovascular disease, and infectious diseases [61]. In-conjunction with biomarkers for neocortical amyloid burden, the CSF Aβ_{1-42} biomarkers presented in this work may help yield a cheap, non-invasive tool for both improving clinical trials targeting amyloid and population screening.

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