



Published in final edited form as:

J Alzheimers Dis. 2024 November ; 102(1): 89–98. doi:10.1177/13872877241283692.

Added value of inflammatory plasma biomarkers to pathologic biomarkers in predicting preclinical Alzheimer's disease

Haley Leclerc¹, Athene KW Lee^{2,3}, Zachary J Kunicki³, Jessica Alber^{1,2,4}

¹Interdisciplinary Neuroscience Program, University of Rhode Island, Kingston, RI, USA

²Butler Hospital Memory & Aging Program, Providence, RI, USA

³Department of Psychiatry and Human Behavior, Alpert Medical School of Brown University, Providence, RI, USA

⁴Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, USA

Abstract

Background: Plasma biomarkers have recently emerged for the diagnosis, assessment, and disease monitoring of Alzheimer's disease (AD), but have yet to be fully validated in preclinical AD. In addition to AD pathologic plasma bio-markers (amyloid- β ($A\beta$) and phosphorylated tau (p-tau) species), a proteomic panel can discriminate between symptomatic AD and cognitively unimpaired older adults in a dementia clinic population.

Objective: Examine the added value of a plasma proteomic panel, validated in symptomatic AD, over standard AD pathologic plasma biomarkers and demographic and genetic (apolipoprotein (*APOE*) $\epsilon 4$ status) risk factors in detecting preclinical AD.

Methods: 125 cognitively unimpaired older adults (mean age = 66 years) who completed $A\beta$ PET and plasma draw were analyzed using multiple regression with $A\beta$ PET status (positive versus negative) as the outcome to determine the best fit for predicting preclinical AD. Model 1 included age, education, and gender. Model 2 and 3 added predictors *APOE* $\epsilon 4$ status (carrier versus non-carrier) and AD pathologic blood biomarkers ($A\beta_{42/40}$ ratio, p-tau181), respectively. Random forest modeling established the 5 proteomic markers from the proteomic panel that best predicted $A\beta$ PET status, and these markers were added in Model 4.

Results: The best model for predicting $A\beta$ PET status included age, years of education, *APOE* $\epsilon 4$ status, $A\beta_{42/40}$ ratio, and p-tau181. Adding the top 5 proteomic markers did not significantly improve the model.

Article reuse guidelines: sagepub.com/journals-permissions

Corresponding author: Jessica Alber, 130 Flagg Road, Kingston, RI 02881, USA. jalber@uri.edu.

Author contributions

Haley Leclerc (Formal analysis; Writing—original draft; Writing—review & editing); Athene K.W. Lee (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing—review & editing); Zachary J. Kunicki (Formal analysis; Methodology; Supervision; Writing—review & editing); Jessica Alber (Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Writing—original draft; Writing—review & editing).

Conclusions: Proteomic markers in plasma did not add predictive value to standard AD pathologic plasma biomarkers in predicting preclinical AD in this sample.

Keywords

biomarkers; early diagnosis; inflammation; plasma proteomics; preclinical Alzheimer's disease

Introduction

Alzheimer's disease (AD) is the fifth leading cause of death for adults over the age of 65 in the United States,¹ and a projected 13.8 million people will have the disease by 2050.² The discovery of preclinical AD,³ a phase of AD during which amyloid- β plaques (A β) accumulate in the brain but older adults are cognitively unimpaired (CU), and do not yet exhibit any clinical symptoms of dementia, led to targeting potential secondary prevention therapeutics in AD, with the eventual goal of treating AD pathology prior to diagnosis, when cognitive symptoms and loss of function are already evident. Currently, one new therapy has been approved by the US Food and Drug Administration (FDA) for removing A β in symptomatic AD patients and another is pending FDA approval. However, clinical trials of preventative AD therapeutics are not cost-effective and have high screen-fail rates, as identifying preclinical disease in the general population requires screening of thousands of individuals.

Current measures to assess for the presence of preclinical AD include A β positron emission tomography (PET) scans using radioactive ligands and cerebrospinal fluid (CSF) testing using lumbar puncture. Secondary prevention treatment, once available, will require point-of-care screening for preclinical AD, which is not sustainable from a public health perspective using A β PET and CSF testing. Both techniques come with relatively high cost, low accessibility, are moderately invasive, and require specialist teams for administration and interpretation. Therefore, there is an unmet need to develop biomarkers for the detection of preclinical AD that are minimally invasive, cost-effective, and can be administered in point-of-care (i.e., primary care provider) settings and analyzed on site or analyzed at a central laboratory to: (1) advance secondary prevention treatment goals; and (2) improve cost and time efficiency of preclinical AD trials of new potential therapeutics.

There are standard demographic factors that increase risk for preclinical and clinical AD: age, gender, and educational attainment. Additionally, the apolipoprotein (*APOE*) ϵ 4 allele has a critical role in A β deposition levels, aggregation of lipids, and regulation of α -synuclein aggregation, neuroinflammation, lipid metabolism, and synaptic plasticity.⁴ *APOE* ϵ 4 status in CU older adults was found to be different between those who were positive (A β +) versus negative (A β -) on A β PET, showing correlation between carrying at least one copy of the *APOE* ϵ 4 allele and A β + status,⁵ and clinical trials of AD prevention therapeutics have focused on *APOE* ϵ 4 as a key target risk factor for enrollment.⁶

Plasma biomarkers have emerged as a potential minimally invasive, cost-efficient, accessible biomarker for both symptomatic and preclinical AD. Unlike A β PET and lumbar puncture, a blood draw can be performed in a point-of-care setting and is relatively inexpensive to process/interpret. Established AD pathology related biomarkers of AD in plasma

include A β _{42/40}, phosphorylated tau181 (p-tau181), phosphorylated tau231 (p-tau231), phosphorylated tau217 (p-tau217), glial fibrillary acidic protein (GFAP), and neurofilament light (NfL). Whereas these biomarkers have been assessed and validated for diagnosis and disease monitoring in symptomatic AD,^{7–12} their use and validation in preclinical AD requires further research.^{13,14}

However, there are many other processes that are synergistic to AD pathogenesis. Notably, the current AD research framework for diagnosis of AD left open the consideration of new biomarkers as the field progressed.¹⁵ Inflammation is a known attribute of AD pathogenesis.¹⁶ Specifically, A β and tau can bind to receptors in astrocytes and microglia, which are key components of the innate immune system, and trigger an immune response that releases inflammatory mediators throughout the brain. When these mediators accumulate, there are higher amounts of neuroinflammation which in turn worsens AD pathogenesis.¹⁷ Inflammation can also interfere with the brain's immune processes and response to further enhance disease progression.¹⁶ Additionally, both vascular (see¹⁸ for a review) and metabolic changes¹⁹ are known contributors to AD pathogenesis. Therefore, plasma biomarkers examining inflammatory, metabolic, and vascular changes hold can be valuable tools for AD diagnosis, assessment, and disease monitoring.

To that end, O'Bryant et al.²⁰ developed a panel composed of inflammatory, metabolic, and vascular proteins that accurately diagnosed symptomatic AD versus healthy control participants in the Texas Alzheimer's Research and Care Consortium, a sample largely recruited through dementia specialty clinics (area under the curve (AUC) = 0.91). The panel has also been validated for detection of symptomatic AD in the Alzheimer's Disease Neuroimaging Initiative sample,²¹ and across assay platforms,²² as well as across 4 large, independent, multi-ethnic and community/clinic based cohorts.²³

The proteomic panel is comprised of: c-reactive protein (CRP), intercellular adhesion molecule1 (ICAM1), vascular cellular adhesion molecule 1 (VCAM1), serum amyloid A (SAA), interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)- α , IL-5, IL-7, Eotaxin-3, thymus and activation related chemokine (TARC), alpha-2-microglobulin (A2 M), beta-2-microglobulin (B2 M), factor VII (FVII), tenascin-C (TNC), fatty acid binding protein-3 (FABP-3), IL-18, pancreatic polypeptide (PPY), thrombopoietin (TPO) and t-lymphocyte secreted protein I-309 (I-309).

In normal aging, both age and the *APOE* ϵ 4 allele confer increased risk for cerebral amyloid accumulation,^{24–28} and eventually clinical AD development, but their predictive value is not specific to AD. Therefore, AD pathologic and proteomic biomarkers are ideally positioned to detect AD risk in a point-of-care setting. Here, our aim was to: (1) apply an blood-based proteomic panel to predict A β PET positivity in CU older adults; and (2) examine the incremental utility of this proteomic panel over using age, *APOE* ϵ 4 status, and AD pathologic plasma biomarkers to predict A β PET status in this population. We hypothesize, due to inflammatory, vascular, and metabolic changes associated with cerebral amyloidosis, that the proteomic panel in plasma will add value to standard demographic risk factors and AD pathologic plasma biomarkers in predicting preclinical disease.

Methods

Participants

This study used a cross-sectional, retrospective research design. Participants were recruited from Butler Alzheimer's Prevention Registry (BAPR), a research data-base that holds over 1100 registrants from the community and contains the following data: basic demographics, medical history, and *APOE* genotype. Registrants can choose to complete optional specimen banking (plasma, cerebrospinal fluid). Inclusion criteria included: (1) member of BAPR; (2) age 55–85 inclusive; (3) willing and able to provide informed consent; (4) completed blood draw within 24 months of A β PET scan; (5) cognitively unimpaired (CU) as determined by brief cognitive screen ($\geq 27/30$ on Mini-Mental State Examination (MMSE) or $\geq 26/30$ on Montreal Cognitive Assessment (MoCA)). Exclusion criteria for this study included: (1) history of brain injury or other known neurologic disease; (2) poorly controlled major depression/psychiatric disorder within the past year; (3) history of schizophrenia or psychosis within the past year; (4) history of alcohol or substance abuse within the past year. Participants that did not have an available numerical value for MoCA or MMSE score ($N=2$) were deemed cognitively normal by existing clinical trial screening protocols in the Butler Hospital Memory & Aging Program and a Clinical Dementia Rating Score (CDR) of 0. $N=125$ participants met these criteria, including $N=82$ A β PET– and $N=43$ A β PET+ (see Table 1). This study was approved by the Butler Hospital Institutional Review Board and written informed consent was obtained from all human participants in accordance with the Declaration of Helsinki.

Procedures

Amyloid PET.—A β PET scans were assessed via previous participation in clinical trials at the Butler Memory & Aging Program. Depending on the source of the A β PET scan, three different ligand tracers were used: florbetapir ($N=89$), florbetaben ($N=17$), and NAV4694 ($N=19$). The prevention studies that utilized florbetapir ligand tracer were evaluated visually by two qualified raters and quantitatively, with A β PET positivity designated with a Standard Uptake Value Ratio (SUVr) cutoff value of 1.10.^{29,30} For florbetaben and 2–18F-fluoro-6-(methylamino)-3-pyridinyl]-1-benzofuran-5-ol (NAV) ligands, binary visual reads (positive versus negative) were conducted by at least two qualified neuroradiologists as part of an AD clinical prevention protocol according to established protocols for each radioactive ligand.^{31,32}

Plasma sample collection and processing.—Blood collection and preprocessing procedures were completed by trained phlebotomy staff according to published pre-analytic standard protocols.³³ Samples were shipped to the O'Bryant Laboratory at the University of North Texas, and processed according to standardized protocol.²³ A customized Hamilton Robotic StarPlus system (Microlab STAR line) was utilized to ensure precision of the prepared samples and plates for assay. Blood samples were processed in duplicate on a multiplex platform using electrochemiluminescence (ECL) technology using the SECTOR Imager 2400 A from Meso Scale Discovery (available: <http://www.mesoscale.com>). Biomarkers used in this study include A2 M, B2 M, SAA, FVII, CRP, svCAM-1, siCAM-1, TNC, FAB3, TPO, TARC, IL-18, PPY, IL-7, I-309, Eoxatin-3, TNF-a, IL-6, IL-5, and IL-10

in addition to A β ₄₂, A β ₄₀, and p-tau181. p-tau217 and p-tau231 plasma assays were not available at the time the analysis was performed.

APOE genotyping.—A portable reverse transcription polymerase chain reaction (PCR) (real time [RT]-PCR) device that analyzes epithelial cells from a cheek swab (Spartan Bioscience) was used for analysis of *APOE* genotype. The Spartan Cube has been found to have 100% concordance with the Clinical Laboratory Improvement Amendments (CLIA) certified gold standard laboratory assays.³⁴ Participants provided cheek swab samples as a part of their participation in the registry. Study staff were trained in cheek swab sample acquisition and processing prior to beginning data collection, either remotely or in person, by Spartan Bioscience, to ensure compliance with procedure and minimize sample contamination.

Statistical analysis

Analyses were performed using R statistical software (version 4.2.1, Vienna, Austria). Plasma data that was below the detectable threshold for each biomarker was excluded. *APOE* genotype was also recoded as either *APOE* ϵ 4 carrier or *APOE* ϵ 4 non-carrier, as the sample contained very few ϵ 4 homozygotes ($N = 13$). Independent samples t-tests were used to compare demographic variables between A β PET + and A β PET– groups. Specific plasma analytes (CRP, sVCAM1, and SAA) were converted to the same scale as the other plasma markers for analytic purposes. Participants missing more than 80% of the proteomic data ($N = 9$) or *APOE* status ($N = 8$) or A β PET ($N = 3$) data were excluded from analyses, leaving a total of 125 participants. Any remaining missing data were handled using a multiple imputation approach.³⁵ Random forest modeling using the randomForest package³⁶ for R was used to examine the accuracy of the plasma biomarkers in the proteomic panel²⁰ in predicting A β PET status. Random forest is a supervised machine-learning based method that employs several decision trees. The RF model has four steps: (1) generate many random subsets (bootstrap samples) from the original data; (2) build a decision tree from each random subset; (3) make a prediction from each decision tree model, and 4) combine the predictions from each individual tree to get the final prediction. The RF model can be used to predict both binary/categorical outcomes and continuous outcomes. In this analysis, it was used to enhance classification of a binary outcome (A β PET + versus A β PET–). To do this, the RF model builds many classification trees, and then combines the predictions from individual trees by a majority vote approach. This method has been shown to perform well in many classification and prediction scenarios,^{37,38} including algorithmic approaches to CSF,³⁹ EEG⁴⁰ and fMRI⁴¹ findings. Importantly, this approach has been used previously with this proteomic panel in order to classify binary/categorical and continuous outcomes.^{21,23,42,43} The number of features entered in Random Forest Modeling can affect the outcome in two ways: selecting many features increases the strength of the individual trees, whereas reducing the number of features leads to a reduction in the correlation among the trees, strengthening the forest as a whole.⁴⁴ Because no existing data optimizes the number of features selected in this population, we compared random forest models with a wide range of features ($N = 20$, $N = 10$, $N = 8$, and $N = 5$) to determine which combination of proteomic markers best predicted A β PET positivity in this population. Statistics used to evaluate model fit included sensitivity, specificity, positive predictive value (PPV), negative

predictive value (NPV), and area under the curve (AUC). Then, multiple regression models were constructed with A β PET status (positive versus negative) as the key outcome variable. In order to assess the incremental utility of plasma biomarkers over *APOE* genotype alone, the following models were created: (1) age, education, gender; (2) age, education, gender, and *APOE* status (carrier versus non-carrier); (3) age, education, gender, *APOE* status (*APOE* ϵ 4 carrier versus *APOE* ϵ 4 non-carrier), A β _{42/40} ratio, p-tau181; (4) age, education, gender, *APOE* status, A β _{42/40} ratio, p-tau181, and the best predictor model identified in the random forest analysis; and for comparison purposes, (5) age, education, gender, *APOE* status (carrier versus non-carrier) and all 21 proteomic markers. Likelihood ratio tests were used to compare the models, accounting for multiple imputation.

Results

Demographics

There were no significant differences between the A β PET + and A β PET – groups in terms of gender, age, years of education, race, and MMSE score (all $p > 0.05$, see Table 1). A β PET + CU older adults had a significantly lower MoCA score (mean = 26.73, SD = 2.12) than A β PET– CU older adults (mean = 27.90, SD = 1.87, See Table 1), although both groups were above the clinical cut-off for cognitive impairment. Finally, as expected, there were more *APOE* ϵ 4 allele carriers in the A β PET + than the A β PET– group ($p < 0.001$, see Table 1).

Proteomic panel: random forest analysis

Random forest modeling was used to determine the most accurate combination of the 20 proteins assayed as part of the proteomic panel to predict A β PET status (positive versus negative). We tested random forest models that included all 20 inflammatory proteins, as well as the top 10, 8, and 5 inflammatory proteins. Models were compared based on sensitivity, specificity, PPV, NPV, and AUC (see Table 2, Figure 1). The model including the top 5 proteins (CRP, SAA, sVCAM1, TNF α , and TARCC) was the best fit with sensitivity of 0.88, specificity of 0.31, PPV of 0.71, NPV of 0.57 and AUC of 0.63. This model was then used to run the remaining multiple regression analyses.

Additive value of proteomic panel to standard pathologic AD plasma biomarkers

Multiple regression analyses were used to examine the best model for predicting A β PET status. In model 1, age, education, and gender were not significant predictors of A β PET results ($p = 0.16$). Model 2 with variables age, education, gender, and *APOE* status was a significant predictor of A β PET status ($p < 0.01$, see Table 4), and showed significant improvement over Model 1 ($p < 0.01$; see Table 3). Model 3 with variables age, education, gender, *APOE* status, A β _{42/40} ratio, and ptau181 was a significant predictor of A β PET status ($p < 0.01$; see Table 4), and showed significant improvement over Model 2 ($D3 = 10.11$, $p < 0.001$, Table 3). Adding the 5-predictor model identified through Random Forest analysis (Table 2; Figure 1) to Model 3 did not significantly improve the model ($p = 0.23$; Table 3), showing that the proteomic panel biomarkers did not significantly improve prediction of A β PET status over *APOE* ϵ 4 status and pathologic AD plasma biomarkers (A β _{42/40}, ptau181) in this sample (Table 4). For comparison purposes, Model 5 shows that

adding all proteomic markers to Model 3 does not significantly improve prediction of A β PET status ($p = 0.09$; Table 3) over *APOE* $\epsilon 4$ status and pathologic AD plasma biomarkers (A $\beta_{42/40}$, ptau181) in this sample (Table 4).

Discussion

In this sample of CU older adults, the best model for predicting A β PET positivity included age, years of education, gender, *APOE* $\epsilon 4$ status, plasma A $\beta_{42/40}$ ratio and plasma p-tau181. Adding the top 5 markers from a 21-item proteomic panel previously validated for discriminating mild cognitive impairment (MCI) and dementia due to AD from CU older adults in dementia clinics²⁰ did not significantly improve the ability of the model to predict A β PET status in CU older adults. This study, to our knowledge, was the first to examine the ability of this proteomic panel to detect preclinical AD.

Inflammation is a well-known and well-studied response to AD pathology; however, other research suggests that inflammatory responses may occur later downstream.⁴⁵ In response to amyloid plaque accumulation, microglia produce inflammatory cytokines leading to neuroinflammation,⁴⁶ and exacerbating A β and tau pathology.⁴⁷ This constant state of inflammation leads to worsening degeneration and contributes to the progression of AD.⁴⁸ Phase I and Phase IIa clinical trials in rodent models of AD have revealed that preventive anti-inflammatory strategies reduce AD neuropathology such as synaptic loss, astrogliosis, and excitotoxicity as well as decreases in cognitive functioning.⁴⁹

The five inflammatory proteins found to be the best fit for predicting A β PET status were CRP, SAA, sVCAM1, TNF α , and TARC. Together, these markers had a high positive predictive value and high sensitivity to amyloid PET status, suggesting potential use for identifying preclinical AD in the general population. However, the low NPV and specificity reduce enthusiasm for use as a screening tool for preclinical AD in CU older adults. All 5 of these inflammatory markers have shown changes in clinical AD^{50–53} compared to CU older adults. Existing research on these biomarkers in preclinical AD is limited (see⁵⁴). TNF α is associated with greater risk of progression to incident MCI in CU older adults,⁵⁵ suggesting that longitudinal monitoring of CU older adults with heightened expression of TNF α may be warranted. In a sample of 1323 CU older adults, Metti et al.⁵⁶ found that extreme CRP variability over a 10-year follow-up period was associated with cognitive decline, specifically in women and *APOE* $\epsilon 4$ non-carriers, which they attributed to greater vascular and metabolic disease burden. In an interesting study, Wang et al.⁵⁷ examined CRP levels and their relationship to AD biomarkers across the lifespan in CU older adults in 4 large cohorts and found that elevated CRP was associated with decreased hippocampal volume across the lifespan but had no relationship to A β PET or CSF A β . They did find that *APOE* $\epsilon 4$ was associated with lower CRP across the lifespan and suggested a lifespan approach to determine whether *APOE* $\epsilon 4$ is associated with diminished inflammatory response across the lifespan, and how this may affect incident dementia risk. In contrast, Oberlin et al.⁵⁸ found that elevated CRP at baseline predicted greater increase over a two-year follow-up period in global and regional A β deposition (as measured by A β PET) in CU older adults, especially in those who were A β PET+ at baseline. Our study was cross-sectional, and

future longitudinal follow-up is warranted to determine whether these proteomic panel markers may predict longitudinal change in cerebral amyloidosis in preclinical AD.

Our results indicate that the inflammatory changes associated with AD may occur later downstream in the pathogenesis of the disease. Alternatively, these results could indicate that the pathological inflammatory processes associated with cerebral amyloidosis are not detectable above a critical threshold using these particular plasma biomarkers in CU older adults. Using a binary outcome (A β PET+ versus A β PET-) in our analysis may limit the generalizability of our results. It is possible that these proteomic markers in plasma may reflect more subtle and/or regional accumulation of cerebral amyloid measured continuously (i.e., using the centiloid scale) in this population. Studies are ongoing to test this hypothesis. Additionally, we compared binary (positive versus negative) reads across 3 different tracers in this study, and although the majority of our sample used the florbetapir tracer ($N=89$), the use of the centiloid scale would eliminate tracer-driven variability from the other two tracers ($N=36$).

Our results confirm previous studies indicating that the *APOE* $\epsilon 4$ allele and p-tau181 measured in plasma are both strong predictors of A β PET positivity in CU older adults. Previous studies have found that the *APOE* $\epsilon 4$ allele predicts A β PET positivity^{24–26} and cognitive, specifically episodic memory decline,^{27,28} one of the first emergent cognitive symptoms in AD, in CU older adults. In a recent publication, plasma p-tau181 was comparable to A β PET in predicting cerebral amyloid accumulation over a 2–10 year follow-up period in CU older adults.⁵⁹ Moreover, plasma p-tau181 predicted A β PET status with high accuracy in CU older adults in the Australian Imaging, Biomarker and Lifestyle (AIBL) study (AUC = 0.808).⁶⁰ Interestingly, in preclinical AD, ptau181 shows faster accumulation over time in *APOE* $\epsilon 4$ carriers than non-carriers,⁶¹ which corroborates work suggesting that p-tau181 increases in preclinical AD in *APOE* $\epsilon 4$ carriers 7–13 years prior to disease onset,⁶² and that longitudinal increase in this biomarker is related to reduction of encoding-related activity in the hippocampus.⁶² Overall, our results support a growing body of literature that both *APOE* $\epsilon 4$ and plasma p-tau181 are accurate predictors of cerebral amyloidosis in CU older adults.

Our study has some notable limitations. First, plasma p-tau217 and p-tau231 assays were not yet publicly available at the time these samples were analyzed. Research shows high discriminative accuracy for A β PET status in these biomarkers between patients with AD compared to other neurodegenerative diseases.¹² Also, GFAP, hypothesized to be a sensitive biomarker for cerebral A β accumulation in CU older adults⁵ was not included in the study, which is an area of opportunity for future research. Additionally, future work should include a larger and more racial/ethnically diverse sample to ensure population-based representation. It should be noted that the participants in the study were community volunteers who signed up for the BAPR online due to interest in AD research, many due to a family history of AD, and therefore *APOE* $\epsilon 4$ carriers could have been over-represented in our sample. Finally, inflammatory response may fluctuate depending on many other factors; examination of these longitudinal fluctuations in these proteomic plasma biomarkers will add to the literature about this panel in preclinical AD. Of note, the processes of inflammation and

AD pathogenesis could be additive or synergistic. Mechanistically, pathologic inflammation could be increasing concentrations of AD pathologic markers such as ptau181, or vice versa.

Future work should examine the timeline of the inflammatory response outside of A β and tau pathologies in preclinical AD to fully grasp this inflammation cascade in this critical phase of AD development. Longitudinal studies examining proteomic markers in plasma from preclinical disease to mild cognitive impairment, to symptomatic AD dementia, will provide much needed information on the inflammation cascade throughout the AD disease continuum.

Acknowledgments

We would like to thank the participants in this study for donating their time to research at the Butler Hospital Memory & Aging Program.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This project was supported by Institutional Development Award Number U54GM115677 from the National Institute of General Medical Sciences of the National Institutes of Health, which funds Advance Clinical and Translational Research (Advance RI-CTR). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: JA receives funding from R21AG074153, R01AG079241, and the Warren Alpert Foundation. AL receives funding from R01AG068990, U24AG057437, U01AG057195 and the Alzheimer's Association.

Data availability

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. 2023 Alzheimer's disease facts and figures. *Alzheimers Dement* 2023; 19: 1598–1695. [PubMed: 36918389]
2. Hebert LE, Weuve J, Scherr PA, et al. Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. *Neurology* 2013; 80: 1778–1783. [PubMed: 23390181]
3. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the national institute on aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; 7: 280–292. [PubMed: 21514248]
4. Uddin MS, Kabir MT, Al Mamun A, et al. APOE And Alzheimer's disease: evidence mounts that targeting APOE4 may combat Alzheimer's pathogenesis. *Mol Neurobiol* 2019; 56: 2450–2465. [PubMed: 30032423]
5. Prins S, de Kam ML, Teunissen CE, et al. Inflammatory plasma biomarkers in subjects with preclinical Alzheimer's disease. *Alzheimers Res Ther* 2022; 14: 106. [PubMed: 35922871]
6. Langlois CM, Bradbury A, Wood EM, et al. Alzheimer's prevention initiative generation program: development of an APOE genetic counseling and disclosure process in the context of clinical trials. *Alzheimers Dement (N Y)* 2019; 5: 705–716. [PubMed: 31921963]

7. Doecke JD, Pérez-Grijalba V, Fandos N, et al. Total A β ₄₂/A β ₄₀ ratio in plasma predicts amyloid-PET status, independent of clinical AD diagnosis. *Neurology* 2020; 94: 15.
8. Ashton NJ, Pascoal TA, Karikari TK, et al. Plasma p-tau₂₃₁: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol* 2021; 141: 709–724. [PubMed: 33585983]
9. Milà-Alomà M, Suárez-Calvet M and Molinuevo JL. Latest advances in cerebrospinal fluid and blood biomarkers of Alzheimer's disease. *Ther Adv Neurol Disord* 2019; 12: 1–23.
10. Karikari TK, Benedet AL, Ashton NJ, et al. Diagnostic performance and prediction of clinical progression of plasma phospho-tau₁₈₁ in the Alzheimer's disease neuroimaging initiative. *Mol Psychiatry* 2021; 26: 429–442. [PubMed: 33106600]
11. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol* 2020; 19: 422–433. [PubMed: 32333900]
12. Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative accuracy of plasma phospho-tau₂₁₇ for Alzheimer disease vs other neurodegenerative disorders. *JAMA* 2020; 324: 772–781. [PubMed: 32722745]
13. Fandos N, Pérez-Grijalba V, Pesini P, et al. Plasma A β _{42/40} ratios as biomarkers for A β cerebral deposition in cognitively normal individuals. *Alzheimers Dement (Amst)* 2017; 8: 179–187. [PubMed: 28948206]
14. Jonaitis EM, Janelidze S, Cody KA, et al. Plasma phosphorylated tau 217 in preclinical Alzheimer's disease. *Brain Commun* 2023; 5: fcad057. [PubMed: 37013174]
15. Jack CR, Bennett DA, Blennow K, et al. NIA-AA Research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 2018; 14: 535–562. [PubMed: 29653606]
16. Heneka MT, Carson MJ, Khoury J, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015; 14: 388–405. [PubMed: 25792098]
17. Novoa C, Salazar P, Cisternas P, et al. Inflammation context in Alzheimer's disease, a relationship intricate to define. *Biol Res* 2022; 55: 39. [PubMed: 36550479]
18. Santos CY, Snyder PJ, Wu W-C, et al. Pathophysiologic relationship between Alzheimer's disease, cerebrovascular disease, and cardiovascular risk: a review and synthesis. *Alzheimers Dement (Amst)* 2017; 7: 69–87. [PubMed: 28275702]
19. Benedet AL, Ashton NJ, Pascoal TA, et al. Plasma neurofilament light associates with Alzheimer's disease metabolic decline in amyloid-positive individuals. *Alzheimers Dement (Amst)* 2019; 11: 679–689. [PubMed: 31673598]
20. O'Bryant SE. A serum protein-based algorithm for the detection of Alzheimer disease. *Arch Neurol* 2010; 67: 1077. [PubMed: 20837851]
21. O'Bryant SE, Xiao G, Barber R, et al. A blood-based screening tool for Alzheimer's disease that spans serum and plasma: findings from TARC and ADNI. *PLoS One* 2011; 6: e28092. [PubMed: 22163278]
22. O'Bryant SE, Xiao G, Zhang F, et al. Validation of a serum screen for Alzheimer's disease across assay platforms, species, and tissues. *J Alzheimers Dis* 2014; 42: 1325–1335. [PubMed: 25024345]
23. O'Bryant SE, Edwards M, Johnson L, et al. A blood screening test for Alzheimer's disease. *Alzheimers Dement (Amst)* 2016; 3: 83–90. [PubMed: 27453929]
24. Pletnikova O, Kageyama Y, Rudow G, et al. The spectrum of preclinical Alzheimer's disease pathology and its modulation by ApoE genotype. *Neurobiol Aging* 2018; 71: 72–80. [PubMed: 30099348]
25. Murphy KR, Landau SM, Choudhury KR, et al. Mapping the effects of ApoE₄, age and cognitive status on 18F-florbetapir PET measured regional cortical patterns of beta-amyloid density and growth. *Neuroimage* 2013; 78: 474–480. [PubMed: 23624169]
26. Morris JC, Roe CM, Xiong C, et al. APOE Predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Ann Neurol* 2010; 67: 122–131. [PubMed: 20186853]
27. Lim YY, Villemagne VL, Pietrzak RH, et al. APOE E₄ moderates amyloid-related memory decline in preclinical Alzheimer's disease. *Neurobiol Aging* 2015; 36: 1239–1244. [PubMed: 25559335]
28. Mormino EC, Betensky RA, Hedden T, et al. Amyloid and APOE e₄ interact to influence short-term decline in preclinical Alzheimer disease. *Neurology* 2014; 82: 176–1777.

29. Clark CM. Use of florbetapir-PET for imaging β -amyloid pathology. *JAMA* 2011; 305: 275. [PubMed: 21245183]
30. Camus V, Payoux P, Barré L, et al. Using PET with 18F-AV-45 (florbetapir) to quantify brain amyloid load in a clinical environment. *Eur J Nucl Med Mol Imaging* 2012; 39: 621–631. [PubMed: 22252372]
31. Joshi AD, Pontecorvo MJ, Lu M, et al. A semiautomated method for quantification of F 18 Florbetapir PET images. *J Nucl Med* 2015; 56: 1736–1741. [PubMed: 26338898]
32. Landau SM, Breault C, Joshi AD, et al. Amyloid- β imaging with Pittsburgh compound B and florbetapir: comparing radiotracers and quantification methods. *J Nucl Med* 2013; 54: 70–77. [PubMed: 23166389]
33. O'Bryant SE, Gupta V, Henriksen K, et al. Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer's disease research. *Alzheimers Dement* 2015; 11: 549–560. [PubMed: 25282381]
34. Lee A, Menard W, Tonini G, et al. Reliability of a rapid APOE assay for Alzheimer's risk assessment and clinical trial screening. *J Prev Alzheimers Dis* 2018; 5: 182.
35. Van Buuren S and Groothuis-Oudshoorn K. Mice: multivariate imputation by chained equations in R. *J Stat Softw* 2011; 45: 1–67.
36. Liaw A and Wiener M. Classification and regression by randomForest, <http://www.stat.berkeley.edu/> (2002).
37. Díaz-Uriarte R and Alvarez de Andrés S. Gene selection and classification of microarray data using random forest. *BMC Bioinformatics* 2006; 7: 3. [PubMed: 16398926]
38. Wu B, Abbott T, Fishman D, et al. Comparison of statistical methods for classification of ovarian cancer using mass spectrometry data. *Bioinformatics* 2003; 19: 1636–1643. [PubMed: 12967959]
39. Finehout EJ, Franck Z, Choe LH, et al. Cerebrospinal fluid proteomic biomarkers for Alzheimer's disease. *Ann Neurol* 2007; 61: 120–129. [PubMed: 17167789]
40. Lehmann C, Koenig T, Jelic V, et al. Application and comparison of classification algorithms for recognition of Alzheimer's disease in electrical brain activity (EEG). *J Neurosci Methods* 2007; 161: 342–350. [PubMed: 17156848]
41. Tripoliti EE, Fotiadis DI and Argyropoulou M. An automated supervised method for the diagnosis of Alzheimer's disease based on fMRI data using weighted voting schemes. In: 2008 IEEE International Workshop on Imaging Systems and Techniques, pp.340–345: IEEE.
42. O'Bryant SE, Xiao G, Barber R, et al. Molecular neuropsychology: creation of test-specific blood biomarker algorithms. *Dement Geriatr Cogn Disord* 2014; 37: 45–57. [PubMed: 24107792]
43. O'Bryant SE, Xiao G, Barber R, et al. A serum protein-based algorithm for the detection of Alzheimer disease. *Arch Neurol* 2010; 67: 1077–1081. [PubMed: 20837851]
44. Breiman L Random forests. *Mach Learn* 2001; 45: 5–32.
45. Zotova E, Nicoll JA, Kalaria R, et al. Inflammation in Alzheimer's disease: relevance to pathogenesis and therapy. *Alzheimers Res Ther* 2010; 2: 1. [PubMed: 20122289]
46. Hansen DV, Hanson JE and Sheng M. Microglia in Alzheimer's disease. *J Cell Biol* 2018; 217: 459–472. [PubMed: 29196460]
47. Kinney JW, Bemiller SM, Murtishaw AS, et al. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimers Dement (N Y)* 2018; 4: 575–590. [PubMed: 30406177]
48. Leng F and Edison P. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? *Nat Rev Neurol* 2021; 17: 157–172. [PubMed: 33318676]
49. Decourt B, Lahiri DK and Sabbagh MN. Targeting tumor necrosis factor alpha for Alzheimer's disease. *Curr Alzheimer Res* 2016; 14: 412–425.
50. Tao Q, Alvin Ang TF, Akhter-Khan SC, et al. Impact of C-reactive protein on cognition and Alzheimer disease biomarkers in homozygous *APOE* e4 carriers. *Neurology* 2021; 97: e1243–e1252. [PubMed: 34266923]
51. Cao X and Chen P. Changes in serum amyloid A (SAA) and 8-OHdG in patients with senile early cognitive impairment. *Med Sci Monitor* 2020; 26: e919586–1.
52. Chen J, Dai AX, Tang HL, et al. Increase of ALCAM and VCAM-1 in the plasma predicts the Alzheimer's disease. *Front Immunol* 2023; 13: 1097409. [PubMed: 36685605]

53. Plantone D, Pardini M, Righi D, et al. The role of TNF- α in Alzheimer's disease: a narrative review. *Cells* 2024; 13: 54.
54. Watermeyer TJ, Raymond V and Ritchie K. Neuroinflammation in preclinical Alzheimer's disease: a review of current evidence. *J Alzheimers Dis Parkinsonism* 2018; 8: 434.
55. Gross AL, Walker KA, Moghekar AR, et al. Plasma markers of inflammation linked to clinical progression and decline during preclinical AD. *Front Aging Neurosci* 2019; 11: 229. [PubMed: 31555121]
56. Metti AL, Yaffe K, Boudreau RM, et al. Trajectories of inflammatory markers and cognitive decline over 10 years. *Neurobiol Aging* 2014; 35: 2785–2790. [PubMed: 24997674]
57. Wang Y, Grydeland H, Roe JM, et al. Associations of circulating C-reactive proteins, APOE ϵ 4, and brain markers for Alzheimer's disease in healthy samples across the lifespan. *Brain Behav Immun* 2022; 100: 243–253. [PubMed: 34920091]
58. Oberlin LE, Erickson KI, Mackey R, et al. Peripheral inflammatory biomarkers predict the deposition and progression of amyloid- β in cognitively unimpaired older adults. *Brain Behav Immun* 2021; 95: 178–189. [PubMed: 33737171]
59. De Meyer S, Schaeverbeke JM, Luckett ES, et al. Plasma pTau181 and pTau217 predict asymptomatic amyloid accumulation equally well as amyloid-PET. *Brain Commun* 2024; 6: fcae162. [PubMed: 39051027]
60. Fowler CJ, Stoops E, Rainey-Smith SR, et al. Plasma p-tau181/A β 1–42 ratio predicts A β -PET status and correlates with CSF-p-tau181/A β 1–42 and future cognitive decline. *Alzheimers Dement (Amst)* 2022; 14: e12375. [PubMed: 36447478]
61. Yakoub Y, Ashton NJ, Strikwerda-Brown C, et al. Longitudinal blood biomarker trajectories in preclinical Alzheimer's disease. *Alzheimers Dement* 2023; 19: 5620–5631. [PubMed: 37294682]
62. Salami A, Adolfsson R, Andersson M, et al. Association of APOE ϵ 4 and plasma p-tau181 with preclinical Alzheimer's disease and longitudinal change in hippocampus function. *J Alzheimers Dis* 2022; 85: 1309–1320. [PubMed: 34924376]

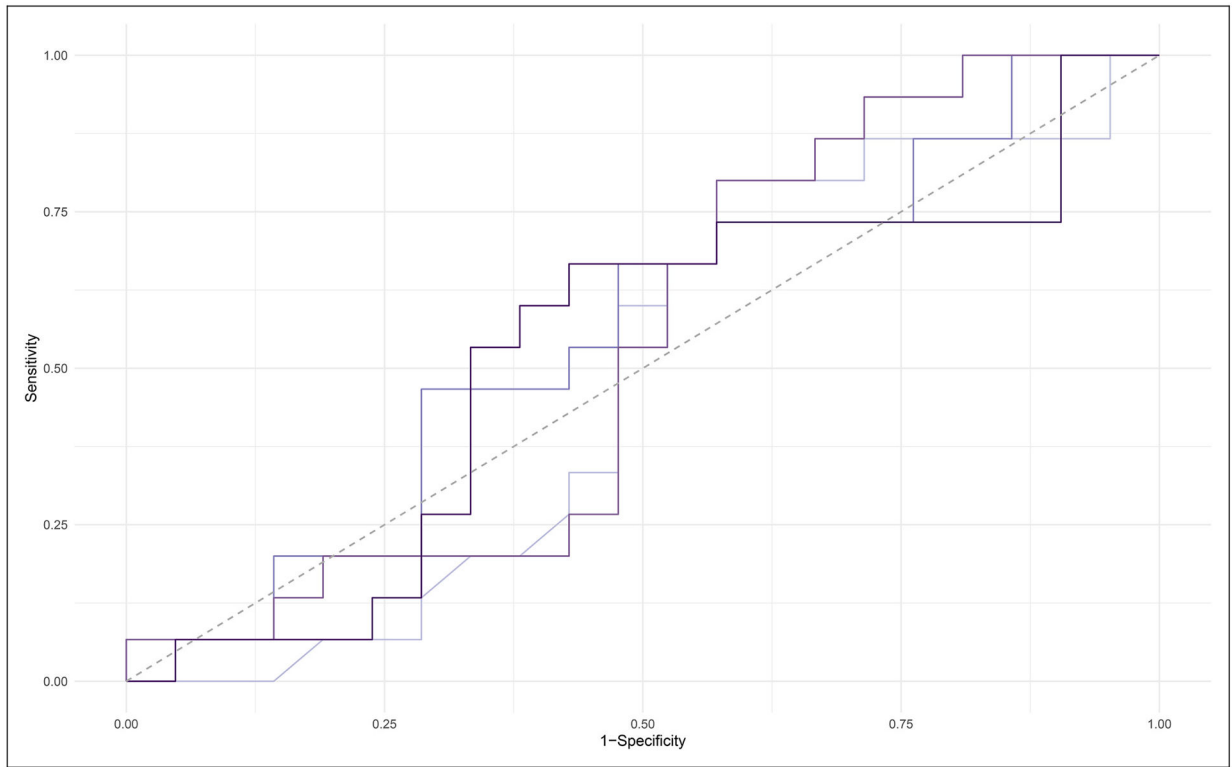


Figure 1. AUC curve for 4 models generated in random forest analysis. Light purple line = 21 predictor model. Dark purple = 10 predictor model. Light blue = 8 predictor model. Dark blue = 5 predictor model.

Table 1.
Demographic characteristics of entire sample, A β PET+ group, and A β PET–group.

Characteristics	Overall (N = 125)	A β – (N = 82)	A β + (N = 43)	p
<i>APOE</i> ϵ 4				<0.001
Carriers	76 (61%)	40 (49%)	36 (84%)	
Non-carriers	49 (39%)	42 (51%)	7 (16%)	
Age:	68 (6)	68 (6)	68 (6)	0.52
Gender:				0.80
Female	82 (66%)	55 (67%)	27 (63%)	
Male	42 (34%)	26 (32%)	16 (37%)	
Other	1 (0.8%)	1 (1.2%)	0 (0%)	
Education (y)	16.46 (2.43)	16.24 (2.55)	16.86 (21.6)	0.16
Race:				0.20
American Indian/Alaska Native	1 (0.8%)	0 (0%)	1 (2.3%)	
Asian	1 (0.8%)	0 (0%)	1 (2.3%)	
Black/African American	2 (1.6%)	2 (2.4%)	0 (0%)	
Native Hawaiian/Other Pacific Islander	0 (0%)	0 (0%)	0 (0%)	
White	119 (95%)	78 (95%)	41 (95%)	
More than One Race	2 (1.6%)	2 (2.4%)	0 (0%)	
MMSE Score (/30)	29.33 (0.89)	29.31 (0.89)	29.73 (0.89)	0.74
MoCA score (/30)	27.42 (2.04)	27.90 (1.87)	26.73 (2.12)	0.04

MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

Results of random forest analysis for predictors (inflammatory plasma biomarkers) of A β PET status in CU older adults.

Table 2.

Model	Sensitivity	Specificity	NPV	PPV	AUC
Model 1–21 Predictor	0.92	0.23	0.70	0.60	0.64
Model 2–10 Predictor	0.84	0.23	0.38	0.43	0.62
Model 3–8 Predictor	0.88	0.31	0.71	0.57	0.60
Model 4–5 Predictor	0.88	0.31	0.71	0.57	0.63

Results were compared using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the curve (AUC).

Table 3.

Model comparison between multiple regression models 1–4.

Model	D3 Statistic	<i>p</i>
Model 1—Age, education, and gender	–	–
Model 2—Model 1 plus <i>APOE</i> ε4 status	18.37	<0.001
Model 3—Model 2 plus Aβ _{42/40} and ptau-181	10.11	<0.001
Model 4—Model 3 plus 5 proteomic markers	0.23	0.95
Model 5—Model 3 plus 21 proteomic markers	1.43	0.09

The D3 statistics are a result of a likelihood ratio test that accounts for multiple imputation. The entry for Model 2 is comparing Models 1 and 2. The entry for Model 3 is comparing Models 2 and 3. The entry for Model 4 is comparing Models 3 and 4.

Table 4.

Multiple regressions (outcome measure A β PET status (positive versus negative) for models 2, 3, and 4 including odds ratio (OR), 95% confidence interval (95% CI), and *p* value for all included model variables.

Variable	Model 2			Model 3			Model 4			Model 5		
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>
Age	1.07	0.99, 1.15	0.08	1.06	0.98, 1.16	0.15	1.05	0.96, 1.15	0.26	1.06	0.94, 1.19	0.36
Education	1.07	0.91, 1.25	0.42	1.05	0.88, 1.25	0.57	1.04	0.88, 1.25	0.63	1.05	0.81, 1.32	0.81
Gender	1.00	0.44, 2.24	>0.99	0.75	0.30, 1.87	0.53	0.77	0.29, 2.03	0.59	1.84	0.49, 6.96	0.36
<i>APOE</i> ϵ 4	7.11	2.60, 19.41	<0.001	7.02	2.37, 20.91	<0.001	7.36	2.37, 22.87	<0.001	14.69	2.45, 88.15	0.004
A β _{42/40}				0.70	0.41, 1.22	0.21	0.68	0.39, 1.21	0.19	0.48	0.21, 1.09	0.08
ptau-181				2.38	2.38, 3.89	<0.001	2.48	1.49, 4.12	<0.001	3.65	1.83, 7.27	<0.001
CRP							1.00	0.98, 1.02	0.86	1.01	0.97, 1.05	0.78
SAA							1.00	1.00, 1.00	0.73	1.00	0.99, 1.01	0.83
sVCAM1							1.08	0.72, 1.61	0.72	1.34	0.57, 3.11	0.50
TNF α							1.15	0.72, 1.82	0.56	1.41	0.71, 2.80	0.32
TARC							1.00	1.00, 1.00	0.83	1.00	1.00, 1.00	0.31
A2M										1.00	1.00, 1.00	0.54
B2M										1.00	0.99, 1.01	0.77
FVII										1.00	1.00, 1.00	0.03
sICAM1										1.00	1.00, 1.00	0.29
TNC										1.00	1.00, 1.00	0.048
FABP3										1.00	1.00, 1.00	0.042
TPO										1.00	1.00, 1.01	0.15
IL18										1.00	1.00, 1.00	0.57
PPY										1.00	1.00, 1.01	0.03
IL7										1.00	0.93, 1.07	>0.99
I309										0.99	0.95, 1.03	0.50
Eotaxin3										1.00	0.97, 1.03	0.77
IL6										1.40	0.52, 3.82	0.50
IL5										2.80	0.83, 9.50	0.10
IL10										1.41	0.26, 7.76	0.69

FABP3, fatty acid binding protein-3; PPX, pancreatic polypeptide; TARC, thymus and activation related chemokine; TNF, tumor necrosis factor.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript