Detection of β-amyloid positivity in Alzheimer’s Disease Neuroimaging Initiative participants with demographics, cognition, MRI, and plasma biomarkers

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### Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| Aβ | β-amyloid |
| AD | Alzheimer’s disease |
| ADAS-Cog | Alzheimer's Disease Assessment Scale – Cognitive subscale |
| ADNI | Alzheimer’s Disease Neuroimaging Initiative |
| AUC | area under curve |
| CDR-SB | Clinical Dementia Rating – Sum of Boxes |
| CI | cognitively impaired |
| CSF | cerebrospinal fluid |
| CU | cognitively unimpaired |
| DL | deep learning |
| FDG | fluorodeoxyglucose |
| LC-MS/MS | liquid chromatography tandem mass spectrometry |
| LONI | Laboratory of Neuro Imaging |
| MMSE | Mini–Mental State Examination |
| MSD | Meso Scale Discovery |
| NFL | neurofilament light |
| NPV | negative predictive value |
| P-tau181 | phosphorylated-tau at threonine-181 |
| PPV | positive predictive value |
| RF | random forest |
| Simoa | Single molecule array |
| SUVR | standardized uptake value ratio |
Abstract

In vivo gold standard for the ante-mortem assessment of brain β-amyloid pathology is currently β-amyloid PET or cerebrospinal fluid measures of β-amyloid$_{42}$ or the β-amyloid$_{42}$/β-amyloid$_{40}$ ratio. The widespread acceptance of a biomarker classification scheme for the Alzheimer’s disease continuum has ignited interest in more affordable and accessible approaches to detect Alzheimer’s disease β-amyloid pathology, a process that often slows down the recruitment into, and adds to the cost of, clinical trials. Recently there has been considerable excitement concerning the value of blood biomarkers. Leveraging multidisciplinary data from cognitively unimpaired participants and participants with mild cognitive impairment recruited by the multisite biomarker study of Alzheimer’s Disease Neuroimaging Initiative, here we assessed to what extent plasma β-amyloid$_{42}$/β-amyloid$_{40}$, neurofilament light, and phosphorylated-tau at threonine-181 biomarkers detect presence of β-amyloid pathology, and to what extent the addition of clinical information such as demographic data, APOE genotype, cognitive assessments, and MRI can assist plasma biomarkers in detecting β-amyloid-positivity. Our results confirm plasma β-amyloid$_{42}$/β-amyloid$_{40}$ as a robust biomarker of brain β-amyloid-positivity (area under curve of 0.80–0.87). Plasma phosphorylated-tau at threonine-181 detected β-amyloid-positivity only in the cognitive impaired with a moderate area under curve of 0.67, while plasma neurofilament light did not detect β-amyloid-positivity in either group of participants. Clinical information as well as MRI–score independently detected PET β-amyloid-positivity both in cognitive unimpaired and impaired (area under curve of 0.69–0.81). Clinical information, particularly APOE ε4 status, enhanced performance of plasma biomarkers in the detection of PET β-amyloid-positivity by 0.06–0.14 units of area under curve for cognitive unimpaired, and by 0.21–0.25 units for cognitive impaired; and further enhancement of these models with an MRI–score of β-amyloid-positivity yielded an additional improvement of 0.04–0.11 units of area under curve for cognitive unimpaired and 0.05–0.09 units for cognitive impaired. Taken together, these multidisciplinary results suggest that when combined with clinical information, plasma phosphorylated-tau at threonine-181 and neurofilament light biomarkers, and an MRI–score could effectively identify β-amyloid+ cognitive unimpaired and impaired (area under curve of 0.80–0.90). Yet, when the MRI–score is considered in combination with clinical information, plasma phosphorylated-tau at threonine-181 and plasma
neurofilament light have minimal added value for detecting β-amyloid-positivity. Our systematic comparison of β-amyloid-positivity detection models identified effective combinations of demographics, APOE, global cognition, MRI, and plasma biomarkers. Promising minimally invasive and low-cost predictors such as plasma biomarkers of β-amyloid$_{42}$/β-amyloid$_{40}$ may be improved by age and APOE genotype.

**Keywords:** β-amyloid, plasma, PET, MRI, Alzheimer’s
Introduction

Alzheimer’s disease (AD), pathologically defined as the presence of plaques of β-amyloid (Aβ) protein, neurofibrillary tangles of tau protein, and neurodegeneration (DeTure and Dickson, 2019), is the major cause of cognitive decline and dementia (2020). Currently, no treatment is approved that has been demonstrated to slow the progress of AD (Aisen, 2019). Historically, AD was diagnosed clinically through neurological and neuropsychological examinations to assess memory impairment and other thinking skills, judge functional abilities, and identify behavior changes, and exclude other causes than AD that could account for the dementia (McKhann et al., 2011). The “gold-standard” method to confirm the presence of AD pathology is pathological examination of brains at autopsy (DeTure and Dickson, 2019). Since the turn of the century, the ability to diagnose AD pathology in living people has been made possible by the development of radioligands for Aβ positron emission tomographic (PET) scans (Klunk et al., 2004; Schilling et al., 2016) and tau PET scans (Marquie et al., 2015; Leuzy et al., 2019), magnetic resonance imaging (MRI) for neurodegeneration (Frisoni et al., 2010), and analysis of cerebrospinal fluid (CSF) for Aβ and tau species (Blennow, 2004; Holtzman, 2011). This has led to an in vivo biological framework of AD including Aβ, tau and neurodegeneration, based on the so called A/T/N system (Jack et al., 2018). Indeed, the descriptive A/T/N system places Aβ+ individuals firmly on the AD continuum while individuals with Aβ- profiles are considered either normal or possessing non-AD pathologic changes (Jack et al., 2018). Many trials, particularly the ones enrolling subjects in earlier stages of disease, are therefore using either Aβ PET imaging or CSF Aβ42 levels as a critical step in clinical trial cohort enrichment (Sperling et al., 2014; Honig et al., 2018).

Despite these advances, PET scans are quite costly and not universally accessible. Even though lumbar punctures are very safe (Peskind et al., 2009), there continues to be reluctance to CSF sample collection in the patient and professional population (Moulder et al., 2017). Therefore, there has been great interest in developing low cost, minimally invasive methods to detect AD Aβ pathology compared to PET scans and or CSF as the “gold standard”. Many publications, (reviewed in Veitch et al.) have evaluated the role of demographics (Insel et al., 2016; Tosun et al., 2016; Jansen et al., 2018; Buckley et al., 2019; Ko et al., 2019; Maserejian et al., 2019),
APOE ε4 (de Rojas et al., 2018; Jansen et al., 2018; Ten Kate et al., 2018; Ba et al., 2019; Buckley et al., 2019), cognition (Mielke et al., 2012; Burnham et al., 2014; Kandel et al., 2015; Burnham et al., 2016; Insel et al., 2016; Kim et al., 2018; Lee et al., 2018; Ba et al., 2019; Brunet et al., 2019; Maserejian et al., 2019; Ansart et al., 2020), and MRI measures (Tosun et al., 2013; Tosun et al., 2014; Tosun et al., 2016; Ten Kate et al., 2018; Petrone et al., 2019; Ansart et al., 2020; Ezzati et al., 2020) to detect AD Aβ pathology. More recently there has been considerable excitement concerning the value of assays of plasma Aβ species and related proteins (Burnham et al., 2014; Kaneko et al., 2014; Burnham et al., 2016; Fandos et al., 2017; Ovod et al., 2017a; Park et al., 2017; de Rojas et al., 2018; Nakamura et al., 2018; Verberk et al., 2018; Westwood et al., 2018; Chatterjee et al., 2019; Chen et al., 2019; Goudey et al., 2019; Lin et al., 2019; Palmqvist et al., 2019a; Palmqvist et al., 2019b; Park et al., 2019; Perez-Grijalba et al., 2019; Vergallo et al., 2019), species of plasma tau, including phosphorylated tau (p-tau) forms (Mielke et al., 2018; Palmqvist et al., 2019b; Barthélemy et al., 2020; Janelidze et al., 2020a; Karikari et al., 2020; Palmqvist et al., 2020; Thijssen et al., 2020), and plasma neurofilament light (NfL) (Palmqvist et al., 2019b; Thijssen et al., 2020) to detect AD Aβ pathology. The first reports of reproducible high precision, high accuracy tests of plasma Aβ42/Aβ40 indicated high sensitivity and specificity for Aβ plaques as measured by mass spectrometry (Ovod et al., 2017b; Nakamura et al., 2018). Subsequently, plasma measures of p-tau at residues 181 (Mielke et al., 2018) and 217 (Barthélemy et al., 2020; Palmqvist et al., 2020) indicated good performance relative to Aβ plaques and tau tangles. The performance of these tests are being evaluated and have been shown to detect PET Aβ-positivity conversion (Schindler et al., 2019), be associated with cognitive decline, and correlate with AD pathology (Janelidze et al., 2020a). If proven useful, these alternative approaches to detect AD Aβ pathology may play an important role in drug discovery and in accelerating identification of risk factors for AD with greater precision.

For optimal and generalizable operationalization of such imputation approaches for the presence of AD Aβ pathology, it is important to assess the independent and added value of each class of predictors (e.g., demographics, APOE ε4, cognition, plasma biomarkers, MRI, etc.) and the differences in their classification performances at different clinical stages. The Alzheimer’s Disease Neuroimaging Initiate (ADNI) is a large, multisite, longitudinal study aimed at
validating biomarkers for AD clinical trials (Weiner et al., 2017). ADNI participants have Aβ PET scans, lumbar punctures for CSF, and blood drawn for plasma studies, therefore allowing for a head-to-head comparison. This study specifically aimed to assess 1) to what extent plasma Aβ42/Aβ40, NfL, and P-tau181 biomarkers detect presence of AD Aβ pathology (i.e., Aβ-positivity); 2) to what extent the addition of demographic data, APOE genotype, and cognitive assessments and 3) MRI can assist plasma biomarkers in detecting Aβ-positivity; and 4) to what extent the stage of clinical diagnosis affects these relationships.

Materials and methods

Study design

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. For up-to-date information, see www.adni-info.org.

Cohort

Subjects of this study were ADNI participants with known PET Aβ status and with plasma biomarker assessments for p-tau181, and NfL, clinical assessments, and structural MRI within six months of Aβ PET imaging. A subset of the main study cohort also had plasma biomarker assessment for Aβ42/Aβ40. The primary focus of the current study was to assess imputation of Aβ positivity from single time-point observations of clinical, neuroimaging, and plasma biomarker data; therefore, a cross-sectional study design was used. Although longitudinal biomarkers, neuroimaging, and clinical data are available for many ADNI participants, we considered only data from the first time-point with complete clinical, neuroimaging, and biomarker assessments for each participant to avoid circular model training and assessment. Clinical assessment closest in time to Aβ PET imaging was used to define cognitively unimpaired (CU) and impaired (CI) diagnostic groups. The diagnostic criteria for ADNI participants were previously described.
Participant selection was made *a priori* from all ADNI subjects based on the availability of complete cross-sectional data as of June 30th, 2020.

**PET Aβ status**

Mean tracer uptake in the cerebellar gray and white matter was computed and used as reference to generate whole-brain standardized uptake value ratio (SUVR) maps of florbetapir PET scans (Jagust *et al.*, 2015). A composite region-of-interest consisting of middle frontal, anterior cingulate, posterior cingulate, inferior parietal, precuneus, supramarginal, middle temporal, and superior temporal regions was used to compute a global SUVR for florbetapir. A threshold of $\text{SUVR} \geq 1.11$ for florbetapir (Landau *et al.*, 2013) was then used to determine PET Aβ status.

**Demographics data**

Age at florbetapir PET imaging, sex, and years of education were included as demographic characteristics of each participant.

**Apolipoprotein E (APOE) genotyping**

APOE genotyping was done by the ADNI Genetics Core using DNA from blood samples, as detailed in Supplementary Material. APOE ε4 carrier status was considered as a predictor of Aβ-positivity in this study.

**Global cognitive assessments**

ADNI participants were assessed with a wide spectrum of clinical and cognitive tests (Weiner *et al.*, 2017). In this study, we limited the global cognitive assessments to the Clinical Dementia Rating – Sum of Boxes (CDR–SB), the Alzheimer's Disease Assessment Scale – Cognitive subscale 13-item (ADAS–Cog), and the Mini–Mental State Examination (MMSE) based on a 30-point questionnaire.
**Plasma sample collection**

Blood samples were obtained by venipuncture in EDTA tubes for plasma, following the ADNI protocol (Kang *et al.*, 2015). Within 60 min, the samples were centrifuged at 3,000 r.p.m. at room temperature, aliquoted and stored at $-80\,^{\circ}\mathrm{C}$. Samples underwent two freeze/thaw cycles. Further details are provided in Supplementary Material.

**Plasma Aβ$_{42}$ and Aβ$_{40}$**

Plasma Aβ isoform concentrations were determined using immunoprecipitation combined with liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described (Ovod *et al.*, 2017b). Plasma aliquots were thawed at 21°C/800 RPM for 10 minutes and centrifuged at 21°C/10000 RCF for 5 minutes prior to immunoprecipitation. Targeted Aβ$_{42}$ and Aβ$_{40}$ isoforms were immunoprecipitated with an anti-Aβ mid-domain antibody (HJ5.1) using a KingFisher (Thermo) automated immunoprecipitation platform. Immuno-enriched fractions were subsequently digested with Lys-N protease, generating Aβ$_{28-42}$ and Aβ$_{28-40}$ species, which were measured by LC-MS/MS (Ovod *et al.*, 2017b). Absolute Aβ isoform concentrations were determined with a 15N-labeled internal standard for each isoform. The total levels of Aβ$_{42}$ and Aβ$_{40}$ were used to calculate the Aβ$_{42}$/Aβ$_{40}$ ratio.

**Plasma p-tau181**

Plasma p-tau181 was analyzed by the Single molecule array (Simoa) technique (Quanterix, Billerica, MA), using an assay developed in the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden (Karikari *et al.*, 2020). The assay uses a combination of two monoclonal antibodies (Tau12 and AT270) and measures N-terminal to mid-domain forms of pTau181 (Karikari *et al.*, 2020). Calibrators were run as duplicates, while plasma samples were measured in singlicate. All the available samples were analyzed in a single batch.

**Plasma NfL**

Plasma NfL was analyzed by the Simoa technique (Quanterix, Billerica, MA). The assay uses a combination of monoclonal antibodies, and purified bovine NfL as a calibrator. Calibrators were
run as triplicates, while plasma samples were measured in singlicate. All the available samples were analyzed in a single batch.

**MRI–score for Aβ-positivity**

3T multimodality MRI data included a 3D MP-RAGE or IR-SPGR T1-weighted MRI with sagittal slices and voxel size of $1 \times 1 \times 1$mm$^3$, as described online (http://adni.loni.usc.edu/methods/documents/mri-protocols). We employed a previously proposed methodology for assessing brain Aβ positivity status (Lang et al., 2019). Briefly, 972 ADNI subjects with structural MRI scans and with known Aβ status based on either CSF or Aβ PET imaging were used to train a deep learning (DL) model. The DL model training cohort included individuals at different clinical stages (CU, subjective memory complaint, early/late MCI, and dementia), but excluding the subjects of the current study with plasma biomarker data. The method yields a probabilistic score of Aβ-positivity between 0 and 1.

**Statistical analysis**

All analyses were performed on CU and CI data separately.

Demographic, clinical, and biomarker characteristics differences between Aβ+ and Aβ-participants were described using two-sample $t$-test and the $\chi^2$ test for continuous and categorical variables, respectively.

Demographic characteristics (age, sex, years of education), *APOE* genotype, cognitive scores (MMSE, ADAS–Cog, and CDR–SB), plasma Aβ$_{42}$/Aβ$_{40}$, p-tau181, and NfL levels, and derived MRI–score were used as inputs to construct random forest (RF) classifiers to detect the Aβ-positivity using florbetapir PET status as the ground-truth. Random forest approach was preselected based on classification performances previously reported (Delgado et al., 2014) and flexibility of RF models to a mixture of numerical (age, years of education, cognitive scores, plasma levels, and MRI–score) and categorical (sex and *APOE* genotype) features. A reference RF classifier was constructed from demographics and cognitive scores, referred as the clinical information here on. A second reference RF classifier was also constructed from MRI–score alone. To assess the added value of each class of variables (i.e., clinical, plasma, and MRI
classes), additional RF classifiers were constructed from 1) each plasma marker alone, 2) each plasma marker jointly with clinical features, 3) MRI-score jointly with clinical features, and 4) each plasma marker jointly with clinical features and MRI-score.

The random forest model construction was repeated 10 times using different random seeds, and the average model performance was reported. Each dataset (CU and CI datasets) was randomly divided into training and test datasets, using non-overlapping 80%/20% split. Each dataset used the same partitioning for all classifiers for an unbiased comparison between classifiers (Vanschoren et al., 2012). The models were built on each training split, and the performance on the test datasets were evaluated, and this process was repeated 10 times. Performance was presented as mean and standard deviation over the model runs. We generated sensitivity-specificity curves based on model classifications on the test data. For each sensitivity-specificity curve, we also computed the area under curve (AUC) values. A confidence interval of 95% was chosen. AUC of two classifiers were compared with DeLong test (DeLong et al., 1988).

Additionally, we computed accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) on each set of model classifications at classifier probability cut-off of 0.5.

Finally, for random forest models with multiple variables the mean decrease in accuracy a variable caused was determined based on the out of bag error estimates. The more the accuracy of the random forest decreases due to the exclusion of a single variable, the more important that variable was deemed for classification of the data.

The main analyses reported below with PET Aβ-positivity as the gold-standard for Aβ-positivity were repeated with CSF Aβ-positivity and results were provided in Supplementary Figure 1. Results from another secondary analysis were also provided in Supplementary Figure 2, where each classifier model was considered in a sub-sample constraint by the plasma Aβ42/Aβ40 cohort where all relevant data was available, therefore a fixed sample size across all classifier models considered in this study. Finally, the main analyses were repeated by restricting clinical information to age and APOE genotype, as reported in Supplementary Figure 3.
All analyses were done using the R language and environment for statistical computing version 4.0.1 (R Foundation for Statistical Computing).

**Data availability**

Data used in this study has been made publicly available by the ADNI in the Laboratory of Neuro Imaging (LONI) database.

**Results**

Plasma Aβ42/Aβ40 results for nine CU and nine CI participants failed quality control at measurement. No outliers (i.e., > 4 standard deviations of the mean) were detected in the plasma Aβ42/Aβ40 measurements. Samples from three CU and one CI participants were measured below the lower limit of quantification of 1.0 pg/mL for plasma p-tau181. We identified additional five CU and five CI participants with outlier values of plasma p-tau181 levels that were discarded from subsequent analyses. Analytical sensitivity for plasma NfL was <1.0 pg/mL, and no sample contained NfL levels in plasma below the limit of detection, but five CUs and 11 CIs were excluded from our analyses due to outlier plasma NfL values. Participants with dementia were excluded for two main reasons. First, 91% of the AD participants (n=235) with plasma NfL and plasma p-tau181 biomarker data were PET Aβ-positive. An unbiased classification performance analysis with a prevalence of 91% Aβ-positivity would have required a sample size greater than 500 (Hanczar et al., 2010). Second, cross-sectional plasma Aβ42/Aβ40 data was only available for non-demented participants. The final main study cohort was composed of 333 CU and 519 CI elderly individuals. Participant characteristics are reported in Table 1.

Thirty-three percent of CU participants in the main study cohort were PET Aβ+. The frequency of APOE ε4 allele was higher among Aβ+ CUs compared to Aβ– CUs. Compared to Aβ– CUs, Aβ+ CUs were older with fewer females and had significantly fewer years of education, greater CDR–SB and ADAS–Cog scores, as well as greater plasma NfL levels (Figure 1). Plasma p-tau181 levels were marginally higher in Aβ+ CUs compared to Aβ– CUs (p=0.057). When controlled for age differences, Aβ– CUs and Aβ+ CUs did not differ in ADAS–Cog scores and plasma NfL levels. Demographic and clinical characteristics of CUs in the plasma Aβ42/Aβ40 sub-cohort did not differ
from those of the main study CUs. Within the plasma $A\beta_{42}/A\beta_{40}$ sub-cohort, $A\beta+$ CUs had lower plasma $A\beta_{42}/A\beta_{40}$ compared to $A\beta-$ CUs (Figure 1; p<10^{-6}).

Fifty-seven percent of CI participants in the main study cohort were PET $A\beta+$. $A\beta+$ CIs were older than $A\beta-$ CIs with fewer years of education and a higher frequency of $APOE\varepsilon 4$ allele. Compared to $A\beta-$ CIs, $A\beta+$ CIs had greater clinical symptoms, with lower MMSE and higher CDR-SB and ADAS–Cog scores. $A\beta+$ CIs had significantly higher plasma p-tau181 and plasma NfL levels than $A\beta-$ CIs (Figure 1). $A\beta-$ vs $A\beta+$ CI group differences in clinical scores and plasma levels were significant after controlling for age differences. Compared to the CIs in the main study cohort, CIs in the plasma $A\beta_{42}/A\beta_{40}$ sub-cohort had lower symptom severity (i.e., mean CDR–SB of 1.4 vs 0.7 with $p<10^{-15}$ and mean ADAS–Cog of 9.2 vs 7.8 with $p=0.002$) and lower plasma NfL levels (39.5pg/ml vs 34.5pg/ml with $p=0.01$). Within the plasma $A\beta_{42}/A\beta_{40}$ sub-cohort, $A\beta+$ CIs had significantly lower plasma $A\beta_{42}/A\beta_{40}$ compared to $A\beta-$ Cis (Figure 1; $p<10^{-10}$).

**Differentiating $A\beta+$ and $A\beta-$ CU participants, Figures 2a-3a and Table 2.** A classifier constructed with only clinical information (i.e., demographics, $APOE\varepsilon 4$ carrier status, and global cognitive assessments) and a classifier constructed with only the MRI–score had similar performances (i.e., DeLong $p=0.06$) with an accuracy of 67-68% in differentiating $A\beta+$ CUs and $A\beta-$ CUs (Supplementary Figure 4). Of these two classifiers, the MRI–score yielded better AUC (0.74 vs 0.69) reflected in higher NPV of MRI–score (76% vs 68%) and poor sensitivity of clinical information (3% vs 46%). When considered alone and together, plasma p-tau181 and plasma NfL did not differentiate $A\beta+$ and $A\beta-$ CUs better than chance (Table 2; column (A)). In contrast, plasma $A\beta_{42}/A\beta_{40}$ alone differentiated $A\beta+$ CUs from $A\beta-$ CUs with an accuracy of 72%, a PPV of 69%, and an NPV of 76%, yielding an AUC of 0.80. The overall performance of plasma $A\beta_{42}/A\beta_{40}$ only classifier was similar to the performance of a classifier using MRI score and clinical information jointly (i.e., AUC of 0.80; DeLong $p=0.53$), with plasma $A\beta_{42}/A\beta_{40}$ having slightly better PPV (69% vs 65%) whereas the multidisciplinary MRI score and clinical information jointly having slightly better accuracy (i.e., 75% vs 72%) and NPV (i.e., 78% vs 76%). All three plasma biomarkers jointly differentiated $A\beta+$ CU and $A\beta-$ CU at an improved accuracy of 77%, a PPV of 77% and an NPV of 80%, yielding an AUC of 0.83, but this was not
significantly different than the performance of plasma Aβ_{42}/Aβ_{40} alone classification (DeLong p=0.09).

When combined with clinical information (Table 2; column (B)), the predictive performance of the plasma p-tau181 and plasma NfL improved but not beyond the performance of the classifier constructed from clinical information alone (i.e., DeLong p=0.18 and p=0.08, respectively). Adding clinical information to the plasma Aβ_{42}/Aβ_{40} classifier yielded better differentiation of Aβ+ CU and Aβ− CU cases with an accuracy of 79%, PPV of 77%, NPV of 81%, and an AUC 0.86, with the greatest improvement in the PPV compared to plasma Aβ_{42}/Aβ_{40} only and clinical information only classifiers. Further enhancing plasma NfL and plasma p-tau181 with the MRI score in addition to the clinical information improved classification accuracy by 5%–8%, PPV by 13%–22%, NPV by 8%–11%, and AUC by 0.10 to 0.14 (DeLong p<10^{-14} and p<10^{-21}, respectively) but this was not better than the classifier constructed with the MRI−score and clinical information (i.e., DeLong p=0.08 and p=0.46, respectively) or the classifier based on plasma Aβ_{42}/Aβ_{40} only (i.e., DeLong p=0.07 and p=0.88, respectively), as reported in Table 2; column (C). Of the three plasma biomarkers, Aβ_{42}/Aβ_{40} in combination with the MRI−score and clinical information performed the best with an accuracy of 83% and AUC of 0.90, with a well-balanced PPV of 84% and NPV of 83%, which was significantly better than the performance of Aβ_{42}/Aβ_{40} alone (i.e., DeLong p<10^{-4}) or in combination with clinical information (i.e., DeLong p=0.02).

The full classifier model including all three plasma biomarkers, the MRI−score, and clinical information had an accuracy of 82%, with a PPV of 90% and NPV of 79%. However, this was not significantly different from the classifier model with plasma Aβ_{42}/Aβ_{40}, MRI−score, and clinical information (DeLong p=0.61), suggesting minimal added value of plasma NfL and plasma p-tau181. The most significant variables in a decreasing order of importance based on mean decrease in accuracy analysis were plasma Aβ_{42}/Aβ_{40}, MRI−score, APOE ε4 status, MMSE, years of education, and sex.

**Differentiating Aβ+ and Aβ− CI participants, Figures 2b-3b and Table 3.** Both clinical information-based and MRI−score-based classifiers performed moderately well in differentiating
Aβ+ and Aβ− CIs with an AUC of 0.81 and 0.76, accuracy of 74% and 67%, PPV of 76% and 70%, and NPV of 73% and 63% (Supplementary Figure 4), respectively. The MRI–score together with clinical information achieved an AUC of 0.88, with an accuracy of 81%, PPV of 82%, and NPV of 80%, performing significantly better than clinical information only (DeLong p<10^{-15}) or MRI–score only (DeLong p<10^{-39}) models. In contrast to CU data, both plasma Aβ42/Aβ40 and plasma p-tau181, but not plasma NfL, separately detected Aβ-positivity in CIs with an average accuracy of 77% and 58%, PPV of 79% and 63%, NPV of 76% and 52%, yielding AUCs of 0.87 and 0.64, respectively. Enhancement with clinical information improved performance metrics of plasma p-tau181 and NfL, but not plasma Aβ42/Aβ40, classifiers by 15 to 23% (Table 3; column (B)). Plasma p-tau181 enhanced with clinical information perform similarly to plasma Aβ42/Aβ40. When further enhanced with the MRI–score in addition to the clinical information, classifier performance metrics for both plasma p-tau181 and plasma NfL increased by an additional 3 to 8%, with plasma p-tau181 performing slightly better with an accuracy of 82%, PPV of 83% PPV, and NPV of 82% (Table 3; column (C)). Similarly, the MRI–score enhanced classification performance of plasma Aβ42/Aβ40 more than clinical information (DeLong p<10^{-4}), reaching an AUC of 0.94 with an accuracy of 86%, PPV of 86%, and NPV of 88%. The full classifier model, including all three plasma biomarkers, MRI–score, and clinical information achieved an AUC of 0.92 and an accuracy of 86%, with a PPV of 88% and NPV of 86%. This was not significantly different than the classifier model with plasma Aβ42/Aβ40, MRI–score, and clinical information (DeLong p=0.31), suggesting minimal added value of plasma NfL and plasma p-tau181. The most significant variables in a decreasing order of importance based on mean decrease in accuracy analysis were plasma Aβ42/Aβ40, MRI–score, APOE ε4 allele, age, and CDR–SB.

Discussion

The major findings of this multicenter biomarker study were (1) of the three plasma biomarkers, when considered separately, Aβ42/Aβ40 consistently differentiated PET Aβ-positivity status both in CU and CI participants, with a slightly better performance in CIs, whereas plasma p-tau181 showed moderate value for differentiating PET Aβ-positivity status in CI participants, and plasma NfL lacked Aβ-positivity stratification value both in CU and CI participants; (2) clinical
information, dominated by \textit{APOE} ε4 status and education in CU participants, and by \textit{APOE} ε4 status and age in CI participants, as well as MRI–score independently differentiated PET Aβ– and Aβ+ both in CU and CI participants; (3) clinical information enhanced performance of plasma biomarkers in differentiating PET Aβ– and Aβ+ participants by 0.06 to 0.14 units of AUC for CUs, and by 0.21 to 0.25 units for CIs; and (4) further enhancement of these models with an MRI–score yielded an additional improvement of 0.04 to 0.11 units of AUC for CUs and 0.05 to 0.09 units for CIs. Taken together the results recapitulate plasma Aβ_{42}/Aβ_{40} as a robust biomarker of brain Aβ-positivity and suggest that when combined with clinical information, plasma p-tau181 and NfL biomarkers, and an MRI–score, could effectively identify Aβ+ individuals with expected greater accuracy in the symptomatic individuals. Interestingly, when the MRI–score is considered in combination with clinical information, plasma p-tau181 and plasma NfL have minimal added value for brain Aβ-positivity stratification in this multicenter ADNI cohort of CU and CI participants.

**Plasma Aβ_{42}/Aβ_{40} detects PET Aβ-positivity.** The first major finding was that plasma Aβ_{42}/Aβ_{40} was a robust biomarker of PET Aβ-positivity independent of clinical diagnosis, whereas plasma p-tau181 detected PET Aβ-positivity only in CIs with a moderate accuracy, and plasma NfL lacked value for stratification of PET Aβ+ and PET Aβ– cases both in CU and CI cohorts. It should be noted that this finding was replicated when the modeling and testing of all classifiers were repeated on the plasma Aβ_{42}/Aβ_{40} sub-cohort to mitigate the potential influence of sample size and sub-cohort characteristics in comparisons of classifiers (Supplementary Figure 2). Of the three plasma biomarkers considered in this study, Aβ_{42}/Aβ_{40} has been the most extensively studied in the literature. Recent studies, particularly the ones using highly sensitive mass spectrometry, have repeatedly reported a strong correlation between plasma Aβ_{42}/Aβ_{40} and the gold-standard CSF and PET Aβ measures (Janelidze et al., 2016; Ovod et al., 2017b; Nakamura et al., 2018; Schindler et al., 2019). Consistent with our findings, plasma Aβ_{42}/Aβ_{40}, especially when combined with age and \textit{APOE} ε4 status, have been shown to accurately stratify Aβ+ individuals (e.g., AUC of 0.80-0.85) in the AD continuum (Palmqvist et al., 2019b; Schindler et al., 2019). The slightly superior performance of plasma Aβ_{42}/Aβ_{40} in this study (cf. Supplementary Figure 3) compared to previous reports of 0.79-0.82 AUC for the detection of
Aβ-positivity in CU participants (Fandos et al., 2017; de Rojas et al., 2018; Chatterjee et al., 2019) and 0.90 AUC for CIs (Lin et al., 2019) might be due to high molecular specificity and detection sensitivity of LC-MS/MS technique used to analyze the ADNI plasma samples. This observation is consistent with the notion that the different assays for plasma Aβ_{42}/Aβ_{40} may have different precision and, in particular, mass spectrometry-based assays compared to immunoassays might be more accurate and robust in measuring levels of plasma Aβ species as biomarker of brain Aβ (Zetterberg, 2019). Another factor contributing to the high performance of the Aβ_{42}/Aβ_{40} ratio, as compared with single biomarkers, is that between-individual differences in basal “total” Aβ secretion is compensated for in the ratio, by dividing with Aβ_{40}, while such differences in plasma NfL and p-tau181 levels, MRI measures, or cognitive abilities might introduce variability in these measures.

Plasma p-tau181 presented a more complex picture as a candidate biomarker of brain Aβ-positivity. Assays for the quantification of plasma p-tau181 are very recently developed (Zetterberg and Blennow, 2020) and are still under extensive investigation to fully understand the role of different plasma tau species as peripheral markers of AD pathophysiology. Compared to the limited number of previously reported evaluations of plasma p-tau181 as a biomarker of brain Aβ-positivity in other research and clinical cohorts (Mielke et al., 2018; Palmqvist et al., 2019b; Barthélemy et al., 2020; Janelidze et al., 2020a; Karikari et al., 2020; Thijssen et al., 2020), ADNI plasma p-tau181 levels measured by the Simoa assay differentiated between PET Aβ+ and PET Aβ– ADNI CI participants with an inferior accuracy (AUC of 0.64). Furthermore, this biomarker had no stratification value for PET Aβ-positivity within the ADNI CU participants (AUC of 0.55). The addition of clinical information to this base model increased AUC for the classification of Aβ+ vs Aβ– by 0.14 to 0.69 in CUs and by 0.21 to 0.85 in CIs. The subsequent addition of an MRI–score to this model further increased AUC for the classification of Aβ+ vs Aβ– by 0.11 to 0.80 in CUs and by 0.05 to 0.90 in CIs, bringing its classification performance to a clinically acceptable level.

Potential sources of the discrepancy between our results and those of other groups may include differences in the plasma analysis assays, diagnostic composition and demographic characteristics of the study cohorts, methodology used to determine ground-truth brain Aβ-
positivity status, and data analytics. One of the earliest plasma p-tau181 studies on a Meso Scale Discovery (MSD) platform reported that plasma p-tau181 as a good biomarker of the elevated brain Aβ with an AUC of 0.7 in CU and 0.85 in MCI participants in their discovery cohort but this study lacked internal validation or replication in an external validation cohort (Mielke et al., 2018). Another study (Barthélemy et al., 2020) reported high specificity of plasma p-tau181, measured by a highly sensitive mass spectrometry assay, for Aβ plaque pathology in their discovery cohort (n=34; including clinically diagnosed CU, MCI, and AD individuals) and then replicated their findings with an AUC of 0.72 to differentiate Aβ− and Aβ+ individuals in an independent replication cohort of CUs, MCIs, and ADs (n=92) but the performance within CU only or MCI only sub-cohorts was not statistically significant. Similarly, a larger multi-cohort study which included individuals with various clinical diagnoses including CU, MCI, and AD reported a stepwise increase in plasma p-tau181 levels, measured on the MSD platform, with both Aβ-positivity and cognitive impairment and achieved an AUC of 0.81 in differentiating Aβ− and Aβ+ individuals, which was increased to 0.84 with the addition of plasma Aβ42/Aβ40 (Janelidze et al., 2020a).

The age of cohort participants may also influence the ability of plasma p-tau181 to detect Aβ-positivity status. For instance, a multi-cohort study used the Simoa assay to measure plasma p-tau181 in four different cohorts (Karikari et al., 2020) and found that plasma p-tau181 biomarker discriminated Aβ+ CU older adults and individuals with CI from Aβ− CU older adults and young adults with an AUC of 0.76–0.88 across cohorts. However, the CU older adults in this study were on average 10 years younger than ADNI participants, raising the question about age-dependent sensitivity of plasma p-tau181 to AD-related Aβ pathology. Similarly, another small cohort study of CU and CI participants, who were on average 13 years younger than ADNI participants, reported an excellent AUC of 0.86 in CU and 0.94, although not internally validated or replicated in an external cohort, in differentiating PET Aβ+ and PET Aβ− CIs with plasma p-tau181 levels (Thijssen et al., 2020). It is highly likely that younger Aβ+ participants might have greater pathophysiological changes than the older ADNI participants in response to Aβ toxicity, which might be a driving factor for increased plasma p-tau181 levels. Indeed, it is well established that younger individuals who are Aβ+ have more brain tau deposition than older individuals who are Aβ+ (Schöll et al., 2017). Furthermore, previous studies found that the
strong correlations between plasma p-tau181 and Aβ PET are often in the Aβ+ but not in Aβ− individuals (Janelidze et al., 2020a) and that increased plasma p-tau181 levels might be initiated by accumulation of Aβ beyond the positivity threshold, and continue to increase as Aβ further accumulates in the brain even during early stages of tau pathology as measured by Braak & Braak staging at autopsy or tau PET during life (Janelidze et al., 2020a; Karikari et al., 2020). Evidence from these recent studies together with the stronger association of plasma p-tau181 with brain Aβ burden in younger cohorts might suggest that plasma p-tau181 is unlikely to be a direct measure of Aβ pathology but instead a marker of tau pathology. Our finding that plasma p-tau181 has moderate stratification accuracy for PET Aβ-positivity only at the symptomatic disease stage suggests that p-tau181 detects Aβ-positivity only once a significant tau pathology, which is closely associated with symptoms, is detectable.

**Plasma NfL was a poor biomarker of PET Aβ-positivity:** The relatively poor performance of plasma NfL in differentiating Aβ+ and Aβ− ADNI individuals, either symptomatic or asymptomatic, is largely consistent with previous literature. Previous studies found no evidence that plasma NfL was related to Aβ or tau pathology as measured by PET or even synaptic dysfunction as measured by fluorodeoxyglucose (FDG)-PET imaging, repeatedly emphasizing that plasma NfL is more likely to be a marker of all cause neurodegeneration (Mattsson et al., 2019; Mielke et al., 2019; Janelidze et al., 2020a; Thijssen et al., 2020). Finally, our finding that plasma p-tau181 and plasma NfL did not improve Aβ-positivity stratification accuracy above and beyond the plasma Aβ42/Aβ40 was consistent with previous studies on other AD research cohorts (Palmqvist et al., 2019b).

**Clinical information and MRI-score independently differentiated PET Aβ+ and Aβ− ADNI individuals:** To date, the most common candidate predictors considered for Aβ-positivity were age, APOE genotype, and measures of cognition, largely because they are easier to collect with widely available standardized protocols. Of these, age has been the most common predictor of elevated brain Aβ followed by the APOE genotype (reviewed in (Ashford et al., 2020)), consistent with the notion that after advanced age, APOE ε4 genotype is a major risk factor for developing AD (Payami et al., 1997). Consistent with the prior knowledge, age and APOE genotype were important predictors of Aβ-positivity for ADNI CU and CI participants (cf.
Supplementary Figure 3). In the main analyses, we observed that the ability of clinical information to differentiate Aβ+ and Aβ− participants improved, especially in terms of sensitivity, with increasing severity of clinical diagnosis. Indeed, measures of global cognition, such as MMSE and CDR−SB, had greater influence in the classifier model for Aβ-positivity within the CI participants. Consistent with our findings, accumulating evidence suggests that elevated Aβ is associated with risk of cognitive worsening and may indicate a pre-symptomatic stage of disease (Roe et al., 2013; Donohue et al., 2017). As the relationships between cognition and AD biomarkers are expected to be subtle, the global measures of cognition may have insufficient sensitivity among CUs to reliable detect pre-symptomatic expression of Aβ pathology, as reflected in our results with extremely low sensitivity of clinical information in detecting Aβ-positivity in CUs.

MRI–score of brain Aβ alone stratified Aβ+ and Aβ− participants with an AUC of 0.74 in ADNI CUs and an AUC of 0.76 in ADNI CIs with a substantially increased sensitivity. When combined with clinical information, MRI–score performed as well as, or, in CIs, even better than, the best performing plasma biomarker, Aβ42/Aβ40. Although structural T1-weighted MRI is not a molecular imaging modality directly targeting quantification of protein accumulation in the brain, MRI has been a gold standard for neurodegeneration (Jack et al., 2004). The evidence for a relationship between Aβ deposition and neurodegeneration has been previously demonstrated in very early AD and even in asymptomatic individuals (Bourgeat et al., 2010; Chételat et al., 2010). In a similar manner to plasma p-tau181, the value of the MRI–score for Aβ-positivity might be a reflection of neurodegenerative processes due to Aβ toxicity, yet we observed that the MRI–score outperformed the plasma p-tau181. The brain Aβ deposition has a spatially distinct signature of cortical atrophy (Bourgeat et al., 2010; Chételat et al., 2010; Tosun et al., 2011) and MRI-based correlates of brain Aβ deposition compared to plasma analytes might have the advantage of capturing this spatial information. Furthermore, although structural T1-weighted imaging has been traditionally considered to reveal fat and water distribution and distinguish tissue types, cellular changes associated with neuropathology might also influence the MRI contrast as well as the MRI intensity quality, such as the gray value distribution, texture features, and spatial heterogeneity (Sørensen et al., 2016; Feng et al., 2019; Ranjbar et al., 2019). Our results also suggest that deep learning, the computational approach used in this study to construct
MRI–scores, might efficiently quantify Aβ toxicity from structural MRI because of its high automatic feature learning and visual pattern recognition abilities (LeCun et al., 2015).

Both clinical information and MRI–score enhanced performance of plasma biomarkers in identifying PET Aβ-positivity. One interesting observation was that although when combined with clinical information and MRI–score, plasma p-tau181 and NfL biomarkers could effectively identify Aβ+ symptomatic individuals, plasma p-tau181 and plasma NfL did not contribute to the detection of brain Aβ above and beyond the classification power of clinical information and MRI–score jointly, particularly in CUs. This is a particularly important criterion in the selection of candidate cost-effective and rapid screening tools for broad implementation in clinical and drug trial settings. Demographics and global cognitive measures are an integral part of the clinical assessment. MRI has long played a role in inclusion and exclusion criteria in patient recruitment and ruling out other causes of cognitive symptoms (Frisoni et al., 2010). Furthermore, MRI has been routinely acquired in clinical trials to identify and monitor adverse events (Cash et al., 2014). Plasma biomarkers, therefore, should have a classification ability as good as or better than clinical information and MRI separately and in combination in order to be a practical non-invasive screener.

Our results in this ADNI study, although limited to CU and CI participants, suggest that plasma Aβ42/Aβ40 but not plasma p-tau181 and plasma NfL might have added value in screening for brain Aβ-positivity. It is also important to emphasize that plasma assays target brain-derived proteins that are present at extremely low concentrations in the peripheral circulation and originate not only in the brain but almost all peripheral cells (Roher et al., 2009). What plasma Aβ measures mean biologically and to what extent the variances seen in plasma Aβ levels reflect brain pathology especially in the CU and CI clinical groups in which greater heterogeneity in comorbid conditions is expected are questions still warrant further investigations. These limitations may make the use of the plasma Aβ biomarkers to predict the AD pathology more difficult at the individual level. Despite the inferior performance of plasma p-tau181 in detecting AD Aβ-positivity observed in this ADNI cohort, this biomarker may have different utility. Plasma p-tau181 can be robustly measured in plasma and is highly specific for AD pathology (Mielke et al., 2018), making it an attractive screening tool for brain Aβ and tau pathologies.
jointly as required for A/T/N biomarker profiling (Jack et al., 2018) linked to differential trajectories of disease progression (Altomare et al., 2019; Jack et al., 2019; Ebenau et al., 2020). Further studies are warranted to better understand the behavior of plasma p-tau181 as a biomarker of the burden of the disease at different disease stages (Lantero Rodriguez et al., 2020). Given that Aβ-positivity assessment using either CSF or PET is independent of clinical diagnosis, clinical stage dependent classifier performance might be a concern if these plasma biomarkers are operationalized in clinical practice. In our analysis, a similar clinical diagnosis-dependent gradual increase in classification performance was observed in Aβ-positivity classifier constructed with clinical information and to a lesser extent with MRI–score.

**Study design-specific considerations**

There are multiple strengths to the study including the large sample size, well-characterized participants, and availability of plasma analytes, Aβ PET imaging, and structural MRI, all assessed within a short period of time. A limitation of this *in vivo* study was the use of Aβ PET as the gold standard for brain Aβ-positivity rather than the true gold standard of neuropathology. A limitation of plasma analyte comparisons is that different techniques were used, namely Simoa for p-tau181 and NfL and LC-MS/MS for Aβ42/Aβ40. Despite the superior specificity, mass spectrometry has the disadvantage of being more expensive and requiring more expertise than immunoassays, which are easily analyzed by laboratories that routinely run blood tests. Another limitation of the study is the potential pre-analytical variability since the blood samples were collected at multiple ADNI sites. Although the collection site as a categorical variable had no significant effect on ADNI plasma levels, multicentre studies of plasma analytes still require further investigation for standardization of protocols to reduce measurement variability (Rozga et al., 2019). We should also note that the current study was limited to plasma p-tau181. Other blood immunoassays targeting tau species, specifically the very recently reported plasma pTau-217, might be promising biomarkers for AD Aβ pathology (Janelidze et al., 2020b). Finally, we should further emphasize that the current study is based on a convenience cohort where the degree of true population representation is not known. Most notable, about 47% of CU and 19% of CI ADNI participants who were CSF p-tau positive were PET Aβ−, suggesting non-AD etiology of their tau pathology that might have particularly impacted the observed plasma p-
tau181 levels (Benussi et al., 2020). Additionally, the PPV and NPV performance of the classifier models considered in this study were limited by the prevalence of the PET Aβ-positivity in the selected ADNI cohort and may not be directly comparable to other studies with different PET Aβ-positivity prevalence.

**Conclusion**

In summary, *in vivo* gold standard for brain Aβ burden assessment is currently Aβ PET or lumbar puncture for CSF Aβ42 (Tapiola et al., 2009; Palmqvist et al., 2016). The widespread acceptance of biomarker classification scheme for the AD continuum (Jack et al., 2018) has ignited interest in more affordable and accessible approaches to detect AD Aβ pathology, a process that often slows down the recruitment into, and adds to the cost of, clinical trials. To this end, our systematic comparison of Aβ-positivity stratification models that use minimally invasive and low-cost measures identified demographics, APOE genotype, global cognitive measures, MR imaging, plasma Aβ measures, plasma p-tau181, and plasma NfL biomarkers, some alone and some in combination, as promising Aβ-positivity classifiers. Advances in ultrasensitive assays for plasma analytes as well as in computational classifier techniques combining multidisciplinary information further promise reduce the difficulty and cost of screening participants with AD Aβ pathology.

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**Conflicts of interest**

Dr. Jack serves on an independent data monitoring board for Roche, has consulted for and served as a speaker for Eisai, and consulted for Biogen, but he receives no personal compensation from any commercial entity. He receives research support from NIH and the Alexander Family Alzheimer’s Disease Research Professorship of the Mayo Clinic.

Dr. Saykin reports grants from NIH Grants, non-financial support from Eli Lilly/Avid Radiopharmaceuticals, other from Bayer Oncology, grants and other from Arkley BioTek, personal fees and other from Springer Nature, outside the submitted work.

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Dr. Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

Dr. Blennow has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

Dr. Bateman cofounded C2NDiagnostics. Washington University and RJB have equity ownership interest in C2N Diagnostics and receive royalty income based on technology (stable isotope labeling kinetics and blood plasma assay) licensed by Washington University to C2N Diagnostics. Dr. Bateman receives income from C2N Diagnostics for serving on the scientific advisory board. Washington University, with Dr. Bateman as coinventor, has submitted the US provisional patent application “Plasma Based Methods for Detecting CNS Amyloid Deposition.” Dr. Bateman consults for Roche, Genentech, AbbVie, Pfizer, Boehringer-Ingelheim, and Merck.

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Figure Captions:

Figure 1. Plasma A) $\text{A}\beta_{42}/\text{A}\beta_{40}$, B) NfL concentrations, and C) $\text{p-tau}181$ concentrations categorized by clinical diagnosis and CSF $\text{A}\beta$-positivity. Plasma $\text{A}\beta_{42}/\text{A}\beta_{40}$ data was available for 173 individuals ($\text{A}\beta-$ CU, $n=50$; $\text{A}\beta+$ CU, $n=37$; $\text{A}\beta-$ CI, $n=40$; $\text{A}\beta+$ CI, $n=46$). Plasma p-tau181 and NfL data included 852 individuals ($\text{A}\beta-$ CU, $n=224$; $\text{A}\beta+$ CU, $n=109$; $\text{A}\beta-$ CI, $n=230$; $\text{A}\beta+$ CI, $n=289$). Unpaired two-samples t-test uncorrected significance levels at ****: $p<0.00001$; ***: $p<0.0001$; **: $p<0.001$; ns: $p \geq 0.5$. CU: Cognitively unimpaired elderly; CI: Elderly individuals with mild cognitive impairment.

Figure 2. Receiver operating characteristic (ROC) analysis of $\text{A}\beta$ positivity prediction in an ADNI cohort of A) cognitively unimpaired (CU) and B) cognitively impaired (CI) elderly individuals. Optimized ROC curves for classifiers constructed separately and jointly with demographic information (age, sex, and years of education), $\text{APOE}$, clinical scores, plasma biomarkers ($\text{A}\beta_{42}/\text{A}\beta_{40}$, p-tau181, and NfL), and structural MRI–score when predicting $\text{A}\beta$-positivity using florbetapir PET as the ground truth in the ADNI study ($n=333$ CUs and $n=519$ CIs). To assess the added value of each class of variables (i.e., clinical, plasma, and MRI classes), additional RF classifiers were constructed from 1) each plasma marker alone, 2) each plasma marker jointly with clinical features, 3) MRI–score jointly with clinical features, and 4) each plasma marker jointly with clinical features and MRI–score. Models including plasma $\text{A}\beta_{42}/\text{A}\beta_{40}$ were tested and validated in a cohort of $n=87$ CUs and $n=86$ CIs due to limited available of plasma $\text{A}\beta_{42}/\text{A}\beta_{40}$ data. Error bars indicate union of 95% CIs from cross-validation iterations.

Figure 3. Classifier performance metrics of $\text{A}\beta$ positivity prediction in A) cognitively unimpaired (CU) individuals and B) individuals with mild cognitively impairment (CI). Area under the curve (AUC) estimates with $\pm 2 \times$ standard variation error bars from cross-validation iterations are shown for classifiers constructed separately and jointly with demographic information (age, sex, and years of education), $\text{APOE}$, clinical scores, plasma biomarkers ($\text{A}\beta_{42}/\text{A}\beta_{40}$, p-tau181, and NfL), and structural MRI–score when predicting $\text{A}\beta$-...
positivity using florbetapir PET as the ground truth in the ADNI study (n=333 CUs and n=519 CIs). To assess the added value of each class of variables (i.e., clinical, plasma, and MRI classes), additional RF classifiers were constructed from 1) each plasma marker alone, 2) each plasma marker jointly with clinical features, 3) MRI–score jointly with clinical features, and 4) each plasma marker jointly with clinical features and MRI–score. Models including plasma $\text{A}\beta_{42}/\text{A}\beta_{40}$ were tested and validated in a cohort of n=87 CUs and n=86 CIs due to limited available of plasma $\text{A}\beta_{42}/\text{A}\beta_{40}$ data. Error bars indicate union of 95% CIs from cross-validation iterations.
Table 1. Sample demographics per clinical diagnostic group

|                     | CU Aβ− | CU Aβ+ | p       | CI Aβ− | CI Aβ+ | p       |
|---------------------|--------|--------|---------|--------|--------|---------|
| **Main cohort**     |        |        |         |        |        |         |
| N                   | 224    | 109    | 0.01    | 230    | 289    | <10−5   |
| Age (years)         | 72.8 ± 6.2 | 74.6 ± 5.3 | 0.01 | 70.3 ± 7.9 | 73.3 ± 6.8 | <10−5 |
| Sex (Female %)      | 52%    | 36%    | 0.005   | 56%    | 56%    | 0.024   |
| Education (years)   | 16.8 ± 2.5 | 15.9 ± 2.8 | 0.003 | 16.3 ± 2.5 | 15.9 ± 2.9 | 0.024 |
| APOE ε4 (carrier %) | 21%    | 43%    | <10−4   | 23%    | 66%    | <10−15  |
| MMSE (score)        | 29.1 ± 1.3 | 28.9 ± 1.1 | <10−4 | 28.4 ± 1.6 | 27.6 ± 1.8 | <10−6 |
| CDR-SB (score)      | 0.06 ± 0.2 | 0.1 ± 0.3  | 0.03   | 1.3 ± 0.8 | 1.6 ± 0.9  | <10−4  |
| ADAS-Cog (score)    | 5.5 ± 3.1 | 6.3 ± 3.0  | 0.02   | 7.8 ± 3.8 | 10.4 ± 4.6  | <10−10  |
| Plasma NfL (pg/ml)  | 35.4 ± 15.8 | 39.4 ± 15.8 | 0.03 | 35.0 ± 18.7 | 43.3 ± 19.8 | <10−5  |
| Plasma p-tau181 (pg/ml) | 14.7 ± 10.6 | 16.9 ± 7.8  | 0.02   | 13.6 ± 8.6 | 21.6 ± 10.7 | <10−14 |
|                     |        |        |         |        |        |         |
| **Plasma Aβ42/Aβ40 subcohort** |        |        |         |        |        |         |
| N                   | 50     | 37     | 0.009   | 40     | 46     |         |
| Age (years)         | 71.9 ± 6.1 | 75.3 ± 5.2 | 0.009 | 70.0 ± 7.9 | 73.1 ± 6.9 |         |
| Sex (Female %)      | 50%    | 33%    |         | 52%    | 51%    |         |
| Education (years)   | 16.8 ± 2.6 | 16.1 ± 2.4 |         | 16.4 ± 2.5 | 16.0 ± 3.0 |         |
| APOE ε4 (carrier %) | 14%    | 51%    | 0.001   | 22%    | 63%    | 0.002   |
| MMSE (score)        | 29.2 ± 1.0 | 28.9 ± 1.0 |         | 28.5 ± 1.3 | 27.6 ± 2.0 | 0.04 |
| CDR-SB (score)      | 0.04 ± 0.1 | 0.11 ± 0.2 |         | 0.8 ± 0.2  | 0.7 ± 0.2  |         |
| ADAS-Cog (score)    | 5.5 ± 2.7 | 6.5 ± 3.1 |         | 7.0 ± 3.0 | 8.4 ± 3.4 |         |
| Plasma NfL (pg/ml)  | 32.1 ± 15.8 | 36.1 ± 12.3 |         | 30.8 ± 11.3 | 37.7 ± 14.7 | 0.04 |
| Plasma p-tau181 (pg/ml) | 13.5 ± 10.1 | 18.8 ± 7.7 | 0.01 | 14.5 ± 10.0 | 18.7 ± 7.6 |         |
| Plasma Aβ42/Aβ40    | 0.12 ± 0.01 | 0.11 ± 0.01 | <10−6 | 0.13 ± 0.01 | 0.11 ± 0.009 | <10−10 |

CU: Cognitively unimpaired elderly; CI: Elderly individuals with mild cognitive impairment; APOE: Apolipoprotein E; MMSE: Mini-Mental State Examination; CDR-SB: Clinical Dementia Rating – Sum of Boxes; ADAS-Cog: Alzheimer's Disease Assessment Scale – Cognitive subscale 13-item; NfL: neurofilament light.
| Table 2. Performance of classifier models in classifying Aβ+ cognitively unimpaired (CU) individuals. To assess the added value of each class of variables (i.e., clinical, plasma, and MRI classes), additional RF classifiers were constructed from 1) each plasma marker alone, 2) each plasma marker jointly with clinical features, 3) MRI–score jointly with clinical features, and 4) each plasma marker jointly with clinical features and MRI–score. |

| (A) Plasma Biomarkers | (B) Clinical information with and without plasma biomarkers | (C) MRI–score with and without clinical information and plasma biomarkers |
|----------------------|-------------------------------------------------|------------------------------------------------------------------|
| AUC                  | Acc    | PPV   | NPV   | Sens   | Spec   | AUC   | Acc   | PPV   | NPV   | Sens   | Spec   | AUC   | Acc   | PPV   | NPV   | Sens   | Spec   |
| MRI score            |        |       |       |       |       |       |        |       |       |       |       |       |       |       |       |       |       |       |
| Clinical information*|        |       |       |       |       |       |        |       |       |       |       |       |       |       |       |       |       |       |
| Aβ42/Aβ40            | 0.80   | 0.72  | 0.69  | 0.76  | 0.64  | 0.77  | 0.69   | 0.68  | 0.45  | 0.68  | 0.03  | 0.98  | [0.60, 0.82] | 0.04  | 0.06  | 0.03  | 0.11  | 0.04  |
|                      | 0.94   | 0.07  | 0.12  | 0.08  | 0.18  | 0.13  | 0.98   | 0.05  | 0.08  | 0.06  | 0.11  | 0.07  | [0.72, 0.87] | 0.02  | 0.06  | 0.02  | 0.09  | 0.05  |
| P-tau181             | 0.55†  | 0.62  | 0.39  | 0.71  | 0.37  | 0.75  | 0.69   | 0.69  | 0.58  | 0.69  | 0.07  | 0.98  | [0.42, 0.63] | 0.04  | 0.06  | 0.04  | 0.07  | 0.06  |
|                      | 0.66   | 0.02  | 0.04  | 0.02  | 0.07  | 0.05  | 0.73   | 0.01  | 0.29  | 0.01  | 0.06  | 0.03  | [0.73, 0.87] | 0.03  | 0.07  | 0.02  | 0.06  | 0.04  |
| NIL                  | 0.54‡  | 0.57  | 0.31  | 0.68  | 0.31  | 0.69  | 0.68   | 0.68  | 0.51  | 0.68  | 0.03  | 0.98  | [0.42, 0.49] | 0.06  | 0.08  | 0.06  | 0.08  | 0.06  |
|                      | 0.64   | 0.03  | 0.05  | 0.02  | 0.08  | 0.04  | 0.79   | 0.01  | 0.33  | 0.01  | 0.02  | 0.02  | [0.71, 0.87] | 0.03  | 0.06  | 0.02  | 0.06  | 0.04  |
| P-tau181 + NIL       | 0.53‡  | 0.60  | 0.34  | 0.69  | 0.26  | 0.76  | 0.65   | 0.69  | 0.53  | 0.72  | 0.25  | 0.90  | [0.42, 0.49] | 0.06  | 0.08  | 0.06  | 0.08  | 0.06  |
|                      | 0.63   | 0.04  | 0.07  | 0.02  | 0.06  | 0.05  | 0.75   | 0.03  | 0.10  | 0.02  | 0.08  | 0.04  | [0.72, 0.88] | 0.03  | 0.08  | 0.02  | 0.08  | 0.05  |
| Aβ42/Aβ40 + p-tau181| 0.83   | 0.77  | 0.77  | 0.80  | 0.70  | 0.83  | 0.85   | 0.81  | 0.82  | 0.83  | 0.73  | 0.87  | [0.72, 0.84] | 0.02  | 0.09  | 0.02  | 0.09  | 0.05  |
|                      | 0.68   | 0.06  | 0.13  | 0.06  | 0.12  | 0.14  | 0.72   | 0.07  | 0.29  | 0.01  | 0.03  | 0.02  | [0.81, 0.90] | 0.06  | 0.08  | 0.07  | 0.14  | 0.04  |

* Demographics: Age, sex, years of education, and APOE ε4 status; Global cognitive assessments: MMSE, ADAS–Cog, and CDR–SB
† 95% confidence intervals
‡ The confidence interval includes the axis y=x, suggesting that the classifier was not better than chance.
| Demographics | MRI score |
|--------------|-----------|
| Age, sex, six years of education, and APOE 4 status | Clinical assessments: MMSE, ADAS–Cog, and CDR–SB |

Table 3. Performance of classifier models in differentiating Alzheimer’s disease (AD) from controls. AUC: Area Under the Curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value; Sens: Sensitivity; CI: 95% confidence intervals.

To assess the added value of each class of biomarkers, the performance of classifier models was further evaluated by including clinical features (A), plasma biomarkers (B), and MRI markers (C). The table below shows the performance metrics with and without each class of biomarkers.

| Class                  | AUC | PPV | Sens | NPV |
|-----------------------|-----|-----|------|-----|
| (A) Plasma biomarkers |     |     |      |     |
| (B) Clinical features |     |     |      |     |
| (C) MRI score         |     |     |      |     |

*The confidence interval includes the area, suggesting that the classifier was not better than chance.

ACCF: American College of Cardiology Foundation.
Figure 1. Plasma a) Aβ42/Αβ40, b) NFL concentrations, and c) p-tau181 concentrations categorized by clinical diagnosis and CSF Aβ-positivity. Plasma Aβ42/Aβ40 data was available for 173 individuals (Aβ- CU, n=50; Aβ+ CU, n=37; Aβ- CI, n=40; Aβ+ CI, n=46). Plasma p-tau181 and NFL data included 852 individuals (Aβ- CU, n=224; Aβ+ CU, n=109; Aβ- CI, n=230; Aβ+ CI, n=289). Unpaired two-samples t-test uncorrected significance levels at ****: p<0.00001; ***: p<0.0001; **: p<0.001; ns: p ≥ 0.5. CU: Cognitively unimpaired elderly; CI: Elderly individuals with mild cognitive impairment.
Figure 2. Receiver operating characteristic (ROC) analysis of Aβ positivity prediction in an ADNI cohort of A) cognitively unimpaired (CU) and B) cognitively impaired (CI) elderly individuals. Optimized ROC curves for classifiers constructed separately and jointly with demographic information (age, sex, and years of education), APOE, clinical scores, plasma biomarkers (Aβ42/Aβ40, p-tau181, and NfL), and structural MRI-score when predicting Aβ-positivity using florbetapir PET as the ground truth in the ADNI study (n=333 CUs and n=519 CIs). To assess the added value of each class of variables (i.e., clinical, plasma, and MRI classes), additional RF classifiers were constructed from 1) each plasma marker alone, 2) each plasma marker jointly with clinical features, 3) MRI-score jointly with clinical features, and 4) each plasma marker jointly with clinical features and MRI-score. Models including plasma Aβ42/Aβ40 were tested and validated in a cohort of n=87 CUs and n=86 CIs due to limited available of plasma Aβ42/Aβ40 data. Error bars indicate union of 95% CIs from cross-validation iterations.
Figure 3. Classifier performance metrics of Aβ positivity prediction in A) cognitively unimpaired (CU) individuals and B) individuals with mild cognitively impairment (CI). Area under the curve (AUC) estimates with ± 2 x standard variation error bars from cross-validation iterations are shown for classifiers constructed separately and jointly with demographic information (age, sex, and years of education), APOE, clinical scores, plasma biomarkers (Aβ42/Aβ40, p-tau181, and NfL), and structural MRI–score when predicting Aβ-positivity using florbetapir PET as the ground truth in the ADNI study (n=333 CUs and n=519 CIs). To assess the added value of each class of variables (i.e., clinical, plasma, and MRI classes), additional RF classifiers were constructed from 1) each plasma marker alone, 2) each plasma marker jointly with clinical features, 3) MRI–score jointly with clinical features, and 4) each plasma marker jointly with clinical features and MRI–score. Models including plasma Aβ42/Aβ40 were tested and validated in a cohort of n=87 CUs and n=86 CIs due to limited available of plasma Aβ42/Aβ40 data. Error bars indicate union of 95% CIs from cross-validation iterations.
Abbreviated Summary: Tosun et al. report a systematic comparison of β-amyloid positivity detection models, identifying effective combinations of demographics, APOE genotype, global cognitive measures, MRI, and plasma biomarkers as promising minimally invasive and low-cost assessments to detect the β-amyloid positivity using florbetapir PET status as the ground-truth.
Aβ PET – radiation exposure; high cost; limited accessibility

How to identify β-amyloid+ participants?

CSF Aβ42 – Invasive lumbar puncture

Structural MRI

Plasma (β-amyloid, phosphorylated-tau at threonine-181, neurofilament light)

“Gold standard” level of brain Aβ

Minimally invasive, cost-effective, scalable methods

Age, sex, education, APOE, cognition

Graphical abstract

138x122mm (300 x 300 DPI)