Accurate risk estimation of β-amyloid positivity to identify prodromal Alzheimer’s disease: Cross-validation study of practical algorithms

Sebastian Palmqvist a,b,*, Philip S. Insel a, Henrik Zetterberg c,d,e,f, Kaj Blennow c,d, Britta Brix g, Erik Stomrud a,h, the Alzheimer’s Disease Neuroimaging Initiative 1, the Swedish BioFINDER study, Niklas Mattsson a,b, Oskar Hansson a,h,**

aClinical Memory Research Unit, Department of Clinical Sciences, Lund University, Malmö, Sweden
bDepartment of Neurology, Skåne University Hospital, Lund, Sweden
cDepartment of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
dClinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
eDepartment of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, United Kingdom
fUK Dementia Research Institute at UCL, London, United Kingdom
gEuroimmun AG, Lübeck, Germany
hMemory Clinic, Skåne University Hospital, Malmö, Sweden

Abstract

Introduction: The aim was to create readily available algorithms that estimate the individual risk of β-amyloid (Aβ) positivity.

Methods: The algorithms were tested in BioFINDER (n = 391, subjective cognitive decline or mild cognitive impairment) and validated in Alzheimer’s Disease Neuroimaging Initiative (n = 661, subjective cognitive decline or mild cognitive impairment). The examined predictors of Aβ status were demographics; cognitive tests; white matter lesions; apolipoprotein E (APOE); and plasma Aβ42/Aβ40, tau, and neurofilament light.

Results: Aβ status was accurately estimated in BioFINDER using age, 10-word delayed recall or Mini–Mental State Examination, and APOE (area under the receiver operating characteristics curve = 0.81 [0.77–0.85] to 0.83 [0.79–0.87]). When validated, the models performed almost identical in Alzheimer’s Disease Neuroimaging Initiative (area under the receiver operating characteristics curve = 0.80–0.82) and within different age, subjective cognitive decline, and mild cognitive impairment populations. Plasma Aβ42/Aβ40 improved the models slightly.

Discussion: The algorithms are implemented on http://amyloidrisk.com where the individual probability of being Aβ positive can be calculated. This is useful in the workup of prodromal Alzheimer’s disease and can reduce the number needed to screen in Alzheimer’s disease trials.

Keywords: Alzheimer’s disease; β-amyloid; Prediction; Diagnostic accuracy; Cerebrospinal fluid; Aβ42; Risk estimation; Position emission tomography; Plasma Aβ42/Aβ40
1. Introduction

β-Amyloid (Aβ) accumulation is believed to be the initial pathology of the most common type of neurological disease leading to dementia, Alzheimer’s disease (AD) [1]. Abnormal levels of Aβ are associated with longitudinal cognitive decline in healthy elderly [2] and progression to AD dementia in subjects with mild cognitive impairment (MCI) [3]. A verified Aβ status can be used to improve the accuracy of AD diagnostics and for including participants in trials of novel AD drugs, as currently used in several clinical trials [4]. Given the devastating symptoms of AD, the high number of affected people, and the tremendous costs for society (US$ 259 billion per year for dementia in the US alone), there will be a great pressure on the health care system to identify persons with abnormal Aβ deposition when disease-modifying AD treatments become available [5].

Brain Aβ can be detected in vivo either by performing a lumbar puncture (LP) and analyzing the levels of the peptide Aβ42 in cerebrospinal fluid (CSF) or by performing a positron emission tomography (PET) scan using a ligand that binds to Aβ fibrils (Aβ PET). There are no significant differences between the two methods in terms of accuracy for identifying AD [6,7], and they are used mostly not only in research but also in clinical practice at some specialized memory clinics. However, because these methods are invasive, costly, and not available in all health care settings, a screening process to select individuals for LP or PET testing, both in clinical practice and clinical treatment trials, would be very useful. Several studies on amyloid prediction tools or blood-based Aβ biomarkers exist, but due to lack of or failed validations, low accuracies, or the usage of advanced technology or extensive neuropsychological testing, none of them are currently being used in clinical or research settings, to the best of our knowledge [8–12].

In the present study, we aimed to develop algorithms that estimate the risk of being Aβ positive using readily available and noninvasive measures and tests. Nondemented subjects with either subjective or objective cognitive symptoms were examined to provide a clinically relevant target population. The models were developed in a training cohort and validated in an independent population. In a second step, we analyzed the added value of including the plasma biomarkers tau, neurofilament light (NFL), and the Aβ42/Aβ40 ratio.

2. Materials and methods

2.1. Participants of the training cohort (BioFINDER)

The Swedish BioFINDER study (Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably) is a prospective study that focuses on identifying key mechanisms and improving clinical diagnostics of AD and other neurodegenerative disorders. Details about the Swedish BioFINDER study design have been published previously [12,13] and are available at http://biofinder.se. In the present study, we used the BioFINDER cohort of prospectively and consecutively included nondemented participants with cognitive complaints. They were enrolled between 2010 and 2015, mostly from primary care centers in the Southern part of Sweden. The inclusion/exclusion criteria are provided in the Supplementary Material. Based on the result of a comprehensive neuropsychological battery and the clinical assessment of a senior neuropsychologist and two physicians specialized in neurocognitive disorders, 54% of the 391 participants were classified as having MCI and 46% as having subjective cognitive decline [14].

2.2. Amyloid outcome measures in BioFINDER

Aβ was measured using 18F-flutemetamol PET if available (n = 241), otherwise CSF Aβ42 was used (n = 150). The scanning [15] and processing [13] procedures have been described previously. The weighted mean standardized uptake value ratio (SUVR) from a global neocortical region of interest [16] relative to a composite reference region (white matter, cerebellum and brainstem [13]) was used to determine the Aβ status. The SUVR cutoff for Aβ positivity was determined using unbiased mixture modeling statistics, which is a well-validated method for determining such a cutoff [13,17,18]. The resulting cutoff for Aβ positivity was >0.738 SUVR.

LP and CSF handling followed a structured protocol [15]. CSF levels of Aβ42 were analyzed using INNOTEST ELISAs (Fujirebio Europe, Ghent, Belgium). The CSF Aβ42 cutoff for Aβ abnormality was determined using the optimized Youden’s Index against Aβ PET in BioFINDER (CSF Aβ42 < 552 ng/L; sensitivity 93%, specificity 84%).

2.3. Predictor variables of Aβ positivity

Different types of predictors were examined in the primary analysis, including demographics (age, education, and sex), apolipoprotein E (APOE) genotype, cognitive test scores, and white matter lesions. The cognitive tests were administered by experienced research nurses who were blinded to the Aβ status of the participants.

APOE genotypes were analyzed from blood samples, and the participants were stratified according to Aβ risk into the following groups (see reference [19] for rationale): (1) ε2/ε2 or ε2/ε3, (2) ε3/ε3, (3) ε2/ε4 or ε3/ε4, and (4) ε4/ε4. APOE ε3/ε3 was the reference category.

Episodic memory function was measured with the delayed recall part of the 10-word list from the Alzheimer’s Disease Assessment Scale–cognition [20]. Cognitive function was also assessed with the Mini–Mental State Examination (MMSE) [21]. Both the total score and the score from the orientation and memory parts of the test were used. The scores from the orientation and memory parts of the MMSE were used based on previous findings showing that
the orientation to time and place and the three-word delayed recall parts can differentiate MCI and dementia due to AD from other causes of cognitive impairment [22,23]. It consists of orientation to place (country, county/state, city, building/place, and floor), orientation to time (year, season, month, day of the week, and date), and three words that are being recalled after a short distraction task.

We also examined A Quick Test of Cognitive Speed (AQT)—color and form score, which is a sensitive test for attention and executive function to account for non-AD-specific cognitive impairment [24,25]. AQT was used alone and as a ratio with the delayed word recall test and MMSE orientation and memory.

Magnetic resonance imaging was performed on a 3-Tesla Siemens Tim Trio scanner (Siemens Medical Solutions, Erlangen, Germany). T2 FLAIR images were used for rating white matter lesions according to the ARWMC scale [26] to account for the impact of cerebrovascular pathology on cognitive impairment.

In a secondary analysis, we added the plasma biomarkers tau, the ratio of Aβ_{42}/Aβ_{40} and NFL, which previously have been tested as AD biomarkers [27–29]. Plasma Aβ_{42} and Aβ_{40} levels were determined using the EUROIMMUN ELISAs (EUROIMMUN, Lubeck, Germany). The total levels of Aβ_{42} and Aβ_{40} were used to calculate the Aβ_{42}/Aβ_{40} ratio. Plasma tau and NFL concentrations were measured on a Simoa HD-1 analyzer using the Human Total Tau kit (Quanterix, Lexington, MA) for tau and an in-house assay based on the same antibodies and standard protein as in the commercially available NF-light kit (UmanDiagnostics, Umea, Sweden) for NFL [30]. All predictor variables were available in all patients, except for plasma NFL and tau (n = 346 of 391 participants).

2.4. Validation cohort—Alzheimer’s Disease Neuroimaging Initiative

A detailed study and data description of the Alzheimer’s Disease Neuroimaging Initiative (ADNI) as well as inclusion/exclusion criteria and MCI definitions can be found on www.adni-info.org and in the Supplementary Material. Only nondemented subjects with cognitive symptoms were selected, which included participants with early and late MCI and participants from the healthy control cohort who had significant memory concerns.

We included only participants with a complete data set of cognitive test, APOE, and Aβ data (Aβ PET or CSF Aβ_{42}). This selection resulted in a population of 661 participants, of which 170 had plasma biomarker data.

Aβ status was based on (in order of preference) (1) Aβ PET using the ligand 11C-florbetapir, (2) Aβ PET using the ligand 11C-Pittsburgh Compound B (PiB), and (3) CSF Aβ_{42} measured using the multiplex xMAP Lumexin platform (Lumexin Corp, Austin, TX, USA) with the INNO-BIA AlzBio3 kit (Innogenetics, Ghent, Belgium) [31,32]. Predefined cutoffs for Aβ positivity were used for florbetapir (>1.11 SUVR) [33], 11C-Pittsburgh Compound B (>1.5 SUVR) [34], and Aβ_{42} (<192 ng/L) [32]. The methods for these three measures have previously been described [32–34].

Plasma Aβ_{42} and Aβ_{40} were measured using the INNO-BIA plasma Aβ immunoassay kit (Fujirebio, Ghent, Belgium) on the Luminex 100 immunoassay platform (Luminex Corp) [35]. The total levels of Aβ_{42} and Aβ_{40} were used to calculate the Aβ_{42}/Aβ_{40} ratio.

2.5. Statistical analysis

Group comparisons were done using the Mann-Whitney U test. In Table 1, we applied Bonferroni correction to adjust for multiple comparisons. P values were thus multiplied by 6 and a value of <0.05 was considered statistically significant. To predict Aβ positivity, the following variables from the training cohort (BioFINDER) were entered in a general linear model: age, gender, presence of APOE ε2/ε2 or ε2/ε3, presence of APOE ε2/ε4 or ε3/ε4, presence of APOE ε4/ε4 (APOE ε3ε3 was not included because it was the reference variable), total MMSE score, the score from the orientation and delayed recall (memory) parts of the MMSE, the 10-word list delayed recall from Alzheimer’s Disease Assessment Scale–cognition (number of errors), years of education, AQT score, 10-word list delayed recall/AQT, MMSE orientation and memory/AQT, and degree of white matter lesions (ARWMC score). Using Aβ status as the dependent variable, the general linear model was fitted to the data using the least absolute shrinkage and selection operator (LASSO) [36]. The LASSO analysis uses a type of forward selection logistic regression that provides more robust predictors because it penalizes the absolute value of the coefficients and shrinks irrelevant coefficients to zero. The LASSO was only used for selecting predictor variables in BioFINDER (the training cohort), it could not be directly applied to the ADNI data (validation cohort) because not all BioFINDER variables were present in ADNI (ARWMC and AQT data). To increase the applicability of an Aβ risk model, we also used a reduced set of variables (but the same population) where we excluded the 10-word list delayed recall, AQT, and white matter lesions assessments because these measures are not always available in all settings. In a final step of Aβ risk analyses, we added plasma tau, plasma NFL, and the plasma Aβ_{42}/Aβ_{40} ratio to the two LASSO models. The selected variables from the LASSO regression (variables with nonzero estimates) were entered in a logistic regression model to calculate the intercept, the coefficients, and the resulting area under the receiver operating characteristics curve (AUC). The Akaike Information Criterion (AIC) was used to assess the model fit in relation to its complexity (number of variables), where a drop of ≥2 indicated a statistically better model [37]. The best model was considered to be the one with the highest AUC and the lowest AIC. The logistic regression models from BioFINDER were then replicated in different
| Variables                        | BioFINDER (training cohort) | ADNI (validation cohort, plasma subset) | ADNI (validation cohort, total population) |
|---------------------------------|-----------------------------|----------------------------------------|---------------------------------------------|
|                                 | $A_b^-$                     | $A_b^+$                                 | $A_b^-$                                    |
|                                 | 197 (50%)                   | 194 (50%)                              | 66 (39%)                                   |
| N                               | 346                         | 104 (61%)                              | 170                                         |
| SCD/MCI                         | 55%/45%                     | 36%/64%                                | 5%/62%/33%                                 |
| SMC/EMCI/LMCI                   | 46%/54%                     | 2%/44%/54%                             | 3%/51%/46%                                 |
| Age (range)                     | 69.8 (60–80)                | 72.1 (60–80)                           | 71.0 (56–89)                               |
| SMC/EMCI/LMCI                   | 5%/62%/33%                  | 2%/44%/54%                             | 3%/51%/46%                                 |
| Sex (women)                     | 45%                         | 44%                                    | 45%                                        |
| Education (years)               | 4%                          | 43%                                    | 46%                                        |
| MMSE (0–30 p)                   | 12.1 (1.0)                  | 11.5 (1.4)                             | 11.8 (1.2)                                 |
| MMSE orientation and delayed recall (0–13 p) | 12.1 (1.0)                  | 11.5 (1.4)                             | 11.8 (1.2)                                 |
| 10-word list delayed recall (0–10 errors) | 4.1 (2.5)                   | 6.0 (2.5)                              | 5.0 (2.6)                                  |
| APOE $^e$/e2 or $^e$/e3         | 13%                         | 2%                                    | 7%                                         |
| APOE $^e$/e3                    | 63%                         | 29%                                    | 46%                                        |
| APOE $^e$/e4 or $^e$/e4         | 22%                         | 50%                                    | 36%                                        |
| APOE $^e$/e4                    | 3%                          | 19%                                    | 11%                                        |
| Plasma $A_b/A_{b_0}$ ratio      | 0.19 (0.06)                 | 0.16 (0.03)                            | 0.17 (0.05)                                |
| Plasma tau (pg/mL)              | 5.3 (2.3)                   | 5.5 (2.7)                              | 5.4 (2.5)                                  |
| Plasma NfL (pg/mL)              | 24.0 (24)                   | 26.7 (17)                              | 25.4 (20.9)                                |
| WML (ARWMC scale, 0–27 p)       | 6.6 (5.7)                   | 6.9 (5.4)                              | 6.8 (5.6)                                  |
| AQT color-form (seconds)        | 79 (25)                     | 85 (29)                                | 82 (27)                                    |

Abbreviations: $A_b$, $\beta$-amyloid; ADNI, Alzheimer’s Disease Neuroimaging Initiative; APOE, apolipoprotein E; ARWMC, age-related white matter changes; BioFINDER, Biomarkers For Identifying Neuro-Degenerative Disorders Early and Reliably; EMCI, early MCI; LMCI, late MCI; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination; NfL, neurofilament light; SCD, subjective cognitive decline; SMC, significant memory concern; WML, white matter lesions.

NOTE. Data are given in mean values (standard deviation) if not otherwise specified. All $P$ values are Bonferroni corrected (multiplied by 6) to adjust for multiple comparisons. Within population comparisons ($A_b^+$ compared with $A_b^-$): $^aP < .05$; $^bP < .01$; $^cP < .001$. Comparison between ADNI and BioFINDER: $^dP < .05$; $^eP < .01$; $^fP < .001$. Comparison between total and plasma populations in ADNI: $^gP < .05$; $^hP < .01$; $^iP < .001$.

*11 cognitively normal participants had progressed to MCI at the present study baseline, and these were approximated as EMCI.
subgroups in BioFINDER and in the independent ADNI cohort for a robust cross-validation. Equations for calculating the individual risk of being Aβ positive were derived from the estimates and intercepts in the different models. The statistics were performed using R, version 3.3 (R Foundation for Statistical Computing, Vienna, Austria, 2013), and SPSS for Mac, version 22 (SPSS Inc., Chicago, IL). The amyloid risk models were implemented online using a R Shiny (version 1.0.0) program.

3. Results

The characteristics of training (BioFINDER) and validation (ADNI) cohorts are described in the Supplementary Material and shown in Table 1.

3.1. Establishing the amyloid prediction models in BioFINDER

The different Aβ prediction models are illustrated in Fig. 1A and Supplementary Table 1. The selected variables from the LASSO regression were age, APOE ε2/ε2ε3, APOE ε2ε4/ε3ε4, APOE ε4ε4, and the 10-word list delayed recall (see Fig. 1 legend for a complete list of examined variables). Hereafter, this is referred to as the “delayed recall” model. In a multivariable logistic regression, coefficients and intercept were established (Supplementary Fig. 1). The resulting area under the ROC curve (AUC) based on the probabilities from the model was 0.83 (95% CI 0.79–0.87) (Fig. 1A, Supplementary Table 1). Because a 10-word list, grading of white matter lesions, and AQT are not always available in all settings, we also ran another LASSO regression using the same population but removed these three measures. The variables selected by the LASSO regression were then age, APOE ε2ε2ε2ε3, APOE ε2ε4/ε3ε4, APOE ε4ε4, and MMSE orientation and memory. This is referred to as the “MMSE model.” In a logistic regression, this model had slightly less AUC than the delayed recall model (AUC 0.81, 95% CI 0.77–0.85), and a comparison of the AICs also favored the delayed recall model (ΔAIC 17).

Next, we reran the aforementioned LASSO analyses but also included the plasma biomarkers Aβ42/Aβ40, NfL, and tau. The selected variables from the analysis were age, APOE ε2ε2/ε2ε3, APOE ε2ε4/ε3ε4, APOE ε4ε4, the 10-word list delayed recall, and plasma Aβ42/Aβ40. This produced the best model with ΔAICs of −8 to −34 compared with the other models and the highest AUC of all models (0.85, 95% CI 0.81–0.89) (Fig. 1A; Supplementary Table 1). When excluding grading of white matter lesions, AQT, and 10-word list delayed recall from the LASSO model, plasma Aβ42/Aβ40 was again selected, in addition to age, APOE ε2ε2ε2ε2ε3, APOE ε2ε4/ε3ε4, APOE ε4ε4, and MMSE orientation and memory. The AUC from the...
logistic regression was 0.83 (95% CI 0.79–0.87), which was favorable compared with the MMSE model without plasma \( A\beta_{42}/A\beta_{40} \) (\( \Delta\text{AUC} 0.02 \) and \( \Delta\text{AIC} -16 \)). In univariate analyses of the selected variables from the LASSO regression, plasma \( A\beta_{42}/A\beta_{40} \) had the highest accuracy (AUC 0.74, 95% CI 0.69–0.79) (Fig. 1B and Supplementary Table 1).

3.2. Replicating the models in ADNI

The BioFINDER models were replicated in both the ADNI subset where plasma \( A\beta_{42}/A\beta_{40} \) values were available (n = 170) and in the total eligible ADNI population (n = 661), that is, the equations in Supplementary Fig. 1 were tested in the ADNI samples (a new model was not fitted in ADNI). The different replications are shown in Fig. 2 and described with exact data in Supplementary Table 2. When replicating the delayed recall model in ADNI, the AUC was 0.82 (95% CI 0.75–0.89) compared with 0.83 in BioFINDER. The AUC was 0.83 (95% CI 0.77–0.89) when replicating the delayed recall model plus plasma \( A\beta_{42}/A\beta_{40} \) (AUC 0.85 in BioFINDER). The MMSE model had an AUC of 0.81 (95% CI 0.75–0.88), equal to its original performance in BioFINDER (AUC 0.81, 95% CI 0.77–0.85). Similar performance was seen when adding plasma \( A\beta_{42}/A\beta_{40} \) to the delayed recall model (AUC 0.83, 95% CI 0.76–0.89, in ADNI compared with 0.83, 95% CI 0.77–0.85, in BioFINDER). In the total ADNI population (n = 661), both the delayed recall and MMSE models had AUC of 0.80 (95% CI 0.77–0.84 and 0.77–0.83, respectively). The performance of the models in the eight different subpopulations in BioFINDER and ADNI (Fig. 2 and Supplementary Table 2) was robust when tested within different age strata or within different groups of cognitive impairment (subjective cognitive decline, early MCI, and late MCI).

3.3. Calculating the individual risk of being amyloid positive

The models were implemented and published on http://amyloidrisk.com where the individual probability of being \( A\beta \) positive can be calculated, including a 95% CI of the predicted probability. The plasma models were not implemented on the website because we believe further research is needed in terms of assay standardization and preanalytical protocols. ROC curves with sensitivity and specificity for each amyloid risk probability is shown in Fig. 3A–D. The highest Youden index (sensitivity + specificity – 1) was produced using a cutoff of 56% probability of amyloid

Fig. 2. (A–D) Replication of the amyloid risk models. The top bar in (A–D) shows the model performance in the original total BioFINDER population, and the error bars represent the 95% CI of the AUC. The other bars show the performance when applying the estimates and intercept from the logistic regression analysis on other populations. Vertical dashed lines have been added at AUC 0.80 for easier comparison between the populations. For model description, see Fig. 1 and Supplementary Fig. 1. Blue bars represent different BioFINDER populations, and red bars represent different ADNI populations. The age stratification was based on the median age of the population. Abbreviations: ADNI, Alzheimer’s Disease Neuroimaging Initiative; AUC, area under the ROC curve; BioFINDER, Biomarkers For Identifying Neuro-Degenerative Disorders Early and Reliably; CI, confidence interval; EMCI, early MCI; LMCI, late MCI; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination; ROC, receiving operating characteristics; SCD, subjective cognitive decline; SMC, significant memory concern.
positivity for the delayed recall model (sensitivity 71%, specificity 83%), 59% probability for the MMSE model (sensitivity 66%, specificity 83%), 43% for the delayed recall model plus plasma $A\beta 42/A\beta 40$ (sensitivity 85%, specificity 71%), and 50% for the MMSE model plus plasma $A\beta 42/A\beta 40$ (sensitivity 75%, specificity 77%).

4. Discussion

In this study, we have developed four different amyloid risk models based on consecutively recruited nondemented patients in BioFINDER ($n = 391$). The models, which included the predictors age, $APOE$ genotype, and parts of the MMSE or a delayed recall test, could accurately predict $A\beta$ positivity (AUCs 0.81–0.83) and were validated in an independent population (ADNI, $n = 170–661$) with similar accuracies. The addition of plasma $A\beta 42/A\beta 40$ to $APOE$, age, and brief cognitive testing increased the accuracy slightly.

There are several previous suggestions on how to estimate $A\beta$ positivity based on MRI measures, neuropsychological tests, $APOE$ genotypes, and blood-based biomarkers [8,9,12,38–41]. For example, we previously found that a combination of demographics, $APOE$, and longitudinal cognitive testing could be used to identify $A\beta$ positivity in cognitively healthy controls [12]. Recently, age and $APOE$ were examined as predictors of $A\beta$ positivity in MCI and subjects without objective cognitive decline [42]. The AUCs in that study were lower (0.74–0.75), and no increase in AUC was seen when MMSE was added. This might be explained by how $APOE$ was coded (only as $\varepsilon 4+/–$) and that they used the total MMSE score, in contrast to the present study where we used four $APOE$ groups based on their different contributing risks to $A\beta$ accumulation [19] and the use of only AD-specific parts of the MMSE score (orientation and memory) [22,23].

A common limitation in many of the previous studies is that the $A\beta$ prediction models have not been validated in...
an independent population. In the present models, we only used biomarkers or measures that previously have been shown to either be associated with Aβ deposition or to predict future development of AD dementia [19,23,27,41], to reduce the risk of random inaccurate findings. The robustness of the models was confirmed by validating them in the independent ADNI population and in eight different subgroups (Fig. 2A–D). Note that the models performed well also in selected populations of individuals with only subjective cognitive symptoms (BioFINDER) and significant memory concerns or early MCI (ADNI), which may be of high interest in clinical trials of novel treatments. This also shows that the high accuracy of the models was not driven by the difference in cognitive status between subjective cognitive decline and MCI (BioFINDER) or early MCI and late MCI (ADNI). The training (BioFINDER) and validation (ADNI) cohorts are different in many ways, which makes it more likely that the established models are indeed generalizable. The differences include, for example, geographic locations (Sweden and North America), education levels (lower in BioFINDER, high in ADNI), cognitive tests in different languages, and the patient selection process (consecutively recruited subjects referred to memory clinics in BioFINDER; selected enrollment in ADNI). Nonetheless, we want to mention potential limitations in these cohorts. The amyloidosis is to a large extent associated with late-onset AD, and the applicability in early-onset AD remains to be tested. The models need further validation in selected primary care populations with individuals who seek medical care due to cognitive complaints (i.e., tested in populations with lower prevalence of Aβ positivity). Finally, the models should be validated in populations where the prevalence of different APOE genotypes differs from the North European/North American populations used in the present study [43].

One popular aim has been to try to identify blood-based AD biomarkers. Plasma biomarker signatures of brain Aβ has, however, been difficult to replicate. Voyle et al. [8] recently performed a large attempt to validate 35 different plasma proteins that had predicted Aβ positivity in previous studies [38–40,44]. Unfortunately, none of the proteins were significantly associated with neocortical Aβ burden in the independent cohort. In the present study, we examined the additive effect of plasma Aβ42/Aβ40, NfL, and tau in our models because these biomarkers have been associated with AD [27–29]. Although levels of NfL were significantly higher in Aβ-positive individuals (Table 1), only plasma Aβ42/Aβ40 was an independent predictor of brain Aβ in addition to age, APOE genotype, and cognitive testing. Plasma Aβ42/Aβ40 was also the predictor with the highest accuracy in the univariate analysis (Fig. 1B). It increased the AUC in both the delayed recall and MMSE models (Fig. 1A and Supplementary Table 1) and increased the AUC when replicated in ADNI (Fig. 2C–D and Supplementary Table 2). However, the clinical relevance of such a small increase in AUC is limited. Also, assay-dependent differences, or possibly preanalytical factors, may have contributed to different levels in the cohorts (Table 1). This highlights the need for an optimal unified analysis method for plasma Aβ42/Aβ40. Promising results with very high accuracies have been seen using mass spectrometry [45,46], but unfortunately this is an advanced and time-consuming technique that cannot be implemented in primary care or large screening settings in the near future.

We propose that the presented models could be useful in mainly two settings, clinical AD trials and primary care. In clinical trials aimed at Aβ-positive subjects, amyloid risk models could reduce the number of unnecessary Aβ PET scans or LPs. In Fig. 4, we illustrate such a scenario using the delayed recall model. Here, we assume that 1000 Aβ-positive subjects are to be included in a clinical trial where Aβ PET is used to verify and assess the Aβ burden. An amyloid risk screening process in a population similar to the BioFINDER cohort could reduce the number of unnecessary (negative) Aβ PET scans by ~90% and reduce the costs by >3.5 million USD [12,47], when using a probability cutoff of >80% for undergoing an Aβ PET scan. In the trial scenario, the objective is thus to increase Aβ prevalence of the eligible population (high specificity). On the other hand, in a primary care workup of cognitive impairment or in a scenario where anti-Aβ drugs have become available, a high sensitivity may be preferred. Here, a probability threshold of around 30% would perhaps be more suitable to ensure a sensitivity of >90% (Fig. 3). To facilitate such a use of the risk models, we have implemented them on http://amyloidrisk.com where age, APOE genotype, and cognitive test score can be entered to calculate the individual probability of being Aβ positive. The website is only intended for research and education until further validation has been conducted, but we believe it can be a useful tool for deciding who should undergo further evaluation with LP or Aβ PET to verify the presence of Aβ pathology.

Acknowledgments

The authors thank Fredrik Kahn, MD, PhD, for valuable input and feedback. Work at the authors’ research center was supported by the European Research Council, the Swedish Research Council, the Marianne and Marcus Wallenberg foundation, the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson’s disease) at Lund University, the Swedish Brain Foundation, the Skåne University Hospital Foundation, the Swedish Alzheimer Association, and the Swedish federal government under the ALF agreement and F. Hoffmann-Roche Ltd. Doses of 18F-flutemetamol injection were sponsored by GE Healthcare. The ADNI data
collection and sharing for this project was funded by the Alzheimer’s Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Alzheimer’s Association; Alzheimer’s Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; Euroimmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer’s Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

S.P., P.S.I., N.M., and E.S. report no conflicts of interest. B.B. is a full-time employee of Euroimmun. K.B. has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Novartis, Pfizer, and Roche Diagnostics. K.B. and H.Z. are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. H.Z. has served at advisory boards of Eli Lilly and Roche Diagnostics and has received travel support from TEVA. O.H. has acquired research support (for the institution) from Roche, GE Healthcare, Biogen, AVID Radiopharmaceuticals, Fujirebio, and Euroimmun. In the past 2 years, he has received
consultancy/speaker fees (paid to the institution) from Lilly, Roche, and Fujirebio.

**Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jalz.2018.08.014.

**RESEARCH IN CONTEXT**

1. Systematic review: We reviewed publications of β-amyloid (Aβ) prediction using PubMed. There are previous prediction models, but they lack adequate accuracy, replicable results, readily available measures, and/or individual risk stratification.

2. Interpretation: Using just age, APOE genotype, and a brief cognitive test, we accurately predicted Aβ positivity in a training cohort (area under the receiver operating characteristics curve = 0.81–0.83, n = 391) and replicated the models in an independent validation cohort (area under the receiver operating characteristics curve = 0.80–0.82, n = 170–661). The individual probability of Aβ positivity can be calculated on http://amyloidrisk.com. This is useful, for example, in the primary care workup of prodromal Alzheimer’s disease or when screening participants in Alzheimer’s disease trials for selecting persons who should be further examined with amyloid PET or cerebrospinal fluid analysis.

3. Future directions: The models need to be replicated in populations with lower prevalence of Aβ positivity (e.g., primary care). The addition of plasma Aβ42/ Aβ40 seems to improve the models, but further standardization of assays and preanalytical protocols is needed.

**References**

[1] Jack CR Jr, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer’s disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 2013;12:207–16.

[2] Donohue MC, Sperling RA, Petersen R, Sun CK, Weiner MW, Aisen PS, et al. Association between elevated brain amyloid and subsequent cognitive decline among cognitively normal persons. JAMA 2017;317:2305–16.

[3] Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer’s disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 2006;5:228–34.

[4] Mattsson N, Carrillo MC, Dean RA, Devous Sr MD, Nikolcheva T, Pesin P, et al. Revolutionizing Alzheimer’s disease and clinical trials through biomarkers. Alzheimer’s & Dementia: Diagnosis, Assess Dis Monit 2015;1:412–9.

[5] Alzheimer’s Association. 2017 Alzheimer’s Disease Facts and Figures. Alzheimers Dement 2017;13:325–73.

[6] Mattsson N, Insel P, Landau SM, Jagust W, Donohue MC, Shaw LM, et al. Diagnostic accuracy of CSF Ab42 and florbetapir PET for Alzheimer’s disease. Ann Clin Transl Neurol 2014;1:534–43.

[7] Palmqvist S, Zetterberg H, Mattsson N, Johansson P, ADNI, Minthon L, et al. Detailed comparison of amyloid PET and CSF biomarkers for identifying early Alzheimer’s Disease. Neurology 2015;85:1240–9.

[8] Boyle P, Baker D, Burnham SC, Covin A, Zhang Z, Sangurdekar DP, et al. Blood protein markers of neocortical amyloid-beta burden: A Candidate Study using SOMAscan technology. J Alzheimers Dis 2015;46:947–61.

[9] Haghighi M, Smith A, Morgan D, Small B, Huang S. Identifying cost-effective predictive rules of amyloid-beta level by integrating neuropsychological tests and plasma-based markers. J Alzheimers Dis 2015;43:1261–70.

[10] Baird AL, Westwood S, Lovestone S. Blood-Based Proteomic Biomarkers of Alzheimer’s Disease Pathology. Front Neurol 2015;6:236.

[11] Tosun D, Ioshi S, Weiner MWAlzheimer’s Disease Neuroimaging I. Neuroimaging predictors of brain amyloidosis in mild cognitive impairment. Ann Neurol 2013;74:188–98.

[12] Insel PS, Palmqvist S, Mackin RS, Nosheny RL, Hansson O, Weiner MW, et al. Assessing risk for preclinical beta-amyloid pathology with APOE, cognitive, and demographic information. Alzheimers Dement (Amst) 2016;4:76–84.

[13] Palmqvist S, Scholl M, Strandberg O, Mattsson N, Stomrud E, Zetterberg H, et al. Earliest accumulation of beta-amyloid occurs within the default-mode network and concurrently affects brain connectivity. Nat Commun 2017;8:1214.

[14] Mattsson N, Insel PS, Palmqvist S, Stomrud E, van Westen D, Minthon L, et al. Increased amyloidogenic APP processing in APOE ε4-negative individuals with cerebral beta-amyloidosis. Nat Commun 2016;7:10918.

[15] Palmqvist S, Zetterberg H, Blennow K, Vestberg S, Andreasson U, Brooks DJ, et al. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: a cross-validation study against amyloid positron emission tomography. JAMA Neurol 2014;71:1282–9.

[16] Landau S, Jagust W. Florbetapir Processing Methods. ida.loni.usc.edu 2015. Helen Wills Neuroscience Institute, UC Berkeley and Lawrence Berkeley National Laboratory, 2015. Available at: https://ida.loni.usc.edu. Accessed November 19, 2018.

[17] Palmqvist S, Mattsson N, Hansson OAlzheimer’s Disease Neuroimaging I. Cerebrospinal fluid analysis detects cerebral amyloid-beta accumulation earlier than positron emission tomography. Brain 2016;139:1226–36.

[18] Toledo JB, Bjerke M, Da X, Landau SM, Foster NL, Jagust W, et al. Nonlinear Association Between Cerebrospinal Fluid and Florbetapir F-18 beta-Amyloid Measures Across the Spectrum of Alzheimer Disease. JAMA Neurol 2015;72:571–81.

[19] Jansen WJ, Ossenkoppele R, Knol DL, Tijms BM, Scheltextes P, Verhey FR, et al. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. JAMA 2015;313:1924–38.

[20] Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer’s disease. Am J Psychiatry 1984;141:1356–64.

[21] Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical procedure for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.

[22] Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.

[23] Palmqvist S, Hansson O, Minthon L, Londos E. Practical suggestions on how to differentiate dementia with Lewy bodies from Alzheimer’s disease with common cognitive tests. Int J Geriatr Psychiatry 2009;24:1405–12.
[23] Palmqvist S, Hertz J, Minthon L, Wattmo C, Zetterberg H, Blennow K, et al. Comparison of brief cognitive tests and CSF biomarkers in predicting Alzheimer’s disease in mild cognitive impairment: six-year follow-up study. PLoS One 2012;7:e38639.

[24] Andersson M, Wiig EH, Minthon L, Londos E. A Quick Test for Cognitive Speed: a measure of cognitive speed in dementia with Lewy bodies. Am J Alzheimers Dis Other Dement 2007:22:313–8.

[25] Palmqvist S, Minthon L, Wattmo C, Londos E, Hansson O. A Quick Test of cognitive speed is sensitive in detecting early response in Alzheimer’s disease. Alzheimers Res Ther 2010;2:29.

[26] Wahlund L, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjögren M, et al. A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke 2001;32:1318–22.

[27] Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, van Westen D, Wahlund L, Barkhof F, Fazekas F, Bronge L, Augustin M, Wiig EH, Minthon L, Londos E. A Quick Test of cognitive speed is sensitive in detecting early treatment response in Alzheimer’s disease. Alzheimers Res Ther 2010;2:29.

[28] Mattsson N, Zetterberg H, Insel PS, Andreasson U, Olsson A, Vanderstichele H, Andreasen N, De Meyer G, Wallin A, Rohrer JD, Woollacott IO, Dick KM, Brotherhood E, Gordon E, Fellows A, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology 2016;87:1329–36.

[29] Olsson A, Vanderstichele H, De Meyer G, Wallin A, Holmberg B, et al. Simultaneous measurement of beta-amyloid(1–42), total tau, and phosphorylated tau (Thr181) in cerebrospinal fluid by the xMAP technology. Clin Chem 2005;51:336–45.

[30] Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarker signature in Alzheimer’s disease neuroimaging initiative subjects. Ann Neurol 2009;65:403–13.

[31] Joshi AD, Pontecorvo MJ, Clark CM, Carpenter AP, Jennings DL, Sadowsky CH, et al. Performance characteristics of amyloid PET with florbetapir F 18 in patients with alzheimer’s disease and cognitively normal subjects. J Nucl Med 2012;53:378–84.

[32] Jagust WJ, Bandy D, Chen K, Foster NL, Landau SM, Mathis CA, et al. The Alzheimer’s Disease Neuroimaging Initiative positron emission tomography core. Alzheimers Dement 2010;6:221–9.

[33] Toledo JB, Vanderstichele H, Figurski M, Aisen PS, Petersen RC, Weiner MW, et al. Factors affecting Abeta plasma levels and their utility as biomarkers in ADNI. Acta Neuropathol 2011;122:401–13.

[34] Tibshirani R. Regression shrinkage and selection via the lasso. J Roy Stat Soc B Met 1996:58:267–88.

[35] Olofsen E, Dahan A. Using Akaikes information theoretic criterion in mixed-effects modeling of pharmacokinetic data: a simulation study. F1000Res 2013;2:71.

[36] Burnham SC, Faux NG, Wilson W, Laws SM, Ames D, Bedo J, et al. A blood-based predictor for neocortical Abeta burden in Alzheimer’s disease: results from the AIBL study. Mol Psychiatry 2014;19:519–26.

[37] Kiddle SJ, Thambisetty M, Simmons A, Riddoch-Contreras J, Hye A, Westman E, et al. Plasma based markers of [11C] PiB-PET brain amyloid burden. PLoS One 2012;7:e44260.

[38] Thambisetty M, Tripaldi R, Riddoch-Contreras J, Hye A, An Y, Campbell J, et al. Proteome-based plasma markers of brain amyloid-beta deposition in non-demented older individuals. J Alzheimers Dis 2010;22:1099–109.

[39] Kandel BM, Avants BB, Gee JC, Arnold SE, Wolk DA. Alzheimer’s Disease Neuroimaging I. Neuropsychological Testing Predicts Cerebrospinal Fluid Amyloid-beta in Mild Cognitive Impairment. J Alzheimers Dis 2015;46:901–12.

[40] Jansen WJ, Ossenkoppele R, Tijms BM, Fagan AM, Hansson O, Kunkel WE, et al. Association of Cerebral Amyloid-beta Aggregation With Cognitive Functioning in Persons Without Dementia. JAMA Psychiatry 2018;75:84–95.

[41] Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, et al. Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/e4) among patients diagnosed with Alzheimer’s disease: a systematic review and meta-analysis. Neuroepidemiology 2012;38:1–17.

[42] Ashton NJ, Kiddle SJ, Graf J, Ward M, Baird AL, Hye A, et al. Blood protein predictors of brain amyloid for enrichment in clinical trials? Alzheimers Dement (Amst) 2015;1:48–60.

[43] Nakamura A, Kaneko N, Villemagne VL, Kato T, Dooce J, Dore V, et al. High performance plasma amyloid-beta biomarkers for Alzheimer’s disease. Nature 2018;554:249–54.

[44] Ovod V, Ramsey KN, Mawuenyega KG, Bollinger JG, Hicks T, Schneider T, et al. Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. Alzheimers Dement 2017:13:841–9.

[45] Blennow K, Mattsson N, Scholl M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer’s disease. Trends Pharmacol Sci 2015;36:297–309.

[46] Valcarcel-Nazco C, Perestelo-Perez L, Molinuevo JL, Mar J, Castillo I, Serrano-Aguilar P. Cost-effectiveness of the use of biomarkers in cerebrospinal fluid for Alzheimer’s disease. J Alzheimers Dis 2014;42:777–88.