Brain amyloidosis ascertainment from cognitive, imaging, and peripheral blood protein measures

ABSTRACT

Background: The goal of this study was to identify a clinical biomarker signature of brain amyloidosis in the Alzheimer’s Disease Neuroimaging Initiative 1 (ADNI1) mild cognitive impairment (MCI) cohort.

Methods: We developed a multimodal biomarker classifier for predicting brain amyloidosis using cognitive, imaging, and peripheral blood protein ADNI1 MCI data. We used CSF β-amyloid 1-42 (Aβ42) ≥192 pg/mL as proxy measure for Pittsburgh compound B (PiB)-PET standard uptake value ratio ≥1.5. We trained our classifier in the subcohort with CSF Aβ42, but no PiB-PET data and tested its performance in the subcohort with PiB-PET but no CSF Aβ42 data. We also examined the utility of our biomarker signature for predicting disease progression from MCI to Alzheimer dementia.

Results: The CSF training classifier selected Mini-Mental State Examination, Trails B, Auditory Verbal Learning Test delayed recall, education, APOE genotype, interleukin 6 receptor, clusterin, and ApoE protein, and achieved leave-one-out accuracy of 85% (area under the curve [AUC] = 0.8). The PiB testing classifier achieved an AUC of 0.72, and when classifier self-tuning was allowed, AUC = 0.74. The 36-month disease-progression classifier achieved AUC = 0.75 and accuracy = 71%.

Conclusions: Automated classifiers based on cognitive and peripheral blood protein variables can identify the presence of brain amyloidosis with a modest level of accuracy. Such methods could have implications for clinical trial design and enrollment in the near future.

Classification of evidence: This study provides Class II evidence that a classification algorithm based on cognitive, imaging, and peripheral blood protein measures identifies patients with brain amyloid on PiB-PET with moderate accuracy (sensitivity 68%, specificity 78%). Neurology® 2015;84:729-737

GLOSSARY

Aβ42 = β-amyloid 1-42; AD = Alzheimer dementia; ADNI1 = Alzheimer’s Disease Neuroimaging Initiative 1; AUC = area under the curve; AVLT = Auditory Verbal Learning Test; BDNF = brain-derived neurotrophic factor; CI = confidence interval; IL-6R = interleukin 6 receptor; IL-13 = interleukin 13; LOOCV = leave-one-out cross-validation; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; NPV = negative predictive value; PiB = Pittsburgh compound B; PPV = positive predictive value; ROC = receiver operating characteristic; ROI = region of interest; SUVR = standard uptake value ratio; SVM = support vector machine; TNF-α = tumor necrosis factor α.

A key breakthrough in Alzheimer dementia (AD) research has been the invention of PET compounds that bind to amyloid deposits in the brain. Randomized secondary prevention trials of anti-amyloid agents that could halt disease progression are presently under way. A vast number of potential participants will need to be screened for these studies. This will expose many amyloid-negative cognitively normal elderly to radiation. Alternatively, blood-based biomarkers

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Supplemental data at Neurology.org
would have the important advantage of being safe, affordable, and easy to administer in large cohorts and/or in rural areas and therefore could have an enormous impact on clinical care and clinical trials alike.

The current standard for identifying brain amyloidosis is amyloid PET imaging. Recently, one research group proposed that CSF β-amyloid 1–42 (Aβ42) levels could serve as reliable indicator of the presence of brain amyloidosis. The pathologically validated cutoff of CSF Aβ42 ≤192 pg/mL for discriminating AD from cognitively normal subjects was found to be a reliable surrogate indicator of the presence of brain amyloidosis (defined as Pittsburgh compound B [PiB]-PET standard uptake value ratio [SUVR] ≥1.5). We hypothesized that we would identify a clinical biomarker signature of brain amyloidosis composed of highly relevant to AD yet simple to measure cognitive, imaging, and peripheral blood protein markers using the Alzheimer’s Disease Neuroimaging Initiative 1 (ADNI1) mild cognitive impairment (MCI) cohort. Using an advanced support vector machine (SVM) approach, we developed a multimodal classifier for predicting brain amyloidosis. Unfortunately, only a small fraction of the ADNI1 MCI cohort received PiB-PET scans. Therefore, we took advantage of the strong agreement between CSF Aβ42 ≤192 pg/mL and PiB SUVR ≥1.5 thresholds and used ADNI1 MCI subjects with CSF Aβ42 data but no PiB-PET biomarker data (n = 151) to develop our classification methodology, which was then tested in the smaller cohort of ADNI1 MCI subjects with PiB-PET data (n = 60). We also assessed the utility of our biomarker signature to predict subsequent clinical progression to AD at 24 and 36 months in all 211 subjects.

METHODS Subjects. Data used to prepare this article were obtained from the ADNI database (http://adni.loni.usc.edu). ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations; subjects have been recruited from more than 50 sites across the United States and Canada. For up-to-date information, see www.adni-info.org. ADNI1 enrolled approximately 400 subjects with amnestic MCI, 200 with mild AD, and 200 normal control subjects, aged 55 to 90 years, between 2005 and 2008. Written informed consent was obtained from all participants. The clinical description of the ADNI1 cohort was recently published. The full list of inclusion/exclusion criteria may be accessed online at http://www.adni-info.org/Scientists/ADNI1Grant.aspx.

ADNI1 enrolled 398 subjects with MCI. The 151 subjects with MCI who provided peripheral blood and CSF, but not PiB-PET data, were selected for inclusion in our training sample. Conversely, the 60 subjects with MCI who provided peripheral blood and PiB-PET imaging constituted our testing sample. MCI progressors were defined as subjects with MCI who progressed to AD at any point of their follow-up. Non-progressors were defined as subjects with MCI who at the respective 24-month and 36-month visit were still diagnosed as MCI.

Standard protocol approvals, registrations, and patient consents. ADNI study sites received approval from their institutional ethical standards committee on human experimentation. Written informed consent was obtained from all research subjects participating in the study or their surrogates when applicable.

Feature selection. We selected the following well-established cognitive measures for inclusion in the classifier models: the Mini-Mental State Examination (MMSE), the most frequently used brief cognitive screening instrument; Trails B, an easy-to-administer measure of executive function; and the Auditory Verbal Learning Test (AVLT) delayed recall, a sensitive word list verbal memory measure.

We selected the following peripheral blood proteins for inclusion in our models: ApoE, a protein involved in cholesterol and Aβ metabolism; brain-derived-neurotrophic factor (BDNF), whose circulating levels are altered in AD; tumor necrosis factor α (TNF-α), a proinflammatory and apoptotic marker elevated in AD; interleukin 6 receptor (IL-6R), the receptor molecule for IL-6—a proinflammatory cytokine; interleukin 13 (IL-13), a cytokine associated with Aβ burden in AD; Apolipoprotein J (ApoJ), a lipoprotein involved in Aβ fibril formation and clearance. Many studies have demonstrated aberrant levels of each of these proteins in peripheral blood in AD, and have found associations between their concentrations and subsequent cognitive decline and/or imaging changes. We applied log_{10} transformations to normalize the distribution of all ADNI1 plasma protein variables.

We also included APOE ε4 genotype and hippocampal volume. APOE, the most established genetic risk factor for sporadic AD, can be reliably determined from peripheral blood samples. Hippocampal atrophy, the most established structural imaging biomarker of AD, is readily detectable in the MCI and even the presymptomatic stages of AD.

PiB biomarker data. Description of PiB-PET acquisition may be found at http://www.adni-info.org. We downloaded the PiB SUVR measures from the University of Pittsburgh from the ADNI Web site in October 2008. Each subject’s PiB SUVR image was sampled with an automated 14 region-of-interest (ROI) template including 9 cortical (anterior cingulate, frontal, sensorimotor, lateral temporal, mesial temporal, parietal and occipital cortex, the occipital pole, and the precuneus) and 3 subcortical (anterior ventral striatum, thalamus, and subcortical white matter) ROIs in addition to the pons and the cerebellum. We derived mean cortical PiB SUVR by averaging the 9 cortical ROI measures. SUVR estimation was done blindly. PiB data were acquired at various time points relative to the baseline MRI. Fourteen subjects had their PiB scan at the time of their ADNI baseline assessments; 40 subjects at the 12-month, one subject at the 18-month, and 5 subjects at the 24-month follow-up assessment. We paired PiB data with the corresponding demographic and hippocampal volumetric variables from the...
same visit. Plasma protein measures were derived at baseline, and therefore preceded PiB scans in some cases.

**Fluid biomarker data.** We downloaded the baseline CSF Aβ42 data from the ADNI Web site (http://adni.loni.usc.edu) in October 2008. The CSF collection and transportation protocols and procedural details on CSF Aβ42 measurements are provided at http://www.adni-info.org and in a recent ADNI publication. 3 Plasma collection, proteomic platforms, and assays are described in detail in reference 10.

**Hippocampal segmentation.** We downloaded and linearly registered the preprocessed baseline 1.5, 3-dimensional T1-weighted scans to the International Consortium for Brain Mapping 53 brain template 31 using a 9-parameter transformation. 32 We resampled the images to an isotropic space of 220 voxels along each axis resulting in a final voxel size of 1 mm3. The hippocampi were segmented with our recently developed and validated automated machine-learning hippocampal segmentation technique (AdaBoost), which uses adaptive boosting 33 as previously described. 34–36 Hippocampal volumes were extracted.

**Statistical methods.** Our analyses were performed retrospectively. We examined the baseline differences in subject demographics, cognitive performance, hippocampal volume, and blood protein measures in the CSF training, PiB-PET testing, and MCI progressor/nonprogressor cohorts using 2-tailed t tests for continuous variables and χ2 tests for categorical variables.

**Support vector machine classifier.** SVMs are a popular machine-learning algorithm. SVMs have been particularly successful in biological classification, because nonlinear kernels introduced to the algorithm allow for nonplanar, multidimensional surfaces to classify patterns of data. We optimized the radial basis function kernel and the SVM cost parameter through grid search using the e1071 package in R (http://cran.r-project.org). Because noncontributory features degrade the classification, we allowed our classifier to rank all features and to iteratively remove those with lower weights until it finds the set of features that yields maximal classification accuracy.

We trained the SVM algorithm in all ADNI1 subjects with MCI who had available baseline protein and CSF Aβ42 data but no PiB-PET imaging (n = 151). The outcome variable was CSF Aβ42 ≤ or >192 pg/mL. The CSF training classifier also included age, sex, and education. Cross-validation used the leave-one-out (LOOCV) approach. We computed receiver operating characteristic (ROC) curve and area under the curve (AUC) to assess the classifier’s prediction performance.

Our PiB classifiers used the subjects with MCI who had available protein and PiB-PET data (n = 60). We ran 2 PiB classifiers: the first one was run with the fixed tuning parameters from the CSF training classifier (PiB testing-without-tuning classifier) and the second where the PiB classifier was allowed to tune its cost and gamma parameters for optimal classification while using LOOCV (PiB testing-with-tuning classifier). The reason for allowing self-tuning while still restricting ourselves to the variables chosen by the CSF training classifier was to further refine the variable list to the ones that are most useful for predicting PiB positivity.

Our primary goal was to identify a clinical biomarker signature of brain amyloidosis in the ADNI1 MCI cohort. The PiB testing-without-tuning classifier results were used assigning level-of-evidence statement because the classifier resulting from PiB testing-with-tuning has incorporation bias. The PiB testing-without-tuning results were assigned as Class II level of evidence, while the PiB testing-with-tuning results were assigned Class IV level of evidence.

Next, we explored how well the CSF training and the PiB testing-with-tuning classifier features performed when predicting future progression from MCI to AD at 24 and 36 months. We ran these classifiers once with fixed parameters and a second time while allowing self-tuning for optimal performance while using LOOCV. Tuning allowed us to detect the optimal classifier performance without restricting ourselves to parameters that worked for predicting closely related but obviously not identical measures (high PiB and low CSF Aβ42).

All classifiers were further subjected to selection bias correction using permutation analyses. The permutation method empirically evaluates the distribution of the test statistic under the null hypothesis. We ran 10,000 permutations of the dependent variable (clinical diagnosis) against the sets of individual biomarker characteristics for each individual classifier and defined a final single corrected p value for each ROC.

We computed sensitivity, specificity, and accuracy for specific biomarker cutpoints and computed normal approximation confidence intervals (CIs) for those parameters.

All classifiers were rerun after excluding APOE ε2 carriers (CSF sample n = 11; PiB sample n = 5). The results can be seen in table e-1 on the Neurology® Web site at Neurology.org.

**RESULTS**

**Demographic and baseline biomarker comparisons.**

**CSF training sample.** Baseline demographic and biomarker comparisons between the MCI group with CSF Aβ42 ≤192 pg/mL and the MCI group with CSF Aβ42 >192 pg/mL are shown in table 1. The groups showed significant differences in cognitive performance and genotype distribution: the CSF Aβ42 ≤192 pg/mL group had poorer AVLT and higher Trails B scores and higher proportion of APOE ε4+ subjects compared with the CSF Aβ42 >192 pg/mL group.

**PiB testing sample.** Baseline demographic and biomarker comparisons between the MCI group with PiB SUVR ≥1.5 and the MCI group with PiB SUVR <1.5 are shown in table 1. Significantly poorer MMSE scores were seen in the PiB SUVR <1.5 group while AVLT and Trails B were similar in the 2 PiB groups. Plasma ApoE and CSF Aβ42 levels were significantly lower in subjects with MCI who had PiB SUVR <1.5; these subjects were also more likely to be APOE ε4+.

**Demographic comparisons of the CSF testing and PiB training samples.** We found no significant differences in age, education, sex, APOE genotype, MMSE score, hippocampal volume, and plasma protein levels between subjects with MCI with CSF Aβ42 ≤192 pg/mL and those with PiB SUVR ≥1.5. Subjects with MCI who had CSF Aβ42 ≤192 pg/mL performed significantly worse on the AVLT and on Trails B relative to subjects with MCI who had PiB SUVR ≥1.5.

Demographic and biomarker variables were similar between subjects with MCI with CSF Aβ42 >192 pg/mL and those with PiB SUVR <1.5 except...
for marginally lower MMSE scores and plasma IL-6R levels in the subjects with MCI from the CSF sample.

A subset of 29 subjects had both PiB-PET and CSF Aβ42 data, which allowed us to compute the accuracy with which CSF Aβ42 predicted PiB SUVR ≥1.5. Twenty of the 21 subjects with PiB SUVR ≥1.5 also had CSF Aβ42 >192 pg/mL (specificity 88%). The overall predictive accuracy of PiB SUVR from CSF Aβ42 was 93%.

### Table 1: Cognitive, demographic, and biomarker comparisons for the training and testing samples

<table>
<thead>
<tr>
<th>Variable</th>
<th>CSF (training) MCI sample</th>
<th></th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>CSF Aβ42 ≤192 pg/mL (n = 114)</td>
<td>74.5 (6.9)</td>
<td>73.7 (9.1)</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>CSF Aβ42 &gt;192 pg/mL (n = 37)</td>
<td>63</td>
<td>73</td>
</tr>
<tr>
<td>Education, y</td>
<td>CSF Aβ42 ≤192 pg/mL (n = 114)</td>
<td>15.6 (3.0)</td>
<td>15.7 (2.9)</td>
</tr>
<tr>
<td>MMSE at baseline</td>
<td>CSF Aβ42 &gt;192 pg/mL (n = 37)</td>
<td>26.6 (1.8)</td>
<td>27.2 (1.7)</td>
</tr>
<tr>
<td>AVLT delayed recall at baseline</td>
<td>CSF Aβ42 ≤192 pg/mL (n = 114)</td>
<td>2.0 (2.6)</td>
<td>4.0 (3.2)</td>
</tr>
<tr>
<td>Trails B at baseline, s</td>
<td>CSF Aβ42 &gt;192 pg/mL (n = 37)</td>
<td>148 (76)</td>
<td>108 (65)</td>
</tr>
<tr>
<td>Mean hippocampal volume, mm³</td>
<td>CSF Aβ42 ≤192 pg/mL (n = 114)</td>
<td>3,718 (665)</td>
<td>3,706 (823)</td>
</tr>
<tr>
<td>APOE genotype, % e4+</td>
<td>CSF Aβ42 &gt;192 pg/mL (n = 37)</td>
<td>64</td>
<td>24</td>
</tr>
<tr>
<td>CSF Aβ42, pg/mL</td>
<td>CSF Aβ42 ≤192 pg/mL (n = 114)</td>
<td>136 (27)</td>
<td>247 (28)</td>
</tr>
<tr>
<td>ApoE protein, log₁₀ μg/mL</td>
<td>CSF Aβ42 &gt;192 pg/mL (n = 37)</td>
<td>1.67 (0.19)</td>
<td>1.71 (0.15)</td>
</tr>
<tr>
<td>BDNF, log₁₀ ng/mL</td>
<td>CSF Aβ42 ≤192 pg/mL (n = 114)</td>
<td>0.27 (0.38)</td>
<td>0.27 (0.44)</td>
</tr>
<tr>
<td>Clusterin, log₁₀ μg/mL</td>
<td>CSF Aβ42 &gt;192 pg/mL (n = 37)</td>
<td>2.49 (0.07)</td>
<td>2.47 (0.43)</td>
</tr>
<tr>
<td>IL-13, log₁₀ pg/mL</td>
<td>CSF Aβ42 ≤192 pg/mL (n = 114)</td>
<td>1.59 (0.18)</td>
<td>1.6 (0.13)</td>
</tr>
<tr>
<td>IL-6R, log₁₀ ng/mL</td>
<td>CSF Aβ42 &gt;192 pg/mL (n = 37)</td>
<td>1.46 (0.15)</td>
<td>1.42 (0.12)</td>
</tr>
<tr>
<td>TNF-α, log₁₀ pg/mL</td>
<td>CSF Aβ42 ≤192 pg/mL (n = 114)</td>
<td>0.82 (0.27)</td>
<td>0.85 (0.29)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>PiB-PET (testing) MCI sample</th>
<th>PiB SUVR &gt;1.5 (n = 41)</th>
<th>PiB SUVR &lt;1.5 (n = 19)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>PiB SUVR &gt;1.5 (n = 41)</td>
<td>75.9 (8.1)</td>
<td>73.6 (8.0)</td>
<td>0.3</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>PiB SUVR &lt;1.5 (n = 19)</td>
<td>61</td>
<td>74</td>
<td>0.4</td>
</tr>
<tr>
<td>Education, y</td>
<td>PiB SUVR &gt;1.5 (n = 41)</td>
<td>16.5 (2.6)</td>
<td>16.3 (2.9)</td>
<td>0.8</td>
</tr>
<tr>
<td>MMSE at baseline</td>
<td>PiB SUVR &lt;1.5 (n = 19)</td>
<td>26.8 (2.3)</td>
<td>28.1 (1.6)</td>
<td>0.01*</td>
</tr>
<tr>
<td>AVLT delayed recall at baseline</td>
<td>PiB SUVR &gt;1.5 (n = 41)</td>
<td>3.2 (4.1)</td>
<td>5.2 (4.7)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Trails B at baseline, s</td>
<td>PiB SUVR &lt;1.5 (n = 19)</td>
<td>121 (63)</td>
<td>105 (69)</td>
<td>0.4</td>
</tr>
<tr>
<td>Mean hippocampal volume, mm³</td>
<td>PiB SUVR &gt;1.5 (n = 41)</td>
<td>3,642 (702)</td>
<td>3,860 (766)</td>
<td>0.3</td>
</tr>
<tr>
<td>APOE genotype, % e4+</td>
<td>PiB SUVR &lt;1.5 (n = 19)</td>
<td>73</td>
<td>16</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>CSF Aβ42, pg/mL</td>
<td>PiB SUVR &gt;1.5 (n = 41)</td>
<td>133 (31)</td>
<td>240 (31)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>ApoE protein, log₁₀ μg/mL</td>
<td>PiB SUVR &lt;1.5 (n = 19)</td>
<td>1.65 (0.18)</td>
<td>1.76 (0.15)</td>
<td>0.03*</td>
</tr>
<tr>
<td>BDNF, log₁₀ ng/mL</td>
<td>PiB SUVR &gt;1.5 (n = 41)</td>
<td>0.25 (0.41)</td>
<td>0.31 (0.28)</td>
<td>0.6</td>
</tr>
<tr>
<td>Clusterin, log₁₀ μg/mL</td>
<td>PiB SUVR &lt;1.5 (n = 19)</td>
<td>2.49 (0.08)</td>
<td>2.48 (0.08)</td>
<td>0.7</td>
</tr>
<tr>
<td>IL-13, log₁₀ pg/mL</td>
<td>PiB SUVR &gt;1.5 (n = 41)</td>
<td>1.62 (0.14)</td>
<td>1.59 (0.13)</td>
<td>0.4</td>
</tr>
<tr>
<td>IL-6R, log₁₀ ng/mL</td>
<td>PiB SUVR &lt;1.5 (n = 19)</td>
<td>1.5 (0.11)</td>
<td>1.49 (0.15)</td>
<td>0.4</td>
</tr>
<tr>
<td>TNF-α, log₁₀ pg/mL</td>
<td>PiB SUVR &gt;1.5 (n = 41)</td>
<td>0.81 (0.28)</td>
<td>0.73 (0.25)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ42 = β-amyloid 1–42; AVLT = Auditory Verbal Learning Test; BDNF = brain-derived neurotrophic factor; IL-6R = interleukin 6 receptor; IL-13 = interleukin 13; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; PiB = Pittsburgh compound B; SUVR = standard uptake value ratio; TNF-α = tumor necrosis factor α.

*Significant values.
Data are mean (SD) or %.

MCI progression to AD. The baseline demographic and biomarker comparisons between subjects with MCI who progressed to AD vs those who did not at month 24 and month 36 can be seen in table 2. At baseline, MCI progressors showed significantly...
lower MMSE and AVLT scores, higher Trails B score, greater proportions of APOE ε4 positivity, and lower plasma ApoE protein concentrations. Hippocampal volumes were marginally smaller in MCI progressors relative to nonprogressors at either time point.

Classifier results. CSF training classifier. The CSF training classifier used age, sex, education, hippocampal volume, APOE genotype, and all plasma proteins (ApoE, BDNF, clusterin, IL-6R, IL-13, and TNF-α) to predict CSF Aβ42 ≤ or >192 pg/mL. Performance optimization through feature ranking and iterative parameter tuning and LOOCV were used. We achieved the best AUC = 0.8 and accuracy of 85% (95% CI = 79%–91%) with Trails B, APOE genotype, AVLT, education, IL-6R, clusterin, mean hippocampal volume, ApoE protein, and MMSE (listed in

### Table 2: Cognitive, demographic, and biomarker comparisons for the MCI progressor and nonprogressor samples

<table>
<thead>
<tr>
<th>Variable</th>
<th>Month 24 sample</th>
<th>Month 36 sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCI_progressor, (n = 138)</td>
<td>MCI_nonprogressor, (n = 192)</td>
</tr>
<tr>
<td>Age, y</td>
<td>74.5 (7.2)</td>
<td>74.8 (7.3)</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td>Education, y</td>
<td>15.7 (2.9)</td>
<td>15.9 (3.0)</td>
</tr>
<tr>
<td>MMSE at baseline</td>
<td>26.8 (1.7)</td>
<td>27.4 (1.7)</td>
</tr>
<tr>
<td>AVLT delayed recall at baseline</td>
<td>1.5 (2.0)</td>
<td>3.9 (3.8)</td>
</tr>
<tr>
<td>Trails B at baseline, s</td>
<td>155 (81)</td>
<td>111 (83)</td>
</tr>
<tr>
<td>Mean hippocampal volume, mm³</td>
<td>3,355 (602)</td>
<td>3,379 (675)</td>
</tr>
<tr>
<td>APOE genotype, % ε4+</td>
<td>67</td>
<td>46</td>
</tr>
<tr>
<td>ApoE protein, log₁₀ μg/mL</td>
<td>1.65 (0.18)</td>
<td>1.7 (0.18)</td>
</tr>
<tr>
<td>BDNF, log₁₀ ng/mL</td>
<td>0.29 (0.39)</td>
<td>0.28 (0.4)</td>
</tr>
<tr>
<td>Clusterin, log₁₀ μg/mL</td>
<td>2.48 (0.07)</td>
<td>2.49 (0.07)</td>
</tr>
<tr>
<td>IL-13, log₁₀ pg/mL</td>
<td>1.61 (0.14)</td>
<td>1.59 (0.19)</td>
</tr>
<tr>
<td>IL-6R, log₁₀ ng/mL</td>
<td>1.45 (0.14)</td>
<td>1.47 (0.13)</td>
</tr>
<tr>
<td>TNF-α, log₁₀ pg/mL</td>
<td>0.82 (0.28)</td>
<td>0.84 (0.26)</td>
</tr>
</tbody>
</table>

Abbreviations: AVLT = Auditory Verbal Learning Test; BDNF = brain-derived neurotrophic factor; IL-6R = interleukin 6 receptor; IL-13 = interleukin 13; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; TNF-α = tumor necrosis factor α.

*Significant values.

Data are mean (SD) or %.

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rank order). The permutation-corrected classifier significance was $p < 0.00001$. Taking into account that CSF $\leq 192$ pg/mL is found in 74% of ADNI subjects with MCI, our classifier achieved sensitivity = 92%, specificity = 65%, positive predictive value (PPV) = 88%, and negative predictive value (NPV) = 74%. Results are shown in panel A of the figure and table e-2. APOE genotype alone achieved accuracy of 76% and AUC = 0.7 (95% CI = 69%–83%).

**PiB testing-without-tuning classifier.** We rigidly entered the 9 features and the optimized cost and gamma parameters selected by the CSF training classifier in the PiB testing classifier. The classifier achieved an AUC of 0.78 in predicting PiB-positive (PiB SUVR $\geq 1.5$) and PiB-negative (PiB SUVR <1.5) cases. Taking into account that a positive amyloid PET scan is found in 63% of ADNI subjects with MCI, our classifier achieved sensitivity = 68%, specificity = 78%, PPV = 84%, and NPV = 59%. The ROC curve is shown in panel C of the figure. At fixed 80% sensitivity, the classifier showed 65% sensitivity. The permutation-corrected classifier significance was $p = 0.19$. APOE genotype alone achieved accuracy of only 28% and AUC = 0.78 (95% CI = 17%–39%).

**PiB testing-with-tuning classifier.** Because we developed a classifier model using a proxy measure for brain amyloidosis (CSF Aβ$_{42}$) that is highly correlated but not identical to the true measure of brain amyloidosis (PiB-PET), we reran the PiB testing classifier using the 9 features selected by the CSF training classifier while allowing the classifier to rank and select from these features and fine-tune its parameters for optimal classification. Using LOOCV, this classifier achieved an accuracy of 83% (95% CI = 74%–93%) and an AUC of 0.78. Taking into account that a positive amyloid PET scan is found in 63% of ADNI subjects with MCI, our classifier achieved sensitivity = 79%, specificity = 83%, PPV = 89%, and NPV = 70%. The ROC curve is shown in panel B of the figure. The features selected by the final model (in rank order) were APOE genotype, MMSE, Trails B, IL-6R, and clusterin (see table e-2). The permutation-corrected classifier significance was $p < 0.00001$. APOE genotype alone achieved accuracy of 77% and AUC = 0.7 (95% CI = 66%–88%).

**Disease-progression classifiers.** We further explored how well the set of 9 variables selected by the CSF training classifier could predict progression from MCI to AD at 24 and 36 months. Running the stringent model using fixed parameters from the CSF training classifier resulted in an AUC = 0.72 at 24 months and AUC = 0.76 at 36 months. Neither classifier produced acceptable accuracy. The ROC curves are shown in panels E and G of the figure. Once we allowed the disease-progression classifiers to rank and select features and fine-tune their
parameters for optimal classification, their performance marginally improved. The 24-month disease-progression classifier achieved an AUC = 0.74 and accuracy of 73% (95% CI = 68%–78%) and the 36-month disease-progression classifier an AUC = 0.77 and accuracy of 74% (95% CI = 69%–79%). The 24-month disease-progression classifier selected (in rank order) AVLT, Trails B, APOE genotype, and IL-6R. The 36-month disease-progression classifier selected (in rank order) AVLT, APOE genotype, and Trails B. The permutation-corrected classifier significance for both classifiers was \( p < 0.00001 \). The results are shown in panels D and F of the figure and table e-2.

**DISCUSSION**

Our data suggest that plasma, imaging, and cognitive measures can be used to potentially predict brain amyloidosis with modest accuracy and confirm the AD relevance of IL-6R and clusterin—the 2 plasma measures that proved useful for this classification.

The LOOCV used by the CSF training classifier is criticized by some for its perceived tendency to inflate the classifier’s performance. A true cross-validation should in theory use nonoverlapping samples. Here, we used the PiB sample as our validation sample. Both the rigidly run and the tuned PiB testing classifiers achieved acceptable but modest accuracies.

For identification of subjects with MCI at greatest risk of disease progression to dementia, the self-tuning classifiers achieved reasonable but modest predictive accuracy. Of note, some but not all of the variables that showed between-group differences (see table 2) were selected. MMSE, for instance, was not chosen, but more challenging tests (AVLT and Trails B) were. IL-6R was the only plasma protein measure that was included.

Several strengths and limitations of this study should be acknowledged. ADNI—the premier longitudinal biomarker study in AD—continues to provide researchers with clinical, cognitive, and biomarker samples free of charge. ADNI uses unified subject assessment, MRI, PiB-PET, CSF, and peripheral blood collection protocols and meticulous data quality control across the sites. Because ADNI uses rigorous exclusion criteria typical of clinical trials, our results have direct implications for clinical trials but may be less applicable to outpatient diagnostic assessments. The lack of a sizable MCI cohort with PiB-PET imaging that would have allowed a true classifier development and more extensive cross-validation is a limitation of this study. There is some etiologic/pathologic uncertainty in the MCI stage because at least 30% of subjects with amnestic MCI have been found to harbor non-AD pathology. However, it is precisely this etiologic heterogeneity that we are trying to overcome with our analyses. We only assessed the classifier performance with the most widely accepted PiB SUVR cutoff of 1.5. If higher levels of clinical trial cohort enrichment for brain amyloidosis, and hence improved cost-effectiveness, are sought, the use of a higher PiB SUVR cutoff value might prove valuable to further reduce the proportion of false-positive cases. Finally, similar to any other microarray technology, proteomic microarray analyses have inherent technical limitations.

An important source of variability for this type of technology is assay time/batch differences between the samples. This could result in future validation issues. Although the biological significance of our plasma analytes has been previously documented, our findings will need to be independently replicated.

**AUTHOR CONTRIBUTIONS**

Liana G. Apostolova: study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content, study supervision. Kristy S. Hwang: analysis and interpretation, critical revision of the manuscript for important intellectual content. David Avila: analysis and interpretation, critical revision of the manuscript for important intellectual content. David Elashoff: study design, analysis and interpretation, critical revision of the manuscript for important intellectual content. Omid Kohannim: study design, critical revision of the manuscript for important intellectual content. Edmond Teng: analysis and interpretation, critical revision of the manuscript for important intellectual content. Sophie Sokolow: analysis and interpretation, critical revision of the manuscript for important intellectual content. Clifford R. Jack: critical revision of the manuscript for important intellectual content, ADNI MRI core supervision. William Jagust: critical revision of the manuscript for important intellectual content, study supervision, ADNI PET core supervision. Leslie Shaw: analysis and interpretation, critical revision of the manuscript for important intellectual content, ADNI Biomarker Core supervision. John Q. Trojanowski: critical revision of the manuscript for important intellectual content, ADNI Biomarker Core supervision. Michael W. Weiner: acquisition of data, critical revision of the manuscript for important intellectual content, overall ADNI supervision. Paul M. Thompson: analysis and interpretation, critical revision of the manuscript for important intellectual content.

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