Multiomics Blood Test for Increasing Asymptomatic AD Patients Proportion in Preventive Clinical Trials

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BACKGROUND

- Recent phase III clinical trials show positive results for anti-amyloid therapies, such as lecanemab and donanemab, in individuals with mild cognitive impairment (MCI), highlighting the increasing importance of assessing drug efficacy during Alzheimer's disease (AD) asymptomatic stage.
- Currently, preventive trials predominantly recruit cognitively unimpaired individuals based on amyloid positivity or APOEε4 allele presence. However, a 65-year-old individual without cognitive impairment has, at most, a 30% lifetime risk of developing AD due to either amyloidosis[1] or being an APOEε4 carrier[2].
- Consequently, a significant proportion of the enrolled participants will not develop AD and will therefore not respond to disease-modifying therapies. This issue attenuates the statistical robustness of preventive clinical trials, potentially limiting their validity and the generalizability of their outcomes.
- It is therefore more important than ever to develop novel blood biomarkers capable of predicting with high specificity which cognitively unimpaired individuals will progress to AD. These tests would facilitate effective preventive clinical trials, focusing on those who would genuinely benefit from disease-modifying AD drugs.

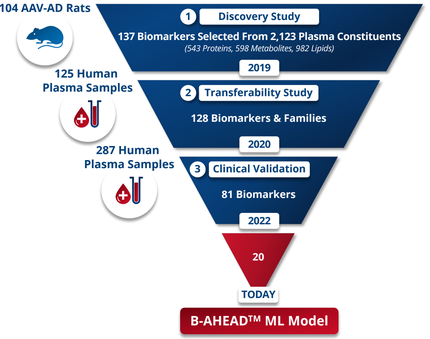
PRELIMINARY RESULTS: BIOMARKERS DISCOVERY

Figure 1. Identification of the Biomarkers

① In the Discovery Study, we leveraged Machine Learning (ML) approaches to pre-identify 137 biomarkers from 104 AAV-AD rats, a gene-transfer based animal model[3]. These potential biomarkers were selected from an initial set of 2,123 plasma components, analyzed via untargeted mass spectrometry.

② During the Transferability Study, we confirmed the existence and relevance of 128 of these biomarkers in humans. This phase included a diverse study group of 50 cognitively normal individuals, 45 individuals in the prodromal stage of AD, and 30 patients with demented AD.

③ Using targeted mass spectrometry, we accurately measured the absolute concentrations of 81 biomarkers (36 metabolites and 45 proteins). Our analysis included 287 human plasma samples gathered from seven independent cohorts across the globe (USA, Europe, Australia), enabling us to build one predictive model.



OBJECTIVE

- This study aimed to build a ML model incorporating novel multiomics biomarkers, pre-identified in a gene-transfer based rat model and assess its performance using retrospective human plasma samples coming from 7 independent cohorts (USA, Europe, Australia).

METHODS

- **Mass Spectrometry:** We developed targeted mass spectrometry assays for 81 biomarkers from the initial set of 128 biomarkers identified in the Transferability Study.
- **Plasma Samples and Clinical Labels:** The study included 287 cognitively impaired participants from 7 cohorts. The clinical labels, healthy controls (HC) or asymptomatic AD, were retrospectively assigned based on cognitive evaluations, after a follow-up period of up to 18 years from blood draw.

Figure 2. Retrospective Plasma Samples: Clinical Label Examples

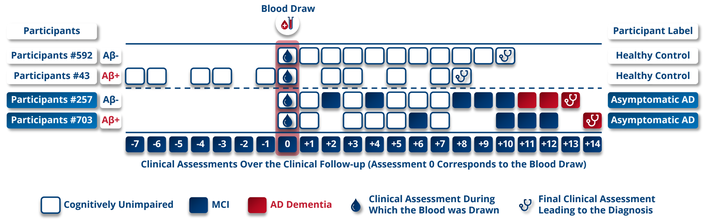
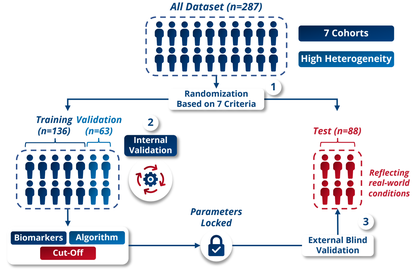


Figure 3. Machine Learning Experimental Design

① **Randomization:** All 287 participants were randomized into 3 distinct sets (training: 47%, validation: 22%, test: 31%), ensuring statistical equivalence across 7 key factors: batch number, clinical label, conversion time, gender, age at baseline, APOE status, amyloid status.

② **Internal Validation:** Using training and validation sets, we defined the best AD biomarkers panel, the ML algorithm, and the optimal cut-off to maximize specificity. With all parameters and hyper-parameters locked in, we established the B-AHEAD™ test.

③ **External Validation:** The robustness and clinical applicability of the B-AHEAD™ test were evaluated through a blinded external validation on the test set.



RESULTS

Figure 4. Identification of the Biomarkers

- During internal validation, we selected 20 blood biomarkers (12 proteins and 8 metabolites) and two covariate (age at blood draw, gender) as the most informative panel to differentiate asymptomatic AD from HC.
- While 65.0% of these biomarkers have been previously reported in the literature as being involved in AD, our study is the first to demonstrate that their combination can be used to predict asymptomatic AD patients among a cognitively unimpaired population using ML algorithms.
- These biomarkers are produced or regulated by peripheral organs.

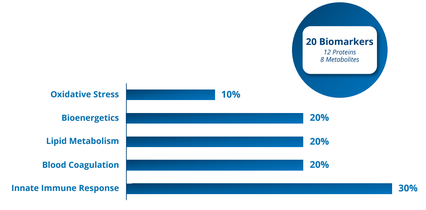


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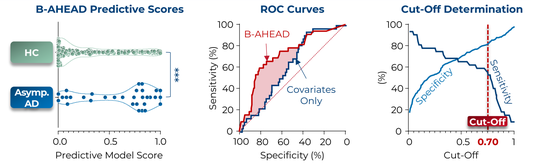
Figure 5. Internal Validation: B-AHEAD™ Predictive Performance

- **B-AHEAD Predicted Symptomatic AD with 80.8% Specificity:** During internal validation, the predictive ML model was calibrated to a cut-off of 0.70, resulting in 80.8% specificity in predicting asymptomatic AD patients from HC, with 59.4% sensitivity (AUROC=71.9%, p<0.0001).



Dataset	Number of Individuals			Results Type	AUROC [95% CI]	Specificity [95% CI]	Sensitivity [95% CI]
	Total	HC	Asymp. AD				
Training + Validation	199	167	32	Mean of 100x 10-fold cross-validations	71.9%*** [0.62-0.82]	80.8% [0.74-0.86]	59.4% [0.42-0.74]

Figure 6. External Validation: B-AHEAD™ Predictive Performance

- **In the external validation, B-AHEAD achieved 81.9% specificity** in predicting asymptomatic AD patients from HC, with 56.3% sensitivity (AUROC=69.1%, p=0.02). These findings strongly argue against model overfitting and affirm the robustness of the B-AHEAD test, thus suggesting its applicability in predicting AD in cognitively unimpaired populations under real-world conditions.

Dataset	Number of Individuals			Results Type	AUROC [95% CI]	Specificity [95% CI]	Sensitivity [95% CI]
	Total	HC	Asymp. AD				
Test	88	72	16	Blind prediction after fitting on training + validation dataset	69.1%*** [0.53-0.85]	81.9% [0.73-0.89]	56.3% [0.33-0.71]

Figure 7. Comparative Analysis with Amyloid (PET or CSF) Tests

- **B-AHEAD tends to outperform the specificity of tests based on the estimation of cerebral amyloid deposits (p=0.06) in the external validation.** When both tests were applied in series, 90.9% specificity and 46.2% sensitivity were achieved.

Dataset	Diagnostic Test	Specificity	Sensitivity
Subpopulation of the test set with known amyloid status at blood draw time (n=68)	Amyloid Assays (PET or CSF)	70.9%	92.3%
	B-AHEAD™	81.8%*	53.8%***
	Amyloid Assays + B-AHEAD™	90.9%***	46.2%***

CONCLUSIONS

- Peripheral multiomics plasma signatures can achieve a level of specificity beyond the reach of amyloid tests, thereby advancing the development of novel blood tests. Specifically, the B-AHEAD blood test represents an innovative generation of assays that can be used alone or in combination with existing tests.
- This test could test could increase the proportion of true asymptomatic AD individuals in preventive clinical trials, thus enhancing the statistical robustness of these clinical trials, potentially augmenting their validity and the generalizability of their outcomes.

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