Multiomics Blood Biomarkers Predict Alzheimer's From Predementia with High Specificity

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BACKGROUND

- Diagnosis of mild cognitive impairment (MCI) or dementia due to Alzheimer's disease (AD) relies on a cognitive evaluation combined with either cerebrospinal fluid (CSF) p-tau/Aβ42 ratio or amyloid positron emission tomography (PET) imaging[1,2].
- Despite their high sensitivity, these biological markers, indicative of cerebral amyloid deposits, have low specificity when predicting AD in patients among the mild cognitive impairment (MCI) population, resulting in a false positive rate of up to 30%[3,4,5]. Current blood tests under development face similar specificity issues as they rely on the same biomarkers.
- Anti-amyloid treatments, such as lecanemab and donanemab, are coming to market and will soon have to be prescribed by neurologists. However, current projections show that treating all patients who have MCI and amyloid positivity will not be feasible due to complex logistics and high treatment costs[6]. In response, clinicians will need to sort through patients to find those most likely to benefit from these therapies.
- It is therefore more important than ever to develop novel blood biomarkers enabling clinicians to quickly identify patients for whom the risk of AD misdiagnosis is very low among the amyloid-positive cognitively impaired population.

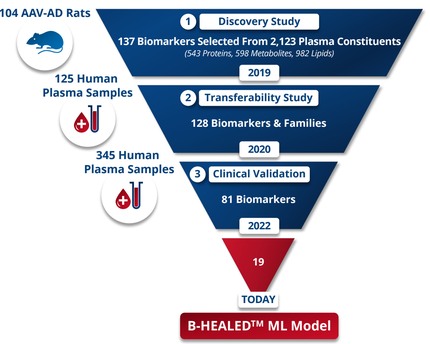
PRELIMINARY RESULTS: BIOMARKERS DISCOVERY

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[7]. 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 345 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 345 cognitively impaired participants from 7 cohorts. The clinical labels, symptomatic AD or Non-AD Brain Disorder (Non-AD BD), were retrospectively assigned based on cognitive evaluations, after a follow-up period of up to 13 years from blood draw.

Figure 2. Retrospective Plasma Samples: Clinical Label Examples

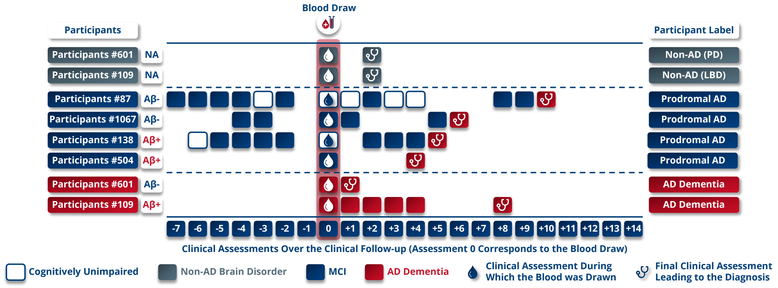
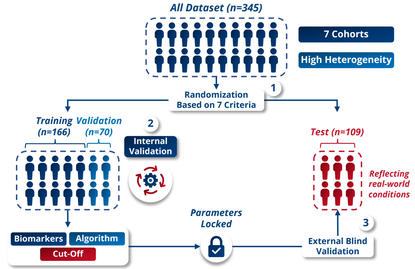


Figure 3. Machine Learning Experimental Design

① **Randomization:** All 345 participants were randomized into 3 distinct sets (training: 48%, validation: 20%, test: 32%), 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-HEALED™ test.

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



RESULTS

Figure 4. Identification of the Biomarkers

- During internal validation, we selected 19 blood biomarkers (13 proteins and 6 metabolites) and one covariate (age at blood draw) as the most informative panel to differentiate prodromal and demented AD from non-AD BD patients.
- While 78.9% 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 AD patients among a cognitively impaired population using ML algorithms.
- These biomarkers are produced or regulated by peripheral organs.

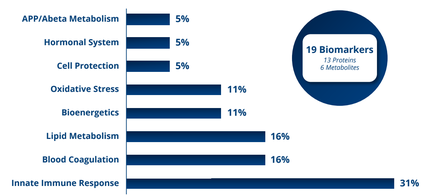


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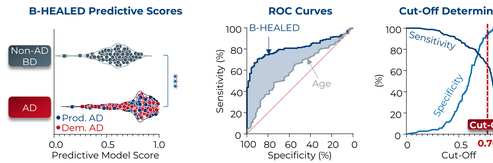
Figure 5. Internal Validation: B-HEALED™ Predictive Performance

- **B-HEALED Predicted Symptomatic AD with 93.0% Specificity:** During internal validation, the predictive ML model was calibrated to a cut-off of 0.76, resulting in 93.0% specificity in predicting AD patients (prodromal AD and demented AD) from non-AD BD patients, with 65.4% sensitivity (AUROC=81.6%, p<0.0001).



Dataset	Number of Individuals				Results Type	AUROC [95% CI]	Specificity [95% CI]	Sensitivity [95% CI]
	Total	Non-AD BD	Prod. AD	Dem. AD				
Training + Validation	236	71	82	83	Mean of 100x 10-fold cross-validations	81.9%*** [0.77-0.87]	93.0% [0.85-0.97]	65.4% [0.58-0.72]

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

- **In the external validation, B-HEALED achieved 92.0% specificity** in predicting AD patients (prodromal AD and demented AD) from non-AD BD patients, with 52.4% sensitivity (AUROC=71.8%, p=0.001). These findings strongly argue against model overfitting and affirm the robustness of the B-HEALED test, thus suggesting its applicability in predicting AD in cognitively impaired populations under real-world conditions.

Dataset	Number of Individuals				Results Type	AUROC [95% CI]	Specificity [95% CI]	Sensitivity [95% CI]
	Total	Non-AD BD	Prod. AD	Dem. AD				
Test	109	25	41	43	Blind prediction after fitting on training + validation dataset	71.8%*** [0.62-0.82]	92.0% [0.75-0.99]	52.4% [0.42-0.53]

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

- **B-HEALED outperforms the specificity of amyloid tests (p=0.01) in the external validation.** When both tests were applied in series, 100% specificity and 39.7% sensitivity were achieved, resulting in 0% false positives considering a 60% AD prevalence in cognitively impaired individuals.

Dataset	Diagnostic Test	Specificity	Sensitivity	False Positives
Subpopulation of the test set with known amyloid status at blood draw time (n=91)	Amyloid Assays (PET or CSF)	78.3%	75.0%	16.2%
	B-HEALED™	91.3%*	54.4%**	9.6%
	Amyloid Assays + B-HEALED™	100.0%***	39.7%***	0.0%***

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-HEALED blood test represents an innovative generation of assays that can be used alone or in combination with existing tests.
- This test could accurately identify a predominantly prodromal AD patient population appropriate for clinical trial recruitment and could assist in the selection of MCI patients for anti-amyloid drug prescription, improving the benefit/risk ratio by significantly reducing false positives compared to sole reliance on amyloid deposit-related tests.

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