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White Paper

Blood-Based Biomarkers to Aid Early Detection of Alzheimer's Disease Pathology with Luminex

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Problem: The Need to Diagnose Alzheimer's Disease Early

Medical researchers and clinicians are increasingly recognizing the need to diagnose Alzheimer's disease (AD) as early as possible. Individuals who appear healthy may demonstrate AD progression at the molecular level, raising the need to begin treatment before symptoms arise.[1] Despite the importance of early detection, as of August 2023, FDA-cleared blood tests do not exist to diagnose AD during presymptomatic and prodromal phases. In the absence of clinical symptoms, diagnosing AD within early phases of the disease relies on observing changes in biomarker levels while AD progresses. Currently, studying AD biomarkers relies on costly imaging techniques and highly invasive sample collection of cerebrospinal fluid (CSF);[2] a non-invasive, accessible, blood-based assay that quantifies biomarkers at a high-throughput rate would address both of these issues.

Along with established AD biomarkers such as amyloid beta peptide (CSF $A\beta_{42}$), plasma proteins, lipids, and autoantibodies (aABs) found in blood are showing promise as potential biomarkers for early detection of AD pathology. Several studies from nearly a decade ago suggested IgG aABs act as a system to clear disease-related debris from blood and lymph.[3] Since those studies, researchers, including those from Durin Technologies, Rowan University, and Brown University, have shown that certain blood- and CSF-derived aABs can indicate pathological processes associated with neurodegenerative disease taking place in patients.[4]

Method: Testing Auto-Antibodies as Alzheimer's Disease Biomarkers with Luminex

Continuing their work to develop aABs as biomarkers, the researchers from Durin Technologies, Rowan University, and Brown University identified 8 aABs as possible markers of early-stage AD.[4] The scientists had previously observed a subset of IgG aABs that accumulate among patients with different diseases, including AD.[5] The 8 separate antigens that bind to their respective aABs were first coupled to Luminex xMAP® microspheres at a concentration of 25 pmol/million beads with the Luminex xMAP® Antibody Coupling Kit. Subsequent reagent and bead-coupling optimization was carried out to improve linearity, robustness, and assay performance with help from the LuminexPLORE Lab.

The levels of these biomarkers relative to non-dementia controls were then measured in banked serum samples from patients at pre-symptomatic, prodromal, and mild-to-moderate phases with the Luminex FLEXMAP 3D® instrument. The patients were classified into a Training Set and a Testing Set to generate a random forest machine learning model for predicting AD progression.

Results: Developing the Alzheimer's Disease Probability Score for Predicting AD Onset

The scientists used the training model to generate a receiver operating characteristic (ROC) curve assessment for detecting AD-related progression in the Testing Set using the aABs (**Figure 1**). An area under the ROC curve of 0.84 was achieved that was further increased to 0.96 after stratifying patients by age. Upon stratifying patients by age, both the sensitivity and specificity of identifying AD-related pathology with aABs further increased. The machine learning data allowed researchers to define an Alzheimer's disease probability score (ADPS) of at least 56 as a metric for increased AD risk among early-stage AD patients (**Figure 2**).

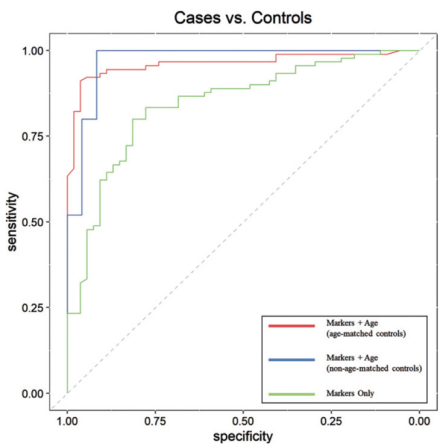

Figure 1. Receiver operating characteristics (ROC) curve evaluating autoantibodies (aAB) biomarkers for detecing Alzheimer's disease (AD)-related pathology among Testing Set subjects.[4]

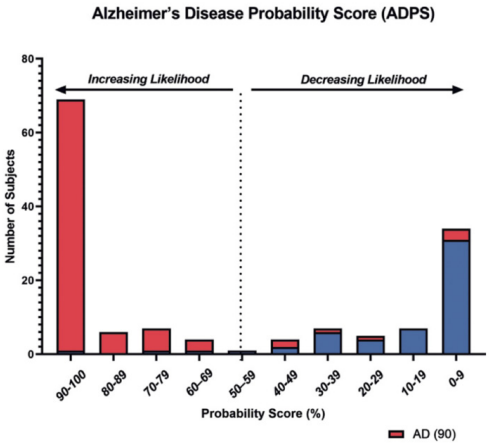

Figure 2. ADPS distribution among Testing Subjects by increasing or decreasing likelihood of having Alzheimer's Disease (AD)-related pathology.[4]

The ADPS was highly robust in classifying AD. In one AD cohort, 31 of 34 prodromal and all 11 mild-moderate AD samples were correctly classified. The results were reproducible with another AD cohort. 10 of 13 prodromal and 2/2 of mild-moderate AD subjects were correctly classified in that cohort (**Table 1**). Finally, the ADPS provided a 96.6% sensitivity for detecting AD-related pathologies among pre-symptomatic AD patients (**Table 2**).

Table 1. Accuracy Metrics of the Eight aABs for Predicting AD-Related Pathology in Cases Compared with Controls, With and Without Age as a Covariate.[4]

Testing Set Subjects	*n*	Threshold	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy %
Markers + Age (age-matched controls)	49	0.65	0.97 (0.93,1)	1 (0.87,1)	0.92 (0.74,0.98)	0.93 (0.77,0.98)	1 (0.85,1.00)	96.0
Markers + Age (non-age-matched controls)	144	0.56	0.96 (0.93,0.99)	0.92 (0.85,0.96)	0.94 (0.85,0.98)	0.97 (0.90,0.99)	0.88 (0.77,0.94)	93.0
Markers	144	0.48	0.84 (0.78,0.91)	0.80 (0.71,0.87)	0.81 (0.69,0.90)	0.88 (0.79,0.93)	0.71 (0.59,0.81)	81.0

Area under the curve (AUC) values at 95% confidence were generated using ROC curve analysis. Threshold values were derived using ROC curves to find the optimal cutoff value corresponding to the largest Youden's J Statistic. Overall accuracy, sensitivity, specificity, PPV, and NPV are derived from probability data with 95% confidence intervals generated using the Wilson score method for binominal proportions.

Table 2. Probability Score Analysis Breakdown Using the Eight aAB Biomarkers and Age as a Covariate.[4]

Testing Set Subjects Correctly Classified	*n*	ADNI Pre-symptomatic	ADNI MCI	ADNI MMAD	NJISA MAP MCI	NJISA MAP AD	NDC
Markers + Age (age-matched controls)	49	7/7	11/11	2/2	4/4	1/1	22/24
Markers + Age (non-age-matched controls)	144	29/30	31/34	11/11	10/13	2/2	51/54
Markers	144	23/30	29/34	11/11	8/13	2/2	45/54

Conclusion: Developing AD Progression Biomarkers Among Pre-Symptomatic Patients

With xMAP® Technology, the researchers were able to develop a blood-based assay to identify aABs as biomarkers for predicting AD among people in the presymptomatic phase. Assay optimization improved analytical sensitivity and specificity while decreasing cross-reactivity and non-specific matrix interference. While the 8 aABs alone were quite accurate in predicting AD-related pathology, adding age as a covariate improved its predictive ability in machine learning models. Multiplex data from the bead-based assay enabled them to quantify autoantibody-based biomarkers to better diagnose disease before the worst of its symptoms arise.

xMAP Technology for Developing your Multiplexed Novel Biomarker Assays

If you want to harness the power of multplex immunoassays to develop and validate novel biomarkers, pair Luminex's xMAP microspheres with your novel antigens or analytes. Assays can be developed by your lab as a homebrew, or with help from our LuminexPLORE Lab assay services. Luminex is an established leader in reliable and proven proteomic and genomic assays in medical research.

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