Multivariate Protein Biomarker Models More Accurately Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone

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INTRODUCTION

BACKGROUND:

- Multiple sclerosis(MS) is a chronic inflammatory demyelinating disease of the central nervous system with various phenotypes and heterogenous disease course. [1]
- While exact pathophysiology of MS remains elusive, both inflammatory and degenerative processes are believed to play a role in the disease mechanism and disability progression. [1,2]
- Identifying disease-specific biomarkers may assist with predicting the diverse disease course and classifying patients to high risk versus low risk for disease activity and progression. [3,4]
- Use of multivariate models reflecting multiple biological pathways that are involved in the complex pathophysiology of MS will most likely increase predictive accuracy of these biomarkers. [4]
- Serum levels of neurofilament light chain (sNfL) are associated with neurodegeneration in Multiple Sclerosis (MS) and correlate with measurements of disease activity (DA), including the presence of gadolinium enhancing (GAD+) lesions.
- The inclusion of additional inflammatory and neurodegenerative protein biomarkers, can provide deeper insights and reveal stronger correlations to radiographic DA than sNfL individually.

OBJECTIVES:

To compare the performance of multivariate protein biomarker models with sNfL individually to classify samples from subjects with and without GAD+ lesions from the Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital (CLIMB) study.

METHODS and MATERIALS

SUBJECTS: A total of 326 serum samples drawn within close-proximity (median interval 1 day) to a contrast-enhanced MRI were measured for 1196 proteins including sNfL using Proximity Extension Assays (PEA) from Olink and 215 proteins using Luminex based immunoassays from Rules Based Medicine (RBM). Samples represented both 113 longitudinal pairs (n=226) and non-paired specimens (n=100) that were categorized by the number of GAD+ lesions per Table 1. 58 samples had been measured previously for 1104 proteins using the Olink platform as a proof of concept study.

Table 1: GAD lesion count distribution for paired and unpaired samples

Sample Group (# of GAD+ lesions)	Sample Pairs	Individual Samples	0 lesions	1 lesion	2 lesions	≥3 lesions
A (0 and ≥1)	98	196	98	66	19	13
B (1 and ≥2)	15	30	0	15	7	8
C (≥ 2)	0	100	0	0	77	23
Totals	113	326	98	81	103	44

STATISTICAL ANALYSIS: Univariate and multivariate machine learning-driven biostatistical techniques were used to classify samples with and without GAD+ lesions. Analysis was performed both on the entire cohort (n=326) and restricted to longitudinal pairs which strictly included a sample with 0 GAD+ lesions. Five-fold cross-validation and regularization (L2) were used in tandem with sequential feature selection to minimize overfitting and ensure generalizability for predicting DA of new samples. Area Under the Curve (AUC) and Accuracy were selected as the key metrics for comparison.

RESULTS-I

FEATURE SELECTION: Exploratory data analysis was conducted to filter noise, reduce dimensionality & avoid collinearity. Univariate significance was combined with multivariate importance from simulated models as shown in Table 2.

P-value	AUC	Protein
6.06E-15	0.746	sNfL
0.015	0.585	CNTN5
0.046	0.552	E-Cad
0.006	0.575	TNC
0.030	0.577	SORT1
0.070	0.574	PDGFC
0.009	0.573	CTSF
0.006	0.582	TNFRSF10A
0.023	0.568	VWA1
0.014	0.569	IL18
2.55E-04	0.62	CCL20
1.39E-04	0.622	CDCP1
2.50E-04	0.627	CXCL9
0.019	0.572	CCL3
0.004	0.588	PODXL2
0.004	0.582	EZR
0.028	0.568	LIF-R
0.082	0.567	GDF-15
0.040	0.556	MFGE8
0.012	0.569	CXCL6
0.020	0.566	YKL-40

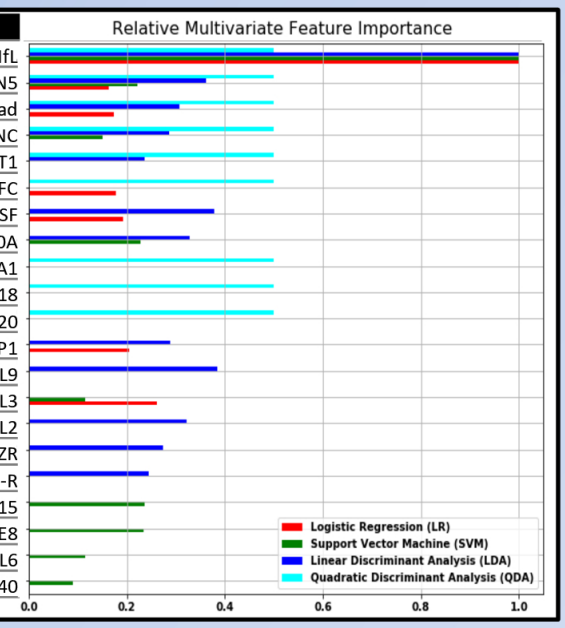

Table 2: Top 21 features ranked by feature importance (across LR, SVM, LDA, and QDA models), shown by accompanying p-value (2 sample, 1-sided homoscedastic t-test) and univariate AUC (trapezoidal integration of TPR, FPR across all 326 samples). sNfL passed multiple hypothesis correction filters (Bonferroni) for paired and unpaired samples while the remaining markers contribute orthogonal signal that were deemed significant explanatory variables (nonzero) through 95% confidence intervals after 100,000 bootstrap iterations. A similar procedure was conducted for the 196 paired samples to identify the strongest shifts (not shown).

RESULTS-II

MODEL-BUILDING: Forward selection, combined with grid search hyperparameter-tuning, as measured by 5-fold stratified cross-validation, achieved strong separation potential across supervised classification models: AUC_{LR} = 0.836 ± 0.066, AUC_{SVM} = 0.834 ± 0.039, AUC_{QDA} = 0.827 ± 0.055, AUC_{LDA} = 0.822 ± 0.065. The highest-performing parsimonious model (a 7-feature logistic regression model) was then validated using 100,000 iterations of repeated 50/50 cross-validation to produce the ROC curves in Fig. 1.

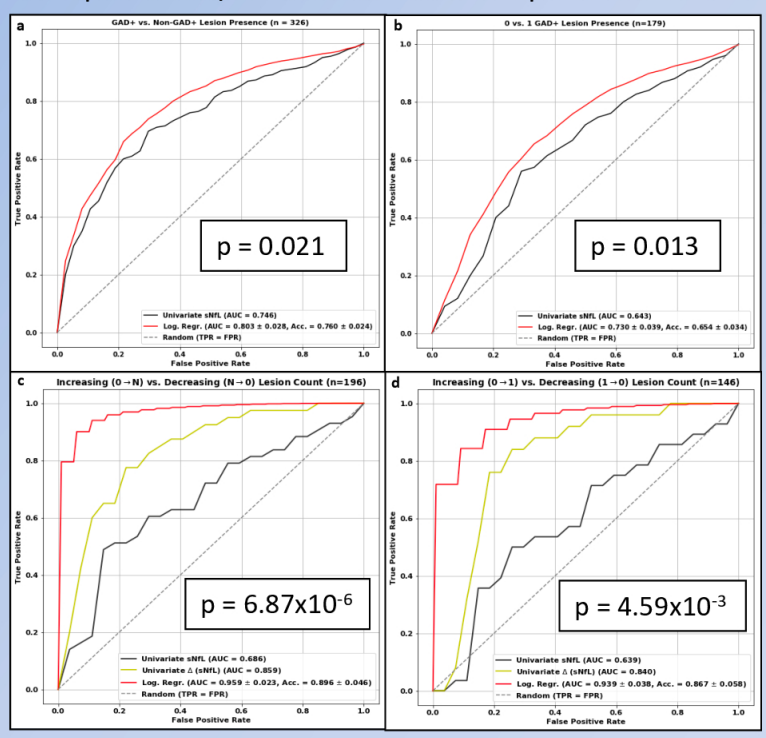

Figure 1. The Receiver Operating Characteristic (ROC) curve visualizes the true and false positive rates of various thresholds to separate the protein levels across samples. The p-value represents the statistical significance of the multivariate model's AUC being significantly greater than the AUC of sNfL (or Δ sNfL). ROC plots b and d reflect the power of the model to discriminate 0 vs. 1 lesions (thereby representing subtle disease activity). Different features were pulled in for the longitudinal analysis; however, in all 4 breakdowns of the study (a-d), logistic regression models showed significantly ($p < 0.05$) improved sensitivity and specificity (as measured by AUC).

Biomarkers that were selected as important features in the multivariate classifier were investigated for relevance and interactions using biological network models. In addition to neurodegeneration, proteins related to inflammatory and immune pathways were identified.

CONCLUSIONS

- Multivariate protein biomarker models representing several biological pathways predicted radiographic DA with greater statistical significance than sNfL alone.
- A multivariate model based on shifts in patient protein levels (between 2 samples, which better controls for age/sex/BMI) was able to strongly predict directionality of lesion activity (AUC=0.96). This not only outperforms sNfL alone, but also improves upon the multivariate model's ability to predict predict lesion presence from an individual MS patient's blood sample (AUC=0.80).
- Further investigation with larger sample numbers and from additional cohorts is warranted.

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DISCLOSURES

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