Differential diagnosis of MS patients versus disease state controls and classification of MS subtypes using serum proteomics

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OBJECTIVE

To evaluate the performance of proteomic multivariate models to classify patient serum samples from different disease states including Multiple Sclerosis (MS), Symptomatic Controls (SC), Inflammatory Neurological Disease Controls (INDC), and Neurodegenerative Controls (NDC). Proteomic multivariate modeling will also be applied to the MS patient samples to evaluate the ability to classify different MS disease courses, Clinically Isolated Syndrome (CIS), Relapsing Remitting MS (RRMS), Primary Progressive MS (PPMS), Secondary Progressive MS (SPMS), from one another.

INTRODUCTION

MS is a complex and heterogeneous disease driven by inflammation, demyelination and neurodegeneration. The pathology of other neuroinflammatory and neurodegenerative diseases share overlapping biological processes and mechanisms with MS. Utilizing blood based multivariate proteomic approaches to accurately distinguish MS from other diseases and symptomatic controls may lead towards enhanced diagnostic capabilities and improve care for patients.

A custom immunoassay panel was developed on the Olink™ platform utilizing Proximity Extension Assay technology and analytically validated in serum to measure the concentration of 19 proteins in serum. Proteins were selected for inclusion into the panel based on results observed in previously reported R&D studies. As these proteins represent different biological pathways involved with MS pathophysiology (immune modulation, neuroinflammation, myelin biology and neuroaxonal integrity), they may also be informative for differential diagnosis applications.

METHODS

We measured the concentrations of the 19 proteins in 334 patient samples from a University Hospital Basel cohort. The cohort consisted of 246 MS patients (120 CIS, 89 RRMS, 23 SPMS, 14 PPMS), 30 SC, 30 INDC, 28 NDC (see Table 1). Univariate and multivariate classifiers that included age and sex as covariates were developed to characterize classification performance for:

1. SC vs the 4 MS subtypes and the other disease states
2. CIS vs other MS subtypes
3. RRMS vs progressive MS (PPMS + SPMS)
4. MS vs other disease states (NDC, INDC)

Table 1. Cohort Characteristics

	N	%	Female (%)	Age (Mean ± SD)
Sample Size	334	100%	65.9%	43.2 ± 15.9
Control:				
Symptomatic Controls (SC)	30	9.0%	70.0%	36.3 ± 15.2
NeuroDegenerative Controls (NDC)	28	8.4%	50.0%	67.7 ± 9.4
Inflammatory Neurological Disease Control (INDC)	30	9.0%	53.3%	53.1 ± 18.7
MS Disease Courses:				
Clinically Isolated Syndrome (CIS)	120	35.9%	71.7%	36.4 ± 12.0
Relapsing Remitting MS (RRMS)	89	26.6%	71.9%	39.4 ± 12.1
Secondary Progressive MS (SPMS)	23	6.9%	56.5%	55.1 ± 9.3
Primary Progressive MS (PPMS)	14	4.2%	57.1%	51.0 ± 8.8

All classifications problems are binary in nature, e.g. for RRMS vs progressive MS, the RRMS is labeled as class 0 and SPMS or PPMS are labeled as class 1.

Univariate Classification: we fitted a logistic regression model that was adjusted for age and sex to individual 19 protein concentrations (Python statsmodels v0.12.2). The top protein with the lowest p-value was subsequently used together with sex and age as features in a cross-validation study (see below). We also identified a model with only sex and age as features.

Multivariate Classification: we implemented a pipeline (Python sklearn v0.24.1) consisting of:

- a standard scaler scaler to standardize features (removing mean and scaling to unit variance);
- feature selector to select the top *k* features according to their ANOVA F-value;
- logistic regression models to classify the different disease states and MS subtypes.

Cross-Validation Study: We used the following hyper-parameters: k = [2, …, 9] for the selector; lasso and ridge penalty terms and regularization strengths with inverses were selected from C = [0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5] for the classifier. To account for data imbalance in the classification training phase, we weighted the input of the classifier inversely proportional to class frequencies in the training phase.

Since we had limited number of subjects at several disease states and MS subtypes, we opted to implement a custom Leave-One-Out (LOO) Cross Validation (CV) study (Python sklearn v0.24.1) that systematically explored all parameter combinations and found the parameter set that resulted in the highest validation F1 score.

Data Imbalance: Because of data imbalance, the classification performance was visualized using the precision-recall curves (versus ROC curves). We identified the confidence interval on the model coefficients in a bootstrap study in which we fitted classifiers with the optimal parameter set (from the CV study) on the bootstrapped training data 1000 times. We also reported the confidence interval on the validation F1 scores and precision-recall curves in separate bootstrap studies.

RESULTS

Multivariate Classification: Performance and Significant Proteins

The top 5 models in terms of validation F1 scores were: SC vs SPMS (F1 = 0.92), SC vs IDC (F1 = 0.82), SC vs PPMS (F1 = 0.77), NDC+INDC vs (RRMS + SPMS + PPMS) (F1 = 0.73), RRMS vs (PPMS + SPMS) (F1 = 0.71). Overall, for classification of symptomatic controls vs MS subgroups, INDC and NDG, NfL was consistently the top protein in terms of effect size and for identification of MS subtypes, CDCP1 and GFAP were among the top choices.

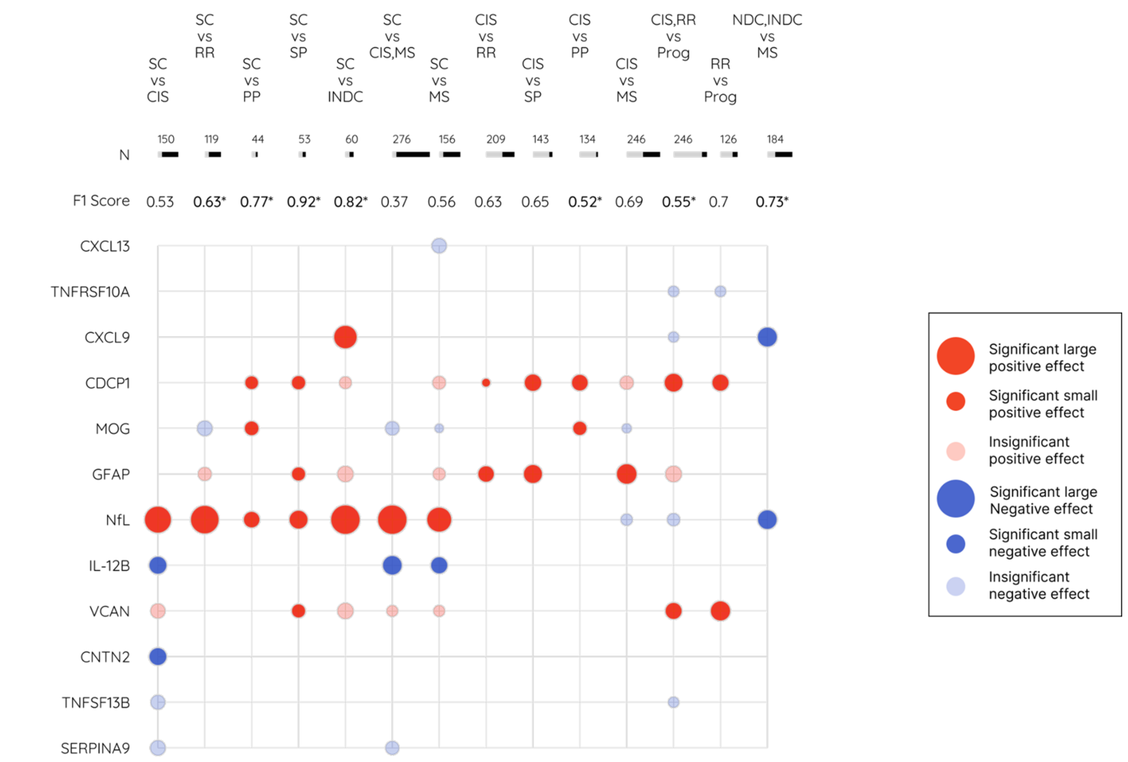

Figure 1. Multivariate classification with adjustment for age and sex: *N* indicates the count of samples for each class, F1 score reports the performance on the validation data and asterisk next to the F1 score indicates statistically significant improvement compared to the univariate classification using the top protein, the radius of each circle is proportional to the estimated coefficient of the corresponding protein in the multivariate classification, red (blue) circles represent proteins with positive (negative) effects in estimating the second class label (e.g. red NfL circle in SC vs CIS).

CONCLUSIONS

Proteomic biomarkers that were selected based on their associations with disease activity also demonstrated promising performance in classifying between different disease states (MS, SC, INDC and NDC) and between MS disease courses (CIS, RRMS, SPMS and PPMS). Among the three selected models, the multivariate models were significantly better than univariate for MS vs NDC + INDC and RRMS vs progressive MS. Several of the classifiers relied on groups with limited sample size and unbalanced datasets. Results in this study demonstrate that the proteins considered for disease activity are also informative for differential diagnostic applications including classification of various disease states and for distinguishing progressive versus non-progressive MS. Further investigation with larger sample numbers and from independent cohorts with age matched subjects is warranted.

RESULTS (continued)

NDC + INDC vs MS (RRMS + PPMS + SPMS)

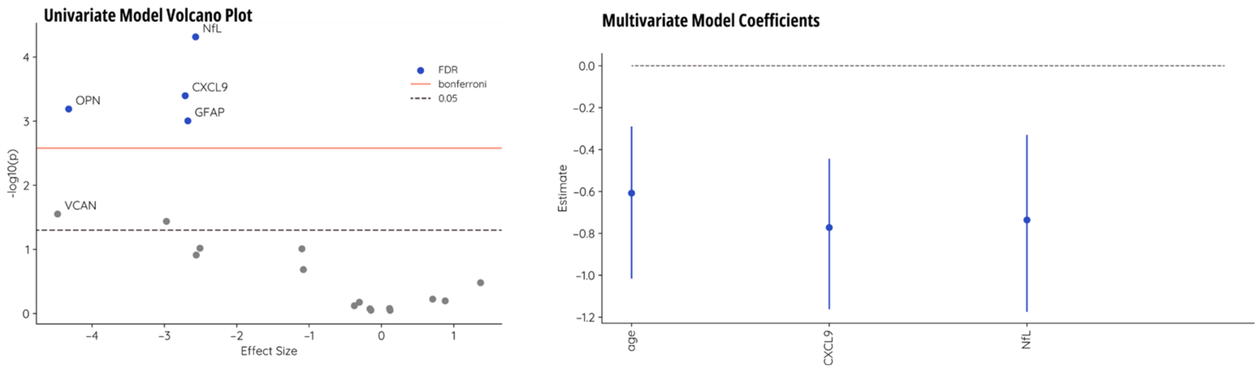

Figure 2. Classification of NDC + INDC vs MS (RRMS + PPMS + SPMS): (left) univariate model with top proteins: NfL, CXCL9, OPN, GFAP, VCAN; (right) for the multivariate model the features age, and serum protein concentration of CXCL9, NfL were statistically significant.

RRMS vs Progressive MS (PPMS + SPMS)

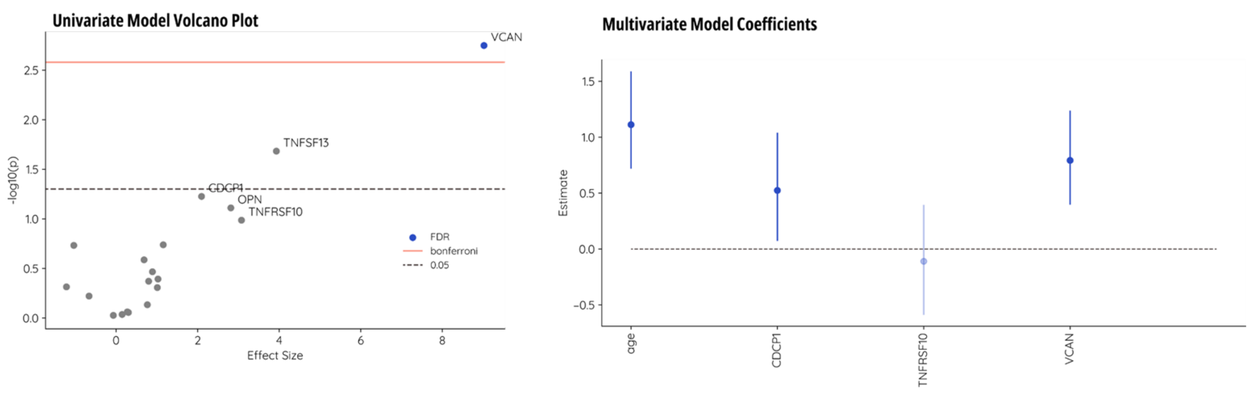

Figure 3. Classification of RRMS vs progressive MS (PPMS + SPMS): (left) univariate model with top proteins: VCAN, TNFSF13, CDCP1, OPN, TNFRSF10; (right) for the multivariate model the features age, and serum protein concentration of CDCP1, VCAN were statistically significant.

CIS vs MS (RRMS + PPMS + SPMS)

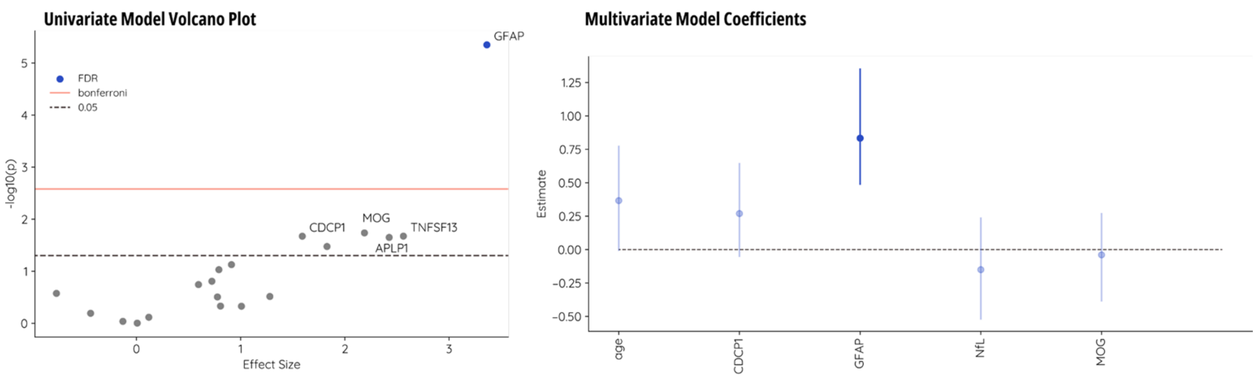

Figure 4. Classification of CIS vs MS (RRMS + PPMS + SPMS): (left) univariate model with top proteins: GFAP, MOG, TNFSF13, CDCP1, APLP1; (right) for the multivariate model serum protein concentration of GFAP was statistically significant.

	F1	Balanced Accuracy	Precision	Recall	Specificity	AUC	NPV
(NDC + INDC) vs MS	0.73 ± 0.04	0.81 ± 0.03	0.69 ± 0.04	0.77 ± 0.05	0.84 ± 0.03	0.77 ± 0.05	0.89 ± 0.02
RRMS vs (PPMS + SPMS)	0.71 ± 0.05	0.80 ± 0.04	0.62 ± 0.05	0.80 ± 0.06	0.78 ± 0.04	0.63 ± 0.08	0.91 ± 0.03
CIS vs MS	0.70 ± 0.03	0.69 ± 0.03	0.67 ± 0.03	0.73 ± 0.04	0.65 ± 0.04	0.72 ± 0.04	0.72 ± 0.03

Table 2. Classifier performance metrics of the multivariate classification models for NDC + INDC vs MS, RRMS vs progressive MS, CIS vs MS.

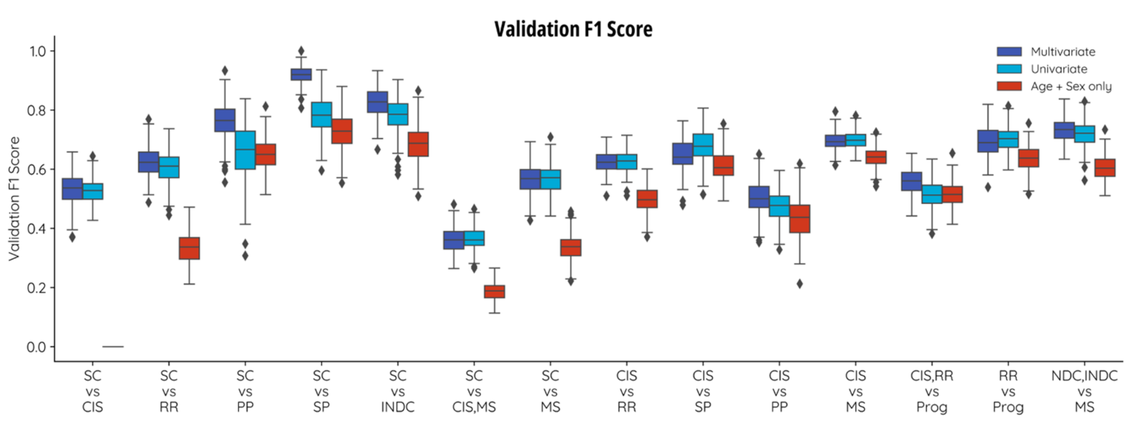

Figure 5. Comparison of the F1 score of the multivariate, univariate with the top protein from the smallest p-value, and a model with age and sex features only.

For question, please contact:
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