# Clinical Disease Activity Status in Subjects with RRMS is Accurately Classified Using Multivariate Serum Protein Biomarker Models

*Ferhan Qureshi[1], Remus Osan[1], Victor Gehman[1], Michael Becich[1], William Hagstrom[1]*
*[1]Octave Bioscience, Inc. 1440 O'Brien Drive Menlo Park CA 94025*
*Contact email: fqureshi@octavebio.com*

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## INTRODUCTION

- Multiple sclerosis (MS) is a disease with considerable heterogeneity, and numerous biological processes are associated with its pathophysiology.[1]
- There is an **unmet clinical need for objective and quantitative measures** to improve decision-making for MS patients and providers.
- Octave Bioscience is creating a range of products for evaluating MS that can address this need, including **serum based proteomic biomarker tests** to measure **disease activity**.
- While some individual biomarkers show promise (*e.g.* Neurofilament Light—NfL, associated with neurodegeneration), incorporating proteins that represent additional biological pathways (inflammatory, immune response, *etc.*) associated with MS may improve the performance of a blood-based biomarker test.[2,3]
- Multiple feasibility studies were performed to investigate the relationship between proteomic biomarkers and several MS clinical endpoints, including this **cross-sectional assessment of MS subjects with clinically defined relapse and remission status**.

## PURPOSE

The purpose of this study was to investigate the performance of **multivariate protein biomarker models** to classify subjects with physician-labeled disease activity status using serum samples obtained from the Accelerated Cure Project (ACP) repository. The primary endpoint for subjects was a clinically defined relapsing stage (**exacerbation**) versus a clinically defined remitting stage (**quiescence** *i.e.* no evidence of disease activity—NEDA).

## METHODS

Serum samples from **124 subjects** with relapsing-remitting MS (**RRMS**) were obtained from the ACP repository and analyzed across two immunoassay platforms. The selected samples exhibited balanced demographic stratification across clinical labels as shown in Table 1.

| Characteristic | Exacerbation (n=60) | Quiescence (n=64) |
|---|---|---|
| Female, % | 77 | 73 |
| Age, years (y) | 38.7 ± 10.1 | 39.3 ± 9.1 |
| Age at Diagnosis, y | 37.5 ± 10.4 | 35.5 ± 9.3 |
| Disease Duration, y | 1.2 ± 2.3 | 3.8 ± 2.1 |

**Table 1. Demographic & clinical characteristics.** All time measurements reported as mean ± standard deviation.

All samples were measured for 1104 proteins, including serum levels of neurofilament light chain (sNfL) using Proximity Extension Assays from Olink and for 215 proteins using Luminex-based immunoassays from Rules Based Medicine. Analytes were filtered based on quality control and performance criteria to reduce dimensionality. Protein biomarker expression was quantified and univariate/multivariate machine learning-driven biostatistical techniques were applied to the data. Area under the curve (AUC) and accuracy were selected as the key metrics for evaluation of model performance. Features were constricted and ranked based on high univariate performance and replication across accuracy-weighted simulations of statistical models.

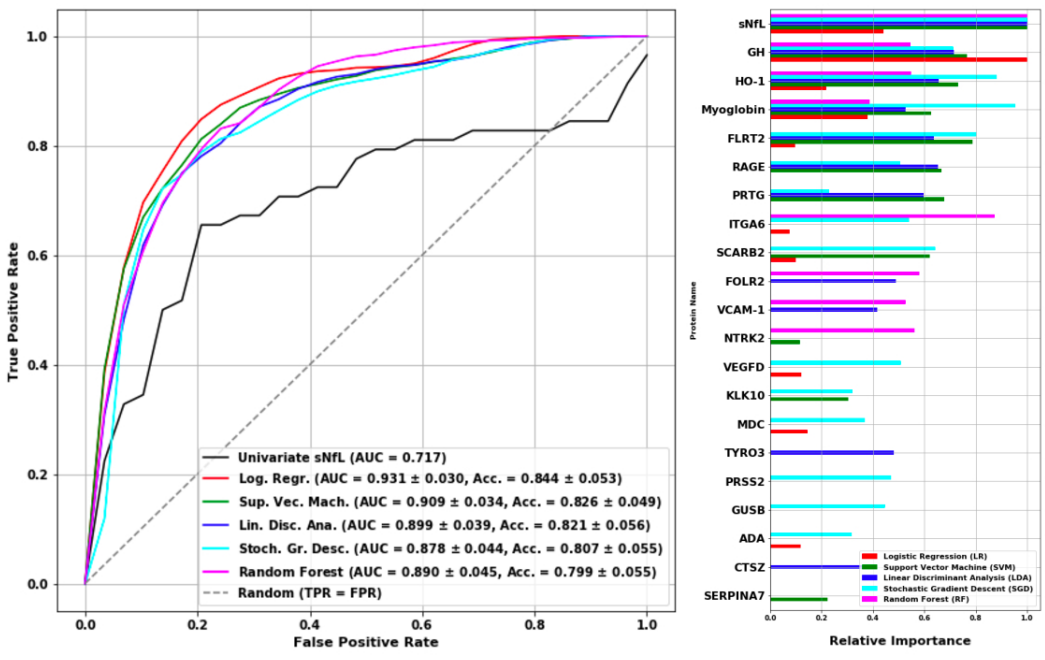
## RESULTS

| Protein | E vs. Q. Diff. (p-value)[A] | E vs. Q (AUC)[B] | Sex Diff. (p-value)[C] | Age ($R^2$)[D] | MS Duration ($R^2$)[E] |
|---|---|---|---|---|---|
| sNfL | $2.51\times10^{-5}$ | 0.717 | 0.481 | 0.041 | 0.008 |
| GH | 0.002 | 0.649 | 0.003 | 0.002 | 0.099 |
| HO-1 | 0.019 | 0.623 | 0.014 | 0.017 | 0.014 |
| Myoglobin | 0.016 | 0.611 | $2.39\times10^{-6}$ | 0.025 | 0.005 |
| FLRT2 | 0.003 | 0.633 | 0.067 | 0.007 | $2.94\times10^{-5}$ |
| RAGE | 0.007 | 0.624 | 0.372 | $1.47\times10^{-4}$ | 0.030 |
| PRTG | 0.021 | 0.619 | 0.101 | 0.006 | 0.016 |
| ITGA6 | 0.010 | 0.620 | 0.462 | 0.001 | 0.019 |
| SCARB2 | 0.001 | 0.659 | 0.303 | 0.040 | 0.001 |
| FOLR2 | 0.002 | 0.665 | 0.002 | 0.009 | 0.018 |
| VCAM-1 | 0.002 | 0.620 | 0.479 | 0.012 | 0.020 |
| NTRK2 | 0.028 | 0.641 | 0.210 | 0.068 | 0.036 |
| VEGFD | 0.012 | 0.616 | 0.017 | 0.008 | 0.022 |
| KLK10 | 0.002 | 0.636 | 0.120 | 0.012 | 0.026 |
| MDC | 0.025 | 0.623 | 0.286 | 0.003 | 0.018 |
| TYRO3 | 0.002 | 0.644 | 0.039 | 0.007 | 0.027 |
| PRSS2 | 0.001 | 0.648 | 0.353 | 0.030 | 0.001 |
| GUSB | 0.001 | 0.654 | 0.029 | 0.001 | 0.068 |
| ADA | 0.014 | 0.641 | 0.105 | 0.008 | 0.007 |
| CTSZ | 0.007 | 0.630 | 0.077 | 0.010 | 0.003 |
| SERPINA7 | 0.014 | 0.617 | 0.019 | 0.001 | 0.033 |

**Table 2: Univariate performance and demographic associations for top 21 proteins**:
A. p-value from 2-sample, 1-sided t-test between Exacerbation (E) and Quiescent (Q) samples
B. AUC calculated by trapezoidal integration of TPR FPR across all 124 samples
C. p-value from 2-sample, 1-sided t-test between male and female samples
D, E. Correlation between protein signal and age/years since diagnosis (Pearson's $R^2$).

sNfL was the highest performing univariate marker, passing multiple hypothesis correction thresholds (Benjamini-Hochberg).

Multivariate statistical approaches that included up to 21 biomarkers achieved performance with **0.930±0.035 AUC**. Five-fold stratified cross-validation was used to guide the initial search while 100,000 bootstrapped 50/50 train/test splits were used to evaluate the final model. Forward selection, combined with a grid search hyperparameter optimization, was used to identify the best classifiers. Our best statistical model significantly outperforms sNfL alone (AUC=0.717): $p=6.9\times10^{-10}$ without sex/disease duration adjustment versus $p=2.5\times10^{-4}$ with adjustment (we hypothesize that the auxiliary features in the multivariate models capture this signal independently of demographic information).




**Figures 1 and 2. Top performing multivariate models and associated features versus sNfL independently.** Left: the receiver-operating characteristic (ROC curve) represents the range of TPR (sensitivity) and FPR (1-specificity) values corresponding to a given classifier. sNfL is shown as the baseline for comparison. Right: normalized multivariate feature importance.

Proteins that were identified as statistically important features in multivariate classifiers were investigated for relevance to biological networks involved in MS neurophysiology. Automated and manual curation of open source ontologies (Uniprot, Pubmed, *etc.*) revealed associations with the following thematic pathways and cellular processes: neurodegeneration, inflammation, immune response, T-cell and B-cell differentiation, astrocyte differentiation, axon guidance and neuron survival/protection.

## CONCLUSIONS

- **Multivariate protein biomarker models** representing several biological pathways involved in the pathophysiology of MS effectively classified clinical disease activity status (exacerbation versus quiescence) with **stronger performance than any single biomarker**.
- **Follow up studies** with larger sample sizes from additional cohorts are warranted to **verify generalizability** of these models and to further **investigate demographic associations with protein signatures**.
- Proteomic experiments investigating various clinical endpoints pertaining to disease activity are underway to guide the development of a **multiplex protein biomarker assay** with the intent to aid in the **monitoring of MS disease activity status in the clinic**.

*Disclosures*: Ferhan Qureshi, Victor Gehman, Michael Becich and William Hagstrom are employees of Octave Bioscience.

## REFERENCES

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**35th Congress of the European Committee for Treatment & Research in Multiple Sclerosis ECTRIMS (2019) | September 11-13 | Stockholm, Sweden**