P025

Proteomic Serum Biomarker Signatures Associated with Patient Reported Outcomes in Multiple Sclerosis

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Background

- The **monitoring of disease progression** among persons with multiple sclerosis (PwMS) in clinical research and practice has typically been undertaken with the Expanded Disability Status Scale (EDSS).

- There **remains a need for a simple, quick, and reproducible assessment of disability that patients can perform at home.**
  - In light of the COVID-19 pandemic, standardized tools for remote patient-monitoring are of increasing importance, especially for patients with modulated immune systems.

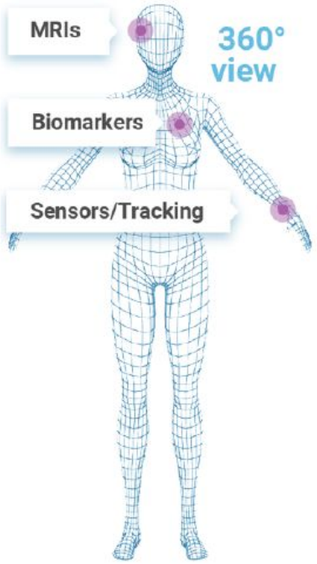
- While several **Patient-Reported Outcome (PRO)** scales exist, widespread adoption has been slow due to lack of corroboration with subclinical measures of disease progression.

- We (University of Pittsburgh and Octave Bioscience) came together to investigate associations between PRO's in a longitudinally tracked MS cohort and Octave's serum biomarkers.



Disclosures: Michael Becich (Young Investigator Educational Grant partially funded by Biogen and Bristol Myers Squibb.), Ferhan Qureshi, and Kelly Leyden are employees of Octave Bioscience.

Methods



- **Octave Bioscience** is developing tools for assessing Disease Activity (DA) and Disease Progression (DP)
  - Octave's **custom assay** measures a panel of 21 proteins whose serum concentrations were associated with radiographic & clinical MS endpoints in independent discovery studies (Chitnis et al., P0063–MS Virtual 2020).
  - Octave leverages neuroradiologist MS expertise and an automated FDA-cleared processing pipeline to enhance the standard of care with an end-to-end **MRI solution.**
  - The Octave Care Program is a **digital service** where patients track their MS between office visits alongside an MS-certified nurse.

- Data Handling
  - Analytical Quality Control (QC) steps were taken such as rerunning any samples that failed acceptability criteria and imputation at limits of quantitation to ensure proper readout of the protein concentrations.
  - To account for variance in PRO assessments, only the **median** from each year was considered.
  - We examined associations with age and sex for each protein and adjusted for background relationships before modeling.

Study Aims: Patient-Reported Outcomes

Objective:

- To examine associations between **serum proteomic biomarker profiles** and **PROs** in PwMS.

Study Design:

- Cross-sectional analysis of observational data from a prospectively collected cohort study from a single academic center.

Outcomes (reflect median measurement from most recent year):

1. **PDDS** - Patient Determined Disease Steps (Fig. 1)
2. **PROMIS** - PRO Measurement Information System[1]
3. **MSRS-R** - Multiple Sclerosis Rating Scale, Revised
    - Composite score including domains of: walking, arms, vision, cognition, speech, swallowing, sensation, bladder/bowel control

Exposures:

- Serum Proteomic Profile (Octave Custom Assay Panel)

Covariates:

- Demographic: Age, Sex, Race/Ethnicity
- Clinical: Disease Duration, ARR, DMT Efficacy, Δtime (Serum - PRO)

Score	Disability level	Description
0	Normal	Mild, sensory symptoms with no limit on activity
1	Mild disability	Minor, noticeable symptoms that have only a small effect on lifestyle
2	Moderate disability	No limitations in walking ability but significant problems that limit daily activities in other ways
3	Gait disability	Interference with activities, especially walking
4	Early cane	Uses a cane or single crutch for walking all or part of the time; can walk 25 feet in 20 seconds without a cane or crutch
5	Late cane	Uses a cane or crutch to walk 25 feet
6	Bilateral support	Needs 2 canes, crutches, or a walker to walk 25 feet
7	Wheelchair/ scooter	Main form of mobility is a wheelchair
8	Bedridden	Unable to sit in a wheelchair for more than 1 hour

Abbreviation: PDDS, Patient-Determined Disease Steps.

Figure 1. PDDS is a validated, self-reported proxy for EDSS (Source: NARCOMS, Fox et al. 2013)

[1] While both PDDS and MSRS-R increase in value as disability increases, the PROMIS scale is inverted so a higher score reflects lower disability.

PROMOTE Study Cohort Characteristics

All patients are enrolled as part of the Prospective Investigation of Multiple Sclerosis in the Three Rivers Region (PROMOTE) study [IRB: STUDY19080007B].

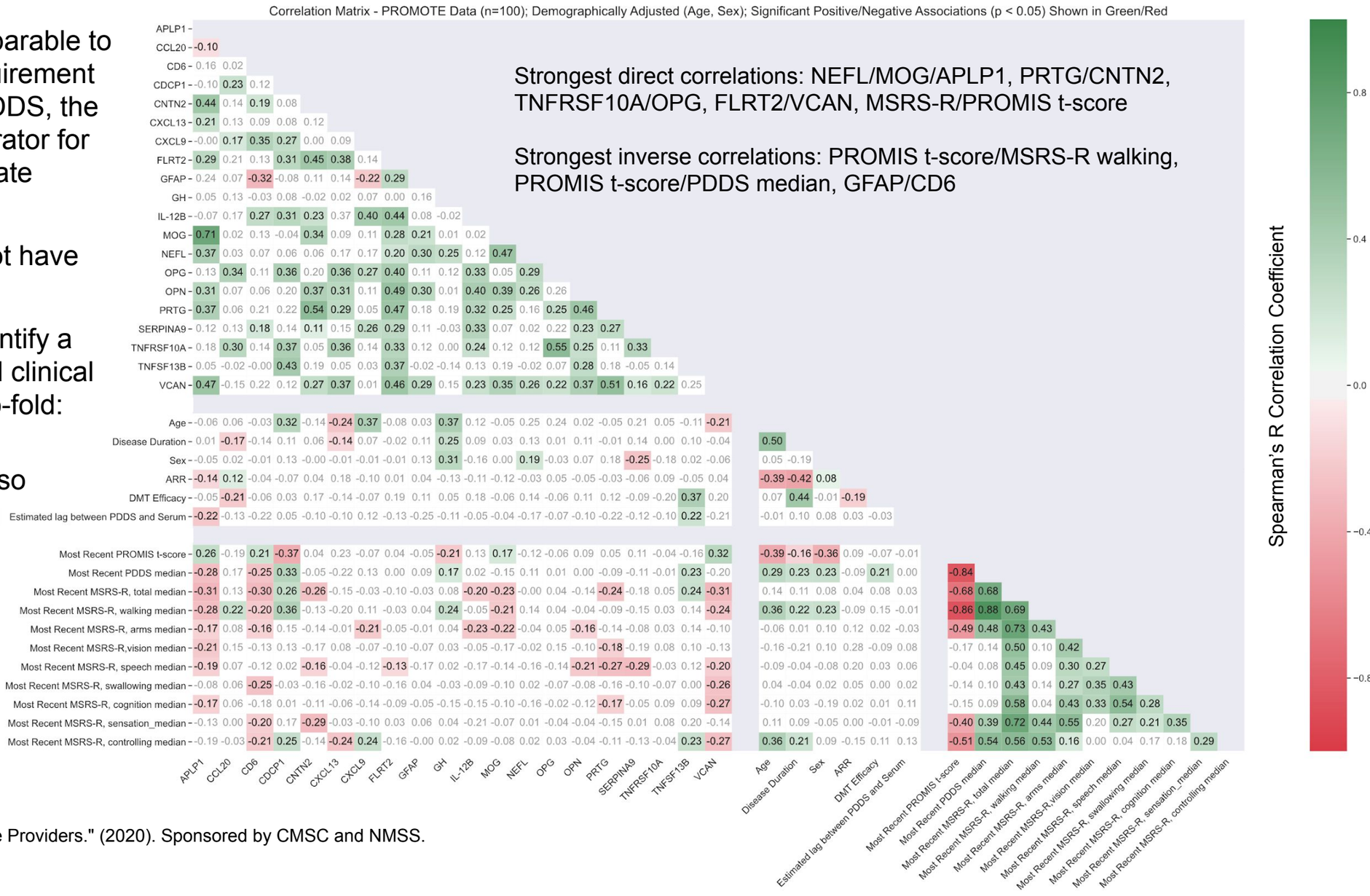
	PDDS ≤ 4	PDDS > 4	All PwMS
# of Women (%)	70 (85%)	11 (61%)	81 (81%)
Age (y, mean ± SD)	45.6 ± 11.5	57.4 ± 10.8	47.8 ± 12.3
Disease Duration (y, mean ± SD)	9.7 ± 8.4	17.3 ± 11.7	11.1 ± 9.5
Self-Reported Race (Ethnicity)	73 White (1 Hispanic)/7 Black/2 Asian	18 White	91 White (1 Hispanic)/7 Black/2 Asian
DMT Efficacy[2] (at time of serum)	41 High (50%) 27 Standard (33%) 14 None (17%)	15 High (83%) 2 Standard (11%) 1 None (6%)	56 High (56%) 29 Standard (29%) 15 None (15%)
Annualized Relapse Rate	0.50 ± 0.68	0.24 ± 0.31	0.45 ± 0.64
PROMIS T-score (median [IQR])	45.9 [40.6, 53.0]	32.4 [29.6, 34.1]	42.6 [36.8, 50.4]
MSRS-R (median [IQR])	7.0 [3.0, 11.0]	9.5 [7.3, 12.8]	7.0 [4.0, 11.0]
Total	82	18	100

These PRO assessments are based on the **most recent measure**. We modeled the serum profiles to differentiate the risk of advancing to each milestone.

[2] For the purposes of this study, natalizumab, mitoxantrone, alemtuzumab, ocrelizumab, cladribine, and ofatumumab are considered high-efficacy therapies and every other approved drug is standard-efficacy. *DMT Efficacy was encoded as a variable with 0=None, 1=Standard Efficacy, 2=High Efficacy at time of serum draw.

Correlation Structure of the Variables

- The threshold of PDDS = 4 (comparable to EDSS = 6) indicates full time requirement for ambulatory assistance. For PDDS, the milestone of 4 is a common separator for classifying severe vs. mild/moderate disability.[3,4,5]
    - PROMIS and MSRS-R do not have an equivalent benchmark.
- Our statistical analysis plan to identify a set of serum protein markers (and clinical features) that predict PROs is two-fold:
    1. **Classification** of PDDS: **logistic regression** with lasso regularization after removing co-linear relationships.
    2. **Regression** of PDDS, PROMIS, and MSRS-R: **ordinary least squares (OLS) generalized linear model** after removing co-linear relationships.



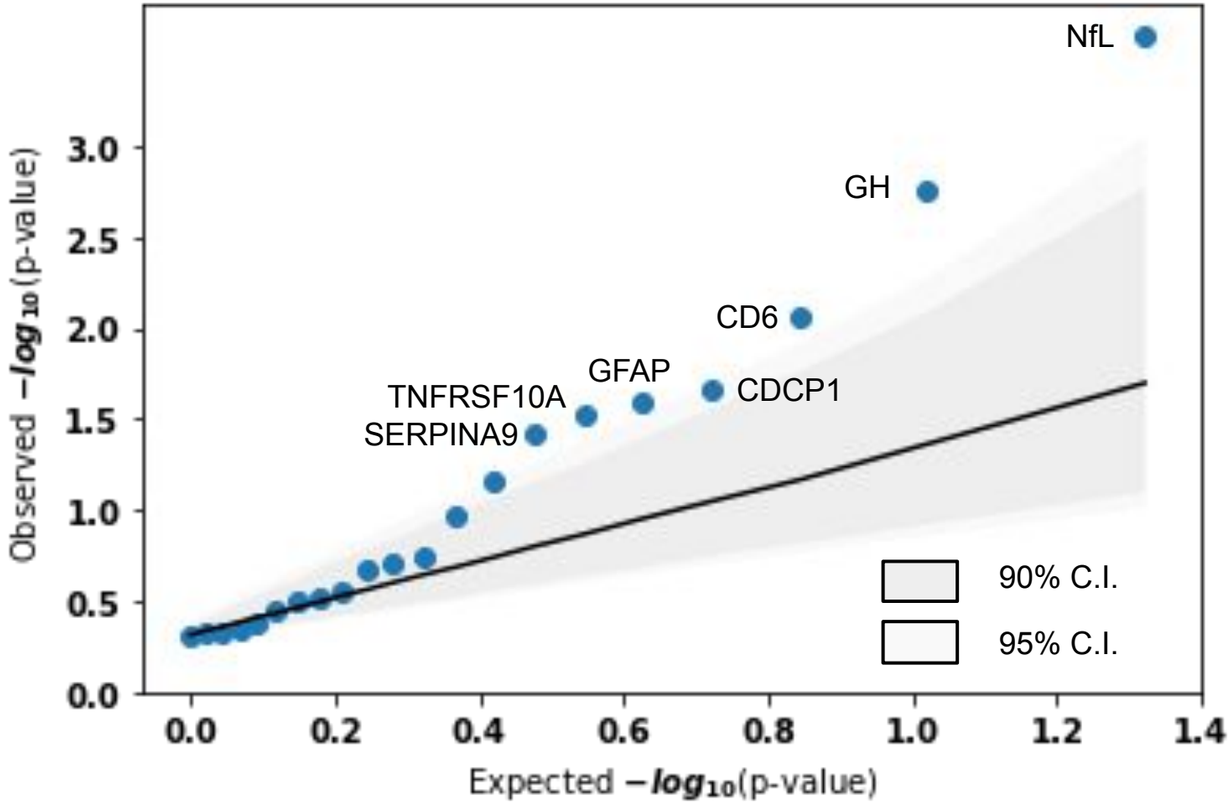
[3] Calabresi, Peter. "Trending in MS: A Webinar for Healthcare Providers." (2020). Sponsored by CMSC and NMSS.
[4] Learmonth et al. 2013
[5] Gray et al. 2009

Univariate Results

Protein	p-value (PDDS > vs. ≤ 4)	R^2 (Ordinal PDDS)	R^2 (Ordinal PROMIS)	R^2 (Ordinal MSRS-R)
APLP1	0.281	0.056	*0.112	0.072
CCL20	0.420	0.039	0.048	0.032
CD6	*0.009	0.051	0.073	0.039
CDCP1	0.022	*0.134	*0.127	0.058
CNTN2	0.303	0.011	0.007	0.068
CXCL13	0.107	0.039	0.030	0.020
CXCL9	0.216	0.010	0.000	0.000
FLRT2	0.362	0.002	0.013	0.029
GFAP	0.026	0.006	0.000	0.012
GH	*0.002	0.064	0.045	0.006
IL-12B	0.316	0.001	0.020	0.020
MOG	0.491	0.021	0.065	0.028
NEFL (NfL)	*2.46E-04	0.027	0.001	0.001
OPG	0.181	0.002	0.003	0.001
OPN	0.452	0.001	0.016	0.019
PRTG	0.466	0.006	0.001	0.059
SERPINA9	0.038	0.019	0.015	0.006
TNFRSF10A	0.030	0.001	0.001	0.009
TNFSF13B	0.069	0.030	0.041	0.031
VCAN	0.196	0.036	*0.114	0.103

- PDDS milestone of 4 (equivalent to EDSS of 6) was used to separate patients into groups of severe vs. mild/moderate disability.
- Results with p < 0.05 highlighted in green
  - Left: Non-parametric Mann-Whitney U test (dichotomized)
  - Right: R^2 reflects Spearman's R correlation (*e.g.* CDCP1 explains 12.7% of the variance in PROMIS).
  - Asterisk (*) indicates that marker was significant at Benjamini-Hochberg multiple hypothesis correction (FDR=0.05)

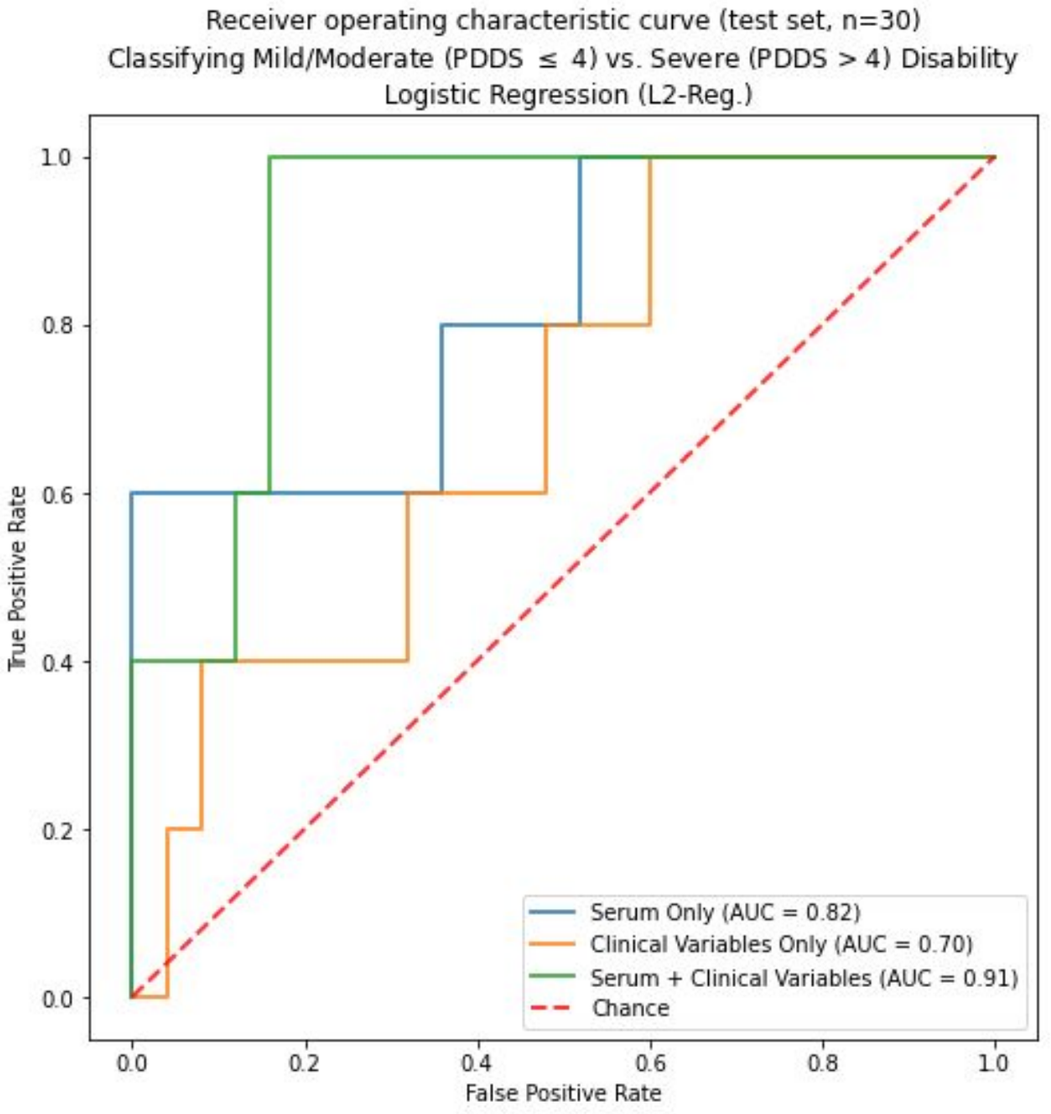
QQ Plot: Expected vs. Observed Significance
Dichotomized PDDS > vs. ≤ 4



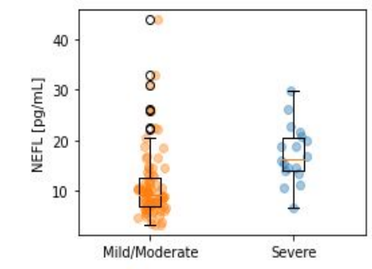
- Quantile-quantile plot of observed versus expected P-values of disability severity.
- The dark gray and light gray areas reflect the confidence interval as generated by 10,000 bootstrapped permutations at a threshold of p=0.10 and p=0.05 respectively.
- The observed p-values for disability severity are outside of the gray areas, suggesting that serum biomarkers (namely NfL, GH, CD6, CDCP1, GFAP, TNFRSF10A, and SERPINA9) are associated with the PDDS severity score beyond chance after accounting for multiple testing burden.

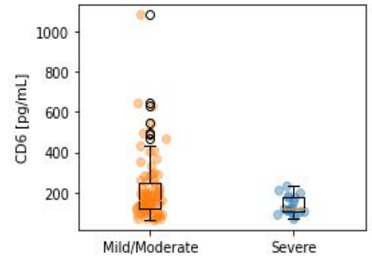
Classification Results (PDDS)

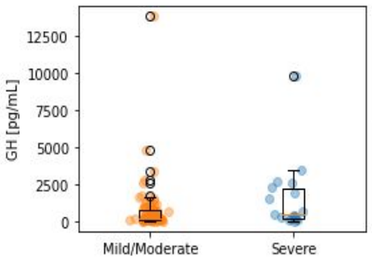
- The **n=100** dataset was divided 70/30 into a **train/validation** and **holdout test set** (stratification was used due to class imbalance).
- We used 5-fold cross-validation to reduce overfitting and determine the best model.
  - We used sequential feature selection to identify the best feature set.
- A cross-validated multivariate logistic regression classifier based on NfL, CD6, GH, and DMT Efficacy was able to separate disease severity at **AUROC: 0.91 ± 0.03**.
  - AUC: 0.88 (Train)/0.91 (Test)
  - Accuracy: 0.86/0.83
  - Sensitivity (Recall): 0.83/0.81
  - PPV (Precision): 0.69/0.66
  - F1-score: 0.76/0.73



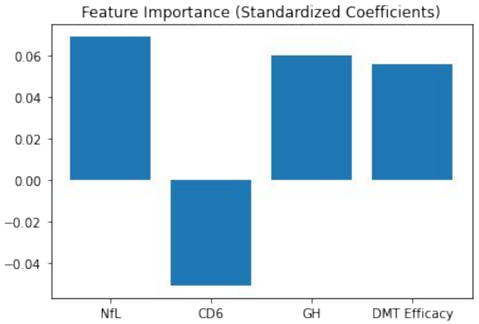
Differential Protein Expressions







NfL, CD6, GH all included in model and significant at MH threshold



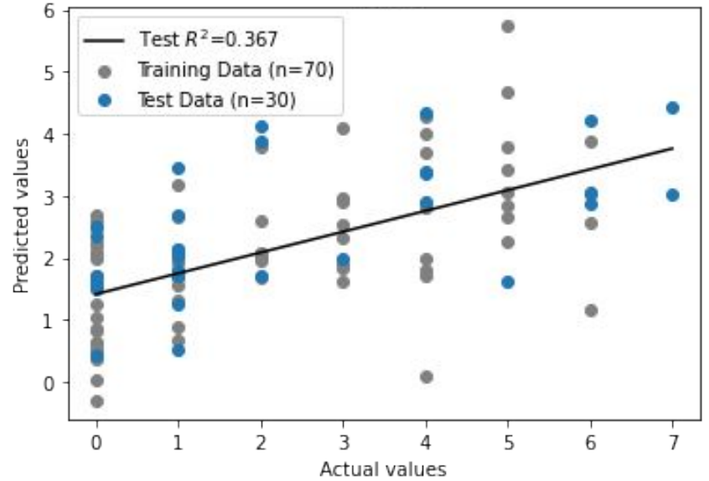
Regression Results (PDDS, PROMIS, MSRS-R)

- Sequential feature selection with 5-fold cross-validation was used to determine best subset of variables for the generalized ordinary least squares (OLS) linear model.
  - We excluded features not important to a model.
  - We divided the dataset 70/30 train/test to evaluate fit.
- The custom proteomic panel and clinical variables showed similar performance in predicting the three ordinal/continuous PROs.
- All models include CDCP1, CD6, Sex, and Disease Duration.

Best **Multivariate Predictor of PDDS** (Train R^2 = 0.335, Test R^2 = 0.367) based on:

- CDCP1
- CD6
- CCL20
- PRTG
- DMT Efficacy
- Sex
- Disease Duration

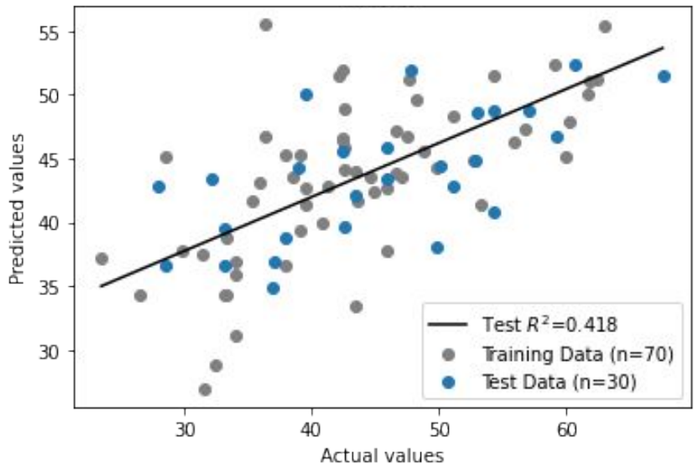
PDDS



Best **multivariate predictor of PROMIS** annualized t-score (Train R^2 =0.423, Test R^2 = 0.418) based on:

- CDCP1
- CD6
- IL-12B
- VCAN
- Age
- Sex
- Disease Duration

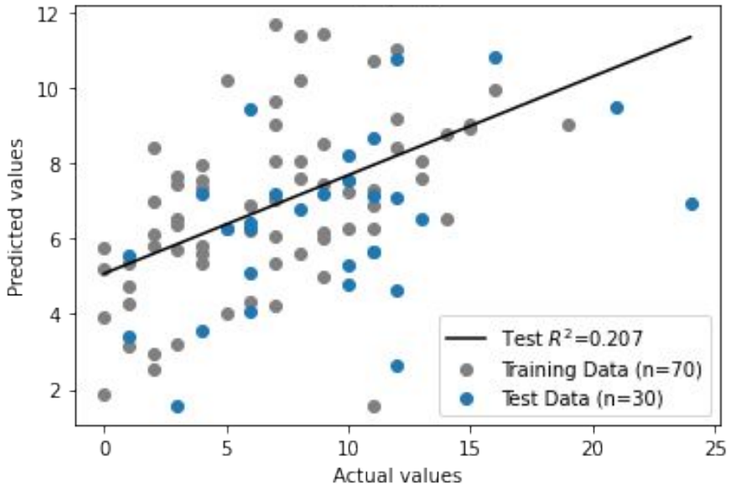
PROMIS



Best **Multivariate Predictor of MSRS-R** (Train R^2 = 0.262, Test R^2 = 0.207) based on:

- CDCP1
- CD6
- IL-12B
- VCAN
- Age
- Sex
- Disease Duration

MSRS-R



Discussion & Conclusions

- Our study shows that the addition of serum protein biomarkers improves the prediction of future MS disability milestone (PDDS ≤ or > 4: AUC > 0.9). Replication analysis is ongoing.
  - More samples (balanced across classes) are necessary to confirm the generalizability of these disease progression results.

- The ability to leverage serum biomarkers and simple clinical features to predict clinically relevant patient-reported outcomes of neurological function will improve patient monitoring in MS in the real world.

- For more information, please contact:
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Thank you for your time!

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