Blood Serum Proteome Correlates of Multiple Sclerosis Disease Progression as evaluated by Clinical and Brain Atrophy Outcomes: A 5-Year Longitudinal Study

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INTRODUCTION

Multiple sclerosis (MS) patients progress through a complex, heterogeneous disease course spanning decades, currently evaluated in a mostly qualitative way. Quantitative measurement of disease progression in the serum proteome would greatly enhance the care for MS patients.

OBJECTIVE

To quantify proteomic correlates of disease progression in two ways: evolution of disability progression (defined by EDSS changes using the standardized definition for MS clinical trials), and development of brain atrophy (measured by MRI brain volumetry). Additionally, we evaluated the performance of a validated multivariate disease activity algorithm relative to the presence and count of Gadolinium enhancing (Gd+) lesions on an MRI associated with the blood draw.

METHODS

We recruited a total of 202 MS patients and collected their data at two time-points, one at baseline and one 6.0 ± 1.0 years later. These comprised of blood serum samples (201/142 patients at baseline/follow up), imaging scans (201/187 patients at baseline/follow up), and the Expanded Disability Status Score (EDSS) clinical assessment (186/196 patients at baseline/follow up).

MS disability progression was defined as::

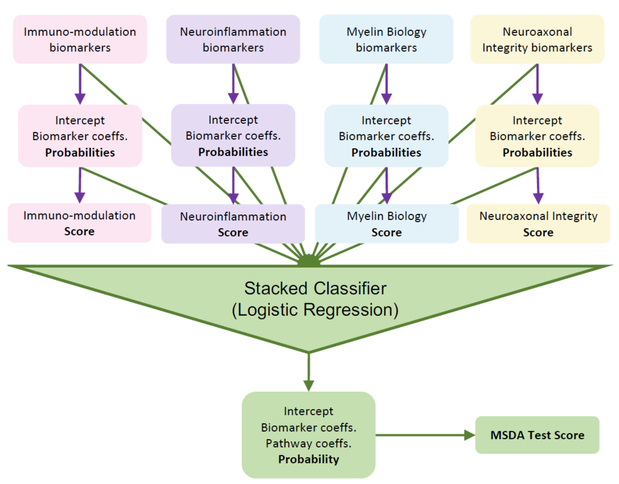
1. baseline EDSS ≤ 0.5 and follow-up EDSS of ≥ 2 or
2. baseline 1 ≤ EDSS ≤ 5 and increase in EDSS ≥ 1 at follow-up or
3. baseline 5.5 ≤ EDSS and increase in EDSS ≥ 0.5 at follow-up.

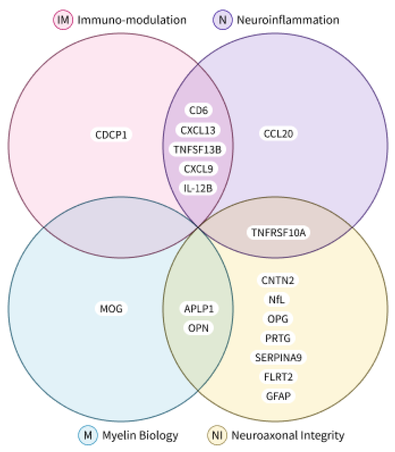
Serum samples were analyzed using a custom immunoassay panel to measure the concentrations of 20 analytically validated protein biomarkers [1]. These proteins were selected for inclusion in the panel based on their associations with MS disease activity and disease progression endpoints observed in previous studies. A stacked classifier logistic regression model that leverages related proteins based on shared biological pathways was applied to determine 4 disease pathway scores (immunomodulation, neuroinflammation, myelin biology and neuroaxonal integrity) and an overall MS disease activity (MSDA) score (Figure 1). Statistical metrics including sensitivity, Negative Predictive Value (NPV), accuracy and odds ratio used during clinical validation to establish and evaluate the DA score thresholds based on Gd+ lesion count for disease activity categories labeled low (L), moderate (M), and high (H) were determined [2]. The 5 scores and the concentrations of individual biomarkers were analyzed relative to the clinical and radiographic disease progression endpoints. For individual proteins, linear mixed effect models that adjusted for age, sex and BMI were utilized after removal of outliers using the non-parametric InterQuartile Range (IQR) method.

Table 1. Cohort Characteristics.

	Time Point	Blood	MRI	EDSS	n	%	Female (%)	Age at Baseline (Mean ± SD)
MS Non-Progressors	Baseline	125	125	126	126	62.4%	74.6%	46.0 ± 11.0
	Follow-up	91	116	126				
MS Progressors	Baseline	55	55	55	55	27.2%	70.9%	48.2 ± 10.8
	Follow-up	34	51	55				
Unknown Progression	Baseline	21	21	5	21	10.4%	85.7%	50.1 ± 12.2
	Follow-up	17	20	15				

Figure 1: MSDA Algorithm Configuration





4 Disease Pathway Scores and Disease Activity Score are Scaled From 1.0 to 10.0 with 0.5 intervals. Thresholds established corresponding to Low, Moderate and High Disease Activity

RESULTS

Figure 1: Box and Whisker Plot of 5 MSDA Test Scores relative to Gd+ Lesion Count

Table 1: MSDA Score Performance by Disease Activity Category versus Gd+ Lesion Count

Low vs Moderate/High Score Thresholds Applied to 0 Gd lesions vs ≥ 1 Gd Lesion								
BNAC (n=313)	**0 Gd**	**≥ 1 Gd**	**Sensitivity***	**Specificity**	**PPV**	**NPV***	**Accuracy**	**Odds Ratio***
L (1.0-4.0)	118	6	0.824	0.423	0.148	0.952	0.470	3.42
M/H (4.5-10.0)	161	28						
Low/Moderate vs High Score Thresholds Applied to 0 and 1 Gd lesions vs ≥ 2 Gd Lesions								
BNAC (n=313)	**0/1 Gd**	**≥ 2 Gd**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy***	**Odds Ratio***
L/M (1.0-7.0)	261	9	0.308	0.870	0.093	0.967	0.850	2.97
H (7.5-10.0)	39	4						

***Statistical metric utilized for establishing and evaluating L/M/H threshold performance in clinical validation study**

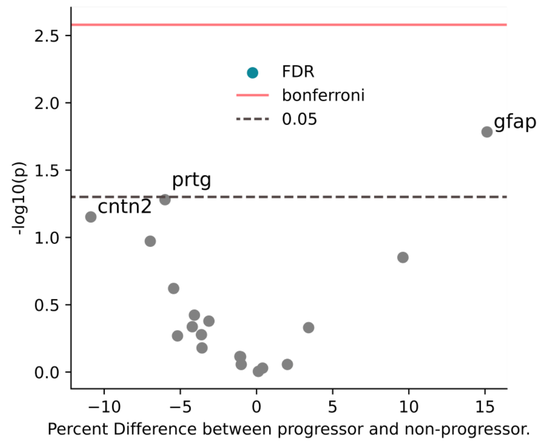
Sensitivity of the Disease Activity score to classify 0 Gd+ lesions versus ≥ 1 lesion was determined to be 0.824 and NPV was determined to be 0.952. Accuracy for distinguishing ≥ 2 Gd+ lesions versus ≤ 1 lesion was determined to be 0.850. Results of these performance metrics are similar to those observed in a prior clinical validation study for which the score thresholds corresponding to Low, Moderate and High DA categories were established. [2] Odds ratios demonstrated that a patient with a Moderate or High DA score is 3.42 times more likely to have ≥ 1 Gd lesions than a patient with a Low DA score and a High score is 2.97 times more likely to have ≥ 2 Gd lesions than a patient with a Low or Moderate score (Table 1).

RESULTS (Continued)

Using a mixed-effects logistic regression model adjusted for age, sex and BMI, associations between the MSDA score and disease pathway scores (trained on Gd+ lesions) with disease progression status were not statistically significant (p>0.05).

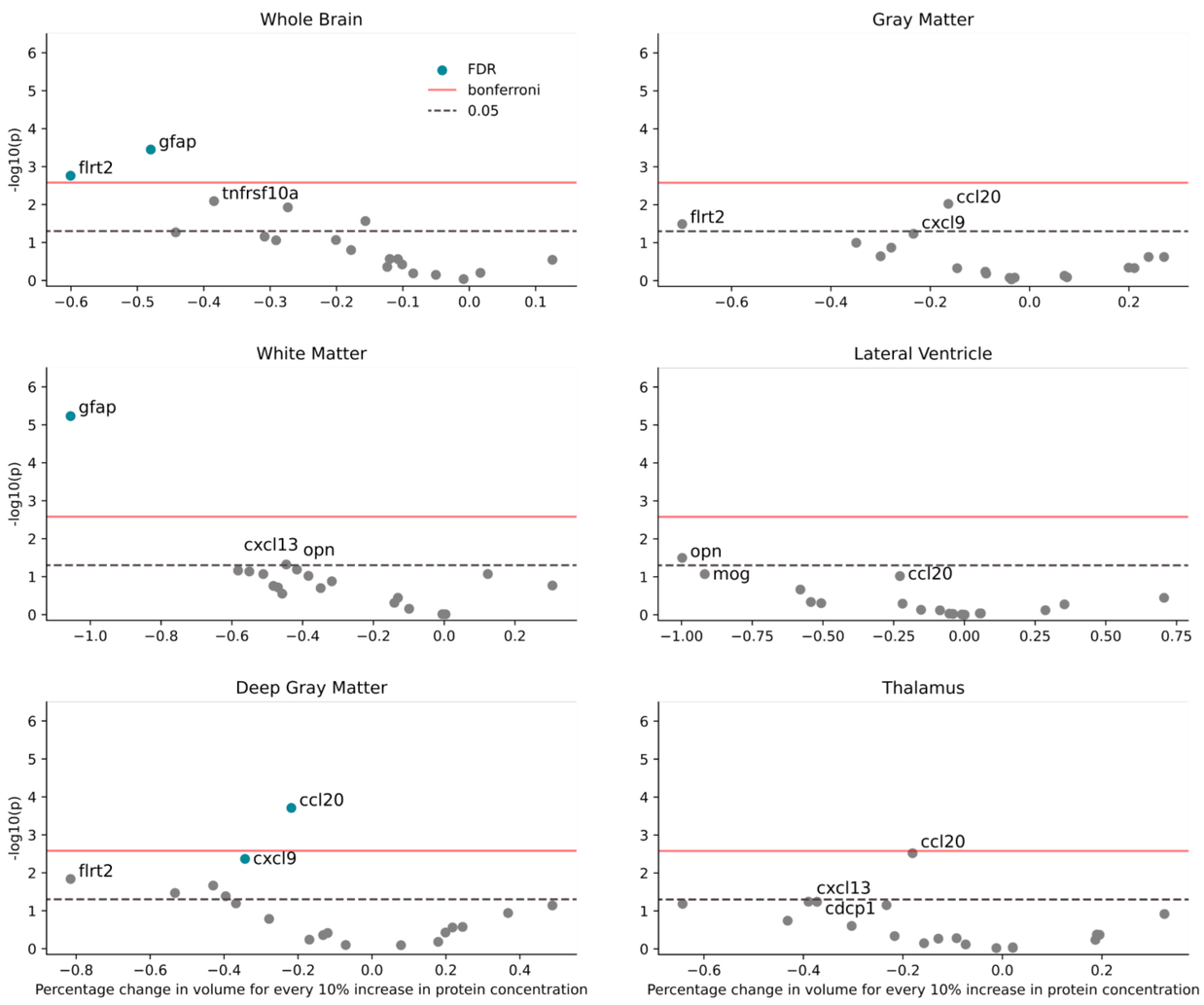
Univariate linear mixed-effects models with subject ID as a random effect (to account for repeated measures), and age, sex, BMI, and progressor status as fixed effects were fit to each protein. The estimated percentage difference between progressor and non-progressor and p-value are shown for each protein in Figure 2 highlighting the top three proteins.

Figure 2: Volcano Plot of Single Protein Association with Progression Status (EDSS)



Univariate linear mixed-effects models with subject ID as a random effect, and protein concentrations, age, sex, and BMI as fixed effects were fit to each MRI endpoint. The estimated percentage change in brain volume for every 10% increase in protein concentration and their corresponding p-values are shown in Figure 3 highlighting the top three proteins.

Figure 3: Volcano Plot of Single Protein Association with MRI Volumetry



CONCLUSIONS

- The MSDA test algorithm replicated performance for accurately categorizing patient disease activity levels (Low, Moderate and High) relative to Gd+ lesions in this independent cohort.
- The MSDA scores which were trained and validated for disease activity endpoints did not associate with clinical and brain atrophy disease progression outcomes. However, measurable effects of disease progression via individual biomarkers were detected in the serum proteome as measured by the assay panel. Serum GFAP had the highest association with MS disability progression defined by EDSS (p<0.05 however not significant after Bonferroni correction). Several individual biomarkers were associated (p<0.05 after Bonferroni correction) with whole brain and regional atrophy measurements including: GFAP (whole brain, white matter volume), FLRT2 (whole brain), and CCL20 (deep gray).
- Extensions to this study will include evaluation of additional progression endpoints at 5 years: optical coherence tomography (OCT) and neuropsychological assessments.
- These results strengthen the evidence for the MSDA test's association with disease activity and broaden our understanding of disease progression correlates through the peripheral proteome.

REFERENCES AND DISCLOSURES

References: [1] Hu W. et al. 2021. Analytical Validation of a Multivariate Proteomic Serum Based Assay for Disease Activity Assessments in Multiple Sclerosis, P010 ACTRIMS 2021 [2] Chitnis T. et al. 2021. Clinical Validation Study Results of a Multivariate Proteomic Serum Based Assay for Disease Activity Assessments in Multiple Sclerosis, P574 ECTRIMS 2021

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