Investigation of Serum Based Proteomic Biomarker Signatures Relative to Steroid Responsiveness and Disease Activity Status in Relapsing Multiple Sclerosis Patients

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INTRODUCTION

Steroids are the primary intervention for relapse treatment in people with MS, although they are not always effective leading to irreversible disability accrual and the need for escalating, invasive treatment options such as plasma exchange. To date, only a few factors influencing steroid response to treat MS relapses have been identified (e.g. vitamin D serum concentration is negatively associated with glucocorticosteroid dose needed for relapse treatment). [1] Serum proteomics have shown increasing promise and utility for disease activity and progression assessments in MS. Identifying proteomic signatures predictive of responsiveness to high dose intravenous glucocorticosteroids (GC) may lead to a minimally invasive and clinically useful tool to help improve outcomes for relapsing MS (RMS) patients.

PURPOSE

We aimed to assess the association of individual proteins and multivariate algorithm scores derived from a custom immunoassay panel in RMS patients relative to three categories of MS disease activity related to GC response: Stable disease without relapse or MRI activity (Stable), in GC responsive (Sensitive) and in GC resistant relapse (Resistant) patients. Additionally, we aimed to assess the association of three disease activity levels (Low, Moderate and High) determined by a validated multivariate model relative to the number of gadolinium enhancing positive (Gd+) MS lesions (0, 1, or ≥ 2 lesions).

METHODS

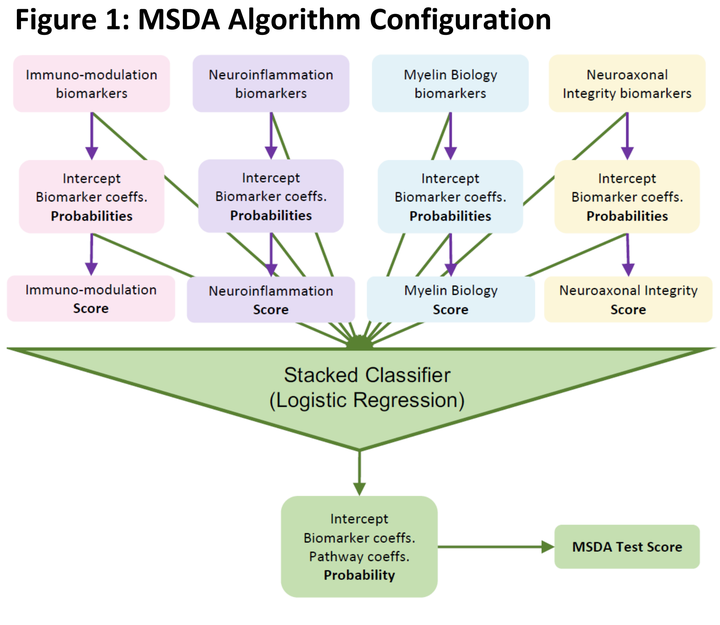
An assay that measures the concentrations of 18 proteins used to determine 4 disease pathway scores (immunomodulation, neuroinflammation, myelin biology and neuroaxonal integrity) and an overall disease activity (DA) score has been analytically and clinically validated. [2, 3] The primary disease activity endpoint utilized for training the score algorithms and validation of the model was the count of Gd+ lesions on an associated MRI. A stacked classifier logistic regression model that leverages related proteins based on shared biological pathways is used to determine the 5 scores (Figure 1). Serum samples were collected from 97 RMS patients (36 Stable, 22 GC Sensitive and 39 GC Resistant) (Table 1). T-tests were used to evaluate the association of MS disease activity categories related to GC response with the DA score, pathway scores, and individual protein concentrations. Additionally, we evaluated statistical metrics including sensitivity, Negative Predictive Value (NPV), accuracy and odds ratio previously used during validation to establish the overall DA score thresholds based on Gd+ lesion count for disease activity categories labeled low (L), moderate (M), and high (H).

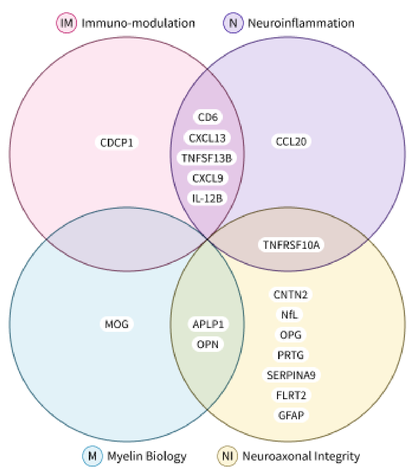
Table 1: Cohort Characteristics

Characteristic	n	Resistant, n = 39	Sensitive, n = 22	Stable, n = 36
Age (Years)[1]	97	35.0 (28.0, 41.0)	33.5 (28.0, 37.5)	33.0 (27.8, 39.5)
Sex[2]	97			
Female		31 (79.5%)	14 (63.6%)	26 (72.2%)
Male		8 (20.5%)	8 (36.4%)	10 (27.8%)
Gd+ lesion count[2]	90			
Gd+ 0		1 (3.1%)	3 (14%)	32 (89%)
Gd+ 1		15 (47%)	11 (50%)	2 (5.6%)
Gd+ ≥ 2		16 (50%)	8 (36%)	2 (5.6%)
Unknown		7	0	0
Disease Duration (Years)[1]	97	1 (0, 9)	3 (0, 9)	4 (2, 8)
DMT Category[2]	97			
None		25 (64%)	14 (64%)	0 (0%)
Platform		10 (26%)	3 (14%)	13 (36%)
High Efficacy		4 (10%)	5 (23%)	23 (64%)
EDSS prior to steroids[3]	93	3.18 (1.0, 9.0)	2.48 (1.0, 4.0)	1.77 (0, 5.5)
Unknown		3	0	1
EDSS after steroids[3]	59	3.27 (1.5, 8.5)	1.75 (0, 3.0)	NA
Unknown		4	0	NA

[1]Median (IQR); [2]n (%); [3]Mean (Min, Max)

Figure 1: MSDA Algorithm Configuration





RESULTS

Table 2: 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

DA Categories	0 Gd	≥ 1 Gd	Sensitivity *	Specificity	PPV	NPV*	Accuracy	Odds Ratio*
L (1.0-4.0)	18	4	0.926	0.500	0.735	0.818	0.756	12.5
M/H (4.5-10.0)	18	50						

Low/Moderate vs High Score Thresholds Applied to 0 and 1 Gd lesions vs ≥ 2 Gd Lesions

DA Categories	0/1 Gd	≥ 2 Gd	Sensitivity	Specificity	PPV	NPV	Accuracy*	Odds Ratio*
L/M (1.0-7.0)	47	11	0.577	0.734	0.469	0.810	0.689	3.77
H (7.5-10.0)	17	15						

*Statistical optimization metric utilized for establishing L/M/H thresholds in prior clinical validation study

CONCLUSIONS

The DA score, pathway scores and several protein biomarkers associated significantly with GC responsiveness. 5 individual biomarkers representing a diverse set of biological pathways, cell types and mechanisms (CCL20, CNTN2, GFAP, NfL and SERPINA9) were associated ($p<0.05$) with the steroid responsiveness group comparison that has the most relevant clinical utility application (Sensitive versus Resistant). Future analyses will include additional multivariate modeling to tailor models specifically for steroid responsiveness and incorporating transcriptomic data for the biomarkers in the panel. This study underlines the utility of the MSDA test to assess MS patients' radiographic disease activity status. A proteomic test validated to predict a relapsed patient's responsiveness to steroids can be a powerful biomarker tool to help improve outcomes for people with MS.

RESULTS (continued)

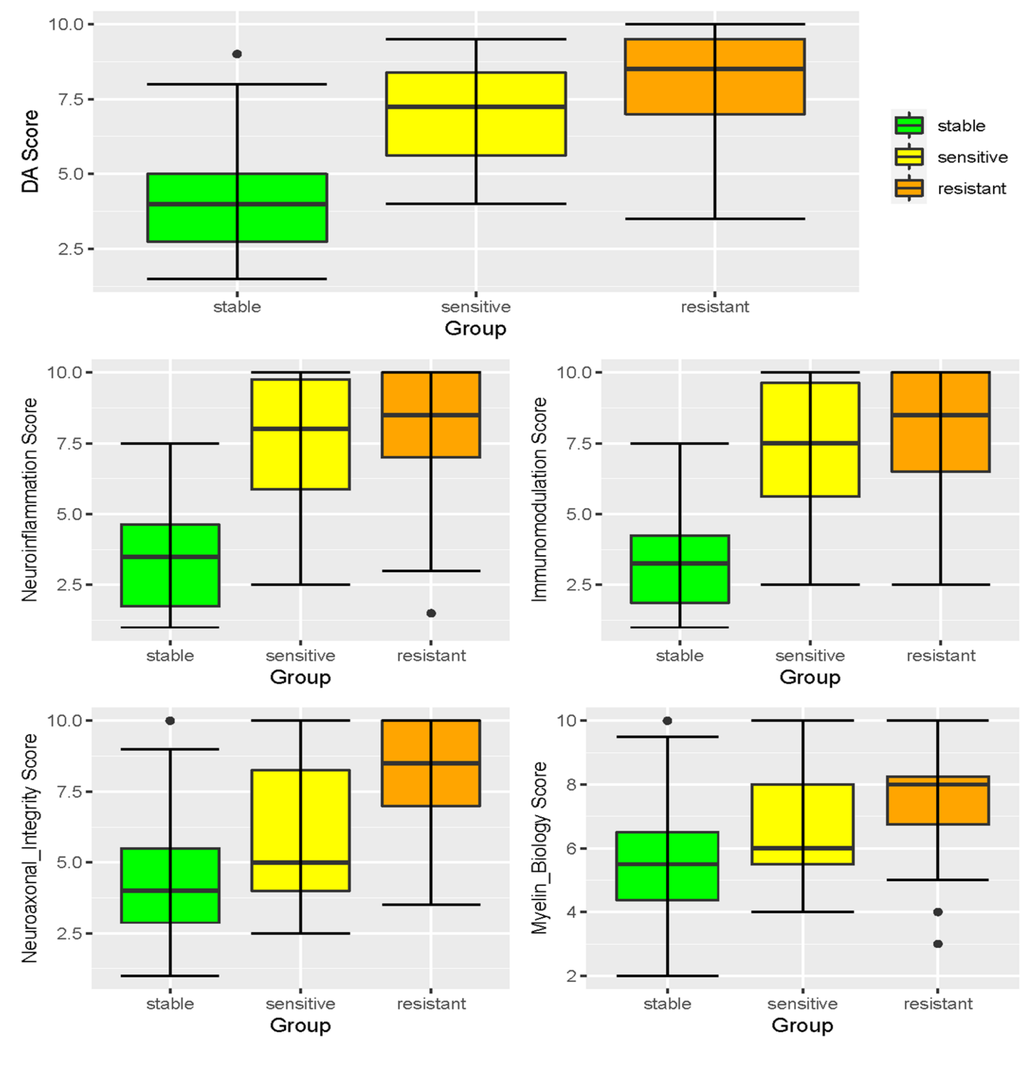
Table 3: Biomarkers and Scores by Steroid Responsiveness Category (p-value)

Biomarker | Score	Stable vs Sensitive	Stable vs Resistant	Sensitive vs Resistant	Stable vs Sensitive and Resistant
APLP1	0.182	0.081	0.889	0.062
CCL20	0.013	0.996	0.012	0.259
CD6	0.026	0.015	0.854	0.006
CDCP1	0.861	0.014	0.056	0.07
CNTN2	0.305	<0.001	0.016	0.001
CXCL13	0.23	0.814	0.161	0.699
CXCL9	0.029	0.63	0.073	0.18
FLRT2	0.023	0.005	0.929	0.002
GFAP	0.166	0.433	0.042	0.935
IL-12B	<0.001	<0.001	0.269	<0.001
MOG	0.774	0.374	0.642	0.443
NfL	0.713	<0.001	0.001	0.002
OPG	0.954	0.131	0.221	0.27
OPN	0.773	0.124	0.113	0.339
PRTG	0.107	0.035	0.862	0.025
SERPINA9	<0.001	0.055	0.039	0.003
TNFRSF10A	0.526	0.578	0.868	0.489
TNFSF13B	<0.001	<0.001	0.314	<0.001
Disease Activity Score	<0.001	<0.001	0.04	<0.001
Immunomodulation Score	<0.001	<0.001	0.275	<0.001
Neuroinflammation Score	<0.001	<0.001	0.521	<0.001
Myelin Biology Score	0.02	<0.001	0.103	<0.001
Neuroaxonal Integrity Score	0.008	<0.001	0.001	<0.001

p-value < 0.0125* | p-value < 0.05

*p-value significance threshold adjusted for multiple comparisons

Figure 2: Box Plots of DA and Pathway Scores by Steroid Responsiveness Category



Four group comparisons were performed to evaluate the 3 disease activity categories relative to GC response. 3 proteins and 4 scores were significant ($p < 0.0125$ after adjustment for multiple comparisons) for stable versus sensitive, 5 proteins and each of the 5 scores were significant for stable versus resistant, 2 proteins and 1 score were significant for sensitive versus resistant and 7 proteins and each of the 5 scores were significant for stable versus sensitive & resistant (Table 3). The Neuroaxonal Integrity score was significant for each of the 4 group comparisons performed (Figure 2). Sensitivity of the Disease Activity score to classify 0 Gd+ lesions versus ≥ 1 lesion was determined to be 0.926 and NPV was determined to be 0.818. Accuracy for distinguishing ≥ 2 Gd+ lesions versus ≤ 1 lesion was determined to be 0.689. Odds ratios demonstrated that a patient with a Moderate or High DA score is 12.5 times more likely to have ≥ 1 Gd lesions than a patient with a Low DA score and a High score is 3.77 times more likely to have ≥2 Gd lesions than a patient with a Low or Moderate score (Table 2). 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.

REFERENCES AND DISCLOSURES

References: [1] Hoepner et al. 2019. Vitamin D increases glucocorticoid efficacy via inhibition of mTORC1 in experimental models of multiple sclerosis.. https://doi.org/10.1007/s00401-019-02018-8) [2] Hu W. et al. 2021. Analytical Validation of a Multivariate Proteomic Serum Based Assay for Disease Activity Assessments in Multiple Sclerosis, P010 ACTRIMS 2021 [3] 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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