Matched Plasma Modification for Calculating a Disease Activity Score in a Serum-Validated Multivariate Proteomic Multiple Sclerosis Disease Activity Test

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INTRODUCTION

- A Multiple Sclerosis Disease Activity (MSDA) Test that measures the concentrations of 18 proteins in serum, and utilizes an algorithm to determine 4 disease pathways scores (immunomodulation, neuroinflammation, myelin biology and neuroaxonal integrity) and an overall Disease Activity (DA) score was analytically and clinically validated [1, 2]. The MSDA test is significantly associated with radiographic and clinical endpoints of disease activity.
- Measurement of matched and simultaneously withdrawn serum and plasma biological samples can result in differential quantification of individual proteins. Determining if the MSDA test also works in plasma samples remains to be established.

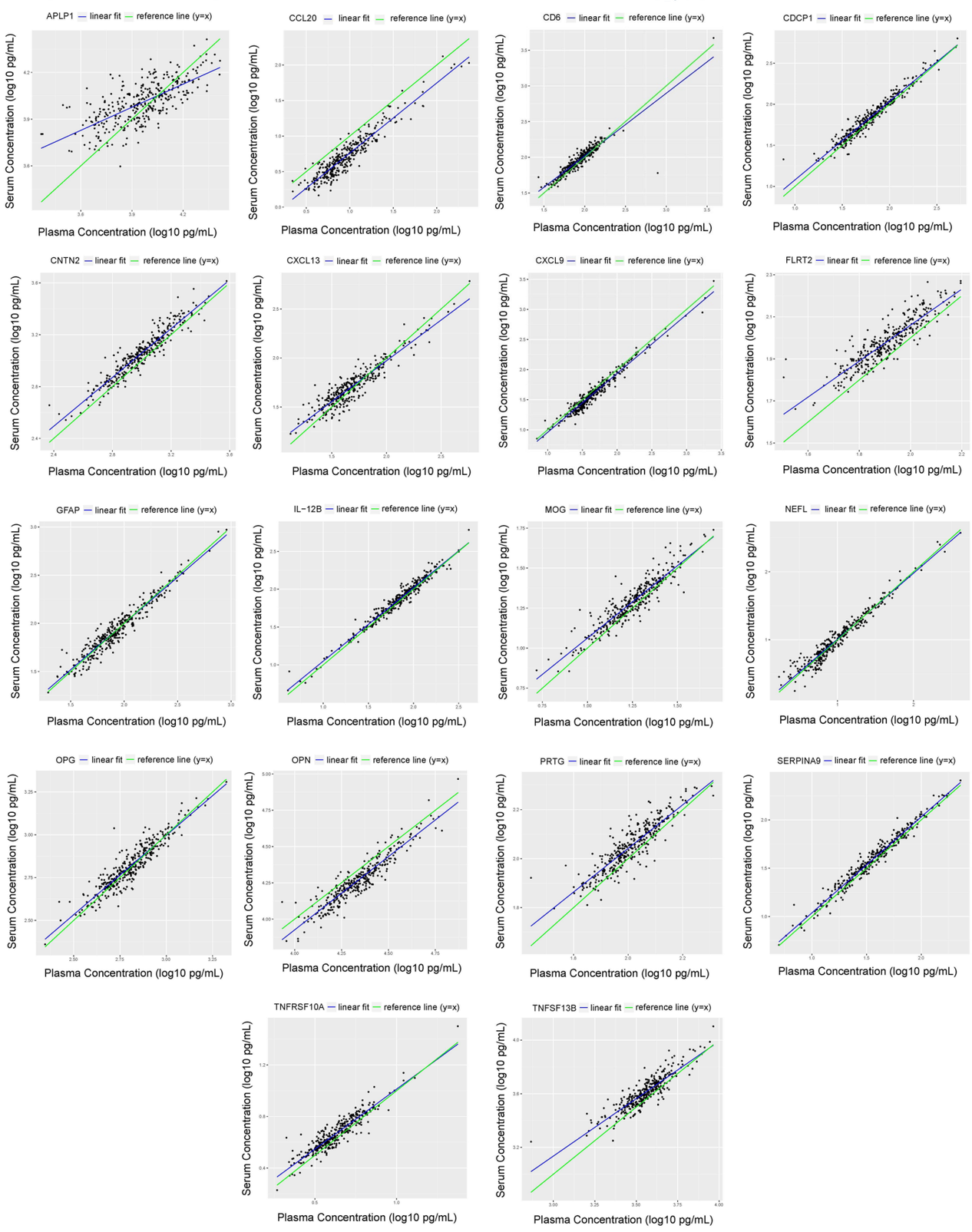
OBJECTIVE

- Validate the MSDA test for use in plasma by devising a process for modifying measured protein concentrations of plasma prior to calculating disease activity and pathway scores.

METHODS

- 336 matched serum and EDTA plasma samples including 248 CIS and MS samples, and 88 control samples (Symptomatic Controls, Inflammatory Neurological Disease Controls, and Neurodegenerative Controls) were assayed across 17 plates. Correlations and median % differences were calculated between plasma and serum concentrations for all 18 biomarkers prior to any adjustments.
- Pre-adjustment Scores (DA and 4 pathways) were calculated for the 248 paired CIS and MS serum and plasma samples for reference.
- 2 methods were tested to adjust plasma values.
    - 1st method - Applying a single uniform factor on plasma concentrations based on the median % difference of concentrations between matched serum and plasma samples.
    - 2nd method - Adjusting plasma concentrations (log10) based on linear regression models from the matched serum and plasma after removal of outliers **(See Figure 1).** One outlier was removed for 5 different Biomarkers (CD6, GH, IL-12B, TNFRSF10A, TNFSF13B).
- Disease Activity scores were calculated from both methods of adjusted plasma values and compared to the serum scores and unadjusted plasma scores to evaluate equivalency.
    - Disease Activity scores and pathway scores range from 1.0-10.0 (with 0.5 increments). DA scores are grouped into 3 categories: Low (1.0-4.0), Moderate (4.5-7.0), and High (7.5-10.0) Disease Activity that were established during validation based on the number of Gadolinium enhancing lesions on an MRI associated with the blood draw.

Figure 1. Linear Regression Models of Individual Biomarkers (log_{10} pg/mL)



CONCLUSIONS

- Calculating DA and pathway scores for the MSDA Test in plasma by adjusting the values using linear regression models provided the closest approximation to scores calculated using serum values.
- This model can be applied to future studies that only have plasma staples available to calculate highly equivalent DA and pathway scores.

RESULTS

- Protein concentrations correlated well between plasma and serum when looking at all 336 matched serum and plasma. 17 out of 18 biomarkers had R^2>0.81 and a Spearman ρ>0.88. **(See Table 1)**
- The median percent differences of concentrations between all 336 paired plasma and serum samples , ranged from -15% to 75% with 17 out of 18 proteins falling within ±20%. **(See Table 2)**
- Comparing the DA scores (248 CIS and MS samples only) between serum and unadjusted plasma , the mean DA score difference was 0.65 with mean pathway scores differences between 0.25 to 0.99. Using the single-factor adjustment, the mean DA Score difference was -0.14 with mean pathway score differences between -0.32 to -0.02. Using the linear regression adjustment, the mean DA Score difference was 0.03 with mean pathway score differences between -0.01 to 0.03 **(See Table 3)**
- A more normal distribution was observed for MSDA score differences across the 248 paired CIS and MS samples when using the linear regression method compared to single-factor adjustment method and no plasma adjustment **(See Figure 2)**
- For the CIS and MS samples, DA score category changes occurred across all 3 models when comparing plasma to serum, changing categories between the low, mid, and high disease categories. **(See Table 4)** The least number of changes was observed when using the linear regression adjustment model. **(See Table 5)**

Table 1. Pearson and Spearman Correlations Between Plasma and Serum Samples

Correlations (Serum vs. Plasma)	R^2	Spearman ρ	Spearman 95% CI
APLP1	0.52	0.69	0.63 to 0.74
CCL20	0.88	0.91	0.89 to 0.92
CD6	0.96	0.93	0.92 to 0.95
CDCP1	0.95	0.97	0.96 to 0.98
CNTN2	0.87	0.94	0.93 to 0.95
CXCL13	0.93	0.88	0.87 to 0.92
CXCL9	0.92	0.96	0.95 to 0.96
FLRT2	0.80	0.89	0.87 to 0.91
GFAP	0.96	0.94	0.93 to 0.95
IL-12B	0.92	0.97	0.97 to 0.98
MOG	0.85	0.92	0.90 to 0.93
NEFL	0.96	0.97	0.96 to 0.97
OPG	0.88	0.91	0.89 to 0.93
OPN	0.87	0.92	0.91 to 0.94
PRTG	0.80	0.88	0.86 to 0.90
SERPINA9	0.97	0.98	0.98 to 0.98
TNFRSF10A	0.90	0.91	0.89 to 0.93
TNFSF13B	0.81	0.88	0.86 to 0.90

Table 2. Median % Difference of Paired Plasma and Serum

Biomarker	Median % Difference
APLP1	-8
CCL20	75
CD6	-7
CDCP1	-9
CNTN2	-13
CXCL13	-5
CXCL9	13
FLRT2	-15
GFAP	0
IL-12B	-5
MOG	-8
NEFL	-3
OPG	-2
OPN	18
PRTG	-9
SERPINA9	-7
TNFRSF10A	-8
TNFSF13B	-12

Table 3. Mean Score Differences (MSDA and 4 Pathway Scores) Comparing 3 Different Plasma Concentration Adjustment Models

Adjustment Model	MSDA Score	Neuroinflammation	Immunomodulation	Neuroaxonal Integrity	Myelin Biology
No Plasma Adjustment	0.65	0.99	0.79	0.41	0.25
Single Factor Adjustment	-0.14	-0.32	-0.07	-0.02	-0.14
Linear Regression Adjjustment	0.03	0.01	-0.01	0.03	0.01

Figure 2. MSDA Score Difference Histograms Comparing 3 Different Plasma Concentration Adjustment Models

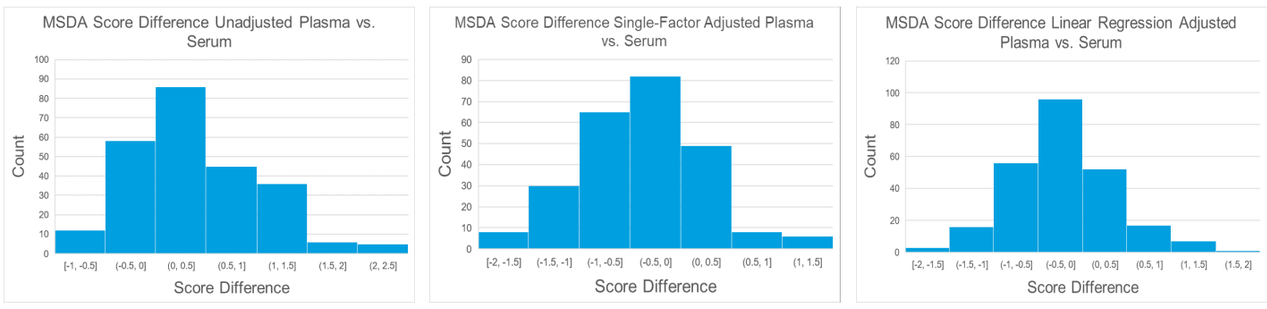


Table 4. Disease Activity Categorization Based on Sample Type/Adjustment

Disease Activity Category	Serum	Unadjusted Plasma	Single-Factor Adjusted Plasma	Linear Regression Adjusted Plasma
Low	59	33	62	53
Mid	114	115	111	118
High	75	100	75	77

Table 5. # of Samples (n=248) Shifting Disease Activity Categories When Compared to Serum

Categorical Changes	Unadjusted Plasma	Single-Factor Adjusted Plasma	Linear Regression Adjusted Plasma
Low to Mid	26	8	12
Mid to Low	0	11	6
Mid to High	25	7	7
High to Mid	0	7	5
Total Categorical Changes	**51**	**33**	**30**

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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