Longitudinal Robustness of Emergent MS Phenotypes from Multiprotein Serum Data

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BACKGROUND

Novel machine learning techniques for measurement of disease activity (DA) and disease progression (DP) through serum proteomics have shown promise in multiple sclerosis (MS) management. Identifying emergent biomarker profiles (pheno-clusters) can enable proteomic-based MS subtyping and support clinical interpretability of a novel MS DA (MSDA) test, developed by Octave Bioscience, Inc [1][2]. Previous research has demonstrated the feasibility and potential clinical utility of proteomic pheno-clustering in MS [3][4].

OBJECTIVE

To characterize the longitudinal stability of pheno-clusters in MS patients identified using unsupervised clustering of serum protein concentration data.

METHODS

Pheno-clustering 137 patient samples were assayed using the MSDA test. The proteomics data was balanced on DMT and grouped into pheno-clusters associated with over or under-expression of 18 proteins, using k-means clustering. We applied the learned grouping to 374 longitudinal samples from an independent cohort (172 patients with ≥2 samples).

Table 1 (left). Summary statistics for the training dataset. Tysabri and non-Tysabri patients were age and sex matched to balance on DMT, making the sample roughly 50% Tysabri.

Table 2 (right). Summary statistics for the longitudinal dataset. Clusters learned from the training data are predicted on this dataset. Patients with at least 2 longitudinal samples were included in the longitudinal stability analysis.

Training Dataset Summary Statistics

		samples	patients
Samples size		137	134
		mean	stddev
Age (years)[1]		48.1	13.4
Disease duration (years)[1]		13.6	10.1
		count	%
Sex[1]:	Female	112	83.6
	Male	22	16.4
DMT[2]:	Tysabri	68	49.6
	Dimethyl fumarate	29	21.2
	Ocrevus	10	7.3
	Glatiramer Acetate	10	7.3
	Aubagio	4	2.9
	Interferon beta	1	0.7
	none	15	10.9

Longitudinal Dataset Summary Statistics

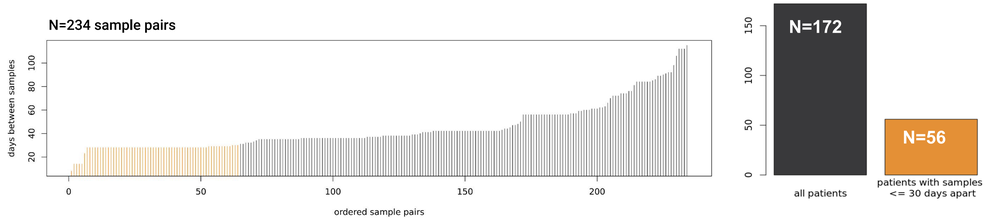
		samples	patients
Sample size		374	172
		mean	stddev
Days between paired samples[2]		44.3	19.9
Age (years)[1]		50.4	12.9
Disease duration (years)[1]		14.5	9.2
		count	%
Sex[1]:	Female	133	77.3
	Male	39	22.7
DMT[2]:	Tysabri	360	96.3
	Ocrevus	11	2.9
	Copaxone	1	0.3
	none	2	0.5

[1]patient-level summary, [2]sample-level summary

Longitudinal Data Analysis 234 paired samples result from the 374 longitudinal samples. For paired samples, we compared mean absolute protein concentration change for sample pairs with different cluster predictions vs. sample pairs with the same cluster prediction. Significance of mean differences was tested via a 2-sided t-test. We also assessed the association between cluster change and time between samples via a logistic regression. We grouped time between samples based on commonly observed values: 1-4 weeks, 5 weeks, 6 weeks, 7-8 weeks, and 9-13 weeks. For all analyses of paired samples, we adjusted inference to account for correlation of sample pairs from the same patients by employing cluster-robust standard errors.

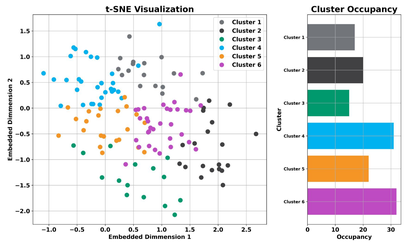
Figure 1 (left): Time between paired samples. Sample pairs ≤ 30 days apart highlighted in orange. As a reference, serum NfL changes tend to be largest following recent DA (<60 days [5]; peaks after 4 weeks [6]).

Figure 2 (Right): Total number of unique patients (grey) and number of unique patients with samples ≤ 30 days apart (orange).



RESULTS

We chose 6 clusters to be able to see clinically relevant distinctions between clusters, while keeping cluster occupancy reasonably high.



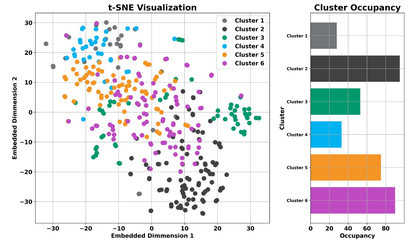
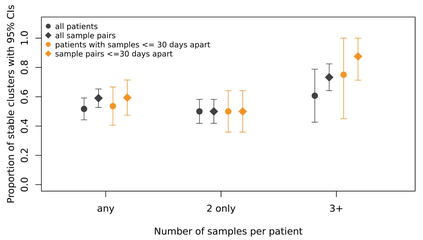


Figure 3 (left). Cluster visualization and occupancy counts of predicted clusters for the 137 samples in the training dataset.

Figure 4 (right). Cluster visualization and occupancy counts of predicted clusters for the 374 samples in the longitudinal dataset.

Longitudinal stability, summarized by proportion of sample pairs with the same cluster prediction, ranged 50-88%. Mean absolute protein concentration change was significantly different, and higher, for more than half of the proteins when comparing sample pairs with different vs. the same cluster predictions.



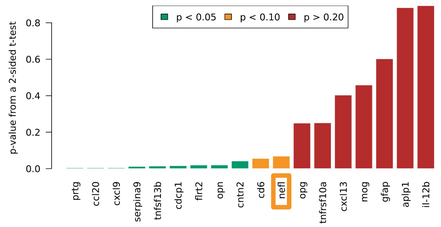


Figure 5 (left): Proportion of sample pairs with the same cluster prediction, ranging 50-88%.

Figure 6 (right): P-values from 2-sided t-tests comparing absolute protein change between samples for groups with and without cluster change.

Note that 4 of the 172 patients switched DMT between samples (sample pairs were 28-41 days apart). All but one of these patients switched clusters.
We did not find evidence for cluster change to be more likely with increased time between samples (logistic regression coefficient p-values >> 0.05).

CONCLUSIONS

- The longitudinal stability of pheno-clusters in a high proportion of patients demonstrates robustness of this methodology.
- Cluster change is associated with shifts in multiple proteins, indicating a multi-protein approach may be more appropriate for detecting subclinical signal.
- The majority of patients (3/4) that switched DMT between longitudinal samples also switched clusters, consistent with previous findings that protein signatures of pheno-clusters may be associated with responses to different DMTs [3].
- Neuroinflammation and progression related protein changes in patients with changing clusters could be indicative of underlying subclinical DA and DP.

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