A Multimodal Approach to Identify relevant biomarkers in MS: May we distinguish different phenotypes at the time of diagnosis?

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Breakthrough research in Autoimmune and Neurodegenerative diseases

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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system (CNS) characterized by significant inter and intra heterogeneity in clinical presentation, disease course, and treatment response.

While many biomarkers offers valuable insights into specific aspects of MS pathophysiology, their individual utility for patient stratification at disease onset is still debated.

AIM

This study aimed to build a prediction model that combines Principal Component Analysis (PCA) and cluster analysis for identifying sets of biomarkers associated with MS at the time of diagnosis and to characterize, if any, distinct MS phenotypes.

METHODS

This was a monocentric, cross-sectional study on treatment naïve patients at the time of MS diagnosis.

Clinical, laboratory, and neuroimaging data were gathered, including optical coherence tomography (OCT) for retinal layer assessment, including peripapillary and macular retinal nerve fiber layer (pRNFL and mRNFL) and the ganglion cell-inner plexiform layer (GCIPL). Serum and cerebrospinal fluid neurofilament light chain (NFL) levels were measured.

The BICAMS battery, which comprises the Symbol Digit Modalities Test (SDMT), California Verbal Learning Test-II (CVLT-II), and Brief Visuospatial Memory Test (BVMT) was used to assess cognitive performance during the diagnostic workflow.

PCA was employed to reduce data dimensionality and reveal key biomarker correlations. Subsequently, K-means clustering was used to classify patients into distinct phenotypic groups.

RESULTS

A total cohort of 71 patients with MS was enrolled , demographic and clinical characteristics are shown in Table 1.

PCA yielded five components with eigenvalues greater than 1.0, collectively accounting for 68.12% of the total variance in the dataset (Fig. 1).

Component 1 was characterized by strong negative coefficients for retinal thickness measurements (GCIPL: -0.82, pRNFL: -0.79, mRNFL: -0.75) and a moderate positive coefficient for serum NFL (0.45) (Fig. 2). Component 2 was primarily characterized by high positive coefficients for NFLs, in both cerebrospinal fluid (CSF NFL: 0.88) and serum (serum NFL: 0.56). (Fig. 2).

K-means clustering of the PCA-transformed data identified two distinct patient subgroups (Cluster 0: n=38; Cluster 1: n=33). Silhouette analysis confirmed that this two-cluster solution provided optimal separation, with an average silhouette coefficient of 0.42 (Fig. 3).

Between-cluster comparisons revealed significant differences in several key variables (Fig. 4). Cluster 1 patients exhibited significantly thicker retinal layers (GCIPL: 72.4 vs. 64.8 µm, p=0.003; mean pRNFL: 98.3 vs. 89.7 µm, p=0.002) and better cognitive performance (mean SDMT z- score: -0.31 vs. -0.89, p=0.01; mean BVMT-R z-score: -0.22 vs. -0.76, p=0.008) compared to Cluster 0 (Fig. 4). Notably, Cluster 1 patients also displayed significantly higher serum NFL levels (mean: 18.6 vs. 12.3 pg/mL, p=0.007), despite their better structural and cognitive profiles (Fig. 4).

Characteristic	All Patients (N=71)	Cluster 0 (n=38)	Cluster 1 (n=33)	p-value*
Age at diagnosis (years)	35.7 ± 9.8	37.2 ± 10.1	34.0 ± 9.3	0.18
Disease duration (months)	24.3 ± 16.5	22.8 ± 15.3	26.1 ± 17.8	0.32
EDSS score	2.0 (1.0-3.5)	2.5 (1.0-3.0)	2.0 (1.0-3.5)	0.09
Relapses in previous 12 months before diagnosis	1.8 ± 1.2	2.1 ± 1.3	1.4 ± 0.9	0.02
Sex (female/male)	48/23	25/13	23/10	0.81
Gadolinium-enhancing lesions (yes/no) at baseline MRI	18/53	12/26	6/27	0.15

*Table 1. Demographic and Clinical Characteristics of MS Patients Overall and by Cluster. *Via chi square or t test as appropriate. EDSS, Expanded Disability Status Scale; MRI, Magnetic resonance imaging.*

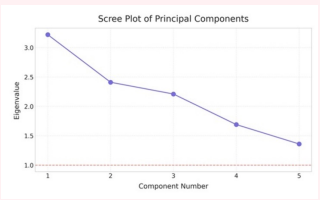

Figure 1. Scree plot showing eigenvalues for each principal component. The horizontal dashed line represents the Kaiser criterion (eigenvalue > 1) for component retention.

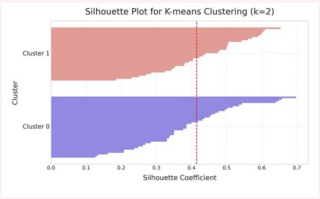

Figure 3. Silhouette plot for the two-cluster solution. Higher silhouette values indicate better cluster assignment. The vertical dashed line represents the average silhouette coefficient (0.42).

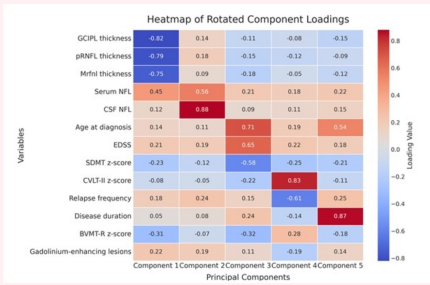

Figure 2. Heatmap of rotated component loadings showing the contribution of each variable to the five principal components. Darker red indicates stronger positive loading, darker blue indicates stronger negative loading. BVMT-R, brief visuospatial memory test-revised; CSF, cerebrospinal fluid; CVLT-II, California verbal learning test-II; EDSS, expanded disability status scale; GCIPL, ganglion cell-inner plexiform layer; mRNFL, macular retinal nerve fiber layer; NFL, neurofilament light chain; pRNFL, peripapillary retinal nerve fiber layer; SDMT, symbol digit modalities test.

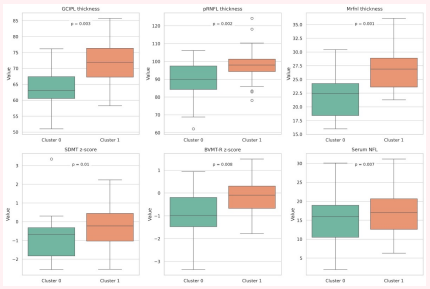

Figure 4. Boxplots comparing key variables between Cluster 0 and Cluster 1. BVMT-R, brief visuospatial memory test-revised; GCIPL, ganglion cell-inner plexiform layer; mRNFL, macular retinal nerve fiber layer; NFL, neurofilament light chain; pRNFL, peripapillary retinal nerve fiber layer; SDMT, symbol digit modalities test.

CONCLUSIONS

These data-driven insights establish a framework for future longitudinal validation studies. Understanding these early disease patterns could inform personalized treatment strategies.

Our results demonstrate the value of multimodal biomarker integration in characterizing MS heterogeneity from disease onset.

REFERENCES

Greenacre M, Groenen PJF, Hastie T, D'Enza AI, Markos A, Tuzhilina E. Principal component analysis. Nature Reviews Methods Primers. 2022;2(1):100. van Dam M, de Jong BA, Willemse EAJ, Nauta IM, Huiskamp M, Klein M, et al. A multimodal marker for cognitive functioning in multiple sclerosis: the role of NfL, GFAP and conventional MRI in predicting cognitive functioning in a prospective clinical cohort. Journal of Neurology. 2023;270(8):3851-61. Spelman T. et al. Treatment Escalation vs Immediate Initiation of Highly Effective Treatment for Patients With Relapsing-Remitting Multiple Sclerosis: Data From 2 Different National Strategies. JAMA Neurol. 2021 Oct 1;78(10):1197-1204

DISCLOSURES

The authors have nothing to disclose.

CONTACT INFORMATION

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