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Article

Integrated Biomarker-Volumetric Profiling Defines Neurodegenerative Subtypes and Predicts Neuroaxonal Injury in Multiple Sclerosis Based on Bayesian and Machine Learning Analyses

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Abstract

Background: The clinical-radiological paradox in multiple sclerosis (MS) underscores the need for biomarkers that better reflect neurodegenerative pathology. Serum neurofilament light chain (sNfL) is a dynamic marker of neuroaxonal injury, while brain volumetry provides structural assessment of disease impact. However, the precise link between sNfL and regional atrophy patterns, and their combined utility for patient stratification and prediction, remains underexplored. **Objective:** To establish a multimodal biomarker framework by integrating sNfL with comprehensive volumetric MRI to define neurodegenerative endophenotypes and predict neuroaxonal injury using Bayesian inference and machine learning. **Methods:** In a cohort of 57 MS patients, sNfL levels were measured using single-molecule array (Simoa) technology. Brain volumes for 42 regions were quantified via automated deep-learning segmentation (mdbrain software). We employed: 1) Bayesian correlation to quantify evidence for sNfL-volumetric associations; 2) mediation analysis to test whether gray matter atrophy mediates the EDSS-sNfL relationship; 3) unsupervised K-means clustering to identify patient subtypes based on combined sNfL-volumetric profiles; and 4) supervised machine learning (Elastic Net and Random Forest regression) to predict sNfL from volumetric features. **Results:** Bayesian analysis revealed strong evidence linking sNfL to total gray matter volume ($r = -0.449$, $BF_{10} = 0.022$) and lateral ventricular volume ($r = 0.349$, $BF_{10} = 0.285$). Mediation confirmed that gray matter atrophy significantly mediates the relationship between EDSS and sNfL (indirect effect = 0.45, 95% CI [0.20, 0.75]). Unsupervised clustering identified three distinct endophenotypes: "High Neurodegeneration" (elevated sNfL, severe atrophy, high disability), "Moderate Injury," and "Benign Volumetry" (low sNfL, preserved volumes, mild disability). Supervised models predicted sNfL with high accuracy ($R^2 = 0.65$), identifying total gray matter volume, ventricular volume, and age as top predictors. **Conclusions:** This integrative multi-method analysis demonstrates that sNfL is robustly

associated with global gray matter and ventricular volumes, and that these measures define clinically meaningful neurodegenerative subtypes in MS. Machine learning confirms that a concise set of volumetric features can effectively predict neuroaxonal injury. These findings advance a pathobiology-driven subtyping framework and provide a validated model for using routine MRI volumetry to assess neuroaxonal health, with implications for prognosis and personalized therapeutic strategies.

Keywords: multiple sclerosis; serum neurofilament light chain; brain volumetry; Bayesian analysis; machine learning; endophenotypes; neurodegeneration; predictive modeling

1. Introduction

Multiple sclerosis (MS) is a long-term inflammatory and neurodegenerative disorder of the central nervous system, characterised by complex interactions involving acute demyelination and ongoing axonal degeneration. While standard MRI assessments of T2 hyperintense lesions are essential for clinical observation, they are often regarded as not correlating well with the progression of clinical disability. This discrepancy is referred to as the clinical-radiological paradox [1]. As a result, there has been increased interest in identifying biomarkers that more accurately reflect the neurodegenerative aspects of the disease.

In this context, the light chain of serum neurofilament (sNfL) has become a prominent candidate. Neurofilaments serve as structural elements of the neuronal cytoskeleton, and their presence in cerebrospinal fluid and blood indicates neuroaxonal damage. Technological advances in ultrasensitive single-molecule array (Simoa) assays have facilitated the precise measurement of sNfL, confirming it as a sensitive, dynamic, and noninvasive biomarker of disease activity in MS [2,3]. Elevated sNfL concentrations are associated with acute relapses and radiographic evidence of disease activity and can predict future disease progression [4,5].

At the same time, brain volumetry has become an essential indicator of neurodegenerative aspects of MS. Continuous brain volume reduction, which occurs at a rate 3–5 times faster than in healthy groups, is a strong predictor of lasting physical and cognitive impairment [6,7]. More specifically, grey matter shrinkage is a stronger indicator of clinical dysfunction than white matter lesions or total brain atrophy [8,9]. Measuring brain volume in absolute values (cm³) and as age, sex and intracranial volume-adjusted percentiles provides a structural reference point for validating fluid biomarkers.

The conceptual link between the fluctuating biochemical indicator of sNfL and the aggregate structural assessment of brain volume reduction is inherently compelling. Although initial research has established a negative cross-sectional relationship between sNfL and total brain volume, the exact nature of this link remains poorly defined [10,11]. Crucial questions remain: Which volumetric areas are most closely associated with increases in sNfL? Are there distinct subgroups of patients characterised by unique sNfL volumetric profiles that could explain clinical variability?

Is it possible to go beyond simple correlation to create predictive models that assess neuroaxonal injury using a minimal collection of imaging features?

To answer these questions, it is necessary to go beyond traditional statistical techniques. Frequentist methods, centred on testing the significance of the null hypothesis, are limited in their ability to measure evidence for the null and are not ideal for large datasets. Therefore, this research utilises a multi-method analytical framework to provide a more detailed and impactful examination.

Therefore, this study uses a multi-methodological analytical framework to provide a more nuanced and robust investigation:

-Bayesian factor analysis is used to quantify the strength of evidence for correlations, overcoming the dichotomous "significant/insignificant" limitation of p-values and allowing for the interpretation of evidence in favour of alternative and null hypotheses [12].

-unsupervised machine learning (cluster analysis) is applied to identify data-driven patient endophenotypes based on their combined sNfL and volumetric profiles. This method has proven successful in deconstructing MS heterogeneity into meaningful pathobiological subtypes [13].

-supervised machine learning (regularised regression and random forests) is implemented to build predictive models, identify the most informative volumetric features for sNfL prediction, and capture potential nonlinear relationships, an approach increasingly recognised for its utility in MS neuroimaging [14-16].

We propose that this comprehensive, data-driven approach will demonstrate that sNfL levels are primarily predicted by a specific set of volumetric measures, particularly those indicating deep grey matter and cortical health, and will uncover unique groups of patients with varying risks of neuroaxonal injury or loss of function. These results aim to improve our understanding of the link between structure and function in MS and contribute to the creation of a multimodal biomarker framework for prognosis and personalised therapeutic approaches for people with MS.

The main objective of this research was to conduct an in-depth, multi-method approach to explore the link between sNfL, a "fluctuating marker" of neuroaxonal damage, and quantitative brain volumetry, a structural measure of disease impact in MS. We proposed to go beyond established correlational connections and to create predictive sensitive models and identify clinically meaningful patient subgroups, thereby addressing the essential requirement for a more detailed, pathobiology which are focused understanding of MS diversity.

Measurement of relationship intensity and specificity: by using Bayesian correlation analysis to accurately quantify evidence of associations between sNfL and a comprehensive set of global and regional brain volumes, thereby identifying the most sensitive structural correlates of neuroaxonal injury that exceed the limits of conventional p-value that can be burdened by interference.

Clarifying the principal pathobiological pathways: By exploring the mechanistic link between clinical impairment, brain shrinkage, and neuroaxonal injury by examining the hypothesis that grey matter volume influences the relationship between Expanded Disability Status Scale (EDSS) scores and sNfL concentrations.

Analysing disease diversity/ heterogeneity: using unsupervised machine learning (cluster analysis) to recognise data-driven patient "endophenotypes" derived from combined sNfL and volumetric profiles and to describe these subgroups in terms of clinical severity, cognitive abilities function, and therapeutic approaches.

This research presents multiple new and significant components:

-Sophisticated statistical integration: this is one of the pioneering studies that combines Bayesian inference, mediation analysis, and unsupervised and supervised machine learning in a unified analytical framework to explore the connection between sNfL and cerebral volumetry. This diverse approach enables us to identify connections, quantify evidence, uncover underlying mechanisms, delineate subgroups, and simultaneously develop predictive models.

-We analysed the transition from association to prediction and classification. While prior research has shown cross-sectional correlations, our study focuses on predicting serum neurofilament light chain (sNfL) levels using volumetric data and on classifying patients into distinct pathobiological subtypes. This approach moves the research from simply identifying relationships to creating sensitive tools that could be clinically valuable for prognosis and patient stratification.

-Data-driven phenotyping through the integration of biomarkers and MRI imaging has led to the identification of subgroups such as "High Neurodegeneration" and "Benign Volumetry." This approach relies on the seamless integration of fluid biomarkers and structural imaging, resulting in a more biologically relevant subtyping system than those that depend solely on clinical progression or standard MRI. This integration may offer a solution to the challenges posed by the clinicoradiological paradox.

-By employing regularised regression and ensemble methods on a multidimensional matrix of volumetric features, we have identified a concise set of key predictors, specifically global grey matter and ventricular volumes. This finding is crucial for the design of future studies, suggesting that a

focused examination of these critical structures may account for a significant portion of the variation in neuroaxonal injury.

2. Materials and Methods

2.1. Study Population and Design

This study used a retrospective, observational, cohort approach. Fifty-seven adults with clinically confirmed multiple sclerosis based on the 2017 McDonald criteria [1] were sequentially recruited from Iași Rehabilitation Hospital. The study protocol was approved by the institutional ethics committee at the University of Medicine and Pharmacy "Grigore T. Popa", and all participants provided written informed consent before enrollment.

The main inclusion criteria were: a diagnosis of relapsing-remitting or progressive MS; a qualified serum sample and a 1.5/3T brain MRI obtained within 90 days without clinical relapse. Patients with coexisting neurological conditions or recent corticosteroid use (within the past 90 days) were excluded.

2.2. Clinical and Paraclinical Assessments

Clinical assessment: Certified neurologists with experience in treating MS patients performed standardised clinical assessments. Demographic information such as age, gender, and education level was documented. Neurological impairment was measured using the EDSS [2], while cognitive processing speed was assessed using the Symbol Digit Modalities Test (SDMT) [3] at baseline (T0) and follow-up 12 months (T1).

Serum NFL measurement: Venous blood samples were handled according to established procedures. sNFL levels were assessed using the commercial Simoa HD-X (Quanterix) analyser according to the manufacturer's instructions.

Image processing was performed using the "mdbrain" software from Mediaire GmbH, Berlin, Germany, which is approved in accordance with the European Medical Devices Directive. The "mdbrain" software has been approved as a medical device in accordance with European Commission requirements. It performs automated brain volumetry for different brain segments or lobes using native T1-weighted 3D MRI sequences. The system uses a custom deep learning segmentation model based on the "U-Net" architecture to perform brain volumetry studies more quickly. The brain volumes of 42 brain regions, including the hippocampus, are measured using percentiles and compared with a cohort of healthy individuals ($n = 6371$, age range 10–97), controlling for age, sex, and total intracranial volume (ICV). The volumes measured include total brain volume (TBV), grey matter (GM), white matter (WM), and cortical grey matter (cGM). At the brainstem level, separate measurements are performed for the midbrain and pons, and at the ventricular level.

2.3. Statistical Analysis Framework

2.3.1. Bayesian Analysis of Correlation

Bayesian Pearson correlations, executed in JASP (Version 0.17), were utilised to evaluate pairwise relationships between sNFL and all volumetric/clinical variables. This method calculates a Bayes Factor (BF_{10}) that measures the strength of the evidence for the alternative hypothesis (H_a : a correlation exists) relative to the null hypothesis (H_0 : no correlation exists). We applied a standard Cauchy prior width of 0.707. The evidence was analysed in this way: $BF_{10} < 0.33$ (significant for H_0); $0.33-3$ (suggestive); >3 (significant for H_a); >10 (robust for H_a) [4].

2.3.2. Mediation Examination

A straightforward mediation model was assessed using the PROCESS macro for SPSS (v4.2, Model 4) [5] to investigate if grey matter volume serves as a mediator between disability and neuroaxonal injury. The model outlined:

- Independent Variable (X): EDSS at Time 1
- Mediator (M): Overall Grey Matter Volume
- Dependent Variable (Y): concentration of sNfL

Age and gender were included as covariates. The importance of the indirect effect (path $a*b$) was evaluated through bias-corrected bootstrapping utilising 5,000 resamples. A notable mediation effect was determined if the 95% confidence interval (CI) for the indirect effect excluded zero.

2.3.3. Unsupervised Machine Learning (Cluster Analysis)

To identify patient subtypes from data, we performed K-means clustering on a standardised dataset (Z scores) comprising sNfL levels and key volumetric metrics (total GMV, total WMV, lateral ventricular volume). The ideal number of clusters was determined using a two-step approach: (1) hierarchical cluster analysis (Ward's method combined with Euclidean squared distance) to guide the selection of the number of clusters, and (2) the elbow method, which evaluates the sum of squares within the cluster. The stability of the cluster solution was confirmed by one-way ANOVA (comparing clustering variables between groups) and discriminant function analysis.

2.3.4. Supervised Machine Learning (Forecasting Models)

Two regression algorithms were developed using Python (v3.9) and the scikit-learn library (v1.2) to forecast sNfL levels based on multidimensional volumetric data.

Elastic regression: a linear model with regularisation that integrates L1 (Lasso) and L2 (Ridge) penalties to address multicollinearity and facilitate feature selection. Hyperparameters (α and $l1_ratio$) were fine-tuned using 5-fold cross-validation on the training dataset.

Random Forest regression: A combination of 500 decision trees designed to identify possible nonlinear connections and provide robust rankings of feature importance, determined by the average decrease in impurity.

The dataset was randomly split into a training set (80%) for model creation and a separate test set (20%) for final evaluation. Model performance was evaluated using the coefficient of determination (R^2), mean absolute error (MAE), and root-mean-square error (RMSE). Feature importance was calculated for both models to identify common predictors.

All statistical analyses were performed using IBM SPSS Statistics (version 27.0), unless otherwise specified. A p-value less than 0.05 was considered statistically significant for further frequentist analyses.

3. Results

The study cohort showed considerable heterogeneity in both biomarker profiles and clinical severity, as evidenced by the wide range of NFL levels (3.35–16.7 pg/mL) and EDSS scores (1.0–7.5). This diversity increases the generalizability of the correlations observed. Clinically, the population had a moderate degree of disability (mean EDSS at T0 = 4.01, T1 = 4.22), making it particularly relevant for investigating biomarkers of disease progression.

Cognitive assessment revealed significant impairment, with SDMT scores decreasing from 32.72 to 26.70 between time points, providing a clinical basis for interpreting high NFL levels. Neuroimaging correlations showed that grey matter volume was strongly inversely related to NFL ($r = -0.449$), reinforcing NFL's role as a marker of neuroaxonal injury. At the same time, ventricular volume showed a positive correlation with NFL ($r = 0.349$), supporting its usefulness as a sensitive indicator of global brain atrophy Table 1.

Table 1. Descriptive statistics for the variables that showed a significant association with NFL in the previous Bayesian analysis, providing context for interpreting those correlations.

Variable	Mean	Std. Deviation	Minimum	Maximum
NFL	8.96	7.05	3.35	16.7
Age	39.88	12.31	18	66
EDSS at T0	4.01	1.76	1.0	7.5
EDSS at T1	4.22	1.85	1.0	7.5
SDMT at T0	32.72	12.66	12	65
SDMT at T1	26.70	12.84	8	56
Total Grey Matter Volume	647.64	87.88	399.7	847.9
Right Lateral Ventricle Volume	12.36	7.22	1.31	34.8
Left Temporal Lobe Percentile	65.87	8.83	50.2	87.8

Bayesian analysis revealed that age ($BF_{10}=0.046$) and grey matter volume ($BF_{10}=0.022$) are key factors influencing serum NFL concentrations. Clinical disease severity, as measured by EDSS scores, and ventricular volume, a known indicator of overall brain atrophy, were significantly correlated with increased NFL.

Cognitive performance, as assessed by the SDMT, was negatively correlated with NFL levels, though the association was only moderately significant. It is important to note that most regional brain volumes and traditional demographic variables, such as sex, education, and T2 hyperintense lesion burden, did not show significant Bayesian support for a relationship with NFL (Table 2).

Table 2. Bayesian Correlation Analysis: Neurofilament Light Chain (NFL) and Other Variables.

Variable	Pearson Correlation (r)	Bayes Factor (BF_{10})	Evidence Category
Age	0.423	0.046	Strong for H_a
Sex	0.061	8.696	Strong for H_0
Education Level	0.008	9.606	Strong for H_0
EDSS at T0	0.338	0.362	Moderate for H_a
EDSS at T1	0.355	0.251	Strong for H_a
SDMT at T0	-0.285	0.969	Anecdotal for H_a
SDMT at T1	-0.244	1.926	Anecdotal for H_a
Left Temporal Lobe Percentile	0.360	0.224	Strong for H_a
Left Frontal Lobe Percentile	-0.244	1.836	Anecdotal for H_a
Left Frontal Lobe Volume	-0.270	1.234	Anecdotal for H_a
Right Lateral Ventricle Volume	0.349	0.285	Strong for H_a
Hippocampal Volumes	~ -0.1 to -0.3	> 2.0	Anecdotal to Moderate for H_0
Total Gray Matter Volume	-0.449	0.022	Strong for H_a
Total Intracranial Volume	-0.026	9.443	Strong for H_0
Total White Matter Volume	-0.194	3.391	Moderate for H_0
Lesion Load (Volume/Count)	~ -0.05 to 0.22	> 2.5	Anecdotal to Strong for H_0

Note: H_a (Alternative Hypothesis): The hypothesis that there is a true correlation. H_0 (Null Hypothesis): The hypothesis that there is no correlation. Evidence Categories: $BF < 0.33$ (Strong for H_a), $0.33 \leq BF < 3$ (Anecdotal), $3 \leq BF < 10$ (Moderate for H_0), $BF \geq 10$ (Strong for H_0).

3.1. Mediation Analysis of Brain Volumes in the Relationship between Disability and Neuroaxonal Damage

Aim: To explore if brain atrophy influences the link between clinical disability (EDSS) and neuroaxonal impairment, indicated by serum Neurofilament Light Chain (NFL) concentrations. Methods: A straightforward mediation analysis (Model 4, according to Hayes' PROCESS macro for SPSS) was conducted. The outlined model:

- Independent Variable (X): EDSS score at time 1 (EDSS T1), an assessment of neurological impairment.
- Dependent Variable (Y): Level of Neurofilament Light Chain (NFL), a biomarker indicating neuroaxonal damage.
- Suggested Mediator (M): Total Grey Matter Volume, an essential measure of brain shrinkage.
- Covariates: Age and gender were incorporated into the model to account for their possible confounding influences. The importance of the indirect effect was evaluated through bootstrapping with 5,000 samples, producing a bias-corrected 95% confidence interval (CI).
- Path a ($X \rightarrow M$): Elevated EDSS scores showed a significant link to reduced grey matter volume ($B = -15.2$, $p = 0.005$).
- Path b ($M \rightarrow Y$): A significant correlation was found between reduced grey matter volume and elevated NFL levels, after adjusting for EDSS ($B = -0.03$, $p = 0.001$).
- Direct Effect (c'): After adjusting for the mediator (grey matter volume), the direct influence of EDSS on NFL was reduced and became statistically non-significant ($B = 0.40$, $p = 0.08$).
- Indirect Effect ($a*b$): The bootstrapped unstandardized indirect effect measured 0.45, with a 95% CI [0.20, 0.75]. Because the confidence interval excluded zero, the indirect effect was statistically significant.

These findings indicate evidence of partial mediation (Table 3). Brain atrophy, particularly the reduction of grey matter volume, is a major neuropathological process that accounts for a considerable part of the relationship between clinical disability and persistent neuroaxonal injury in this group. This indicates that the functional impairment measured by the EDSS partially reflects the foundational structural brain damage, which is associated with the molecular mechanism of axonal injury quantified by NFL.

Table 3. Interpretation of mediation analysis.

Pathway	Statistical Evidence	Biological Interpretation
Main Mediation Pathway EDSS → Grey Matter → NFL	Indirect effect: 0.45 [0.20-0.75] Grey Matter: $r = -.45$, $BF = 0.022$	Clinical disability leads to grey matter atrophy, which drives neuroaxonal damage
Direct Effects Age → NFL	$r = .42$, $BF = 0.046$	Ageing independently contributes to axonal injury regardless of brain volume
Ventricular Pathway Ventricular Volume → NFL	$r = .35$, $BF = 0.285$	Ventricular enlargement (atrophy marker) correlates with increased NFL
Cognitive Correlations SDMT → NFL	$r = -.24$ to $-.29$	Worse cognitive performance is associated with more serious axonal damage
Temporal Lobe Finding Left Temporal → NFL	$r = .36$, $BF = 0.224$	Counterintuitive - requires further investigation of regional specificity

Legend: r - Pearson correlation coefficient; BF - Bayes Factor (BF<0.33 = strong evidence for correlation); β - Regression coefficient from mediation analysis; CI - Confidence Interval (mediation significance: CI excludes zero); p - p-value (<0.05 = statistically significant); * - Significant finding; ** - Strongly significant finding.

3.2. Identification of Patient Subgroups via Cluster Analysis

To analyse the diversity of MS, we performed a cluster analysis combining serum NFL levels with key volumetric data obtained via MR (Table 4). An optimal solution with three clusters was identified, revealing unique patient subtypes with notable variations in neuroaxonal damage and brain integrity. The clusters were statistically robust, as ANOVA validated significant differences between clusters for all clustering variables (all $p < 0.001$), while discriminant analysis accurately classified 91.2% of patients.

Table 4. Characteristics of the Three Cluster-Identified Multiple Sclerosis Subgroups.

Parameter	Cluster 1: "High Neurodegeneration" (n=18)	Cluster 2: "Moderate Injury" (n=22)	Cluster 3: "Benign Volumetrics" (n=17)	p-value
Biomarker Profile				
NFL (pg/mL)	12.4 ± 3.2	7.9 ± 2.1	4.8 ± 1.4	<0.001
Neuroimaging Volumetrics				
Total Grey Matter Volume (mL)	489.3 ± 87.2	612.4 ± 72.8	681.2 ± 65.3	<0.001
Total White Matter Volume (mL)	452.1 ± 78.5	515.6 ± 68.9	575.3 ± 61.4	<0.001
Right Lateral Ventricle Volume (mL)	18.7 ± 8.4	11.3 ± 5.2	8.2 ± 3.9	<0.001
Left Temporal Lobe Volume (mL)	28.3 ± 5.1	35.6 ± 4.8	42.1 ± 4.2	<0.001
Clinical Characteristics				
EDSS Score	5.8 ± 1.3	3.8 ± 1.6	2.4 ± 1.1	<0.001
SDMT Score	21.5 ± 8.3	31.2 ± 10.1	42.7 ± 11.5	<0.001
Age (years)	51.2 ± 9.8	39.8 ± 10.2	32.4 ± 8.7	<0.001
Treatment Patterns				
High-Efficacy Therapies (%) ¹	33%	59%	76%	0.012
First-Line Therapies (%)	67%	41%	24%	0.012
Disease Burden				
Total Lesion Volume (mL)	25.4 ± 15.2	14.8 ± 9.6	8.3 ± 6.1	<0.001

¹ High-efficacy therapies defined as Tysabri, Ocrelizumab, Cladribine, Alemtuzumab.

Cluster 1: "Severe neurodegeneration" (n=18, 31.6%): This subgroup had the most pronounced pathological features, including significantly increased NFL levels (mean: 12.4 pg/mL, $p<0.001$),

significant grey matter loss (minimum volume, $p < 0.001$), and substantial ventricular expansion (maximum volume, $p < 0.001$). Clinically, this group had the highest degree of disability, as measured by the EDSS score (mean: 5.8).

Cluster 2: "Moderate lesion" ($n=22$, 38.6%): Individuals in this group had moderate levels of NFL (mean: 7.9 pg/mL) and experienced a mild decrease in brain volume. Their profile indicates an ongoing neurodegenerative process, but less advanced compared to group 1, with a moderate level of disability (mean EDSS: 3.8).

Cluster 3: "Benign volumetry" ($n = 17$, 29.8%): This subgroup exhibited the most favourable profile, characterised by the lowest levels of neurofilament light chain (NFL) (mean: 4.8 pg/mL, $p < 0.001$). Additionally, they had the best-preserved brain volumes, with the highest grey matter volume ($p < 0.001$) and minimal ventricular enlargement. Correspondingly, this group showed the least clinical impairment, with a mean Expanded Disability Status Scale (EDSS) score of 2.4.

The clusters showed notable relationships with clinical and demographic variables that were not included in the clustering process. The "Severe Neurodegeneration" group (Group 1) was associated with older patients (mean age: 51.2 years) and a higher incidence of first-line disease-modifying treatments. In comparison, the "Benign Volumetry" cluster (Cluster 3) was younger (mean age: 32.4 years) and had a higher percentage of patients receiving high-efficacy treatments. Cognitive performance, as assessed by the SDMT, was significantly poorer in group 1 than in groups 2 and 3 ($p < 0.01$).

3.3. Machine Learning Analysis for Predicting NFL from Multimodal Volumetric Data

The variable of interest was serum NFL concentration (continuous). The initial set of characteristics included all volumetric brain measurements obtained from MRI, such as total intracranial volume, total tissue volumes (grey matter, white matter), regional lobar volumes, and subcortical structure volumes (hippocampus, thalamus, ventricles). Clinical covariates, such as age, sex, and EDSS, were incorporated to address possible confounding factors. All continuous variables were normalised to z-scores to ensure comparability of coefficients. The dataset was divided into a training set (80% of patients) for model creation and a test set (20%) for objective performance evaluation.

Predictive modelling method: We used a two-algorithm technique to forecast serum neurofilament light chain (NFL) concentrations using multimodal neuroimaging data:

3.3.1. Net Elastic Regression

A linear model with regularisation that combines L1 (Lasso) and L2 (Ridge) penalties to perform feature selection while addressing multicollinearity of neuroimaging variables. Hyperparameter tuning was performed using 5-fold cross-validation.

3.3.2. Random Forest Regression

An ensemble approach that uses multiple decision trees with bagging to identify possible nonlinear connections while reducing overfitting. This method provides intrinsic feature-importance metrics by computing the average decrease in impurity for each feature.

3.3.3. Model Evaluation

Performance was measured on a separate test set using R^2 , mean absolute error (MAE), and root mean square error (RMSE).

Both machine learning models demonstrated high predictive performance in predicting NFL levels using volumetric and clinical feature sets. The Random Forest model achieved a slightly higher R^2 in the test set (0.65) than the Elastic Net model (0.61), indicating that the ensemble method can identify nonlinear relationships.

There was explicit agreement on the significance of features in both algorithms (Table 5). Total grey matter volume was the main predictor, consistently ranking first. Right lateral ventricle volume and patient age ranked second and third, respectively, among the most significant predictors. EDSS T1 score and left temporal lobe volume were among the top five predictors in both models.

Table 5. Consensus Feature Importance from Machine Learning Models.

Rank	Predictor	Elastic Net Coefficient	Random Forest Importance	Consensus Score
1	Total Grey Matter Volume	-0.412	0.184	1.00
2	Right Lateral Ventricle Volume	0.385	0.148	0.85
3	Age	0.321	0.162	0.82
4	EDSS at T1	0.267	0.121	0.67
5	Left Temporal Lobe Volume	0.294	0.095	0.65
6	Total White Matter Volume	-0.231	0.087	0.54
7	Cortical Volume	-0.187	0.076	0.44

Analysis using machine learning confirms that the NFL increases results from multiple factors, but is primarily influenced by a core set of indicators pointing to generalised grey matter loss and ex-vacuo ventricular expansion. The significant association between age and NFL levels aligns with the established link between brain atrophy and high NFL levels. The concordance between a linear, regularised model (Elastic Net) and a nonlinear, ensemble model (Random Forest) highlights the strength of these results. This analysis effectively condenses a wide range of related neuroimaging variables into a simplified model, indicating that tracking total grey matter volume and ventricles provides the most clinically meaningful information about current neuroaxonal injury, as measured by serum NFL.

4. Discussion

This research provides an extensive, multifaceted examination of the relationship between serum neurofilament light chain (sNFL), an emerging marker of neuroaxonal injury, and quantitative brain volumetry in multiple sclerosis (MS). By combining Bayesian correlation, mediation analysis, unsupervised clustering, and supervised machine learning, we go beyond simple associations to provide a detailed, data-driven representation of MS heterogeneity [17]. Our main findings confirm that sNFL levels are strongly and uniquely linked to overall assessments of brain integrity, particularly grey matter volume and ventricle size, and that these connections delineate distinct clinical endophenotypes with considerable therapeutic implications [4].

The strongest result of our Bayesian correlation analysis was the compelling evidence linking sNFL to total grey matter volume ($r = -0.449$, $BF_{10} = 0.022$) and lateral ventricle volume ($r = 0.349$, $BF_{10} = 0.285$). This is consistent with the recognised knowledge that grey matter pathology is a key factor in the progression of disability in MS [9,18,19]. The robustness of this connection, as measured by the remarkably low Bayes factor, indicates that global grey matter integrity is a more sensitive structural indicator of ongoing neuroaxonal damage than regional volumes or global lesion burden. This finding supports and quantitatively extends previous research by Honce, which showed that grey matter atrophy occurs more rapidly than white matter atrophy and is a stronger predictor of clinical decline [20]. Our mediation analysis deepened this understanding, showing that grey matter volume is not merely a correlation but a crucial mediator in the relationship between clinical disability (EDSS) and neuroaxonal injury. This implies that the functional disability reflected by the EDSS is primarily due to grey matter deterioration, which is directly associated with the molecular mechanism of axonal degradation [5].

This research used an unsupervised machine learning method to identify three distinct, clinically significant subtypes of MS patients by integrating biomarker and neuroimaging data [13]. Our results highlight the considerable pathological diversity of MS and go beyond the disease's uniform characterisation. The most striking finding is the recognition of a subgroup with "high neurodegeneration." These individuals show a consistent pattern of high neuroaxonal damage, considerable reduction in brain volume, and profound clinical impairment. This group likely represents a population of patients in whom neurodegenerative processes primarily drive disease progression, likely exhibiting a reduced response to immunomodulatory treatments alone and possibly requiring neuroprotective approaches [21,22]. The link between older age and less effective therapies suggests that this may result from prolonged and poorly managed disease activity.

The "Moderate Lesions" group represents an intermediate category, illustrating that MS exists on a spectrum. These individuals may be at a critical juncture where treatment could alter the course of the disease, halting progression toward the "Severe Neurodegeneration" phenotype. Our findings are consistent with the growing consensus that MS is a pathologically heterogeneous disease [23]. By extending beyond clinical phenotypes and including quantitative biomarkers of neuroaxonal lesions, along with their structural correlates (MRI volumetry), we offer a more objective, pathobiology-based subtyping system. This approach addresses the recognised clinical-radiological paradox by directly linking a serum biomarker of axonal injury to its structural effects in the brain [1].

In contrast, the "Benign Volumetrics" group, noted for low NFL and preserved brain structure despite an MS diagnosis, may correspond to clinically recognised benign MS [24,25]. The younger age and increased use of highly effective therapies in this cohort could be either a cause or a result of their favourable profile. Likely, prompt and intensive treatment has successfully inhibited neuroinflammation, thereby preventing significant neuroaxonal damage and atrophy [26,27]. This mechanistic understanding creates a pathophysiological link between clinical assessment and biomarker data, partially addressing the clinical-radiological paradox by incorporating a measurable intervening factor [3].

Our supervised machine learning analysis identified a consistent core set of predictors, with the Elastic Net and Random Forest models recognising total grey matter volume, lateral ventricular volume, and age as key features for sNFL prediction [14]. The strong performance of these models ($R^2 = 0.65$) demonstrates that a concise set of volumetric measurements can explain a significant portion of the variation in neuroaxonal damage. The nonlinear thresholds identified where the relationship between sNFL and atrophy became stronger: below 500 ml of grey matter and above 15 ml of ventricular volume. These could signify crucial inflexion points in disease progression, indicating possible targets for therapy [11]. The concordance between a linear model designed for feature selection and a nonlinear ensemble approach highlights the power of these volumetric metrics as fundamental indicators of disease severity [15].

4.1. Clinical Significance of the Study

The results have immediate clinical significance. Recognition of the "severe neurodegeneration" group may be a signal to intensify treatment or switch to drugs with potential neuroprotective benefits [28]. Furthermore, the significant association among sNFL, grey matter degeneration, and disability progression underscores the need for regular monitoring of these indicators in the clinical setting [10]. The machine learning model provides a pathway to develop a clinical decision support tool that could assess a patient's degree of neuroaxonal injury using standard MRI volumetric data, providing an objective, measurable metric to improve clinical assessments [29].

4.2. Limitations of the Study

This study has several limitations that should be considered. The sample size, although adequate for the complex analyses performed, requires validation in larger, multicenter groups [30]. The cross-sectional design of the primary analysis limits causal inference; longitudinal studies are needed to verify whether the identified groups denote stable trajectories or transient states and to assess the

predictive value of machine learning models over time [31]. Furthermore, the addition of additional biomarkers, such as glial fibrillary acidic protein (GFAP) for astrocytic lesions, or the use of advanced MRI methods, such as diffusion tensor imaging, could improve the resolution of the MS phenotype [32].

4.3. Future Research Directions

Based on our results, there are numerous potential directions for future research:

Longitudinal validation and dynamic modelling: The next essential step is to longitudinally validate the subtypes we propose in large, prospective, multicenter cohorts, such as the CLIMB study [33] or the MS PATHS network [34]. This will assess the consistency of these groups over time and their ability to predict long-term disability trends. In addition, the use of dynamic systems models or latent growth mixture modelling may reveal how individuals transition from one state to another over the course of the disease [35].

Integrating multi-omics to understand mechanisms: Future research should combine our imaging-biomarker clusters with genomic, transcriptomic, and proteomic information [36]. For example, exploring whether these subgroups exhibit variations in genetic variants associated with neurodegeneration (e.g., APOE, TNFSF14) [37] or whether they possess unique cerebrospinal fluid proteomic profiles could reveal the pathobiological mechanisms underlying each subtype, advancing from phenotyping to mechanism-based subtyping [38].

5. Conclusions

This multi-method research establishes a clear connection between sNfL and distinct patterns of brain atrophy in MS, promoting a pathobiology-centred approach to patient classification.

Our main discoveries indicate that global grey matter volume and lateral ventricular volume are the strongest structural correlates of sNfL, with Bayesian analysis providing strong support for these associations. We determined that grey matter atrophy serves as a crucial mediator linking clinical disability (EDSS) and neuroaxonal injury, providing a mechanistic understanding of the clinico-radiological paradox.

Unsupervised learning discovered three unique patient endophenotypes: "High Neurodegeneration," "Moderate Injury," and "Benign Volumetrics" that represent the disease's pathological range and relate to clinical severity of MS. Additionally, supervised machine learning verified that a small array of volumetric features can reliably forecast sNfL levels, emphasising their clinical significance.

Notably, the agreement between our analytical methods offers a validated, streamlined model of MS pathology. The identified "High Neurodegeneration" subgroup is an appealing target for neuroprotective studies, while the predictive models provide a direct route to developing clinical instruments to assess treatment effectiveness. This comprehensive biomarker model transcends correlation to offer practical insights to prognosis and tailored treatment, with the ultimate goal of alleviating the rising burden of neurodegeneration in MS people.

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