

Original article

A real-world data of serum neurofilament light chain in a large cohort of Egyptian multiple sclerosis patients: Hospital-based study

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ABSTRACT

Background: Serum neurofilament light chain (sNFL) is a promising biomarker for neuroaxonal injury in multiple sclerosis (MS). Traditional clinical and radiological examinations often fail to capture the underlying neurodegeneration, particularly in the absence of clinical relapses or gadolinium-enhanced lesions. This study aims to assess sNFL levels in real-world MS patients who have no evidence of activity, to evaluate the potential of sNFL as a biomarker for smoldering-associated worsening (SAW).

Materials and Methods: A cross-sectional study, involved 162 MS patients without evidence of disease activity and 40 healthy, age, sex, and education matched controls (HCs). Patients were classified according to MS subtype, DMT status, and type. sNFL levels were measured using an enzyme-linked immunosorbent assay (ELISA) and levels were compared in each group.

Results: sNFL levels were significantly higher in MS patients compared to (HCs) ($p < 0.001$). Median sNFL levels were lowest in the clinically isolated (CIS) group and steady increase in RRMS and reaching the highest levels in the SPMS group ($p < 0.001$). Despite a slight decrease in sNFL levels in patients who started DMT for a year or less than in the naïve group, sNFL levels were highest in patients who were on DMTs for longer durations ($p = 0.003$). EDSS score was the sole independent predictor of sNFL levels ($B = 0.415$, $p = 0.002$). A cut-off value of 23.25 pg/ml was set to distinguish cases and HCs (92 % specificity and 90 % sensitivity), and 75.48 pg/ml was set to distinguish progressive forms (70.00 % sensitivity and 78.30 % specificity).

Conclusion: sNFL is sensitive for detecting subclinical neurodegeneration in the absence of relapse or gadolinium-enhanced lesions, supporting the utility of sNFL measurements into routine clinical practice to improve monitoring and management of MS.

1. Introduction

Multiple sclerosis (MS) is a complex neurological disorder characterized by neuroinflammation, demyelination, and axonal damage (Visva and Oliveria, 2019). The traditional classification of MS based on clinical presentation and disease progression patterns is being challenged as our understanding of the neuropathological mechanisms of MS evolves suggesting a continuum of disease rather than distinct clinical types or stages (Yamamura, 2023). Neurodegeneration and axonal loss occur independently of demyelination, even years before the first

clinical event as highlighted by the detection of elevated serum neurofilament light chain (sNFL) levels in asymptomatic patients up to six years before the diagnosis of MS (Bjornevik et al., 2020). In individuals with radiologically isolated syndrome (RIS), white matter microstructural changes with altered brain connectivity and thalamic atrophy were found indicating an underlying degenerative process that is masked clinically by the brain's compensatory and reserve capacities, the failure of which will lead to the appearance of the first clinical event; the clinically isolated syndrome (CIS) (Azevedo et al., 2015; Benito-León et al., 2023). In patients with relapsing-remitting MS (RRMS), diffuse

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axonal damage in the normal-appearing white matter (NAWM) unrelated to focal neuroinflammatory lesions was found during early disease stages (Bao et al., 2022). While the current disease-modifying treatments (DMTs) target neuroinflammation and disability accumulation from relapses “Relapse-associated worsening (RAW)”, patients even those on highly efficacious DMTs continue to experience progression of their symptoms even in the absence of clinical or radiological evidence of relapse; “progression independent of relapse activity (PIRA)” (Kappos et al., 2020). RAW and PIRA both contribute to disability accumulation, with RAW being more prominent in the early stages, and PIRA taking precedence as the disease advances (Lublin et al., 2022). This subtle deterioration in both physical and cognitive symptoms in PIRA is better explained by the recently introduced term “smoldering-associated-worsening” (SAW) caused by a more diffuse and ongoing smoldering pathological process of the entire CNS, that the current treatments do not fully address (Scalfari et al., 2024). This shift in perspectives when viewing MS as a disease continuum of two parallel processes; neuroinflammation and neurodegeneration requires a quantitative rather than a qualitative method for proper risk assessment, tailored management, and treatment monitoring as the current conventional clinical and radiological tools fail to capture this smoldering process (Filippi and Preziosa, 2020).

Serum neurofilament light chain (sNFL) has emerged as a potential biomarker in this context. Neurofilaments are major structural elements within the neuronal axis, that are highly stable with a low turnover rate under normal conditions. However, in pathogenic conditions where neuroaxonal damage occurs, they leak into the extracellular space, the cerebrospinal fluid, and eventually into the blood, making them a specific biomarker for ongoing degeneration (Abdelhak et al., 2023). Increased NFL has been found in a variety of neurodegenerative, traumatic, and ischemic brain illnesses in addition to inflammatory conditions. Increased levels of sNFL were found when patients with >30 different neurological diseases were compared to healthy controls. Therefore, elevated NFL levels are indicative of axonal injury in general, regardless of the cause (Yuan and Nixon, 2021). Several studies suggested that sNFL levels correlate with both disease activity and progression in MS, supporting its use as a prognostic marker (Ashfaq, 2022; Brune et al., 2022; Cantó et al., 2019a; Gafson et al., 2020; Håkansson et al., 2018). Thebault et al. found that a two-fold increase in the baseline sNFL level was associated with a 1.46 times increased risk of relapse, particularly in patients with lower baseline sNFL (Thebault et al., 2022). In longitudinal studies, sNFL levels increased during the month before the appearance of new Gd+ lesions and then decreased back to baseline, suggesting a temporal association and that sNFL could be a valuable tool for monitoring subclinical disease activity (Bar-Or et al., 2022). An average elevation of 35 % in sNFL levels was found 3 months following either a clinical relapse or the appearance of a new Gd+ lesion compared to remission samples (Rosso et al., 2020). Several studies have also found a significant reduction in sNFL levels after DMT initiation which correlated with improved clinical and radiological outcomes, suggesting its utility as a biomarker for treatment monitoring (Hernández et al., 2024). When controlling for clinical and radiological activity, higher sNFL levels correlated with worsening disability, reflecting the underlying degeneration and the risk for progression (Satyanarayan et al., 2023).

While sNFL shows promise as a biomarker for predicting relapses and monitoring disease activity and progression in MS, its implementation in clinical practice requires further validation. Most of those findings were driven from patients with certain profiles or on specific DMTs not reflecting real-world MS cohorts. Moreover, due to the temporal association with disease activity, response to treatment, and other confounding factors challenges remain to set up standardized cut-off values that showed great variability among studies with different methodological designs.

Here, this study aims to provide real-world data on sNFL levels in MS patients in remission (without evidence of either clinical or radiological

activity) to assess the subclinical neurodegeneration in the absence of confounding acute disease activity in different stages of the disease as well as in different DMT statuses using the available enzyme-linked immunosorbent assay (ELISA) as a non-invasive, cost-effective, and replicable tool for quantification of the underlying baseline neurodegeneration.

2. Materials and methods

2.1. Participants

This real-life cross-sectional study was conducted at Al-Eman MS Unit, Assiut governorate, Egypt. All patients with a confirmed diagnosis of MS according to the 2017 revised McDonald criteria who were attending the unit for either diagnosis or during the follow-up visits from the 1st of December 2023 to the end of July 2024 were consecutively recruited as shown in Fig. 1.

Inclusion criteria: Age between 18 and 55 years old, both sexes were included, and without any evidence of either new clinical or radiological activity in the preceding 3 months “as sNFL levels transiently increase up to 3 months following a relapse (Rosso et al., 2020)”. For the newly diagnosed patients, sample withdrawal is 3 months after remission.

Exclusion Criteria: Patients were excluded if they had an active relapse, new T2, or the presence of Gd+ lesions in the preceding 3 months. Patients were also excluded if they had any confounding factor that might contribute to sNFL level elevation such as an associated systemic disease, body mass index (BMI) ≥ 30 , or were on long-term treatment other than DMT. Pregnant and lactating patients were also excluded.

Patients were classified according to different MS subtypes into CIS, RRMS, SPMS, and PPMS (Thompson, 2018), where CIS and RRMS were collectively grouped as non-progressive MS while PPMS and SPMS were grouped as Progressive MS. Patients were also classified according to their DMT status into DMT-Naïve, received DMT for <12 months, and received DMT for >12 months “12 month period was chosen because it is a generally accepted time after which most DMTs become effective (Giovannoni, 2018; Giovannoni et al., 2015)”. For the treated group of patients, the DMTs were divided according to their efficacy into low efficacy (including Interferons, Dimethyl fumarate, and Teriflunomide), moderate efficacy (Fingolimod), and high efficacy (Ocrevus and Rituximab).

Healthy controls (HC): 40 age and sex-matched healthy individuals were recruited. They were not related to any MS patients, had no medical conditions nor were on any medications.

2.2. Demographic and clinical data

Data obtained from each patient included demographic data, clinical data including disease duration, the total number of relapses, DMT history, current EDSS score, current scores of the 9-hole pig test (9-HPT), the 25-foot-walk test (25-FWT), and symbol digit modality testing (SDMT). Revision of all available radiological examinations for the presence of any new radiological activity and lesion distribution including the presence of spinal cord lesions, as well as the number of oligoclonal bands in the cerebrospinal fluid (CSF OCB) were also reported.

2.3. Measurement of human neurofilament, light polypeptide (sNFL), levels in serum

Blood samples were obtained from both patients and HCs using standard serum separator tubes, allowed to clot prior to centrifugation at 1000×g for 20 min, then separated and stored in aliquots at -80°C . The concentrations of NFL in serum samples were quantified using commercially available ELISA kits (DL Sci & Tech Development Co., Ltd., China). For each ELISA, standards were created by reconstituting

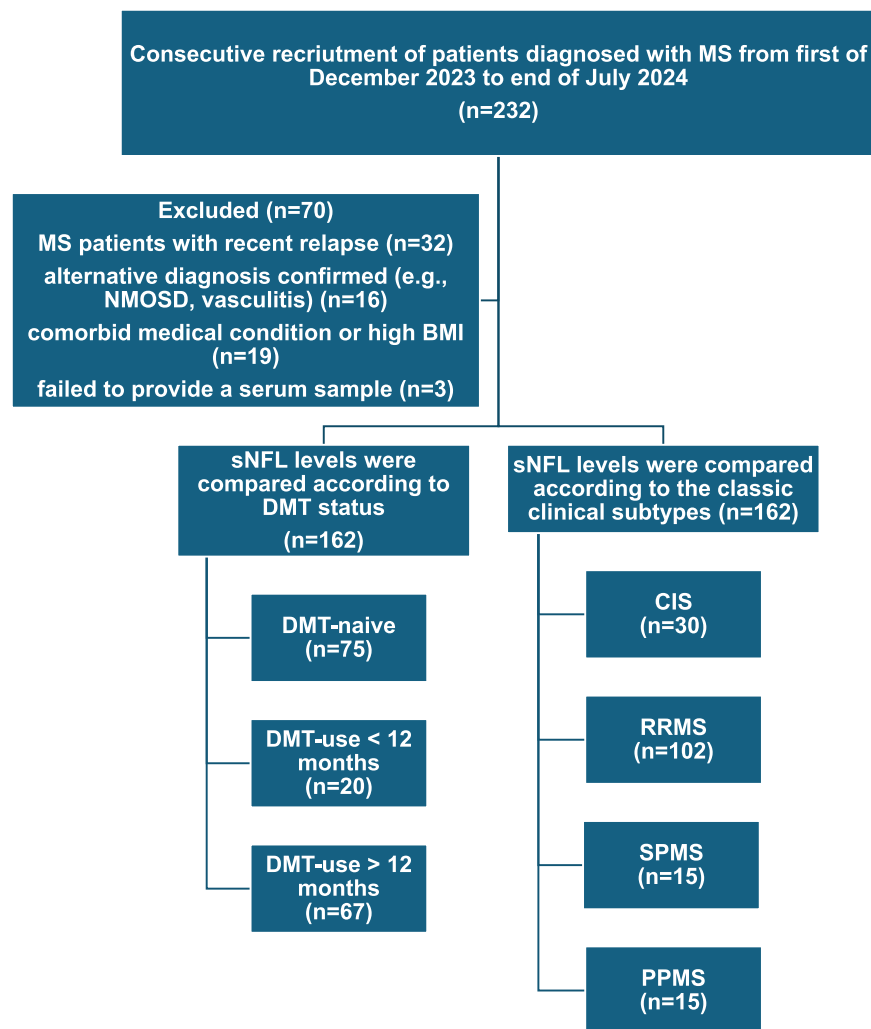


Fig. 1. Flowchart of patient recruitment and sNFL comparison according to different groups.

the corresponding proteins and establishing a seven-point standard curve, including a blank. Each assay involved the addition of 100 μ L of standards, samples, and blanks to a pre-coated 96-well plate, followed by incubation at 37 °C for 2 h. Detection Reagent A was then introduced, and the plate was incubated for an additional hour, followed by a washing step. Detection Reagent B was added next and incubated for another hour, followed by further washing. TMB Substrate Solution was added to each well and incubated for 15–25 min at 37 °C, shielded from light, and the reaction was terminated by adding Stop Solution. The absorbance was read at 450 nm, and the concentration of each analyte was determined by comparing the optical density values to the standard curves. All samples were analyzed in duplicate to ensure precision, and the assays were conducted according to the manufacturer's guidelines.

2.4. Statistical analysis

Statistical calculations were performed with IBM SPSS Statistics 26 software (IBM Corp, Armonk, NY). Since sNFL levels showed a non-normal distribution, the results were presented as median NFL levels and interquartile range and nonparametric tests, including the Mann-Whitney test and the Kruskal-Wallis test were used for the comparison of 2 groups and multiple groups respectively. Correlations between serum NFL levels and different parameters were performed with the Spearman rank correlation coefficient. Linear regression and multiple linear regression analysis were done with sNFL as the dependent variable. A p-value of ≤ 0.05 was considered statistically significant. The

receiver operating characteristic (ROC) curve estimation was performed with the assumption of nonparametric distribution. To calculate optimal cutoffs that maximize both sensitivity and specificity Points closest to the left upper corner were chosen as cut-off values.

3. Results

3.1. Descriptive data and clinical characteristics

Two hundred and two consecutive serum samples were analyzed for sNFL level including 162 MS patients and 40 HCs. Serum sNFL concentrations were significantly higher in patients with MS than in HCs ($p < 0.001$). Age, sex, and the number of educational years did not significantly influence serum NFL concentrations of both patients and HCs ($P = 0.113$, 0.645, and 0.134 respectively). Differences in demographic and clinical data of MS patients and healthy controls are shown in Table 1.

3.2. Correlation between serum NFL concentrations and different clinical, radiological and laboratory data in MS patients

As shown in Table 2, there was a statistically significant moderate positive correlation between sNFL levels and EDSS and the 25-FWT. A statistically significant weak positive correlation was also found between sNFL levels and disease duration, total number of relapses, 9-HPT score, and SDMT scores, while a statistically significant weak negative

Table 1
Demographic and clinical data of MS patients versus Healthy controls.

	Total MS patients (n = 162)	Control (n = 40)	P-value
sNFL, median (IQR)	53.5(48.9)	9.3 (8.5)	<0.001
Age, median (IQR)	31.0 (14.0)	31.5 (11.8)	0.958
Sex, n (%)			0.379
Females	125 (77.2)	18 (69.2)	
Males	37 (22.8)	8 (30.8)	
Educational years, median (IQR)	12.0 (3.0)	11.5 (4.5)	0.055
Disease duration in years, median (IQR)	3.0(5.0)	NA	
Total number of relapses, median (IQR)	3.0 (2)	NA	
EDSS, median (IQR)	1.5 (1.0)	NA	
Presence of spinal cord lesions (in 110 patients), n (%)	71 (64.5)	NA	
Positivity of CSF OCB (in 117 patients), n (%)	109 (93.2)	NA	
DMT status, n (%)		NA	
Naive	73 (45.1)		
DMT<12 months	22 (13.6)		
DMT>12 months	67 (41.4)		
DMTs used at the time of sampling (in 87 patients), n (%)		NA	
Interferons	4 (4.6)		
Dimethyl fumarate	4 (4.6)		
Teriflunomide	36 (41.4)		
Fingolimod	10 (11.5)		
Ocrevus	7 (8.0)		
Rituximab			

IQR: Inter Quartile Range, EDSS: Expanded Disability Status Score, DMT: Disease-Modifying Therapy, CSF OCB: cerebrospinal fluid oligoclonal bands.
* P-value <0.05 is considered significant.

Table 2
Correlation between sNFL levels and different demographic and clinical data in MS patients.

	sNFL (pg/ml)	
	Correlation coefficient	P-value
Age	0.155	0.050
Educational years	-0.228	<0.001*
Disease duration in years	0.360	<0.001*
Total number of relapses	0.319	<0.001*
EDSS score	0.542	<0.001*
9-hole pig test	0.344	<0.001*
25-foot walk test	0.431	<0.001*
Spinal cord lesions (in 110 patients)	0.119	0.214
Number of CSF OCB (in 117 patients)	0.005	0.956

* P-value <0.05 is considered significant.

correlation was found with the total number of educational years.

3.3. sNFL levels in different MS subtypes

Although sNFL levels were higher in all MS subtypes compared to HCs, subgroup comparison according to MS subtypes showed that patients with CIS had a significantly lower sNFL level (median 36.8 pg/ml) compared to either RRMS, SPMS, or PPMS subgroups ($P < 0.001$). A steady increase in sNFL was found in RRMS patients (median 60.9 pg/ml) reaching the highest levels in the SPMS and PPMS subgroups (93.6 and 75.6 pg/ml respectively). However, a significant difference in sNFL levels was found only between RRMS and SPMS subgroups ($P < 0.001$). Other differences between different MS subtypes are shown in Table 3.

3.4. sNFL levels in different DMT status

In untreated patients, median sNFL concentrations were 45.5 pg/ml.

while in patients treated with DMTs for 12 months or less, sNFL levels were lower than in the untreated patients (median 37.4 pg/ml) but without significant difference ($P = 0.266$). The highest levels of sNFL were found in patients treated for >12 months that were significantly different from either naïve or recently treated patients ($P = 0.006$ and 0.004 respectively). Other differences between different treatment groups are shown in Table 4.

3.5. sNFL levels in different DMT use

No significant difference in sNFL levels in patients using different DMTs even when DMTs were categorized according to their efficacy (Table 5 and Table 6).

The linear regression analysis was conducted to determine the association between various clinical factors and serum neurofilament light chain (sNFL) levels in MS patients. The results are summarized in Table 7. Univariate linear regression analysis with sNFL levels as the dependent variable showed that higher sNFL levels were associated with longer disease duration, higher relapse rate, progressive forms of the disease, longer duration of DMT use, higher EDSS scores, poorer manual dexterity, worse walking performance, and poor cognitive performance. Receiver-operating characteristic curve (ROC) for sNFL levels with cut-off points to distinguish cases and HCs as well as patients with clinical progressive disease were shown in Fig. 2.

4. Discussion

This cross-sectional study aims to assess the baseline sNFL levels (i.e., not related to relapse activity) in a large, randomized group of MS patients of different clinical subtypes, treatment durations, and under different DMTs to capture the variability of sNFL in real-world scenarios.

Our results demonstrate significantly higher sNFL concentrations in MS patients compared to HCs ($p < 0.001$), emphasizing its potential as a diagnostic biomarker. Importantly, demographic factors such as age, sex, and years of education did not significantly influence sNFL concentrations, highlighting its specificity to disease pathology rather than external variables. This aligns with previous studies that have demonstrated the robustness of sNFL as a biomarker, largely unaffected by such external variables (Aktas O, 2020; Bittner et al., 2020). On the other hand, studies that found a correlation between sNFL levels and age attempted to define a 'normal' range across different age groups yet inconsistent normative values were obtained across different populations (Beltran, 2024; Disanto et al., 2017a). However, the age-dependency of sNFL may not be a relevant confounder in clinical practice when dealing with MS patients without comorbidities as the physiological increase in sNFL levels in healthy individuals may be obscured by the accelerated degeneration and axonal loss seen in younger MS patients (Freedman et al., 2024)

A moderate positive correlation between sNFL levels and disability scores (the EDSS score and the 25-foot walk test) suggests that higher sNFL levels are associated with greater disability and poorer functional outcomes. These correlations highlight the relevance of sNFL in reflecting the current clinical status of MS patients in line with recent studies connecting sNFL with neuroaxonal damage and disability progression in MS (Barro et al., 2018; Thebault et al., 2020). The significant correlations with the EDSS score and functional tests suggest that sNFL could be a valuable tool in routine clinical assessments to monitor disease progression (Bittner et al., 2021).

The weak correlations observed with the total number of educational years, disease duration, total number of relapses, and 9-hole peg test, indicate that while sNFL levels do reflect broader aspects of disease impact, the strength of these associations is less pronounced. These findings align with recent studies that have explored the multi-faceted nature of MS and the role of sNFL in capturing various clinical and cognitive dimensions of disease activity (Barro et al., 2018; Disanto et al., 2017a).

Table 3
Subgroup comparison between different MS subtypes.

	CIS	RRMS	SPMS	PPMS	P-value
Number, n (%)	30.0 (18.5)	102.0 (63.0)	15.0 (9.3)	15.0 (9.3)	
sNFL, median (IQR)	36.8 (14.8) [4.4–92.8]	60.9 (42.9) [5.5–134.0]	136.8 (93.6) [17.8–261.1]	75.6 (57.7) [3.51–301.2]	<0.001*
Age, median (IQR)	28.0 (7.3)	31.5 (13.3)	38.0 (17.0)	39.0 (16.0)	<0.001*
Sex, n (%)					0.310
Females	12.0 (73.3 %)	82.0 (80.4 %)	12.0 (80.0 %)	9.0 (60.0 %)	
males	8.0 (26.7 %)	20.0 (19.6 %)	3.0 (20.0 %)	6.0 (40.0 %)	
Educational years, median (IQR)	12.0 (2.0)	12.0 (3.0)	8.0 (6.0)	11.0 (5.0)	0.055
Disease duration in years, median (IQR)	0.3 (0.9)	3.5 (4.0)	7.0 (7.0)	6.0 (5.0)	<0.001*
Total number of relapses, median (IQR)	1.0 (0.0)	3 (2)	4.5 (3.0)	4.0 (5.0)	<0.001*
EDSS, median (IQR)	1.0 (1.0)	1.5 (1.0)	4.5 (3.0)	4.0 (2.5)	<0.001*
9-hole pig test, median (IQR)	24.0 (6.0)	25.0 (8.0)	32.0 (16.0)	41.0 (11.0)	<0.001*
25-foot walk test, median (IQR)	12.0 (3.3)	16.0 (8.0)	40.0 (36.0)	34.0 (26.0)	<0.001*
SDMT, mean (SD)	30.5 (10.3)	29.3 (11.3)	18.8 (14.0)	17.4 (9.4)	0.007*

IQR: Inter Quartile Range, EDSS: Expanded Disability Status Score, DMT: Disease-Modifying Therapy, CSF OCB: cerebrospinal fluid oligoclonal bands.

* P-value <0.05 is considered significant.

Table 4
Subgroup comparison between different DMT statuses.

	DMT-naïve patients	Patients on DMT for <12 months	Patients on DMT for >12 months	P-value
Number, n (%)	75.0 (45.1)	20.0 (13.6)	67.0 (41.4)	
sNFL, median (IQR)	45.5 (45.5)	37.4 (58.2)	69.4 (58.2)	0.003*
Age, median (IQR)	32.0 (15.5)	29.0 (16.3)	31.0 (13.0)	0.909
Sex, n (%)				0.370
Females	51.0 (81.3)	16.0 (80.0)	48.0 (71.6)	
males	14.0 (18.7)	4.0 (20.0)	19.0 (28.4)	
Educational years, median (IQR)	12.0 (3.0)	12.5 (2.0)	11.0 (4.0)	0.051
Disease duration in years, median (IQR)	1.0 (5.0)	2.0 (2.0)	5.0 (4.0)	<0.001*
Total number of relapses, median (IQR)	2.0 (2.0)	2.0 (2.0)	4.0 (2.0)	<0.001*
EDSS, median (IQR)	1.5 (1.0)	1.0 (0.0)	2.0 (2.0)	<0.001*
9-hole pig test, median (IQR)	25.0 (7.0)	24.8 (7.0)	29.0 (13.0)	0.040*
25-foot walk test, median (IQR)	15.0 (8.0)	14.0 (4.0)	22.0 (18.5)	<0.001*

IQR: Inter Quartile Range, SD: Standard deviation, EDSS: Expanded Disability Status Score, DMT: Disease-Modifying Therapy.

* P-value <0.05 is considered significant.

When sNFL levels were compared across MS subtypes, a significantly higher sNFL levels were found in all MS subtypes compared to HCs. Our data shows a stratified increase in sNFL levels from CIS (median 36.8 pg/ml) to RRMS (median 60.9 pg/ml), SPMS (median 93.6 pg/ml), and PPMS (median 75.6 pg/ml), with significant differences between CIS and other subtypes ($P < 0.001$). This stratification aligns with the progressive and cumulative nature of axonal damage in MS, where CIS represents an earlier stage with less cumulative damage (Bridel et al., 2021; Kuhle et al., 2024).

Table 5
sNFL level in MS patients according to the different DMTs used.

DMT	Interferon	Dimethyl fumarate	Teriflunomide	Fingolimod	Ocrevus	Rituximab	P-value
Number, %	26.0 (29.9)	4.0 (4.6)	4.0 (4.6)	36.0 (41.4)	10.0 (11.5)	7.0 (8.0)	0.231
sNFL level, median (IQR)	61.3 (59.9)	29.4 (116.3)	33.4 (39.8)	66.4 (43.8)	93.5 (43.3)	57.6 (140.0)	

IQR: Inter Quartile Range, SD: Standard deviation, *P-value <0.05 is considered significant.

The steady increase in sNFL levels from RRMS (median 60.9 pg/ml) to SPMS (median 93.6 pg/ml) underscores sNFL utility in distinguishing different stages of disease progression. The significant difference in sNFL levels between RRMS and SPMS ($P < 0.001$) suggests that sNFL can identify transition point in the disease course, supporting its role in disease staging and monitoring (Bittner et al., 2021; Bridel et al., 2021; Disanto et al., 2017b).

The lack of significant difference between PPMS and RRMS and between PPMS and SPMS is in line with what has already been reported in the literature (Williams et al., 2020). In RRMS, repeated relapses contribute to ongoing axonal damage and disability accumulation, even during periods of clinical remission sNFL levels can remain elevated, reflecting underlying neurodegenerative processes (Ferreira-Atuesta et al., 2021). While the initial stages of SPMS are often preceded by a relapsing-remitting phase characterized by episodic inflammation, the transition to a progressive phase involves a shift towards chronic neurodegeneration, reduced inflammatory activity, and increased rates of axonal damage. PPMS, on the other hand, presents a continuous neurodegenerative process, leading to sustained sNFL elevation over time. Thus, despite the differing patterns of clinical activity, the cumulative axonal damage in both subtypes may result in comparable sNFL levels (Bhan et al., 2018).

To understand the impact of treatment on sNFL we compared naïve patients, patients treated for at least 12 months, and those treated for more than one year. In untreated patients, higher median sNFL levels (45.5 pg/ml) compared to those treated for 12 months or less (median 37.4 pg/ml) were observed, although this difference was not statistically significant ($P = 0.266$). A significant finding was the highest sNFL levels

Table 6
sNFL level in MS patients according to the different DMTs efficacy used.

DMT efficacy	Low efficacy	Moderate efficacy	High efficacy	P-value
number	34.0 (39.1)	36.0 (41.4)	17.0 (19.5)	0.079
sNFL level, median (IQR)	46.5 (50.5)	66.4 (43.8)	82.2 (74.6)	

footer 1IQR: Inter Quartile Range, SD: Standard deviation, *P-value <0.05 is considered significant.

Table 7
Univariate and multivariate linear regression analysis between log sNFL levels and different clinical parameters.

	Univariate regression			Multivariate regression		
	P-value	B	95 % CI	P-value	B	95 % CI
Log disease duration	<0.001*	0.341	0.115–0.289	0.059		
Log the total number of relapses	0.001*	0.262	0.130–0.488	0.246		
Progressive forms	<0.001*	−0.356	−0.169–0.076	0.942		
DMT status	<0.001*	−0.316	−0.174–0.069	0.098		
Log EDSS score	<0.001*	0.475	0.436–0.802	<0.001*	0.599	0.417–0.782
Log 9-hole pig test	0.003*	0.243	0.216–1.010	0.269		
Log 25-foot-walk test	<0.001*	0.331	0.286–0.753	0.725		

EDSS: Expanded Disability Status Score, DMT: Disease-Modifying Therapy, CI: Confidence Interval at 95 %, *P-value <0.05 is considered significant.

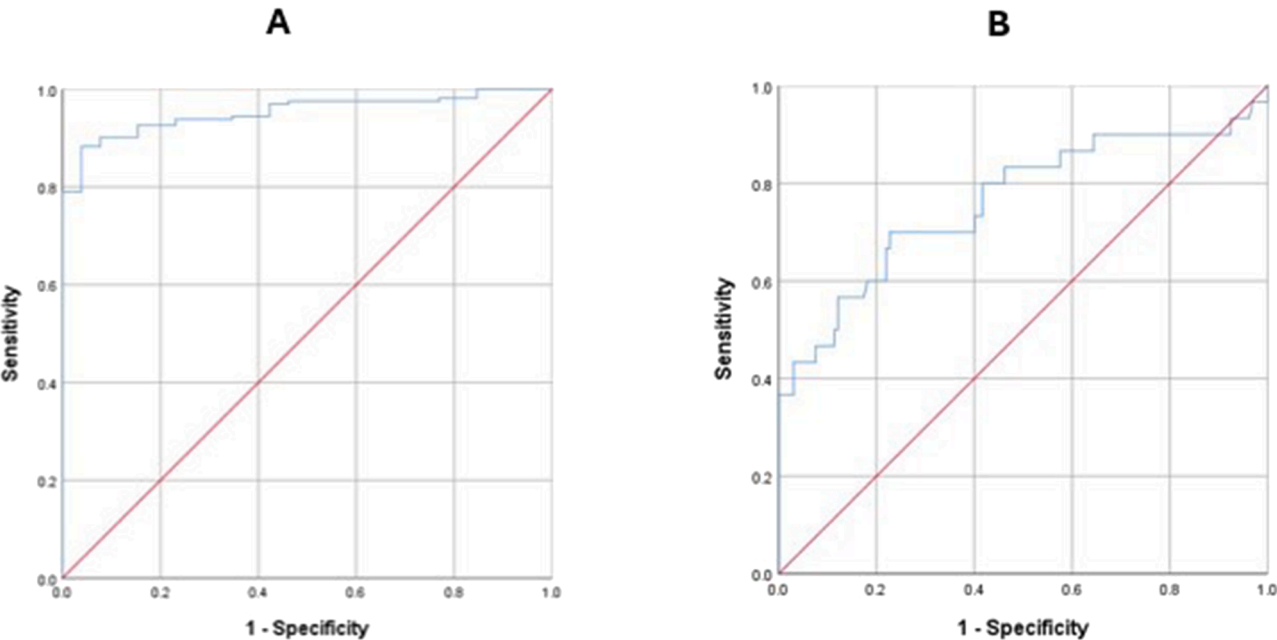


Fig. 2. Receiver-operating characteristic curve (ROC) of sNFL.
A: The ROC curve for sNFL distinguishing between MS patients and HCs. The area under the curve (AUC) for serum NFL was 0.95, indicating excellent discrimination ability of sNFL levels between cases and controls (95 % CI: 0.923 –0.982). The optimal cutoff point for sNFL to distinguish between cases and controls is 23.25. At this cutoff point, the specificity was 92 % and the sensitivity was 90 %.
B: The ROC curve for sNFL distinguishing between progressive and non-progressive MS. The area under the curve (AUC) is 0.79, indicating good discrimination ability of sNFL levels between progressive and non-progressive MS (95 % CI: 0.649–0.875). The optimal cutoff point for sNFL to distinguish between progressive and non-progressive MS is 75.48. At this cutoff point, the sensitivity was 70.00 %, and the specificity was 78.30 %.

in patients treated with DMTs for >12 months ($P = 0.006$ and 0.004 , respectively). While the presence of SPMS patients in this group partially explains this, additional factors such as long-term neuroaxonal damage accumulation may contribute. This highlights the neuroprotective limitations of current DMTs, which effectively control inflammation but do not adequately address ongoing degeneration (Amin and Hersh, 2023). DMTs do not typically promote remyelination or repair of existing axonal damage and, therefore, can't balance the underlying neurodegenerative processes causing the sustained high sNFL levels (Piehl, 2021).

A high proportion of patients on first-line therapies was observed in the current study which is reflective of the real-world clinical practice in resource-constrained settings in contrast to the high-income countries, where second-line or high-efficacy DMTs dominate. No significant differences in sNFL levels were observed among patients using different DMTs, even when categorized according to their efficacy. In contrast to other studies that found a significant reduction in sNFL levels in patients treated with more efficacious DMTs (Barrero Hernández et al., 2024; Kapoor et al., 2019; Kuhle et al., 2018, 2019). However, in the current study, all participants were in remission with their DMTs effectively controlling the disease activity, this implied that patients with longer

disease duration often have accumulated significant axonal damage with persistently elevated sNFL levels even with the most efficacious DMTs (Steffen et al., 2023). Moreover, patients with more aggressive disease phenotypes who are receiving the high efficacy DMTs tend to have higher baseline sNFL levels, which remain elevated despite treatment due to the previously accumulated damage (Barrero Hernández et al., 2024).

When the Diagnostic Accuracy of sNFL was evaluated, it was robust, with an area under the curve (AUC) of 0.95 for distinguishing MS cases from controls. The optimal cutoff point of 23.25 pg/ml provided high sensitivity (90 %) and specificity (92 %), demonstrating excellent discrimination ability. This finding corroborates previous studies highlighting sNFL's high diagnostic accuracy in differentiating MS from healthy individuals (Bridel et al., 2021; Thebault et al., 2021). For distinguishing progressive from non-progressive MS, the AUC was 0.79, indicating good discriminative ability. The optimal cutoff point of 75.48 pg/ml provided a sensitivity of 70 % and specificity of 78.3 %. These results suggest that while sNFL is effective in identifying progression, its discriminative power is slightly lower compared to its diagnostic accuracy for MS as a whole. Nonetheless, these findings support sNFL's utility in clinical practice for diagnostic and prognostic purposes

(Barrero Hernández et al., 2024; Satyanarayan et al., 2023).

Multivariate linear regression analysis identified EDSS score as the sole independent predictor of sNFL levels, underscoring the close association between clinical disability and neuroaxonal injury in MS. EDSS scores provide a cumulative measure of disability, capturing the history of disease progression and the extent of irreversible damage. Similarly, sNFL levels indicate ongoing neurodegeneration and axonal injury. The cumulative nature of both measures likely explains the strong correlation between EDSS and sNFL levels (Bittner et al., 2021; Cantó et al., 2019b).

5. Limitations of the study and future directions

The main limitation of our study was the inability to use the single molecule array 'SIMOA' for the measurement of sNFL levels as it is not available in Egypt, and we had to use the less sensitive ELISA technique instead. Moreover, our cut-off levels did not consider confounding factors such as hematic volume; the implementation of these factors and the increase in the number of HCs involved might further tailor the reference levels and increase the robustness of identification of patients with elevated sNFL, possibly allowing stratification of patients having mildly, moderately and highly elevated. Additionally, the relatively small sample size in certain MS subgroups might limit the statistical power and generalizability of these findings therefore, larger, multicenter studies are needed to validate these subgroup comparisons. Lastly, the cross-sectional nature of the study, as well as the lack of correlation with radiological volumetric data. However, to our knowledge this was the first study to investigate the quantification of the underlying neurodegeneration unrelated to disease activity in a real-world sample of Egyptian MS patients.

6. Conclusion

This study provides compelling evidence for the utility of sNFL as a quantitative biomarker for MS in daily practice. Elevated sNFL levels in MS patients without evidence of disease activity and even in the light of long-term DMT use reflect cumulative axonal damage and disability progression. A broader therapeutic approach that includes both anti-inflammatory and neuroprotective strategies will hold promise for better managing neuroaxonal damage and reducing sNFL levels in MS patients.

8. Ethics approval

The study was approved by the Institutional Review Board of the faculty of medicine at Assiut University, Assiut, Egypt (IRB local approval number: 17,300,883). All participants provided written informed consent.

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Data availability

Data can be made available to qualified investigators upon reasonable request to the corresponding author.

CRedit authorship contribution statement

Eman M. khedr: Writing – review & editing, Visualization,

Supervision, Resources, Project administration, Methodology, Conceptualization. **Ahmed Nasreldein:** Writing – review & editing, Formal analysis. **Hussein Bahey El-Deen:** Writing – original draft, Formal analysis, Data curation. **Mohamed A El-Mokhtar:** Resources, Methodology, Investigation. **Doaa M. Mahmoud:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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