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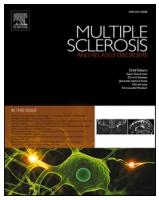

Original article

Real-world analytical and clinical evaluation of the Siemens Atellica® immunoassay for plasma neurofilament light chain in multiple sclerosis

Valerio Nicolella [a,b,*], Carmela Polito [c], Vincenzo Criscuolo [a], Rosa Sirica [c], Mariano Fiorenza [c], Federica Novarella [a], Domenica Sorvillo [c], Antonio Carotenuto [a,d], Maria Petracca [e], Roberta Lanzillo [a,d], Giuseppe Castaldo [f,g], Vincenzo Brescia Morra [a,d], Marcello Moccia [d,f,1], Daniela Terracciano [c,1]

[a] Department of Neuroscience, Reproductive Science and Odontostomatology, Federico II University of Naples, Naples, Italy
[b] Department of Precision Medicine in Medical, Surgical and Critical Care, University of Palermo, Palermo, Italy
[c] Department of Translational Medical Sciences, Federico II University of Naples, Naples, Italy
[d] Multiple Sclerosis Unit, Policlinico Federico II University Hospital, Naples, Italy
[e] Department of Human Neuroscience, Sapienza University of Rome, Rome, Italy
[f] Department of Molecular Medicine and Medical Biotechnology, Federico II University of Naples, Italy
[g] Centre for Advanced Biotechnology (CEINGE), Naples, Italy

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ABSTRACT

Background: Plasma neurofilament light chain (pNfL) is an established biomarker of neuroaxonal injury in multiple sclerosis (MS), but analytical variability across platforms may limit clinical comparability.
Objective: We compared pNfL values obtained using Lumipulse® and Atellica® assays, and evaluated the clinical correlates (associations with disability status, disease activity, MRI outcomes, and treatments) of Atellica® pNfL in a real-world MS cohort.
Methods: Baseline plasma samples from people with MS were analysed using both immunoassays. Agreement was assessed using Passing–Bablok regression, Lin's concordance correlation coefficient, and Bland–Altman analysis. Associations between Atellica® pNfL and demographic and clinical variables were evaluated using multivariable regression.
Results: We included 394 people with MS (mean age 41.0 ± 13.0 years; 61.5% female; median EDSS 2.5). Lumipulse® and Atellica® pNfL values showed strong association, with Passing–Bablok regression indicating proportional bias (slope 0.78; 95% CI 0.70–0.86; intercept 3.24; 95% CI 2.46–3.90) and moderate concordance ($\rho c = 0.49$). Higher Atellica® pNfL levels were independently associated with older age and greater disability, but not with short-term clinical or radiological activity.
Conclusions: Atellica® and Lumipulse® showed strong association but only moderate concordance in pNfL measurements. These findings indicate that the two platforms cannot be considered interchangeable. Atellica® pNfL reflects disability status and represents a clinically meaningful biomarker when interpreted within assay-specific frameworks in real-world MS care.

1. Background

Neurofilament light chain (NfL) is a neuron-specific cytoskeletal protein, that can be measured in blood and acts as a biomarker of neuroaxonal injury in a broad spectrum of neurological disorders (Bar-Or et al., 2025; Khalil et al., 2024; La Civita et al., 2025). In recent years, several immunoassays for the quantification of NfL have been developed (Booth et al., 2025; Nicolella et al., 2024; Ziemssen et al., 2025). However, direct comparisons between assays are limited, and most available studies have been conducted in selected cohorts (e.g., clinical trials with specific age ranges and lack of significant comorbidity burden), leaving important questions about assay comparability and

* Corresponding author.
E-mail address: vnicolella@live.com (V. Nicolella).
[1] Equally contributed

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generalizability unresolved (Andreasson et al., 2023; Ashrafzadeh-Kian et al., 2024).

In multiple sclerosis (MS), NfL offers valuable insights into both disease activity and therapeutic response (Arroyo Pereiro et al., 2024; Barro et al., 2023; Kuhle et al., 2019), thereby providing clinicians with a dynamic measure of disease burden that complements magnetic resonance imaging (MRI) and clinical assessments (Gaetani and Schoonheim, 2022; Williams et al., 2022). Beyond its role in monitoring, NfL has shown promise in guiding treatment decisions, with recent studies indicating that its integration into routine practice may substantially improve individualized management of people living with multiple sclerosis (pwMS) (Freedman et al., 2024; van Lierop et al., 2024). Consequently, harmonization of assay platforms has become crucial, with the establishment of robust and universally accepted conversion standards being essential to guarantee that NfL applications are consistent, reproducible, and clinically meaningful across laboratories and clinical settings (Benkert et al., 2022; Nicolella et al., 2025; Vermunt et al., 2022; Ziemssen et al., 2025).

Recently, the Atellica® IM NfL assay has undergone both analytical and clinical validation for prognostic use in patients with relapsing multiple sclerosis (pwRMS) in routine practice, and has completed conformity assessment for CE marking in accordance with the European Union's in vitro diagnostic (IVD) medical device regulations (Ziemssen et al., 2025). In addition, a plasma NfL (pNfL) threshold has been established as the optimal cutoff for risk stratification of pwRMS based on the risk of active MRI (Ziemssen et al., 2025). However, evidence regarding the use of this platform and cut-off in real-world clinical settings is currently lacking.

Hereby, we aim to compare the performance of the Siemens Healthineers Atellica® IM NfL and the Fujirebio Lumipulse® G NfL blood assays in plasma, and to evaluate demographic and clinical correlates of pNfL levels measured with Atellica® in a real-world cohort of pwMS.

2. Methods

2.1. Study design and population

This is a retrospective analysis of longitudinally collected data conducted at the MS Clinical Unit of the Federico II Policlinico University Hospital, Naples, Italy. The study was approved by the Campania-3 Ethics Committee (158/25). All patients signed informed consent authorizing the use of anonymized data in line with data protection regulation (GDPR EU 2016/679). The present study was performed in accordance with good clinical practice and the Declaration of Helsinki.

We included consecutive people aged >18 years old with a diagnosis of MS, from September 2023 to July 2025, regardless of age, Expanded Disability Status Scale (EDSS), or treatment status (Montalban et al., 2025; Nicolella et al., 2024). Patients were asked to participate in the study at their scheduled neurological consultation, and blood was drawn.

2.2. Demographic and clinical variables

At baseline (time of blood drawn for pNfL measurements), we collected the following variables: age, sex, smoking (ever or never smoker), cardiovascular comorbidities (high blood pressure, high cholesterol, diabetes, atrial fibrillation, stroke, coronary disease and/or related medications), MS disease duration (time from reported onset to assessment), expanded disability status scale (EDSS), descriptor of disease progression (relapsing, progressive), current disease modifying treatment (DMT), and duration of current DMT.

For statistical purpose, DMTs were categorised into monoclonal antibodies (alemtuzumab, natalizumab, ocrelizumab, and ofatumumab), oral therapies (cladribine, dimethyl fumarate, fingolimod, siponimod, ozanimod, ponesimod, and teriflunomide), and injectables (peg-interferon beta, interferon beta, and glatiramer acetate) (Henderson et al., 2023).

Clinical relapses were defined as new, worsening, or recurrent neurological symptoms lasting at least 24 h in the absence of infection, fever, or adverse reaction to prescribed medication. MRI activity was defined as new or enlarging T2 lesions and/or T1 gadolinium-enhancing lesions, as documented in the official radiology report and confirmed by a neuroradiologist (Montalban et al., 2025). EDSS progression was defined as an increase of 1.5 points if baseline EDSS was 0, 1 point if baseline EDSS was between 1.0 and 5.5, or 0.5 points if baseline EDSS was > 5.5 (Kalincik et al., 2015). Relapses and active MRI lesions were jointly combined to define evidence of disease activity 2 (EDA-2). EDA-2 status together with EDSS progression were jointly combined to define evidence of disease activity 3 (EDA-3) (Lublin, 2023). Both EDA-2 and EDA-3 status were assessed in the 6 months preceding baseline (time of blood draw for pNfL measurements) and during subsequent clinical visits.

2.3. Neurofilament light chain measurements

Fasting blood samples were obtained on the same day as the clinical assessments. Blood samples were collected by venipuncture in BD Vacutainer™ tubes containing ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant and processed within 2 h of collection. Samples were centrifuged at 2000 × g for 10 min at room temperature to obtain plasma. Immediately after centrifugation, plasma was carefully separated and aliquoted into multiple polypropylene tubes to avoid repeated freeze–thaw cycles.

Separate aliquots were prepared for each analytical platform at the time of initial sample processing. All measurements were performed according to the manufacturers' instructions. Whenever possible, paired plasma aliquots were analysed on the Lumipulse® and Atellica® platforms within the same analytical session. Overall, 312/394 paired samples (79.2%) were measured during the same analytical session. For the remaining 82/394 paired samples (20.8%), measurements were performed in separate analytical sessions after storage at −80 °C, with a median interval of 14 days between the two platform measurements (IQR: 7–28 days; range: 3–61 days). All samples were thawed only once before analysis, and separate aliquots prepared at the time of initial processing were used for each platform in order to minimize pre-analytical variability.

Both assays were performed according to the manufacturers' instructions and under routine clinical laboratory quality assurance procedures. Internal quality controls provided by the manufacturers were run at the beginning of each analytical session to verify assay performance.

The analytical measuring ranges, limits of detection and assay characteristics were consistent with those reported in the manufacturers' documentation and previous validation studies of the Lumipulse® G NfL Blood assay and the Atellica® IM NfL assay (Table 1) (Ashrafzadeh-Kian et al., 2024; Dargvainiene et al., 2025; Furlan et al., 2026; La Civita et al., 2025; Nicolella et al., 2024; Ziemssen et al., 2025).

All measurements were performed in singlicate, reflecting standard routine laboratory practice for automated immunoassays. The laboratory participates in external quality assessment programs for immunoassays and follows standardized procedures for calibration verification, instrument maintenance, and analytical validation.

Classification of pwMS was based on pNfL values and age, using cut-offs suggested by Simren and colleagues (normality thresholds specific for age ranges: 5–<18, 18–<50, 50–<60, 60–<70, and >70 years) and Ziemssen and colleagues (risk cut-offs specific for age range 18–<55) (Simren et al., 2022; Ziemssen et al., 2025). The thresholds proposed by Simrén et al. were originally developed using the Simoa platform; in the present study, they were applied without recalibration to assess their robustness and clinical performance across different analytical platforms in a real-world setting. However, this approach may introduce

Table 1
NfL assays characteristics.
Analytical characteristics were derived from manufacturer-provided documentation, technical specifications, and previously published studies (Ashrafzadeh-Kian et al., 2024; Furlan et al., 2026).

Manufacturer & Analyzer	Assay Type	LOD (pg/mL)	Analytical Measuring Range (pg/mL)	Precision (%CV)	IVD / RUO Status	Matrix
Fujirebio Lumipulse® G1200	CLEIA	3.0	2.0–5000	Intra: 4.1; Inter: 5.5	IVD	Serum, EDTA/Li-hep plasma, CSF
Siemens Atellica® IM	CLIA	1.3	2.5–3000	Intra: 2.1; Inter: 6.9	IVD	Serum, EDTA plasma

some degree of uncertainty in patient classification due to known inter-assay variability.

2.4. Statistical analyses

Mean (and standard deviation) were calculated for age, MS disease duration, follow-up duration, current DMT duration. Median (and range) were calculated for EDSS. Number (and percent) were calculated for sex, smoking status, presence of cardiovascular comorbidities, descriptor of disease progression, current DMT, EDSS progression, and EDA-3.

To compare Siemens Healthineers Atellica® IM Neurofilament Light Chain and Lumipulse® G NfL blood assays, we performed Passing–Bablok regression using Lumipulse® as the independent variable and Atellica® as the dependent variable. Additionally, we used a univariable linear regression model to evaluate the association between pNfL measurements obtained with the two assays. Agreement between the Siemens Atellica® IM and Fujirebio Lumipulse® assays was evaluated using Lin's concordance correlation coefficient together with Bland–Altman limits of agreement. We used the chi-square test and Cohen's kappa statistic to compare the cut-off values proposed by Simrén and colleagues and by Ziemssen and colleagues when applied to both the Atellica® and Lumipulse® assays (Simren et al., 2022; Ziemssen et al., 2025).

Then, we compared the cut-off values proposed by Simrén and colleagues with those proposed by Ziemssen and colleagues in pwMS aged 18–55 years ($N = 292$). We also applied the cut-off values proposed by Ziemssen and colleagues to both the Atellica® and Lumipulse® assays. For each comparison, chi-square tests were used to determine the presence of significant associations, while Cohen's kappa coefficients were calculated to quantify the level of agreement between the cut-off values (Simren et al., 2022; Ziemssen et al., 2025).

A direct Lumipulse®-to-Atellica® conversion equation was derived using paired measurements based on the Passing–Bablok regression model within the observed measurement range.

To evaluate the clinical utility of pNfL measured with Atellica®, we used different univariable linear regression models including each demographic and clinical variable, in turn, as independent variable (age, sex, smoking, presence of cardiovascular comorbidities, MS disease duration, descriptor of disease progression, EDSS, EDA-3 in the previous 6 months, EDA-3 during follow-up, current DMT group, DMT duration), and pNfL (raw) values as the dependent variable. The same models were then run including the full set of covariates (age, sex, disease duration, EDSS, presence of cardiovascular comorbidities, smoking, and descriptor of disease progression). To estimate the impact of pNfL above or below the cut-off value proposed by Ziemssen and colleagues, we used a multivariable Cox regression models including pNfL dichotomized by the proposed cut-off served as the independent variable and, in turn, EDA-2 and EDA-3 status during follow-up as dependent variable. Covariates for the model were age, sex, disease duration, EDSS, presence of cardiovascular comorbidities, smoking, and descriptor of disease progression.

Results were reported as coefficients (Coeff), 95% confidence intervals (95% CI), and p-values, as appropriate. Agreement between assays were assessed using Passing–Bablok regression and Bland–Altman plots, while descriptive statistics and variable distributions were summarized in tables. Statistical analyses were performed using Stata version 15.0 (StataCorp, College Station, TX, USA) and R version 4.6.0 (R Foundation for Statistical Computing, Vienna, Austria) Results were considered statistically significant if $p < 0.05$.

3. Results

We included 394 pwMS (mean age 41.02 ± 12.97 years; 61.54% females), with a mean disease duration of 13.45 ± 10.13 years and a median EDSS of 2.5 (range 1.0–8.0). Demographic, clinical, and treatment variables are reported in Table 2.

3.1. NfL measurements

The mean Lumipulse® pNfL value was 14.28 ± 18.74 pg/mL (range: 2.00–247.21 pg/mL), whereas the mean Atellica® pNfL value was 13.63 ± 10.33 pg/mL (range: 2.90–99.80 pg/mL).

Lumipulse® pNfL and Atellica® pNfL measurements were significantly associated (Coeff = 1.05; 95% CI: 0.90–1.20; $p < 0.01$). Passing–Bablok regression analysis demonstrated a significant relationship between Lumipulse® and Atellica® pNfL measurements, with an estimated slope of 0.78 (95% CI 0.72–0.84) and an intercept of 3.24 (95% CI 2.46–3.90) (Fig. 1A).

Agreement between the Siemens Atellica® IM and Fujirebio Lumipulse® assays was quantified using Lin's concordance correlation coefficient (ρc = 0.49; 95%CI= 0.43–0.55; $p < 0.01$) and Bland–Altman analysis (mean difference = 0.65 pg/mL; limits of agreement: –29.30 to +30.60 pg/mL; $p < 0.01$) (Fig. 1B). Given this systematic method-dependent bias, we derived a directional conversion model to estimate Atellica® values from Lumipulse® measurements: Atellica®

Table 2
Demographic, clinical and treatment features.

	N = 394
Age, years	41.02±12.97
Sex, females (%)	240(61.54%)
Cardiovascular risk factors, n (%)	73(18.53%)
Ever Smoking, n (%)	93(23.60%)
Disease duration, years	13.45±10.13
Follow-up duration, years	0.81±0.71
EDSS, median(range)	2.5(1.0–8.0)
Descriptor of disease progression	
Relapsing	303(76.90%)
Progressive	91(23.10%)
DMT	
Current DMT duration, years	1.98±2.37
DMT group	
No DMT	4(1.02%)
Oral DMT	*242(61.42%)*
Monoclonal antibodies	*143(36.29%)*
Injectable DMTs	*5(1.27%)*
EDA-3 at baseline, n(%)	*12(3.05%)*
EDA-2 at baseline, n(%)	*2(0.51%)*
`*EDA-3 during follow-up, n(%)*	*30(7.62%)*
Relapse (%)	6(1.52%)
MRI Activity(%)	9(2.28%)
Confirmed EDSS Progression(%)	15(3.81%)

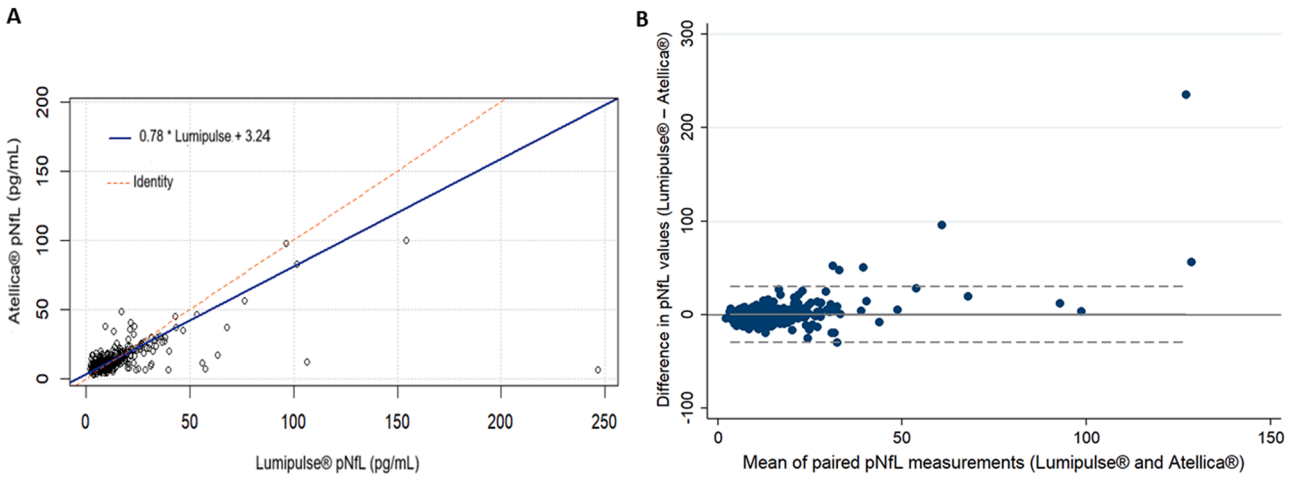

Fig. 1. Plasma neurofilament methods comparison. (A) Passing–Bablok regression comparing Lumipulse® and Atellica® pNfL measurements, with regression line (Atellica = 3.24 + 0.78 × Lumipulse) and line of identity. (B) Bland–Altman plot showing mean difference and limits of agreement between methods.

pNfL = 3.24 + 0.78 × Lumipulse® pNfL.

Classification of pNfL using Lumipulse® and Atellica® immunoassays based on different suggested cut-offs is reported in Table 3. Pearson's chi-square test indicated a significant association between the cut-off values proposed by Simrén and colleagues when applied to both the Atellica® and Lumipulse® assays ($\chi^2(1, N = 394) = 8.45$; $p < 0.01$), while Cohen's kappa indicated moderate agreement between the assays ($\kappa = 0.47$; 95%CI= 0.38–0.55; $p < 0.01$) (Simren et al., 2022).

When looking at pwMS aged 18–55 years ($N = 292$), the chi-square test indicated a significant association between the cut-off values proposed by Simrén and colleagues and Ziemssen and colleagues ($\chi^2(1, N = 292) = 97.24$; $p < 0.01$), while Cohen's kappa indicated moderate agreement between the assays ($\kappa = 0.50$; 95%CI= 0.41–0.59; $p < 0.01$) (5, 22). Also, the chi-square test indicated a significant association between the cut-off values proposed by Ziemssen and colleagues when applied to both the Atellica® and Lumipulse® assays ($\chi^2(1, N = 292) = 41.12$; $p < 0.01$), while Cohen's kappa indicated moderate agreement between the assays ($\kappa = 0.37$; 95%CI= 0.25–0.50; $p < 0.01$).

Table 3
Cross-comparison of pNfL classification across analytical platforms and cut-off definitions.
Sections A and B show inter-assay agreement between Lumipulse® (rows) and Atellica® (columns) using age-adjusted thresholds proposed by Simrén and colleagues and Ziemssen and colleagues(18–55 years), respectively. Section C shows comparison between classifications obtained using Simrén and colleagues and Ziemssen and colleagues cut-offs within the Atellica® assay in patients aged 18–55 years.

A. Inter-assay Simrén and colleagues (Brain Comm 2022)		Atellica®		
Lumipulse®		NfL below	NfL above	Total
	NfL below	184 (46.7%)	52 (13.2%)	236 (59.9%)
	NfL above	65 (16.5%)	93 (23.6%)	158 (40.1%)
	Total	249 (63.2%)	145 (36.8%)	394 (100%)
B. Inter-assay *Ziemssen and colleagues) (18–55y.o.) (MSJ 2025)*		Atellica®		
Lumipulse®		NfL below	NfL above	Total
	NfL below	290 (65.1%)	37 (12.7%)	328 (77.7%)
	NfL above	29 (9.9%)	36 (12.3%)	65 (22.2%)
	Total	219 (75.0%)	73 (25.0%)	292 (100%)
C. Cut-off comparison (Atellica®, 18–55 y.o.)		*Ziemssen and colleagues (MSJ 2025)*		
		Below	*Above*	Total
Simrén and colleagues Brain Comm 2022	Below	157 (53.7%)	70 (24.0%)	227 (77.7%)
	Above	0 (0.0%)	65 (22.3%)	65 (22.3%)
	Total	157 (53.7%)	135 (46.3%)	292 (100%)

3.2. Clinical utility

Looking at demographic and clinical variables, higher pNfL levels measured using Atellica® were associated with older age (Coeff = 0.32; 95%CI= 0.25–0.39; $p < 0.01$), presence of cardiovascular comorbidity (Coeff = 5.80; 95%CI= 3.23–8.37; $p < 0.01$), longer disease duration (Coeff = 0.34; 95% CI= 0.24–0.43; $p < 0.01$), higher EDSS (Coeff = 2.15; 95%CI: 1.60–2.69; $p < 0.01$), and progressive course (Coeff = 6.25; 95%CI= 3.90–8.60; $p < 0.01$). However, we did not find significant associations for EDA-2 and EDA-3 status at baseline (EDA-2 Coeff = −6.91; 95%CI= −21.30, 7.48; $p = 0.346$; EDA-3 Coeff = 3.14; 95%CI= −2.82, 9.09; $p = 0.301$). When including the full set of covariates (age, sex, disease duration, EDSS, cardiovascular comorbidities, smoking status, and descriptors of disease progression), higher pNfL was confirmed to be associated with older age (Coeff = 0.18; 95% CI= 0.06–0.29; $p < 0.01$) and higher EDSS (Coeff = 1.49; 95% CI= 0.55–2.42; $p < 0.01$) (Table 4; Fig. 2A and Fig. 2B).

On Cox regression models, pNfL levels above the cut-off were not associated with an increased risk of subsequent disease activity (EDA-2 HR = 0.47; 95% CI= 0.13, 1.65; $p = 0.238$; EDA-3 HR = 0.99; 95% CI= 0.46, 2.13; $p = 0.986$). This lack of association persisted after adjusting for the full set of covariates, with identical estimates (EDA-2 HR = 0.49; 95% CI 0.12, 1.98; $p = 0.315$; EDA-3 HR = 0.47; 95% CI= 0.18, 1.21; $p = 0.116$) (Fig. 2C and Fig. 2D).

4. Discussion

In our real-world cohort of pwMS, we observed a moderate concordance between pNfL values measured with the Lumipulse® and Atellica® platforms, and confirmed established clinical associations for pNfL also for the Atellica® immunoassay.

Real-world implementation of pNfL requires careful considerations over inter-platform agreement and correlations. Strong correlations have been reported between blood-based NfL measurements across different assay platforms (Andreasson et al., 2023; Ashrafzadeh-Kian et al., 2024; Vecchio et al., 2024). Ashrafzadeh-Kian and colleagues

Table 4
Atellica® pNfL and demographic, clinical and treatment correlates. Coefficients (Coeff), 95% confidence intervals (95% CI), and p-values from linear regression models including, in turn, demographic, clinical, and treatment features as independent variables, and pNfL levels as the dependent variable. In the adjusted models, covariates were age, sex, disease duration, EDSS, cardiovascular comorbidities, smoking status, and descriptors of disease progression. Significant results ($p < 0.05$) are reported in bold.

	Unadjusted models				Adjusted models			
	Coeff	95% CI Lower	Upper	p Value	Coeff	95% CI Lower	Upper	P Value
Age	0.32	0.25	0 .39	<0.01	0.18	0.06	0.29	<0.01
Cardiovascular comorbidity								
yes vs no	5.80	3.23	8.37	<0.01	1.00	−1.73	3.74	0.470
Sex								
Males vs Females	0.05	−2.07	2.18	0.961	0.08	−1.86	2.02	0.934
Disease Duration	0.34	0.24	0.43	<0.01	0.08	−0.05	0.21	0.242
EDSS	2.15	1.60	2.69	<0.01	1.49	0.55	2.42	<0.01
DMT duration	−0.00	−0.02	0.01	0.619	−0.00	−0.02	0.01	0.633
DMT group								
No DMT *(reference)*								
Oral DMTs	−5.67	−15.91	4.57	0.277	−2.68	−12.11	6.74	0.576
Monoclonal antibodies	−5.32	15.62	4.98	0.310	−4.13	−13.60	5.33	0.391
Injectable DMTs	−10.86	−24.84	2.77	0.118	0.391	−19.53	5.42	0.267
Disease course								
progressive vs relapsing	6.25	3.90	8.60	<0.01	−1.09	−5.56	1.61	0.280
EDA2 baseline	−6.91	−21.30	7.48	0.346	−10.15	−23.31	3.01	0.130
EDA3 baseline	3.14	−2.82	9.09	0.301	−2.12	−7.88	3.65	0.471
EDA3 follow-up	−2.21	−7.39	2.97	0.403	−1.59	−6.50	3.32	0.525
EDA2 follow-up	−0.06	−3.92	3.80	0.977	−2.38	−6.07	1.32	0.207

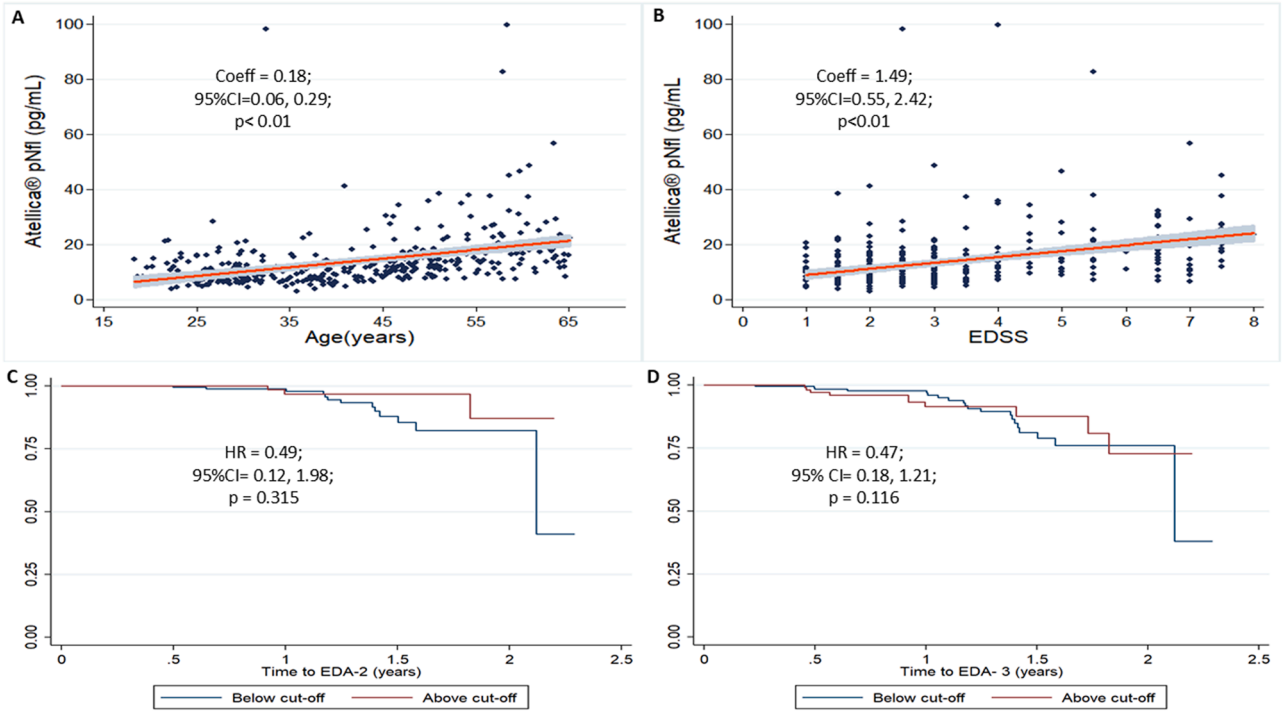

Fig. 2. Clinical utility of Atellica® pNfL. Scatterplots (grey shades represent confidence intervals) show the associations between plasma neurofilament light chain (pNfL) levels and age (A) and EDSS (B). Kaplan–Meier survival curves illustrate the association between pNfL levels above the proposed cut-off and the occurrence of subsequent radiological or clinical disease activity. (EDA-2) (C), and subsequent disease activity (EDA-3) (D). Coefficients (Coeff), Hazard Ratio (HR), 95% confidence intervals (95% CI), and p-values are presented for significant and non-significant associations.

showed a robust correlation between Lumipulse® and Atellica® pNfL levels in a cohort of patients with amyotrophic lateral sclerosis (ALS) and MS (Ashrafzadeh-Kian et al., 2024). Consistent with these findings, we observed a significant association between pNfL values measured with the Lumipulse® and Atellica® platforms. However, the linear and Passing-Bablok equations derived in the two studies differ, largely reflecting differences in the underlying patient cohorts. In line with these differences, we derived an exploratory cross-platform conversion equation based on Passing–Bablok regression (Atellica® = 3.24 + 0.78 × Lumipulse®), reflecting the presence of proportional bias between assays. Despite the numerical divergence between the two equations, the Atellica® assay demonstrated robust analytical performance in capturing clinical aspects of neuroaxonal injury in MS, independently of potential confounders. In addition, the concordance coefficient was moderate (ρc = 0.49) and variability remained substantial (–29.30 to +30.60 pg/mL). The relatively wide

limits of agreement observed in the Bland–Altman analysis are likely to be clinically relevant, particularly in the context of the pNfL concentrations observed in our cohort, as these differences may influence patient classification and interpretation at commonly used clinical thresholds. Proportional bias was also observed, as indicated by a Passing–Bablok slope significantly different from 1 and a positive correlation between inter-assay differences and mean values in the Bland–Altman analysis, suggesting that uncertainty would be mostly around clinical thresholds, rather than in pNfL values significantly above or below normality levels.

To further evaluate agreement, Cohen's kappa statistic was applied to assess the concordance of the cut-off values proposed by Simrén and colleagues when implemented across both assays (Simren et al., 2022). The moderate agreement observed ($\kappa = 0.47$) suggests that, while these thresholds capture comparable trends in pNfL classification, differences persist that may limit their direct interchangeability. The observed level of concordance can be considered moderate and likely reflects intrinsic differences in assay calibration, dynamic range, and signal amplification, which are well documented across blood NfL platforms (Sheth et al., 2025). Importantly, both assays showed highly consistent clinical associations, supporting their clinical validity when used within assay-specific interpretative frameworks. In particular, looking at clinical associations, disability status was the strongest correlate of Atellica® pNfL. While pNfL is conventionally related to relapsing activity, in our population (long disease duration and utilization of high-effective treatments) pNfL might as well reflect progressive status (Willard et al., 2025).

Our aim was not to propose novel cut-off values, but to test the real-world performance and cross-platform comparability of currently available, clinically validated thresholds. Considering the analytical characteristics of the two platforms Lumipulse® (a highly sensitive CLEIA system with flexible mono-test cartridges) and Atellica® (high-throughput modular platform with advanced automation), the observed moderate agreement indicates that age-adjusted cut-offs capture similar biological patterns while still being influenced by platform-specific analytical features. This finding reinforces the need for harmonization strategies rather than direct interchangeability of absolute values. Nevertheless, the overall consistency supports the integration of the Atellica® assay into routine practice (Freedman et al., 2024). From a translational perspective, the Atellica® platform offers the advantage of full automation and high-throughput integration within routine laboratory workflows, facilitating large-scale implementation of blood NfL testing in clinical neurology.

In the validation work of the Atellica® assay, Ziemssen and colleagues established clinical utility of pNfL in MS, in particular for prediction of active MRI (Ziemssen et al., 2025). In our study, we found significant associations between Atellica® pNfL levels and disability status, as measured by EDSS. However, we did not observe higher pNfL concentrations in patients with evidence of disease activity (EDA-3 nor EDA-2) during the previous 6 months, a time that would fall within the predicted half-life of blood NfL, nor in relation to subsequent clinical activity (Thebault et al., 2021; Vermunt et al., 2022). Blood NfL primarily reflects recent neuroaxonal injury and shows its strongest prognostic value within 6–12 months (Benkert et al., 2022; Ziemssen et al., 2025). With a mean follow-up of 0.81 years, our timeframe was broadly aligned with this window. However, our population is rather heterogeneous with relatively few patients reaching clinical and radiological outcomes (3.05%), with subsequently low statistical power to detect associations with future disease activity. Accordingly, our findings should be interpreted as an absence of detectable association under the present study design and population, rather than as evidence of lack of clinical utility. Moreover, our MS population was older than that examined by Ziemssen and colleagues, and this difference in age distribution may have contributed to the variability observed in circulating NfL levels, as age is a well-recognized determinant of blood NfL levels, reflecting cumulative neuroaxonal injury as well as comorbid conditions that increase with aging (Khalil et al., 2020; Sastre-Garriga et al., 2025; Ziemssen et al., 2025). Importantly, Ziemssen and colleagues did not account for demographic variables such as cardiovascular comorbidities, which are known to influence NfL values and may act as confounders (Fitzgerald et al., 2022; Nicolella et al., 2025).

Limitations of our study include the fact that we enrolled pwMS predominantly treated with moderate-to-high efficacy DMTs, which may have influenced both circulating pNfL levels and clinical outcomes (Foong et al., 2025). Also, previous studies have been conducted in heterogeneous clinical contexts. Simrén and colleagues focused on case–control discrimination, whereas Ziemssen and colleagues evaluated pNfL primarily as a marker of disease activity (Simren et al., 2022; Ziemssen et al., 2025). These differences in study design and intended clinical use limit the direct comparability with our real-world MS cohort and may influence the generalizability of cross-platform performance. In addition, our analyses relied on a single baseline pNfL measurement, while longitudinal intra-individual changes are known to provide greater sensitivity for detecting disease activity (Benkert et al., 2022). Our analyses were not extended to additional NfL cut-offs proposed in the literature, as these thresholds have already been evaluated in previous studies (Nicolella et al., 2025; Ziemssen et al., 2025). Body mass index (BMI) is a recognized determinant of circulating NfL concentrations (Benkert et al., 2022). Beyond reflecting metabolic and inflammatory status, BMI may also influence the volume of distribution and thereby affect measured NfL levels (Benkert et al., 2022; Beydoun et al., 2022; Nicolella et al., 2024). Because BMI could not be obtained consistently in real-world clinical practice, it was not included in the present analysis. This absence represents a limitation, as residual confounding cannot be fully excluded. Nonetheless, findings from Søndergaard and colleagues indicate that, despite the correlation between NfL and BMI, age-based correction alone is often sufficient for clinical use, supporting the robustness of our approach when BMI data are unavailable (Søndergaard et al., 2024). Moreover, the consecutive inclusion of individuals with MS resulted in a highly heterogeneous cohort with respect to age, disease phenotype, disability level, and treatment status, thereby limiting the ability to derive clinically generalizable conclusions (Van Lierop et al., 2024). This variability may also contribute to the lack of association between pNfL levels above the cut-off and the risk of subsequent disease activity. However, the inclusion of older patients and individuals with comorbidities reflects the clinical complexity of routine MS care (Sastre-Garriga et al., 2025). This approach allowed us to confirm that age and disability remain the dominant determinants of pNfL levels even after adjusting for cardiovascular risk factors and treatment exposure, supporting the robustness of Atellica® pNfL in real-world settings (Fitzgerald et al., 2022; Vermunt et al., 2022). In addition, not all patients had MRI, further weakening the strength of the associations between MRI measures and circulating NfL concentrations (Gaetani and Schoonheim, 2022; Williams et al., 2022). Also, the cut-offs and decision thresholds applied in this study were originally developed on analytical platforms different from the one used for our measurements, and, in particular, the age-adjusted cut-offs proposed by Simrén and colleagues were established for the Simoa platform (Simren et al., 2022; Ziemssen et al., 2025). Despite evidence from recent analytical comparisons showed that Atellica and Simoa exhibit strong correlation, absolute NfL concentrations may differ between these platforms due to different calibration and assay designs (Andreasson et al., 2023; Ashrafzadeh-Kian et al., 2024). As a result, thresholds developed for Simoa may not translate seamlessly to Atellica and could introduce some uncertainty in interpretation. Applying these thresholds without recalibration may result in some degree of uncertainty in patient classification, particularly at the individual level (Andreasson et al., 2023; Sotirchos et al., 2023).

Although preanalytical handling was standardized and separate aliquots were used for each platform, approximately 20% of paired samples were not analysed during the same analytical session. Therefore, we cannot completely exclude that analytical timing, calibration status,

reagent lots, or session-related variability may have contributed, at least in part, to the observed inter-platform discordance and to the width of the Bland–Altman limits of agreement. However, our findings are consistent with previous head-to-head comparisons of blood NfL immunoassays, which reported strong correlations but non-negligible differences in absolute concentrations across analytical platforms, supporting the need for platform-specific interpretation and harmonization strategies (Ashrafzadeh-Kian et al., 2024).

Another important limitation concerns the derivation of a cross-platform conversion equation. The moderate concordance and relatively wide limits of agreement suggest that the two assays cannot be considered fully interchangeable. Therefore, application of this conversion at the individual patient level should be approached with caution. We propose interpreting this equation as an exploratory conversion model, useful for describing the relative behavior of the two platforms within our dataset, but not yet sufficient to ensure clinical interchangeability. This interpretation is consistent with the existing literature, which highlights several technical differences inherent to assay architecture that limit direct comparability across platforms (Andreasson et al., 2023; Booth et al., 2025).

In conclusion, Atellica® pNfL showed robust analytical performance and consistent clinical associations in our cohort. However, the moderate agreement with Lumipulse® measurements and the limited predictive value of age-adjusted cut-offs observed in this study indicate that cross-platform interchangeability cannot be assumed. These findings support the need for further harmonization and validation, and suggest that pNfL values should be interpreted within assay-specific and clinically contextualized frameworks.

CRediT authorship contribution statement

Valerio Nicolella: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. **Carmela Polito:** Data curation, Investigation, Methodology, Writing – original draft. **Vincenzo Criscuolo:** Investigation, Methodology, Writing – original draft. **Rosa Sirica:** Investigation, Methodology, Writing – original draft. **Mariano Fiorenza:** Investigation, Methodology, Writing – original draft. **Federica Novarella:** Investigation, Methodology, Writing – original draft. **Domenica Sorvillo:** Methodology, Writing – original draft. **Antonio Carotenuto:** Supervision, Visualization, Writing – review & editing. **Maria Petracca:** Supervision, Validation, Writing – review & editing. **Roberta Lanzillo:** Supervision, Validation, Writing – review & editing. **Giuseppe Castaldo:** Resources, Supervision, Writing – review & editing. **Vincenzo Brescia Morra:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Marcello Moccia:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Software, Visualization, Writing – review & editing. **Daniela Terracciano:** Conceptualization, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

Authors declare no potential conflict of interest in relation to this manuscript.

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Data availability

Data are available upon reasonable request to the corresponding author.

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