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Neurofilament light chain and Alzheimer pathology biomarkers in elderly people with multiple sclerosis

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ABSTRACT

Objectives: Neurofilament light chain (NfL) conventionally reflects disability worsening in multiple sclerosis (MS), but does not necessarily imply MS-specific mechanisms and could be affected by biological or pathological aging, including Alzheimer disease (AD) pathology. We aim to evaluate clinical correlates of plasma NfL (pNfL) in elderly patients with MS, and its associations with AD plasma biomarkers.
Methods: We recruited consecutive people with MS >65 years, and collected expanded disability status scale (EDSS), current disease modifying treatment (DMT) and years of exposure to DMTs. Cognitive function was assessed using the Symbol Digit Modalities Test (SDMT), the California Verbal Learning Test-II (CVLT-II), and the Brief Visuospatial Memory Test-Revised (BVMTR). Plasma biomarkers (Aβ40, Aβ42, p-Tau181, and pNfL) were measured using LUMIPULSE assay kits on the CLEIA analyser LUMIPULSE G600II.
Results: We included 83 elderly adults (age 68.8 ± 3.1 years; females 65.8 %) with MS (EDSS 5.5 (2.0–8.0)). On linear regression models adjusted for age, sex, BMI, smoking status, and cardiovascular risk factors, there were no associations between pNfL and EDSS, type and duration of DMT exposure, SDMT, CVLT, and BVMRT. Higher pNfL was associated with lower plasma Aβ40 (Coeff = −0.40; 95 %CI = -0.66, −0.14; $p = 0.003$), plasma Aβ42 (Coeff = −4.46; 95 %CI = -7.92, −1.00; $p = 0.01$), and Aβ42/Aβ40 ratio (Coeff = −2612.06; 95 %CI = -4114.76, −1109.36; $p = 0.001$). Also, lower Aβ42/Aβ40 ratio was associated with impaired CVLT (Coeff = −0.021; 95 % CI = -0.036, −0.006; $p = 0.007$).
Interpretation: pNfL in elderly people with MS is associated with biomarkers of amyloid accumulation, which in turn are associated with cognitive impairments, suggesting overlapping pathologies between MS and AD.

1. Background

Over recent decades, there has been substantial progress in the management of multiple sclerosis (MS), leading to life expectancy nearly equivalent to the general population [1]. As such, aging has become part of MS natural history.

Aging significantly affects the course of MS. Elderly people with MS (PwMS) are more likely to experience disease progression, compared to their younger counterparts [1]. Indeed, as age progresses, the body's ability to repair, remyelinate, and maintain other physiological functions becomes less robust [2] Aging is associated with metabolic changes and related oxidative stress, causing cell death and contributing to

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neurodegeneration [2]. Another feature is inflammaging, characterized by chronic, low-grade systemic inflammation, with an overall increase in pro-inflammatory cytokines and inflammatory markers [3–5]. Along with MS-specific considerations, aging carries additional risks from comorbidities that can affect the health of the central nervous system directly (e.g., Alzheimer disease (AD)) or indirectly (e.g., vascular diseases) [6]. In particular, based on two previous studies using administrative data, AD might be much more common in MS, compared with the general population [7,8].

Measuring neurodegeneration in vivo is nowadays possible using blood-based biomarkers. Neurofilament light chain (NfL) is neuron-specific component of cytoskeletal proteins that is released into the bloodstream following neuroaxonal injury, and can be detected using ultra-sensitive immunoassays. In PwMS, NfL levels are generally interpreted as a marker of inflammatory disease activity, including clinical relapses and active MRI. However, NfL levels are not specific of MS-related pathology, and are expected to increase as a consequence of age-related brain changes and comorbidities [9,10]. Elevated blood NfL levels have been detected in individuals with subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia, along with changes of other biomarkers specific of AD pathology including p-tau, β-amyloid 42 (Aβ42), and β-amyloid 40 (Aβ40) [11,12].

In our study, we aim to: (1) evaluate the clinical correlates of NfL in PwMS over 65 years of age; (2) assess the possible correlation between NfL and AD pathology biomarkers (p-tau, Aβ42, Aβ40); and (3) define whether AD pathology biomarkers contribute to explain clinical characteristics conventionally thought to be related to MS.

2. Methods

2.1. Study design and population

This is a cross-section study conducted at the MS Clinical Unit of the Federico II University Hospital, Naples, Italy. The study was approved by the Federico II Ethics Committee (332/21). All patients signed informed consent authorizing the use of anonymized data in line with data protection regulation (GDPR EU2016/679). The present study was performed in accordance with good clinical practice and Declaration of Helsinki.

We included consecutive PwMS (from Jul 2023 to Feb 2024) based on the following criteria: 1) diagnosis of MS [13]; 2) age $>$ 65 years (World Health Organisation definition of an elderly person); 3) consent to the study. We excluded PwMS if they had: 1) previous diagnosis of dementia; 2) treatment with medications that are primarily prescribed for cognitive impairment (e.g., memantine, rivastigmine).

Patients were asked to participate to the study at their scheduled neurological consultation and blood drawn. Participation to the study did not interfere with the planned clinical assessment, and, thus, only a subset of patients fell within the timeframe for the annual neuropsychological evaluation during the enrolment period.

2.2. Demographic and clinical variables

Demographic and clinical variables were age, sex, height and weight (from which we calculated the body mass index (BMI)), smoking (defined as any regular use of tobacco containing products, and classified into ever or never smoker based on self-reported—i.e., auto-anamnestic—information), cardiovascular comorbidities (high blood pressure, high cholesterol, diabetes, atrial fibrillation, stroke, coronary disease and/or related medications). We further classified patients into normal weight (BMI $<$ 25), overweight (BMI 25–30) and obese (BMI $>$ 30).

MS clinical variables were expanded disability status scale (EDSS), descriptor of disease progression (relapsing, progressive), current disease modifying treatment (DMT), previous DMTs and overall duration of exposure to DMTs. 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). We also collected relapses, EDSS and MRI results from the 12 months before inclusion in the study.

A subset of patients underwent the Brief International Cognitive Assessment for MS (BICAMS) neuropsychological battery, which includes the following tests: the Symbol Digit Modalities Test (SDMT), evaluating attention and information processing speed; the California Verbal Learning Test-II (CVLT-II), evaluating memory and verbal learning; and the Brief Visuospatial Memory Test-Revised (BVMTR), evaluating visuo-spatial learning. Based on Italian age, sex, and education-adjusted references, we defined the presence of impaired or normal results for each test and for any of the BICAMS tests [14,15].

2.3. NfL and AD pathology biomarkers

Fasting blood samples were obtained on the same day of the clinical assessments. Plasma samples were taken at the same time in BD Vacutainer™ anti-coagulated ethylene-diamine-tetra-acetic acid (EDTA).

Blood samples were centrifuged within 3 h after draw at 1100 rpm × 10 min, aliquoted into polypropylene tubes, and stored at −80 °C.

Plasma NfL (pNfL), Aβ40, Aβ42, and p-Tau181 were sequentially measured from the same sample aliquot using Lumipulse™ assay kits (Fujirebio, Tokyo, Japan), on the chemiluminescent enzyme immunoassay (CLEIA) analyser Lumipulse G system (Fujirebio, Tokyo, Japan) (measuring ranges: Aβ40 0.10–5000 ng/L; Aβ42 0.10–1000 ng/L; p-Tau181 0.05–60 ng/L). We have also calculated the Aβ42/Aβ40 ratio, that provides better accuracy towards amyloid accumulation than single biomarkers [16]. For NfL, we calculated age- and BMI– adjusted z scores and percentiles [17].

The Fujirebio Lumipulse® G blood assay is a fully automated CLEIA method for quantifying plasma and serum biomarkers. It uses a two-step sandwich format with proprietary antibodies (details not publicly disclosed). The system supports both simplex and duplicate runs, in batch or random-access mode. We ran the samples in simplex and in a single batch. Analytical characteristics of the assay have been previously reported elsewhere [18].

2.4. Statistical analyses

Mean (and standard deviation) (age, BMI, overall time of exposure to DMTs, SDMT, CVLT, BVMRT, pNfL, Aβ40, Aβ42, p-Tau181, and Aβ42/Aβ40 ratio), median (and range) (EDSS), and number (and percent) (sex, smoking, presence of cardiovascular comorbidities, descriptor of disease progression) were calculated.

To evaluate the associations between pNfL levels in elderly adults with MS ($>$65 years of age) and different clinical variables (descriptor of disease progression, EDSS, type of DMT, duration of exposure to DMTs, impaired SDMT, impaired CVLT, impaired BVMRT, and impairment of any of the BICAMS tests) (aim 1), we used linear regression models including pNfL as dependent variable, and clinical factors, in turn, as independent variables. To evaluate the associations between pNfL and AD pathology biomarkers (Aβ40, Aβ42, p-Tau181, Aβ42/Aβ40 ratio) (aim 2), we used linear regression models including pNfL as the dependent variable, and other biomarkers, in turn, as independent variables. Finally, to evaluate the associations between AD pathology biomarkers and clinical variables (aim 3), we used linear regression models and preferred the use of Aβ42/Aβ40 ratio (dependent variable) due to its much accurate diagnostic properties.

Covariates were age, sex, BMI categories, smoking, and cardiovascular comorbidities. [9] Results were presented as coefficients (Coeff), 95 % confidence intervals (95 %CI), and p-values, with statistical significance set at $p < 0.05$. Variables' and residuals' distributions were assessed through graphical and statistical methods. All analyses were

conducted using Stata 15.0 (StataCorp, College Station, TX, USA).

3. Results

We included 82 PwMS (age 68.9 ± 3.2 years; 65.8 % females), with median EDSS of 5.5 (ranging from 2.0 to 8.0). Out of 82 patients, BMI was available for 61, and cognitive test scores for 31. No one had relapses, new MRI lesions or EDSS progression in the previous year. Demographic, clinical, treatment and laboratory variables are reported in Table 1.

On linear regression models adjusted for age, sex, BMI, smoking status, and cardiovascular risk factors, we found no significant associations between pNfL and descriptors of disease progression (Coeff = −48.67; 95 %CI = -109.42, 12.07; $p = 0.115$), EDSS (Coeff = 1.36; 95 % CI = -19.68, 16.95; $p = 0.883$), type of DMT (injectables Coeff = −42.88; 95 %CI = -226.39, 140.62; $p = 0.643$; oral DMTs Coeff = 11.46; 95 %CI = -159.48, 184.42; $p = 0.885$; monoclonal antibodies Coeff = −30.21; 95 %CI = -199.30, 138.87; $p = 0.723$), duration of exposure to DMTs (Coeff = −0.16; 95 %CI = -0.52, 0.19; $p = 0.36$), impaired SDMT (Coeff = 1.15; 95 %CI = -7.30, 9.60; $p = 0.78$), impaired CVLT (Coeff = −0.51; 95 %CI = -8.75, 7.72; $p = 0.89$), impaired BVMRT (Coeff = 0.91; 95 %CI = -5.19, 7.02; $p = 0.76$), and impairment of any of the BICAMS tests (Coeff = −26.9; 95 %CI = -110.949, 57.11; $p = 0.51$).

On linear regression models adjusted for age, sex, BMI, smoking status, and cardiovascular risk factors, we found significant associations between higher levels of pNfL and lower levels of plasma Aβ40 (Coeff = −0.40; 95 %CI = -0.66, −0.14; $p = 0.003$) (Fig. 1A) and lower levels of plasma Aβ42 (Coeff = −4.46; 95 %CI = -7.92, −1.00; $p = 0.01$) (Fig. 1B), but no significant association with plasma p-Tau181 (Coeff = 0.36; 95 % CI = -10.94, 11.67; $p = 0.949$) (Fig. 1C). Also, we found significant associations between higher levels of pNfL and lower Aβ42/Aβ40 ratio (Coeff = −2612.06; 95 %CI = -4114.76, −1109.36; $p = 0.001$) (Fig. 1D).

On linear regression models adjusted for age, sex, BMI, smoking status, and cardiovascular risk factors, we found lower Aβ42/Aβ40 ratio in people with impaired CVLT (Coeff = −0.021; 95 %CI = -0.036, −0.006; $p = 0.007$) (Fig. 2A), and with impairment of any of the BICAMS tests (Coeff = −0.021; 95 %CI = -0.036, −0.006; p = 0.007) (Fig. 2B), but not significant associations with descriptor of disease progression (Coeff = 0.004; 95 %CI = -0.004, 0.01; $p = 0.35$), EDSS (Coeff = 0.0005; 95 %CI = -0.002, 0.003; $p = 0.71$), type of DMT (injectables Coeff = −0.01; 95 %CI = -0.03, 0.01; $p = 0.410$; oral DMTs Coeff = −0.006; 95 %CI = -0.03, 0.01; $p = 0.642$; monoclonal antibodies Coeff = −0.003; 95 %CI = -0.02, 0.02; $p = 0.755$), duration of exposure to DMTs (Coeff = −0.00002; 95 %CI = -0.00008, 0.00002; $p = 0.332$), impaired SDMT (Coeff = 0.0003; 95 %CI = -0.0007, 0.001; p = 0.71), and impaired BVMRT (Coeff = −0.002; 95 %CI = -0.026, 0.022; $p = 0.849$).

Table 1
Demographic, clinical, treatment and laboratory variables.

	N	
Demographic variables		
Age, years	82	68.9 ± 3.2
Sex, females	82	54 (65.8 %)
Cardiovascular disease	82	59 (71.9 %)
Ever Smoking	82	16 (19–5 %)
BMI	61	26.7 ± 3.7
Normal weight (%)		34.40 %
Overweight (%)		45.90 %
Obese (%)		19.70 %
MS clinical variables		
Disease duration, years	82	19.2 ± 11.6
EDSS, median (range)	82	5.5 (2–8)
Descriptor of disease progression	82	
Relapsing		21 (25.6 %)
Progressive		61 (74.4 %)
SDMT	31	44.4 ± 9.7
SDMT impairment		3 (9.7 %)
CVLT	31	35.4 ± 8.9
CVLT impairment		19 (61.3 %)
BVMRT	31	46.6 ± 12
BVMRT impairment		5 (16.0 %)
Treatment variables		
DMT	82	
No DMT		2 (2.4 %)
Oral DMT		25 (30.5 %)
Monoclonal antibodies		45 (54.9 %)
Injectable DMTs		10 (12.2 %)
Duration of exposure to DMTs, years	82	14.7 ± 7.2
Laboratory variables		
pNfL, pg/ml	82	33.59 ± 116.94
pNfL >90th percentile derived from z scores	61	15 (24.5 %)
p-Tau181, pg/ml	82	2.72 ± 2.37
Aβ40, pg/ml	82	334.98 ± 99.32
Aβ42, pg/ml	82	21.72 ± 7.28
Aβ42/ Aβ40 ratio	82	0.06 ± 0.01

4. Discussion

In our cohort of elderly people with MS, there is high prevalence of memory deficits, as measured by the CVLT (impaired in 61.3 %), compared with deficits in attention processing speed, assessed with the SDMT (impaired in 9.7 %). These results align with previous findings suggesting that cognitive alterations in MS may change with age and disease duration, progressively involving domains affected by aging, such as memory, rather than those traditionally associated with MS, such as processing speed [19–21]. This shift in cognitive impairment characteristics suggests a complex interplay between MS pathology and age-related neurodegenerative changes [2]. Furthermore, the lack of association between pNfL levels and conventional MS-related clinical variables implies that, in elderly PwMS, neuroaxonal loss may not be driven solely by MS activity, but may also be influenced by concurrent neurodegenerative changes, including AD. In keep with this, our study showed that in people with MS above 65 years of age, neuroaxonal loss (i.e., higher levels of pNfL) was associated with evidence of amyloid accumulation (i.e., low levels of Aβ42 and Aβ40, and low Aβ42/Aβ40 ratio), which was in turn associated with memory impairments.

Previous studies have shown that, in PwMS in younger age spans, elevated levels of serum NfL are strongly associated with both clinical and radiological activity, with peaks observed during relapses [22]. High serum NfL levels may also serve as a predictive marker for disease progression, including the transition from relapsing to progressive phases of MS [23]. Additionally, elevated pNfL levels have been linked to structural MRI changes, such as reductions in brain and spinal cord volume, underscoring its potential as a biomarker for tracking overall neuroaxonal loss in MS [24]. Monitoring NfL levels during therapy could, therefore, help assess treatment efficacy [25,26]. However, evidence on NfL has accumulated mostly from clinical trials with stringent age ranges, inclusion criteria (e.g., active disease) and exclusion of comorbidities, and from real-world studies only minimally including elderly people with MS. Intriguingly, our population of elderly PwMS had no relapses and MRI activity in the year before the assessment, and, thus, reflects a clinically realistic MS population in terms of inflammatory activity. As such, understanding the utility of NfL in different age ranges, and independently of disease activity, is essential to ensure the reliable application of NfL across all age groups, thus optimizing clinical management also for elderly PwMS [27]. Age appears to be an important factor affecting NfL levels, as shown by the correlation between NfL levels and age [28]. This relationship may be attributed to the increased prevalence of comorbidities, particularly cardiovascular conditions, in older populations, which contributes to neurodegenerative changes and vascular damage, potentially elevating NfL levels. It is therefore crucial differentiating NfL elevations attributable to MS pathology, from concomitant conditions.

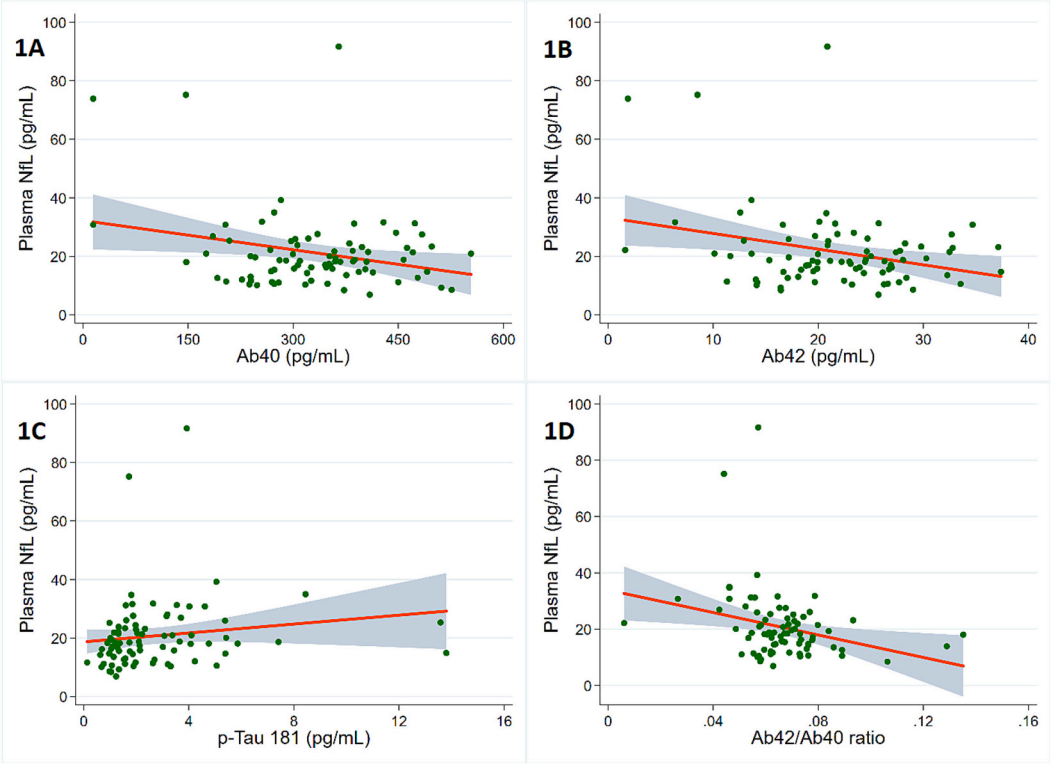

Fig. 1. Scatter plots showing associations between pNfL and AD pathology biomarkers.

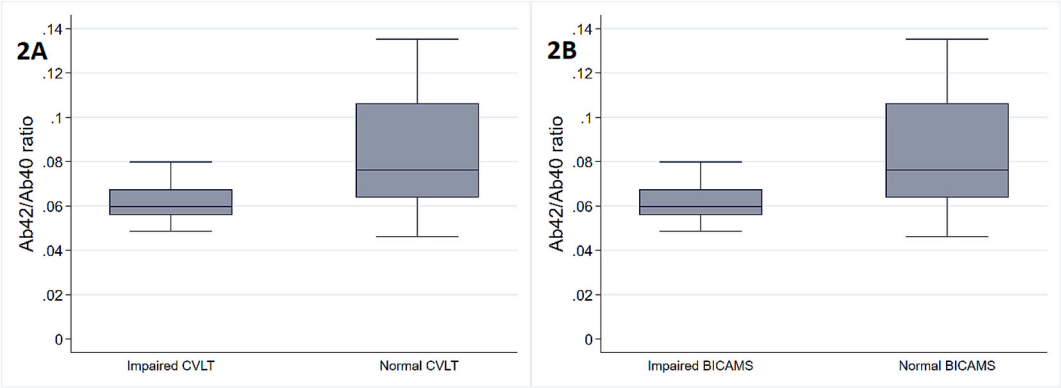

Fig. 2. Box-and-whisker plots showing associations between AD pathology biomarkers and neuropsychological tests.

Our findings reveal a significant association between elevated pNfL levels and evidence of amyloid accumulation (low levels of Aβ42 and Aβ40, and low Aβ42/Aβ40 ratio). In particular, elevated pNfL was associated with lower Aβ40 and Aβ42. Interestingly, reduced CSF levels of Aβ can occur as a consequence of altered amyloid metabolism in MS lesions with active inflammation and demyelination, and, accordingly, have been associated with more severe disease progression and accelerated brain atrophy in MS [29–31]. However, this does not seem our case, considering that none of our patients had recent relapses, new MRI lesions or EDSS progression in the previous year. Also, the association between high pNfL levels and low Aβ42/Aβ40 ratio further supports the presence of pathological amyloid accumulation (i.e., Aβ42 is prone to aggregation), suggesting this is a driver of neuroaxonal injury in elderly people with MS [32]. Nevertheless, Brier and colleagues recently showed low rates of amyloid accumulation in elderly PwMS compared with controls. Authors suggested that genetic or biological factors associated with MS might confer a degree of protection against the development of amyloid pathology, highlighting a complex interaction between MS and AD-related mechanisms [33]. Additionally, Brier and colleagues hypothesized that exposure to DMTs might play a protective role by modulating immune mechanisms involved in amyloid aggregation [33]. However, the latter does not seem confirmed by our study, where we did not find associations between AD pathology biomarkers and exposure to DMTs. Anyway, we did not assess rates of amyloid accumulation, nor included controls, but evaluated clinical correlates of amyloid pathology biomarkers. Also, we excluded PwMS with dementia and, indeed, we did not find an association between pNfL and p-Tau181, (tau pathology typically emerges in later stages of neurodegeneration, while amyloid accumulation is often the earliest event) [34], and, thus, our results reflect a different population from Brier and colleagues. More importantly, we did not assess fulfillment of AD criteria, but rather evaluated AD pathology biomarkers; future studies should consider the inclusion of amyloid PET imaging for ATN classification [35].

While our study provides some useful information, it is important to

acknowledge several limitations. Larger sample size with full neuropsychological testing and longitudinal design would be needed to establish causal relationships. The long-term implications — such as whether amyloid accumulation contributes to the development of clinical AD or affects MS progression — remain speculative and require further investigation. Also, our study is limited by the use of plasma biomarkers (that are less sensitive than CSF), and by the recruitment in a MS clinical centre (thus biasing the population towards treated and less disabled patients). Newer blood-based biomarkers (e.g., p-Tau 217) are definitely more specific and should be considered in the future. [36,37] Additionally, the impact of DMTs on amyloid accumulation, particularly through their influence on the inflammatory processes of MS [38], warrants further research in the future and might have biased the results. Moreover, additional variables, including BMI, smoking and MRI data with burden of cerebrovascular disease, were not systematically collected, but would have been a valuable addition to better characterize the relationships between vascular conditions and biomarkers. Smoking may be underestimated because based on autoanamnestic information.

In conclusion, our study focused on clinical aspects of elderly people with MS, and showed that cognitive changes have a different profile, when compared with younger patients, that might be related to concomitant amyloid accumulation and related neurodegeneration. Given the complexity of neurodegenerative processes in older adults, the use of NfL as a biomarker in elderly people with MS requires careful consideration. Our results have significant implications for the interpretation of clinical and laboratory results in elderly PwMS, and deserve longitudinal extension.

CRediT authorship contribution statement

Federica Novarella: Writing – original draft. **Valerio Nicolella:** Investigation. **Mariano Fiorenza:** Methodology. **Fabrizia Falco:** Conceptualization. **Isabel Monteiro:** Investigation. **Giuseppe Corsini:** Investigation. **Davide Ranucci:** Investigation. **Antonio Carotenuto:** Writing – review & editing. **Maria Petracca:** Writing – review & editing. **Roberta Lanzillo:** Writing – review & editing. **Elena Salvatore:** Investigation. **Giuseppe Castaldo:** Methodology. **Vincenzo Brescia Morra:** Supervision. **Daniela Terracciano:** Investigation. **Marcello Moccia:** Project administration.

Declaration of competing interest

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

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Data availability

Data is available upon request to the corresponding author.

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