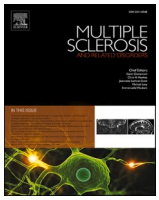
Multiple Sclerosis and Related Disorders 88 (2024) 105748

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard



Original article

Prevalence of elevated sNFL in a real-world setting: Results on 908 patients with different multiple sclerosis types and treatment conditions

Cecilia Irene Bava [a,b,c,*], Paola Valentino [a,1], Simona Malucchi [c], Rugiada Bottero [c], Serena Martire [b,d], Alessia Di Sapio [b,c], Antonio Bertolotto [a]

[a] NICO - Neuroscience Institute Cavalieri Ottolenghi, Orbassano, Italy
[b] CRESM Biobank, University Hospital San Luigi Gonzaga, Orbassano, Italy
[c] Department of Neurology and CRESM, University Hospital San Luigi Gonzaga, Orbassano, Italy
[d] Department of Neuroscience "Rita Levi Montalcini", University of Turin, Italy

ARTICLE INFO

Keywords:
Multiple sclerosis
Neurofilament-light chain
Real-world
NFL
Treatment

ABSTRACT

Background: In the field of research for new validated surrogate biomarkers of treatment efficacy, disease activity and progression in Multiple Sclerosis (MS), serum neurofilament light-chain (sNFL) are actually the best candidate for MS patient monitoring. However, before they can be implemented in clinical practice, their usefulness as additional red flag routine measure must be demonstrated. To tackle the problem, this real-life cross-sectional study at the Regional Referring Center for Multiple Sclerosis (CRESM) aims to characterize sNFL levels and prevalence of elevated sNFL, according to our age-dependent cut-off values, in a large group of patients with different types of MS and treatment conditions.

Methods: 908 serum samples from as many MS patients being admitted at CRESM for diagnostic definition and/or during routinary treatment monitoring were consecutively collected between January 2019 and January 2020. sNFL levels were measured by single molecule array (Simoa™) technology on SR-X instrument using NF-light assays (Quanterix); results were interpreted using previously published cut-off values.

Results: Primary and Secondary Progressive MS (PPMS, SPMS) forms demonstrate higher levels and prevalence of elevated sNFL (PPMS= 32 %, SPMS= 21 %) compared to the Relapse and Remitting one (RRMS = 12 %). Besides, naïve samples of RRMS and PPMS subtypes showed higher prevalence of elevated sNFL (RRMS naïve= 31 %, PPMS naïve=67 %) compared to samples from patients treated for more than 12 months (RRMS treat>12m= 9 %, PPMS treat>12m= 19 %); treated SPMS patients demonstrated higher sNFL levels and a prevalence (22 %) of elevated sNFL compared to RRMS treated patients. Focusing on RRMS, no statistical difference was found between groups of patients treated for whatever time (up to or more than 60 months) and with either DMT type (high or low-efficacy DMT). Finally, RRMS patients treated with all DMTs for more than 12 months, with the exception of teriflunomide and alemtuzumab showed a prevalence of elevated sNFL in the range of 5–10 %.

Conclusion: in a real-world setting comprising about 1000 MS patients, sNFL quantification was elevated in 5-to-67 % of patients, in different MS forms and treatment conditions. Elevated levels of sNFL must be considered a red-flag suggesting the need of a further clinical monitoring in any circumstance, as it can be indicative of new inflammation, ongoing degeneration or co-morbidities. This study supports the introduction of sNFL quantification in everyday patient management.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune neurodegenerative disease of the central nervous system (CNS) characterized by inflammation, demyelination and neuronal loss (Kölliker Frers et al., 2022; Sotirchos et al., 2023). Traditionally, the clinical course of the disease have been classified in three different forms: Relapse-Remitting MS (RRMS), Primary Progressive MS (PPMS) and Secondary Progressive MS (SPMS), for which different therapeutic approaches are available (Disanto et al., 2017; Kölliker Frers et al., 2022), even if this classification

* Corresponding author.
E-mail address: c.bava@sanluigi.piemonte.it (C.I. Bava).
[1] Dipartimento di Scienze Cliniche e Biologiche, Università degli Studi di Torino, Torino, Italy.

https://doi.org/10.1016/j.msard.2024.105748
Received 29 December 2023; Received in revised form 18 June 2024; Accepted 23 June 2024
Available online 29 June 2024

has recently been called into question by the latest scientific acquisitions in the neuropathological and neuroimaging fields (Granziera et al., 2023; Kuhlmann et al., 2023; Yamamura, 2023).

For all disease modifying therapies (DMTs), the definition of therapeutic response and efficacy are of utmost importance for the identification of (non) responsive patients and their management (Sotirchos et al., 2023). Nowadays, monitoring is mainly performed through magnetic resonance imaging (MRI) and clinical evaluation (Akgün et al., 2019; Hyun et al., 2020; Sotirchos et al., 2023). Both of these reliable approaches are however not able to highlight neuroinflammation and neurodegeneration as a whole or to reflect past events. Moreover, both approaches are difficult to standardize, and MRI is a high-cost procedure and not always readily available (Akgün et al., 2019; Hyun et al., 2020; Kuhle et al., 2019; Thebault et al., 2020).

Thus, several efforts have been made in the field of disease monitoring biomarkers, to longitudinally monitor patients with a more personalized approach, to evaluate subclinical neuro-axonal damage, and to support clinical evaluation (Disanto et al., 2017). Among all, serum neurofilament-light chain (sNFL) is consolidated to be the most promising biomarker for DMTs efficacy (Akgün et al., 2019; Delcoigne et al., 2020; Disanto et al., 2017; Hyun et al., 2020; Ning and Wang, 2022; Novakova et al., 2017; Thebault et al., 2020; Valentino et al., 2021; Varhaug et al., 2019).

Particularly, sNFL correlates with age and its levels are higher in MS patients than in healthy controls, especially in Progressive MS (PMS) forms. Also, sNFL is known to lower in patients treated with DMTs (Disanto et al., 2017; Kölliker Frers et al., 2022; Kuhle et al., 2019; Ning and Wang, 2022; Novakova et al., 2017; Piehl et al., 2018; Sotirchos et al., 2023; Thebault et al., 2020).

However, although the correlation between sNFL and therapeutical outcomes is well established at group level, their implementation in clinical practice is the main current need to address. Undoubtedly, reference values are needed to reliably interpret the result at a single-patient level and correctly apply the biomarker in clinical practice. Several research groups, including ours, have elaborated cut-off values taking into account also confounding factors, such as age and body mass index (BMI) (Benkert et al., 2022; Disanto et al., 2017; Hviid et al., 2020; Hyun et al., 2020; Manouchehrinia et al., 2020; Ning and Wang, 2022; Valentino et al., 2021; Vermunt et al., 2022). Furthermore, several efforts are still needed for large-cohort real-life studies, multicentric validation and standardization between different laboratories (Bittner et al., 2021; Sen et al., 2023; Thebault et al., 2020).

In the Regional Referring Center for Multiple Sclerosis (CRESM; Piedmont, Italy) we were able to address the current issues in the field thanks to the previously elaborated normative values and the elevated number of patients (more than 2000) that regularly come to our Center for check-ups and therapy administration.

In this context, we implemented sNFL quantification as an additional clinical practice measure testing 908 MS patients in order to evaluate the use of sNFL as additional red-flag measure during routine monitoring and management of a large cohort, to understand whether a single sNFL determination could reveal in which patients could a serial sNFL monitoring in clinical practice be useful, also considering long-time stable patients and those treated with high-efficacy DMTs.

Specifically, the objective of this real-life cross-sectional study was to characterize sNFL levels and the prevalence of elevated sNFL concentration, according to our age-dependent cut-off values (Valentino et al., 2021), in groups of patients categorized according to different types of MS (RR, PP and SP) and treatment condition (treated or not, type and time of treatment).

2. Methods

2.1. Participants and samples

2.1.1. MS patients

The present real-life cross-sectional study was conducted at the Regional Referring Center for MS (CRESM).

908 serum samples were consecutively collected between January 2019 and January 2020 from MS patients being admitted at CRESM for diagnostic definition and/or during routine treatment/disease monitoring.

Patient inclusion criteria were: age between 18 and 59 years-old; diagnosis of RRMS, PPMS, SPMS according to revised Mc Donald criteria (Forsberg et al., 2023; Iaffaldano et al., 2022; Thompson et al., 2018); availability of clinical and medical records (DMT and timing), ability to provide informed consent. Exclusion criteria was ongoing pregnancy.

Patient data are summarized in Table 1 and 2.

The cohort comprised 791 RRMS, 47 PPMS and 70 SPMS patients (Table 1).

Out of these, RR/PP-naïve were defined as those patients without previous treatment history, in which blood was taken immediately prior the diagnostic lumbar puncture (LP) or therapy start (RR-naïve $n = 59$; PP-naïve $n = 9$; no SP-naïve).

Patients were also grouped according to their current treatment condition. RR-treat<12 m included RRMS patients treated for less than 12 months (median 6 months, range 1–8 months; $n = 42$). RR/PP/SP-treat≥12 m included patients with RR/PP/SPMS treated with various DMTs for more than 12 months (RR-treat≥12 m, range 11–288 months, $n = 626$; PP-treat≥12 m, range 11–66 months, $n = 32$; SP-treat≥12 m, range 12–128 months, $n = 55$). 12 months is a generally accepted period of time after which we are confident that DMTs has become effective (Giovannoni, 2018; Giovannoni et al., 2015). For Alemtuzumab this period was considered 12 months after the last course of infusions, or 24 months after the start of treatment (Giovannoni, 2018; Giovannoni et al., 2015; Katsavos and Coles, 2018).

The cohort of RR-treat≥12 m patients included the following low-efficacy DMTs (LE-DMT) (Table 2): Interferon-Beta (IFN; $n = 92$); Glatiramer Acetate (GA; $n = 40$); Dimethyl Fumarate (DMF; $n = 130$); Teriflunomide (TERIFL; $n = 43$).

Natalizumab (NAT; $n = 108$); Alemtuzumab (ALEM; $n = 34$); Fingolimod (FING; $n = 110$); anti-CD20 ($n = 66$), Azathioprine ($n = 2$), autologous Hematopoietic Stem Cells Transplant ($n = 1$) were included in the group of high efficacy DMTs (HE-DMT).

Besides, RR-treat ≥12 m were divided for years of treatment and DMT type: more in details, between the first and the fifth year of treatment, (11–60 m; range 11–60 months; LE-DMT $n = 262$; HE-DMT $n = 239$) and after the fifth year (>60 m; ≥61 months; LE-DMT $n = 45$, HE-DMTs $n = 80$).

2.1.2. Healthy participants (HC)

sNFL levels from 73 HCs, previously used to define age-related cut-off values (Valentino et al., 2021), were considered as a control group in this study. Particularly, age-dependent cut-offs were calculated as the [sNFL mean + 3*standard deviation] of each age group, resulting in the ultimate following cut-offs:

- <40 years-old: 10 pg/ml
- 40–49 years-old: 11 pg/ml
- 50–59 years-old: 16 pg/ml

In the same paper, inter-assay variability of the test was evaluated on three serum samples tested in five different assay runs on independent days, resulting in an average coefficient of variation (CV) of 10.2 %. Consequently, borderline levels were adopted to guarantee a conservative approach in daily clinical practice and distinguish clear-positive

Table 1
Patient characteristics, median sNFL (pg/ml) and number / prevalence of elevated sNFL.

	PPMS			SPSM		RRMS							
	TOTAL	*PP-naive*	*PP-treat ≥12m*	*TOTAL*	*SP-treat ≥12m*	*TOTAL*	*RR-naive*	*RR-treat <12 m (1–8 m)*	*RR-treat ≥12 m*	*LE-DMT 11–60m*	*LE-DMT >60m*	*HE-DMT 11–60m*	*HE-DMT >60m*
n samples	47	9	32	70	55	791	59	42	626	262	45	239	80
Age, mean (range), y	50 (34–59)	50 (39–59)	51 (34–59)	49 (27–59)	49 (27–59)	41 (18–59)	38 (18–59)	38 (18–59)	42 (18–59)	43 (19–59)	44 (28–59)	40 (18–58)	42 (18–59)
Sex (M/F), n	25/22	7/2	15/17	26/44	22/33	248/543	24/35	8/34	197/429	91/171	15/30	71/168	21/59
Disease duration median (years), y	7 (1–17)	1 (1–2)	10 (3–17)	15 (1–42)	16 (3–42)	9 (0–45)	1 (0–27)	4 (0–32)	10 (1–45)	9 (1–37)	13 (3–38)	8 (1–45)	17 (5–34)
sNFL median (range), pg/ml	10.8 (3.5 - 77.9)	14.4 (6.7 - 77.9)	10.7 (3.5 - 26.9)	8.3 (2.4 – 37.6)	8.5 (2.9 – 37.6)	6.5 (1.4 – 441.5)	8.5 (3.0 - 441.5)	6.5 (1.6 – 158.4)	6.4 (1.4 - 81.4)	6.6 (1.5 - 36.0)	6.4 (1.3 – 24.7)	6.1 (1.4 –81.4)	6.2 (1.7 – 22.4)
elevated sNFL – n samples (%)	15 (32)	6 (67)	6 (19)	15 (21)	12 (22)	96 (12)	18 (31)	7 (17)	54 (9)	26 (10)	3 (7)	20 (8)	5 (6)

Table 2
RRMS patient treated with different DMTs: characteristics, median sNFL (pg/ml) and number / prevalence of elevated sNFL.

	IFN	GA	DMF	*TERI*	*NAT*	*ALEM*	*FING*	*ANTI-CD20*
n samples	92	40	130	43	108	34	110	66
Age, mean (range), y	40 (19 - 59)	45 (28 - 59)	42 (22 - 59)	48 (35 - 59)	36 (18 - 58)	40 (24 - 56)	42 (19 - 59)	44 (22 - 58)
Sex (M/F), n	33/59	13/27	46/84	15/28	30/78	13/21	30/80	17/49
Disease duration median (range), y	8 (1–38)	11 (3–29)	10 (1–37)	17 (1–33)	8 (1–32)	10 (3–37)	12 (1–34)	13 (2–45)
sNFL median (range), pg/ml	8.5 (3.0 – 441.5)	6.7 (3.3 – 21.3)	6.5 (1.5 – 36.0)	8.7 (3.8 – 26.5)	4.8 (1.4 – 22.4)	6.5 (3.3 – 81.4)	6.5 (1.7 – 20.8)	7.2 (2.3 – 30.5)
elevated sNFL – n samples (%)	9 (10)	2 (5)	13 (10)	5 (12)	6 (6)	6 (18)	7 (6)	6 (9)

values from others that could arise from assay-derived fluctuations. For each sample, we calculated its NFL range between [sNFL value ± 10 % sNFL value]: samples defined as borderline were those who comprised the age-specific cut-off within the calculated range.

Cut-off levels were used to classify patient samples as having "normal" (lower than age-appropriate cut-off), "borderline" (as defined above) and "elevated" (above age-appropriate cut-off) sNFL.

HCs were selected from CRESM Biobank, the institutionalized biobank for MS and other autoimmune neurological diseases at CRESM (San Luigi Hospital deliberation n°56/2020). Inclusion criteria were age between 18 and 59 years-old, and absence of family history and individual's own neurological or autoimmune disease.

2.2. sNFL measurement

Blood samples for both HCs and individuals with MS were collected in serum tubes (BD Vacutainer, Becton, Dickinson and Company) and processed within two hours from collection according to CRESM Biobank standard procedures and international guidelines (Marnetto et al., 2020; Teunissen et al., 2010).

Blood samples were centrifuged at 3000 x g 10 min, and serum supernatant stored at −80 °C in coded aliquots until analysis, to avoid repeated freeze-thaw cycles.

NFL levels were measured by single molecule array (Simoa™) on SR-X instrument (Lambert et al., 2018) using NF-light assays (Quanterix). In each assay session, samples were run in single together with a titration curve and two internal controls provided in the kit, as well as two homemade pooled controls (with high and low titer). Samples were analyzed following manufacturer's instruction. sNFL levels were interpreted according to previously defined age-dependent reference values and inter-assay variability, set at 10 % (Valentino et al., 2021). Particularly, we considered as normal those samples for which NFL quantification range comprising [value ± 10 %value] was all below the specific age-dependent cut-off level; we considered borderline those samples for which NFL quantification range comprising [value ± 10 % value] comprised the specific age-dependent cut-off level; we considered elevated those samples for which NFL quantification range comprising [value ± 10 %value] was all above the specific age-dependent cut-off level.

2.3. Ethical committee approval

The study was approved by the Ethical Committee of San Luigi Gonzaga University Hospital (approvals number 7262/2019 and 18,390/2019). All participants provided written informed consent.

2.4. Statistical analysis

Statistical analysis was performed using Python version 3.11.5. Normality of distribution and homogeneity of variances were evaluated by Shapiro-Wilk test and Levene's test. Kruskal-Wallis test with Dunn Post-hoc test was performed to test differences in sNFL levels between groups. Chi-square test and Fisher's exact test was used to compare categorical variables, as appropriate. p value < 0.05 was considered statistically significant.

3. Results

In this cross-sectional study, we analyzed sNFL from patients categorized by disease type and treatment condition (treated or not, type and timing of treatment). We compared sNFL levels as well as prevalence of pathologically elevated sNFL in each category, considering

previously published age-dependent cut-off values and inter-assay variability (Valentino et al., 2021; see methods Section 2.1.2).

4. sNFL in different MS subtypes

We compared sNFL levels in HC and MS patients according to disease subtype (Fig. 1, Table 1).

At a group level, sNFL levels were higher in all MS subtypes compared to HCs (Kruskal-Wallis test, $p < 0.0001$). Both PP and SP MS patients showed higher levels of sNFL than RRMS patients (Kruskal-Wallis test, $p < 0.0001$). No statistical difference was highlighted between PPMS and SPMS groups.

When comparing each individual value with the respective age-dependent cut-off, the prevalence of elevated sNFL was 12 % in RRMS patients, 32 % in PPMS patients and 21 % in SPMS patients. There was a significantly higher prevalence of elevated sNFL in PPMS compared to RRMS patients (chi-square analysis $p < 0.0001$) and in SPMS compared to RRMS participants (chi-square analysis $p = 0.0219$).

Borderline values were 6 % in RRMS patients and 11 % in both PPMS and SPMS patients.

5. sNFL in different disease types and treatment conditions

We compared sNFL levels in MS patients according to their disease type and treatment condition, namely treatment-naïve patients (RR/PP-naïve) and patients treated for more than 12 months (RR/PP/SP-treat ≥12 m), as described in method section (Fig. 2, Table 1).

In RRMS, sNFL values in naïve patients were significantly higher than in treated patients (Kruskal-Wallis test, $p < 0.0001$). Moreover, RR-treat ≥12 m group showed significantly lower sNFL levels compared to SP-treat≥ 12 m patients (Kruskal-Wallis test, $p < 0.0001$). Similarly, sNFL levels in PP-naïve were higher than in PP-treat ≥12 m, but the difference was not statistically significant.

When considering cut-off values, both naïve groups demonstrated a higher prevalence of elevated sNFL compared to the corresponding treated group (RR-naïve=37 %, RR-treat ≥12m=11 %, chi-square analysis $p < 0.0001$), (PP-naïve=67 %, PP-treat ≥12 $m = 22$ %, Fisher's exact test=0.0362). RR-treat ≥12 m also demonstrated a lower prevalence of elevated sNFL compared to SP-treat ≥12 m patients (25 %, chi-square analysis $p = 0.0009$). Moreover, borderline values were: 8 % in RR-naïve, 6 % in RR-treat ≥12 m, 0 % in PP-naïve, 16 % in PP-treat ≥12 m and 15 % in SP-treat ≥12 m participants.

6. sNFL in DMTs treated patients

The influence of DMTs on sNFL levels was evaluated in treated RRMS patients, comparing them to RR-naïve patients (Figs. 3 and 4, Table 1 and 2).

6.1. sNFL along therapy follow-up

To assess the impact of DMTs, sNFL were measured and compared in RR-naïve and in treated patients: both RR-treat <12 m and RR-treat ≥12 m participants were considered (Fig. 3; Table 1).

Compared to RR-naïve individuals, sNFL levels at a group level were reduced in both RR-treat <12 m (Kruskal-Wallis test, $p = 0.0065$) and RR-treat ≥12 m groups (Kruskal-Wallis test, $p < 0.0001$). No difference between the two treated groups was found (Fig. 3A).

Then, to further evaluate the impact of the years of treatment on sNFL levels, RR-treat ≥12 m participants were also divided in different time ranges (treated for less or more than 60 months) and DMT types, as indicated in the method section (Fig. 3B). No difference was found between sNFL levels in the group of patients treated for less or more than 60 months, when comparing the two groups of LE-DMT and HE-DMT treated patients.

Based on sNFL reference ranges, 31 % of RR-naïve patients showed elevated sNFL, compared to 17 % of RR-treat <12 m patients, and 9 % of RR-treat ≥12 m patients (Fig. 3C). The prevalence of elevated sNFL was significantly higher in RR-naïve compared to RR-treat ≥12 m patients only (chi-square analysis $p < 0.0001$). Furthermore, borderline sNFL prevalence was 8 % in RR-naïve, 5 % in RR-treat <12 m and 6 % in RR-treat ≥12 m participants.

When considering years of treatment and DMT type, the prevalence of elevated sNFL was 10 % LE-DMTs 12–60 m, 7 % in LE-DMT >60 m, 8 % in HE-DMT 12–60 m and 6 % in HE-DMT >60 m participants; borderline NFL values were 5 % in LE-DMT 12–60 m, 9 % in LE-DMT >60 m, 5 % in HE-DMT 12–60 m and 5 % in HE-DMT >60 m participants (Fig. 3D). No significant difference in prevalence of elevated sNFL was found between groups of the same-efficacy DMTs.

6.2. sNFL in patients treated with different DMTs

In order to establish the impact of different DMTs on sNFL levels, we focused on patients treated for more than 12 months (Fig. 4; Table 2).

Compared to RR-naïve individuals, IFN (Kruskal-Wallis test, $p = 0.0003$), GA (Kruskal-Wallis test, $p = 0.0110$) DMF (Kruskal-Wallis test,

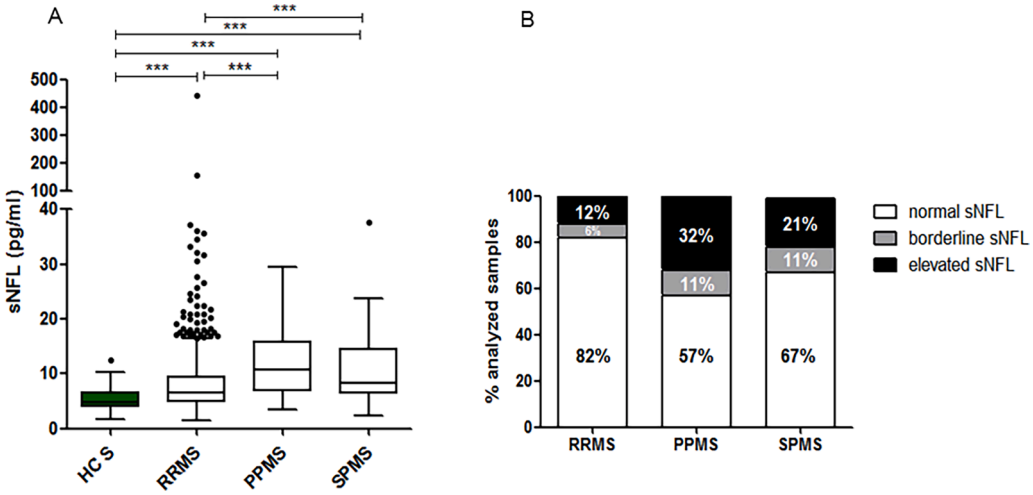


Fig. 1. sNFL levels and prevalence of elevated sNFL in different MS subtypes. (A)sNFL were higher in progressive MS patients, compared to RRMS ones. (Kruskal-Wallis test, pvalue< 0.0001). (B) According to cut-off levels, both PPMS and SPMS group showed a significantly higher prevalence of elevated sNFL compared to the RRMS one (PPMS chi-square analysis $p < 0.000l$, SPMS chi-square analysis $p = 0.0219$).

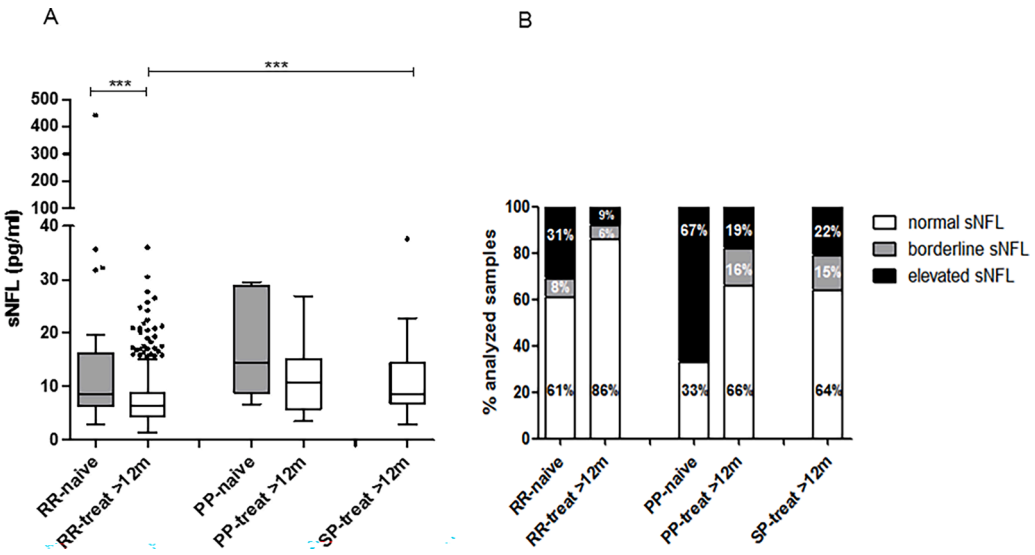


Fig. 2. sNFL levels and prevalence of elevated sNFL in naïve versus treated patients of different disease subtypes. (A) RRMS naïve patients demonstrated significantly higher sNFL levels compared to treated ones (Kruskal-Wallis test $p < 0.0001$). For PPMS participants, the difference was not statistically significant. RR-treat ≥12 m patient' sNFL levels were significantly lower than SP-treat ≥12 m group (Kruskal-Wallis test $p < 0.0001$). (B) According to cut-off levels, all naïve patients showed a higher prevalence of elevated sNFL compared to the treated groups (RRMS chi-square analysis $p < 0.0001$; PPMS Fisher's exact test = 0.0362). RR-treat ≥12 m individuals' prevalence was also significantly lower than SP-treat ≥12 m group (chi-square analysis $p = 0.0009$).

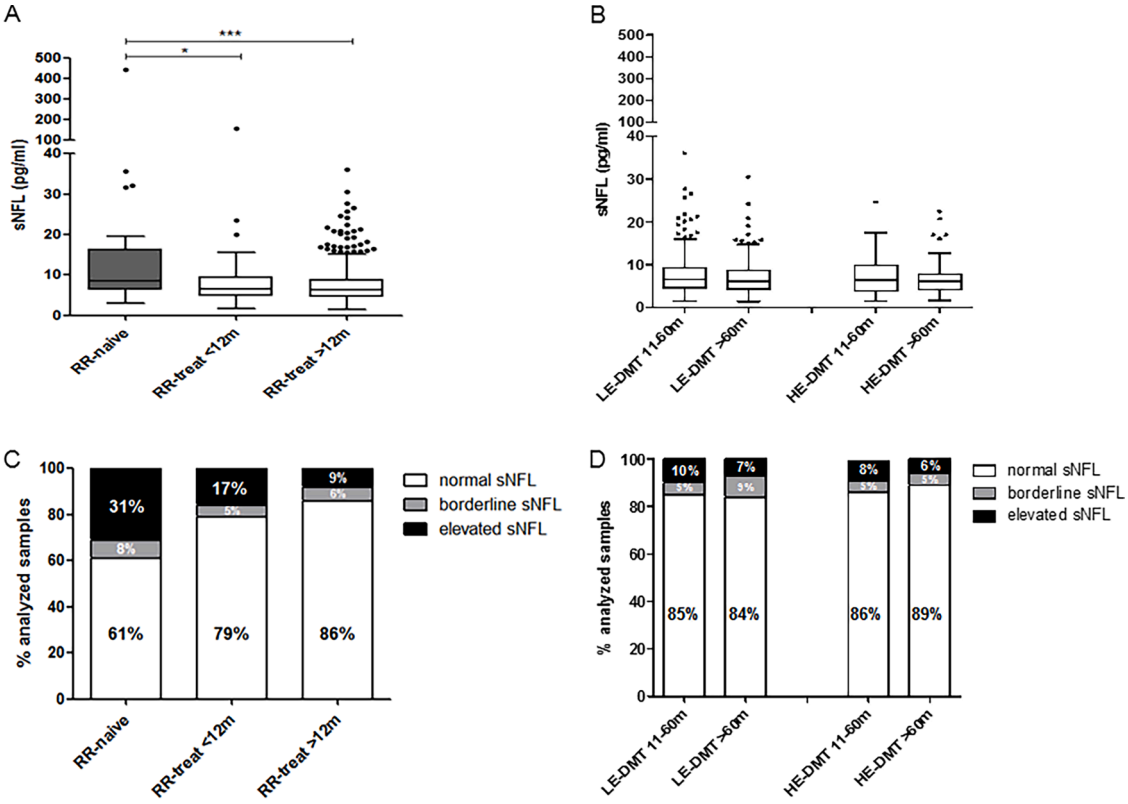


Fig. 3. sNFL levels and prevalence of elevated sNFL along therapy follow-up. (A) RR-naïve patients showed higher sNFL levels compared to treated ones (RR-treat <12 m, Kruskal-Wallis test, $p = 0.0065$; RR-treat≥12 m, Kruskal-Wallis test $p < 0.0001$). (B) No difference in sNFL levels was found in individuals treated far less or more than 60 months, with either LE-DMT or HE-DMT. (C). According to cut-off levels, the prevalence of elevated sNFL was significantly higher in RR- naïve individuals compared to RR-treat≥12 m patients only (chi-square analysis $p < 0.0001$). (C) The prevalence of elevated sNFL levels in patients treated far less or more than 60 months was not statistically different, in either LE-DMT or HE-DMT groups.

$p < 0.0001$), NAT (Kruskal-Wallis test, $p < 0.0001$), FING (Kruskal-Wallis test, $p = 0.0005$) and anti-CD20 (Kruskal-Wallis test, $p = 0.0370$) groups showed significantly lower sNFL levels.

Based on reference values, compared to RR-naive the prevalence of elevated sNFL was lower in IFN (10 %, chi-square analysis $p = 0.0016$), in GA (5 %, Fisher's exact test $p = 0.0016$), in DMF (10 %, chi-square analysis $p = 0.0004$), in TERIFL (12 %, chi-square analysis $p = 0.0434$), in NAT (6 %, chi-square analysis $p < 0.001$), in FING (6 %, chi-

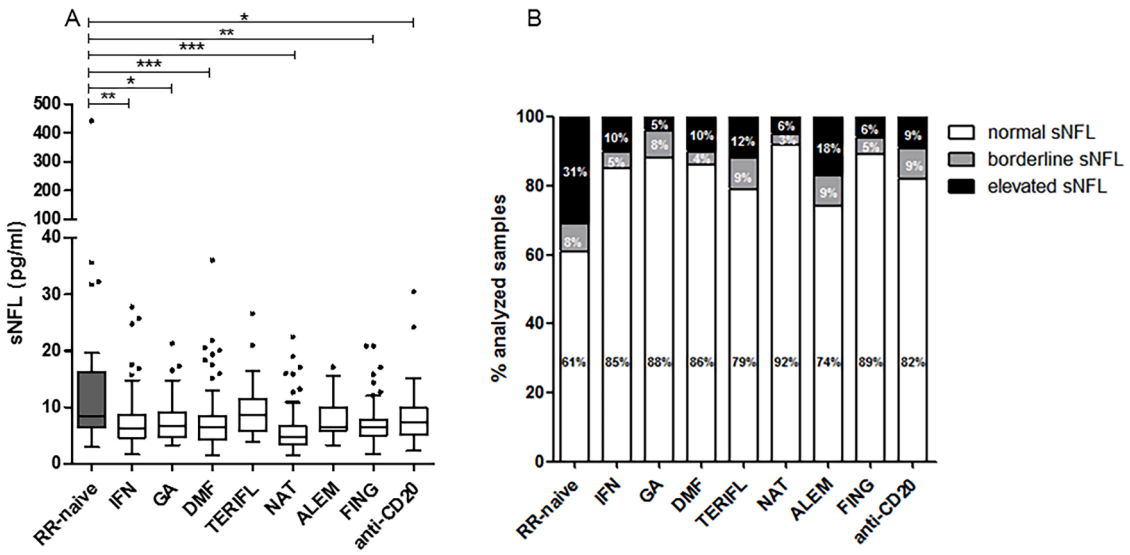


Fig. 4. sNFL levels and prevalence of elevated sNFL in RRMS patients treated with different DMTs. (A) Compared to RR-naïve individuals, IFN (Kruskal-Wallis test, $p = 0.0003$), GA (Kruskal-Wallis test, $p = 0.0110$) DMF (Kruskal-Wallis test, $p < 0.0001$), NAT (Kruskal-Wallis test, $p < 0.0001$), FING (Kruskal-Wallis test, $p = 0.0005$) and anti-CD20 (Kruskal-Wallis test, $p = 0.0370$) groups showed significantly lower sNFL levels. (B) According to cut-off levels, IFN (chi-square analysis $p = 0.0016$), GA (Fisher's exact test $p = 0.0016$), DMF (chi-square analysis $p = 0.0004$), TERIFL (chi-square analysis $p = 0.0434$), NAT (chi-square analysis $p < 0.001$), FING (chi-square analysis $p < 0.0001$) and anti-CD20 (chi-square analysis $p = 0.0048$) individuals demonstrated significantly less elevated sNFL compared to RR- naïve.

square analysis $p < 0.0001$) and in anti-CD20 (9 %, chi-square analysis $p = 0.0048$) groups.

Also, 5 % of IFN, 8 % of GA, 4 % of DMF, 9 % of TERIFL, 3 % of NAT, 9 % of ALEM, 5 % of FING and 9 % of anti-CD20 patients demonstrated borderline sNFL levels.

7. Discussion

sNFL has been widely proposed as a valid and easily quantifiable biomarker in MS. Indeed, many studies established sNFL correlation with the disease course and treatment efficacy, making sNFL the most promising longitudinal biomarker in MS patient monitoring (Akgün et al., 2019; Delcoigne et al., 2020; Disanto et al., 2017; Hyun et al., 2020; Kölliker Frers et al., 2022; Kuhle et al., 2019; Ning and Wang, 2022; Novakova et al., 2017; Piehl et al., 2018; Sotirchos et al., 2023; Thebault et al., 2020; Varhaug et al., 2019). Nevertheless, in order to implement sNFL in clinical practice, several efforts are needed in terms of cut-off definition and harmonization, application in multicentric / real-life studies, and analytical standardization (Sen et al., 2023; Thebault et al., 2020).

In this context, our real-life cross-sectional study aims to assess sNFL levels and the prevalence of elevated sNFL in a large cohort of 908 MS patients of different clinical phenotypes (RR, PP and SP) and in different treatment conditions (treated or not, and according to the type and duration of treatment), which samples were consecutively collected from January 2019 to January 2020. Ultimately, we wanted to test sNFL usefulness as additional supportive measure in clinical practice, as a red-flag in MS patients management. Particularly, we wanted to assess the informativeness of one single sNFL measurement to understand in which patients might be worth performing longitudinal sNFL quantification in daily clinical practice. Results interpretation was carried out using age-dependent cut-off levels previously defined and published by our group (Valentino et al., 2021; see methods Section 2.1.2). Moreover, we defined as borderline those samples for which [sNFL quantification ± 10 %] range included the age-specific cut-off value, considering the previously calculated and published inter-assay variability of 10 % (Valentino et al., 2021). These values should be carefully interpreted and deserve a further follow-up measurement for ultimate clinical decision-making (Sotirchos et al., 2023).

We measured sNFL levels in all RR/PP/SPMS patients (independent from treatment condition) to assess the impact of different disease subtypes on the biomarker's levels. In our cohort we confirmed that sNFL levels as well as the prevalence of elevated sNFL are higher in both PPMS and SPMS compared to RRMS patients. Lack of significant difference between PPMS and SPMS is in line with what already reported in literature (Varhaug et al., 2019).

To understand the impact of treatment on sNFL we compared naïve and treated patients for at least 12 months.

Naïve samples of RRMS and PPMS subtypes showed higher prevalence of elevated sNFL compared to patients treated for more than 12 months. Moreover, treated SPMS patients demonstrated higher sNFL levels and a higher prevalence of elevated sNFL compared to RRMS treated patients, in line with literature (Saraste et al., 2021; Sen et al., 2023).

When focusing on PPMS patients, the naïve cohort was undoubtedly limited, with 9 cases among which 6 showed elevated sNFL. Again, sNFL demonstrate to be useful to identify the plausible ongoing chronic neurodegeneration in this group, likely more prominent in those with elevated sNFL. On the other hand, at the time of blood-draw, treated PPMS patients were on anti-CD20 therapy, which is known to be highly effective in reducing inflammatory activity and clinical attacks: elevated sNFL in 19 % of these cohort of patients is probably indicative of persistent axonal degeneration (Bar-Or et al., 2023; Williams et al., 2021). Again, sNFL is an useful red flag measure even in patients treated with highly efficacious DMTs (Bar-Or et al., 2023; Bittner et al., 2021; Disanto et al., 2017; Kuhle et al., 2019; Leppert et al., 2022; Lorscheider et al., 2016; Masanneck et al., 2022; Meier et al., 2023; Ning and Wang, 2022; Novakova et al., 2017; Sen et al., 2023; Williams et al., 2021).

Similarly, in the 55 SPMS patients who were mainly treated with off-label rituximab (RTX) for at least 12 months (as siponimod was still unavailable at the time of blood draw), the relevant prevalence of elevated sNFL (22 %) can be attributed to ongoing neurodegeneration: sNFL dosing may be useful in highlighting these patients.

To test sNFL impact on monitoring therapy efficacy along follow-up and specific DMTs effectiveness we then focused on RRMS patients, which is the most plentiful subtype and for which therapeutical options

are wider in choice (Preziosa et al., 2020).

Considering RRMS patients, no clear differences of elevated sNFL prevalence was observed between naïve and short treated patients (RR-treat<12 m group). The great majority of patients in this group was treated for up to 6 months (median 6 months, range 1–8 months), which surely include the period of time in which DMTs are not fully effective (Giovannoni, 2018; Giovannoni et al., 2015; Kuhle et al., 2019; Sejbaek et al., 2019). On the contrary, still elevated sNFL along time after the settlement period might indicate poor DMT efficacy and subclinical activity to look further into: sNFL levels have to be contextualized within each specific case (Berger et al., 2020; Bittner et al., 2021; Ferreira-Atuesta et al., 2021; Novakova et al., 2017).

Interestingly, 6–10 % of RR-treat ≥12 m patients demonstrated elevated sNFL despite DMT type (LE-DMT or HE-DMT) and duration of treatment (up to or more than 60 months). This is apparently in contrast with previously carried-out phase III clinical trials comparing LE-DMT and HE-DMT (ASCLEPIOS-I, n.d.; ASCLEPIOS-II, n.d.; CARE-MS-I, n.d.; CARE-MS-II, n.d.; Freeman et al., 2022; Granqvist et al., 2018; Harding et al., 2019; Kuhle et al., 2019; OPERA-I, n.d.; OPERA-II, n.d.; RIFUND-MS, n.d.; Signori et al., 2020; TENERE, n.d.), which showed that HE-DMTs are more efficient in NFL lowering.

However, as we are referring to a clinical context, the concept of "randomization" is excluded and each patient's therapy is personally tailored to their clinical prognostic factors, outcomes, as well as convenience and preferences, making the clinicians' ability in considering all these factors crucial in clinical practice (Berger et al., 2020; Brown et al., 2018; Manzano et al., 2020; Singer et al., 2021). This a priori selection of treatment for the individual subject leads to a comparable prevalence of sNFL among patients treated with HE-DMTs and LE-DMTs.

Also, when focusing on time, our cohort showed that in patients treated for more than 5 years with both LE-DMT and HE-DMT, the prevalence of elevated sNFL was similar, without a statistical difference, to those treated for less time. From a practical point of view, 6–7 % of patients showed elevated sNFL even after more than 5 years of treatment with either LE-DMTs or HE-DMTs.

Lastly, to assess levels and prevalence of elevated sNFL in each DMTs, we divided RR-treat ≥12 m into DMT-specific groups. As therapeutic choices are individually carried out for each patient based on his personal history and case, it is important to contextualize treatments. The great majority of DMTs showed a prevalence of elevated sNFL of 5–10 %, with two exceptions: TERIFL, which demonstrated a slightly more elevated sNFL prevalence (12 %), and ALEM, which showed a definitely higher prevalence (18 %) compared to other DMTs.

TERIFL is a LE-DMT effective on brain atrophy (Miller, 2021). Especially, as siponimod was not yet available at the time of blood draws (G.U. Serie Generale n°122, 2020), clinicians at CRESM decided upon effectively treating with TERIFL older patients (mean age 48 years old; mean age for other LE-DMT: 40–45 years old), probably already in a slowly progressing phase of the disease, which can account for the higher levels of sNFL.

Instead, ALEM is an immunoricostitutive HE-DMT generally chosen for hyperaggressive patients (Akgün et al., 2019; Katsavos and Coles, 2018; López-Real et al., 2023; Russo et al., 2022; Saccà et al., 2019). At the time of blood draw, ALEM was chosen at CRESM for patients with high inflammatory disease activity and/or failing several therapies, thus reasonably presenting higher risk of neurodegeneration(Maggi et al., 2021; Pfeuffer et al., 2021).

Limitations of our work include the non-longitudinal nature of it, as well as the lack of correlation with clinical data. Moreover, our cut-off levels did not consider confounding factors as BMI and 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 a stratification of patients having mildly, moderately and highly elevated.

8. Conclusion

In conclusion, our real-life study highlights the potential of sNFL quantification as additional serial and objective information in patient routinary monitoring: one single detection already revealed groups of patients in all conditions which was worth of more clinical in-depth analysis and contextualization, in line with literature (Berger et al., 2020; Brown et al., 2018; Gross and Corboy, 2019; Harding et al., 2019).

Normal sNFL in MS patients treated with both LE-DMT and HE-DMT suggests treatment effectiveness, as already reported in literature (Akgün et al., 2019; Delcoigne et al., 2020; Disanto et al., 2017; Hyun et al., 2020; Ning and Wang, 2022; Novakova et al., 2017; Thebault et al., 2020; Varhaug et al., 2019). On the other hand, elevated sNFL must be considered a red-flag suggesting the need of a further, more in depth clinical assessment as it can be indicative of new inflammation, ongoing degeneration or co-morbidities.

Indeed, since a single sNFL dosage already highlighted a prevalence of elevated sNFL levels in any considered category despite MS category and treatment type, results support the development of further longitudinal studies and the introduction of serial sNFL measurement in regular clinical practice to be contextualized within the set of available clinical and neuroimaging data.

Role of the funding source

This work was supported by Roche, by the Italian Ministry of Health (grant number RF-2013–02357497), by FISM - Fondazione Italiana Sclerosi Multipla (cod. 2020/S/5) and financed or co-financed with the 'Cinque per mille' public funding.

Funding sources had no involvement in study design, collection, analysis, and interpretation of data, nor in the writing of the report and in the decision to submit the article for publication.

Funding

This work was supported by Roche, by the Italian Ministry of Health (grant number RF-2013–02357497), by FISM - Fondazione Italiana Sclerosi Multipla (cod. 2020/S/5) and financed or co-financed with the 'Cinque per mille' public funding.

CRediT authorship contribution statement

Cecilia Irene Bava: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Paola Valentino:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Simona Malucchi:** Writing – original draft, Resources. **Rugiada Bottero:** Writing – original draft, Resources. **Serena Martire:** Writing – original draft, Methodology, Formal analysis, Data curation. **Alessia Di Sapio:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Antonio Bertolotto:** Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Antonio Bertolotto reports financial support was provided by Roche SpA. Antonio Bertolotto reports financial support was provided by Italian Multiple Sclerosis Association. Paola Valentino reports a relationship with Biogen that includes: speaking and lecture fees and travel reimbursement. Paola Valentino reports a relationship with Novartis that includes: speaking and lecture fees and travel reimbursement. Paola Valentino reports a relationship with Roche SpA that includes: speaking and lecture fees and travel reimbursement. Paola Valentino reports a relationship with Merck & Co Inc that includes: funding grants. Paola

Valentino reports a relationship with Quanterix Corp that includes: funding grants. Simona Malucchi reports a relationship with Biogen that includes: speaking and lecture fees and travel reimbursement. Simona Malucchi reports a relationship with Merck & Co Inc that includes: speaking and lecture fees and travel reimbursement. Simona Malucchi reports a relationship with Novartis that includes: speaking and lecture fees and travel reimbursement. Simona Malucchi reports a relationship with Roche SpA that includes: speaking and lecture fees and travel reimbursement. Serena Martire reports a relationship with Biogen that includes: board membership, consulting or advisory, speaking and lecture fees, and travel reimbursement. Serena Martire reports a relationship with Novartis that includes: board membership, consulting or advisory, speaking and lecture fees, and travel reimbursement. Antonio Bertolotto reports a relationship with Almirall that includes: board membership, consulting or advisory, and funding grants. Antonio Bertolotto reports a relationship with Bayer that includes: board membership and consulting or advisory. Antonio Bertolotto reports a relationship with Biogen that includes: board membership, consulting or advisory, funding grants, speaking and lecture fees, and travel reimbursement. Antonio Bertolotto reports a relationship with Genzyme Corporation that includes: board membership and consulting or advisory. Antonio Bertolotto reports a relationship with Novartis that includes: funding grants, speaking and lecture fees, and travel reimbursement. Antonio Bertolotto reports a relationship with Sanofi that includes: speaking and lecture fees and travel reimbursement. Antonio Bertolotto reports a relationship with Associazione San Luigi Gonzaga ONLUS that includes: funding grants. Antonio Bertolotto reports a relationship with Fondazione per la Ricerca Biomedica ONLUS that includes: funding grants. Antonio Bertolotto reports a relationship with Mylan Pharmaceuticals Inc that includes: funding grants. Antonio Bertolotto reports a relationship with Italian Multiple Sclerosis Association that includes: funding grants. Rugiada Bottero reports a relationship with Novartis that includes: consulting or advisory. Rugiada Bottero reports a relationship with Sanofi that includes: consulting or advisory. Rugiada Bottero reports a relationship with Merck that includes: consulting or advisory. Alessia Di Sapio reports a relationship with Biogen that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Alessia Di Sapio reports a relationship with Novartis that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Alessia Di Sapio reports a relationship with Roche that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Alessia Di Sapio reports a relationship with Sanofi that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Alessia Di Sapio reports a relationship with Alexion that includes: speaking and lecture fees and travel reimbursement. Alessia Di Sapio reports a relationship with Merck that includes: travel reimbursement. Alessia Di Sapio reports a relationship with Genzyme Corporation that includes: travel reimbursement. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank CRESM Biobank for sample and data collection, Da Col family and Associazione San Luigi Gonzaga ONLUS for the donation that allowed SR-X system purchase.

References

Akgün, K., Kretschmann, N., Haase, R., Proschmann, U., Kitzler, H.H., Reichmann, H., Ziemssen, T., 2019. Profiling individual clinical responses by high-frequency serum neurofilament assessment in MS. Neurol. Neuroimmunol. NeuroInflammation 6, 1–12. https://doi.org/10.1212/NXI.0000000000000555.

ASCLEPIOS-I, n.d. Efficacy and Safety of Ofatumumab Compared to Teriflunomide in Patients With Relapsing Multiple Sclerosis (ASCLEPIOS I) [WWW Document]. 2016. URL https://clinicaltrials.gov/study/NCT02792218.

ASCLEPIOS-II, 2016. Efficacy and Safety of Ofatumumab Compared to Teriflunomide in Patients With Relapsing Multiple Sclerosis. n.d. (ASCLEPIOS II) [WWW Document]. URL. https://clinicaltrials.gov/study/NCT02792231.

Bar-Or, A., Thanei, G.A., Harp, C., Bernasconi, C., Bonati, U., Cross, A.H., Fischer, S., Gaetano, L., Hauser, S.L., Hendricks, R., Kappos, L., Kuhle, J., Leppert, D., Model, F., Sauter, A., Koendgen, H., Jia, X., Herman, A.E., 2023. Blood neurofilament light levels predict non-relapsing progression following anti-CD20 therapy in relapsing and primary progressive multiple sclerosis: findings from the ocrelizumab randomised, double-blind phase 3 clinical trials. EBioMedicine 93, 104662. https://doi.org/10.1016/j.ebiom.2023.104662.

Benkert, P., Meier, S., Schaedelin, S., Manouchehrinia, A., Yaldizli, Ö., Maceski, A., Oechtering, J., Achtnichts, L., Conen, D., Derfuss, T., Lalive, P.H., Mueller, C., Müller, S., Naegelin, Y., Oksenberg, J.R., Pot, C., Salmen, A., Willemse, E., Kockum, I., Blennow, K., Zetterberg, H., Gobbi, C., Kappos, L., Wiendl, H., Berger, K., Sormani, M.P., Granziera, C., Piehl, F., Leppert, D., Kuhle, J., Aeschbacher, S., Barakovic, M., Buser, A., Chan, A., Disanto, G., D'Souza, M., Du Pasquier, R., Findling, O., Galbusera, R., Hrusovsky, K., Khalil, M., Lorscheider, J., Mathias, A., Orleth, A., Radue, E.W., Rahmanzadeh, R., Sinnecker, T., Subramaniam, S., Vehoff, J., Wellmann, S., Wuerfel, J., Zecca, C., 2022. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. Lancet Neurol. 21, 246–257. https://doi.org/10.1016/S1474-4422(22)00009-6.

Berger, T., Adamczyk-Sowa, M., Csépány, T., Fazekas, F., Fabjan, T.H., Horáková, D., Ledinek, A.H., Illes, Z., Kobelt, G., Jazbec, S.Š., Klímová, E., Leutmezer, F., Rejdak, K., Rozsa, C., Sellner, J., Selmaj, K., Štourač, P., Szilasiová, J., Turčáni, P., Vachová, M., Vanecková, M., Vécsei, L., Havrdová, E.K., 2020. Factors influencing daily treatment choices in multiple sclerosis: practice guidelines, biomarkers and burden of disease. Ther. Adv. Neurol. Disord. 13, 1–13. https://doi.org/10.1177/1756286420975223. https://, 10.1177/.

Bittner, S., Oh, J., Havrdová, E.K., Tintoré, M., Zipp, F., 2021. The potential of serum neurofilament as biomarker for multiple sclerosis. Brain 144, 2954–2963. https://doi.org/10.1093/brain/awab241.

Brown, H., Gabriele, S., White, J., 2018. Physician and patient treatment decision-making in relapsing-remitting multiple sclerosis in Europe and the USA. Neurodegener. Dis. Manag. 8, 371–376. https://doi.org/10.2217/nmt-2018-0023.

CARE-MS-I, n.d. Comparison of Alemtuzumab and Rebif® Efficacy in Multiple Sclerosis, Study One (CARE-MS I) [WWW Document]. 2007. URL https://clinicaltrials.gov/study/NCT00530348.

CARE-MS-II, n.d. Comparison of Alemtuzumab and Rebif® Efficacy in Multiple Sclerosis, Study Two (CARE-MS II) [WWW Document]. 2007. URL https://clinicaltrials.gov/study/NCT00548405.

Delcoigne, B., Manouchehrinia, A., Barro, C., Benkert, P., Michalak, Z., Kappos, L., Leppert, D., Tsai, J.A., Plavina, T., Kieseier, B.C., Lycke, J., Alfredsson, L., Kockum, I., Kuhle, J., Olsson, T., Piehl, F., 2020. Blood neurofilament light levels segregate treatment effects in multiple sclerosis. Neurology 94, e1201–e1212. https://doi.org/10.1212/WNL.0000000000009097.

Disanto, G., Barro, C., Benkert, P., Naegelin, Y., Schädelin, S., Giardiello, A., Zecca, C., Blennow, K., Zetterberg, H., Leppert, D., Kappos, L., Gobbi, C., Kuhle, J., 2017. Serum Neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann. Neurol. 81, 857–870. https://doi.org/10.1002/ana.24954.

Ferreira-Atuesta, C., Reyes, S., Giovanonni, G., Gnanapavan, S., 2021. The evolution of neurofilament light chain in multiple sclerosis. Front. Neurosci. 15, 1–13. https://doi.org/10.3389/fnins.2021.642384.

Forsberg, L., Spelman, T., Klyve, P., Manouchehrinia, A., Ramanujam, R., Mouresan, E., Drahota, J., Horakova, D., Joensen, H., Pontieri, L., Magyari, M., Ellenberger, D., Stahmann, A., Rodgers, J., Witts, J., Middleton, R., Nicholas, R., Bezlyak, V., Adlard, N., Hach, T., Lines, C., Vukusic, S., Soilu-Hänninen, M., van der Walt, A., Butzkueven, H., Iaffaldano, P., Trojano, M., Glaser, A., Hillert, J., 2023. Proportion and characteristics of secondary progressive multiple sclerosis in five European registries using objective classifiers. Mult. Scler. J. - Exp. Transl. Clin. 9 https://doi.org/10.1177/20552173231153557.

Freeman, L., Longbrake, E.E., Coyle, P.K., Hendin, B., Vollmer, T., 2022. High-efficacy therapies for treatment-naïve individuals with relapsing–remitting multiple sclerosis. CNS Drugs 36, 1285–1299. https://doi.org/10.1007/s40263-022-00965-7.

Giovannoni, G., 2018. Disease-modifying treatments for early and advanced multiple sclerosis: a new treatment paradigm. Curr. Opin. Neurol. 31, 233–243. https://doi.org/10.1097/WCO.0000000000000561.

Giovannoni, G., Turner, B., Gnanapavan, S., Offiah, C., Schmierer, K., Marta, M., 2015. Is it time to target no evident disease activity (NEDA) in multiple sclerosis? Mult. Scler. Relat. Disord. 4, 329–333. https://doi.org/10.1016/j.msard.2015.04.006.

Granqvist, M., Boremalm, M., Poorghobad, A., Svenningsson, A., Salzer, J., Frisell, T., Piehl, F., 2018. Comparative effectiveness of rituximab and other initial treatment choices for multiple sclerosis. JAMA Neurol. 75, 320–327. https://doi.org/10.1001/jamaneurol.2017.4011.

Granziera, C., Derfuss, T., Kappos, L., 2023. Time to change the current clinical classification of multiple sclerosis? JAMA Neurol. 80, 128–130. https://doi.org/10.1037/a0024238.

Gross, R.H., Corboy, J.R., 2019. Monitoring, switching, and stopping multiple sclerosis disease-modifying therapies. Contin. Lifelong Learn. Neurol. 25, 715–735. https://doi.org/10.1212/CON.0000000000000738.

Harding, K., Williams, O., Willis, M., Hrastelj, J., Rimmer, A., Joseph, F., Tomassini, V., Wardle, M., Pickersgill, T., Robertson, N., Tallantyre, E., 2019. Clinical outcomes of escalation vs early intensive disease-modifying therapy in patients with multiple sclerosis. JAMA Neurol. 76, 536–541. https://doi.org/10.1001/jamaneurol.2018.4905.

Hviid, C.V.B., Knudsen, C.S., Parkner, T., 2020. Reference interval and preanalytical properties of serum neurofilament light chain in Scandinavian adults. Scand. J. Clin. Lab. Invest. 80, 291–295. https://doi.org/10.1080/00365513.2020.1730434.

Hyun, J.W., Kim, Y., Kim, G., Kim, S.H., Kim, H.J., 2020. Longitudinal analysis of serum neurofilament light chain: a potential therapeutic monitoring biomarker for multiple sclerosis. Mult. Scler. J. 26, 659–667. https://doi.org/10.1177/1352458519840757.

Iaffaldano, P., Lucisano, G., Guerra, T., Patti, F., Onofrj, M., Brescia Morra, V., Zaffaroni, M., Pozzilli, C., Cocco, E., Sola, P., Salemi, G., Inglese, M., Bergamaschi, R., Gasperini, C., Conte, A., Salvetti, M., Lus, G., Maniscalco, G.T., Totaro, R., Vianello, M., Granella, F., Ferraro, E., Aguglia, U., Gatto, M., Sangalli, F., Chisari, C.G., De Luca, G., Carotenuto, A., Baroncini, D., Colombo, D., Nica, M., Paolicelli, D., Comi, G., Filippi, M., Amato, M.P., Trojano, M., 2022. Towards a validated definition of the clinical transition to secondary progressive multiple sclerosis: a study from the Italian MS Register. Mult. Scler. J. 28, 2243–2252. https://doi.org/10.1177/13524585221114007.

Katsavos, S., Coles, A., 2018. Alemtuzumab as treatment for multiple sclerosis. Cold Spring Harb. Perspect. Med. 8, 1–10. https://doi.org/10.1101/cshperspect.a032029.

Kölliker Frers, R.A., Otero-Losada, M., Kobiec, T., Udovin, L.D., Aon Bertolino, M.L., Herrera, M.I., Capani, F., 2022. Multidimensional overview of neurofilament light chain contribution to comprehensively understanding multiple sclerosis. Front. Immunol. 13, 1–19. https://doi.org/10.3389/fimmu.2022.912005.

Kuhle, J., Kropshofer, H., Haering, D.A., Kundu, U., Meinert, R., Barro, C., Dahlke, F., Tomic, D., Leppert, D., Kappos, L., 2019. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology 92, E1007–E1015. https://doi.org/10.1212/WNL.0000000000007032.

Kuhlmann, T., Moccia, M., Coetzee, T., Cohen, J.A., Correale, J., Graves, J., Marrie, R.A., Montalban, X., Yong, V.W., Thompson, A.J., Reich, D.S., Amato, M.P., Banwell, B., Barkhof, F., Chataway, J., Chitnis, T., Comi, G., Derfuss, T., Finlayson, M., Goldman, M., Green, A., Hellwig, K., Kos, D., Miller, A., Mowry, E., Oh, J., Salter, A., Sormani, M.P., Tintore, M., Tremlett, H., Trojano, M., van der Walt, A., Vukusic, S., Waubant, E., 2023. Multiple sclerosis progression: time for a new mechanism-driven framework. Lancet Neurol. 22, 78–88. https://doi.org/10.1016/S1474-4422(22)00289-7.

Lambert, J., Chang, L., Song, L., Patel, P.P., Shan, D., Johnson, J., Rissin, D., 2018. Comparison of two platforms quantitating Fg/Ml neurological biomarkers using single molecule arrays and digital elisa: the benchtop reader Sr-X™ and the fully automated analyzer Hd-1™. Alzheimer's Dement 14, 2018. https://doi.org/10.1016/j.jalz.2018.06.783.

Leppert, D., Kropshofer, H., Häring, D.A., Dahlke, F., Patil, A., Meinert, R., Tomic, D., Kappos, L., Kuhle, J., 2022. Blood neurofilament light in progressive multiple sclerosis: post hoc analysis of 2 randomized controlled trials. Neurology 98, E2120–E2131. https://doi.org/10.1212/WNL.0000000000200258.

López-Real, A.M., Gonzalez, I., Solar, D.M., Oterino, A., Costa, E., Pato, A., Llaneza, M.A., García-Estévez, D.A., Rodriguez-Regal, A., Rodriguez, M., Peña, J., 2023. Alemtuzumab treatment in real clinical practice: experience in a multicenter cohort. Mult. Scler. Relat. Disord. 75 https://doi.org/10.1016/j.msard.2023.104762.

Lorscheider, J., Buzzard, K., Jokubaitis, V., Spelman, T., Havrdova, E., Horakova, D., Trojano, M., Izquierdo, G., Girard, M., Duquette, P., Prat, A., Lugaresi, A., Grand'maison, F., Grammond, P., Hupperts, R., Alroughani, R., Sola, P., Boz, C., Pucci, E., Lechner-Scott, J., Bergamaschi, R., Oreja-Guevara, C., Iuliano, G., Van Pesch, V., Granella, F., Ramo-Tello, C., Spitaleri, D., Petersen, T., Slee, M., Verheul, F., Ampapa, R., Amato, M.P., Mccombe, P., Vucic, S., Sánchez Menoyo, J.L., Cristiano, E., Barnett, M.H., Hodgkinson, S., Olascoaga, J., Saladino, M.L., Gray, O., Shaw, C., Moore, F., Butzkueven, H., Kalincik, T., 2016. Defining secondary progressive multiple sclerosis. Brain 139, 2395–2405. https://doi.org/10.1093/brain/aww173.

Maggi, P., Kuhle, J., Schädelin, S., Van Der Meer, F., Weigel, M., Galbusera, R., Mathias, A., Lu, P.J., Rahmanzadeh, R., Benkert, P., La Rosa, F., Bach Cuadra, M., Sati, P., Théaudin, M., Pot, C., van Pesch, V., Leppert, D., Stadelmann, C., Kappos, L., Du Pasquier, R., Reich, D.S., Absinta, M., Granziera, C., 2021. Chronic white matter inflammation and serum neurofilament levels in multiple sclerosis. Neurology 97, E543–E553. https://doi.org/10.1212/WNL.0000000000012326.

Manouchehrinia, A., Piehl, F., Hillert, J., Kuhle, J., Alfredsson, L., Olsson, T., Kockum, I., 2020. Confounding effect of blood volume and body mass index on blood neurofilament light chain levels. Ann. Clin. Transl. Neurol. 7, 139–143. https://doi.org/10.1002/acn3.50972.

Manzano, A., Eskytė, I., Ford, H.L., Bekker, H.L., Potrata, B., Chataway, J., Schmierer, K., Pepper, G., Meads, D., Webb, E.J., Pavitt, S.H., 2020. Impact of communication on first treatment decisions in people with relapsing-remitting multiple sclerosis. Patient Educ. Couns. 103, 2540–2547. https://doi.org/10.1016/j.pec.2020.05.014.

Marnetto, F., Valentino, P., Caldano, M., Ficorilli, A., Paudice, A., Bertolotto, A., 2020. BB-CRESM: a structured institutional biobank for quality research in multiple sclerosis. Mult. Scler. J. 26, 43–117. https://doi.org/10.1177/1352458520974938.

Masanneck, L., Rolfes, L., Regner-Nelke, L., Willison, A., Räuber, S., Steffen, F., Bittner, S., Zipp, F., Albrecht, P., Ruck, T., Hartung, H.P., Meuth, S.G., Pawlitzki, M., 2022. Detecting ongoing disease activity in mildly affected multiple sclerosis patients under first-line therapies. Mult. Scler. Relat. Disord. 63 https://doi.org/10.1016/j.msard.2022.103927.

Meier, S., Willemse, E.A.J., Schaedelin, S., Oechtering, J., Lorscheider, J., Melie-Garcia, L., Cagol, A., Barakovic, M., Galbusera, R., Subramaniam, S., Barro, C., Abdelhak, A., Thebault, S., Achtnichts, L., Lalive, P., Müller, S., Pot, C., Salmen, A., Disanto, G., Zecca, C., D'souza, M., Orleth, A., Khalil, M., Buchmann, A., Du Pasquier, R., Yaldizli, Ö., Derfuss, T., Berger, K., Hermesdorf, M., Wiendl, H., Piehl, F., Battaglini, M., Fischer, U., Kappos, L., Gobbi, C., Granziera, C., Bridel, C., Leppert, D., Maleska Maceski, A., Benkert, P., Kuhle, J., 2023. Serum glial fibrillary acidic protein compared with neurofilament light chain as a biomarker for disease progression in multiple sclerosis. JAMA Neurol. 80, 287–297. https://doi.org/10.1001/jamaneurol.2022.5250.

Miller, A.E., 2021. An updated review of teriflunomide's use in multiple sclerosis. Neurodegener. Dis. Manag. 11, 387–409. https://doi.org/10.2217/nmt-2021-0014.

Ning, L., Wang, B., 2022. Neurofilament light chain in blood as a diagnostic and predictive biomarker for multiple sclerosis: a systematic review and meta-analysis. PLoS One 17, 1–18. https://doi.org/10.1371/journal.pone.0274565.

Novakova, L., Zetterberg, H., Sundström, P., Axelsson, M., Khademi, M., Gunnarsson, M., Malmeström, C., Svenningsson, A., Olsson, T., Piehl, F., Blennow, K., Lycke, J., 2017. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 89, 2230–2237. https://doi.org/10.1212/WNL.0000000000004683.

OPERA-I, n.d. A Study of Ocrelizumab in Comparison With Interferon Beta-1a (Rebif) in Participants With Relapsing Multiple Sclerosis (OPERA I) [WWW Document]. 2011. URL https://clinicaltrials.gov/study/NCT01412333.

OPERA-II, n.d. A Study of Ocrelizumab in Comparison With Interferon Beta-1a (Rebif) in Participants With Relapsing Multiple Sclerosis (OPERA II) [WWW Document]. 2011. URL https://clinicaltrials.gov/study/NCT01247324.

Pfeuffer, S., Ruck, T., Pul, R., Rolfes, L., Korsukewitz, C., Pawlitzki, M., Wildemann, B., Klotz, L., Kleinschnitz, C., Scalfari, A., Wiendl, H., Meuth, S.G., 2021. Impact of previous disease-modifying treatment on effectiveness and safety outcomes, among patients with multiple sclerosis treated with alemtuzumab. J. Neurol. Neurosurg. Psychiatry 92, 1007–1013. https://doi.org/10.1136/jnnp-2020-325304.

Piehl, F., Kockum, I., Khademi, M., Blennow, K., Lycke, J., Zetterberg, H., Olsson, T., 2018. Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. Mult. Scler. J. 24, 1046–1054. https://doi.org/10.1177/1352458517715132.

Preziosa, P., Rocca, M.A., Filippi, M., 2020. Current state-of-art of the application of serum neurofilaments in multiple sclerosis diagnosis and monitoring. Expert Rev. Neurother. 20, 747–769. https://doi.org/10.1080/14737175.2020.1760846.

RIFUND-MS, n.d. RItuximab Versus FUmarate in Newly Diagnosed Multiple Sclerosis. (RIFUND-MS) [WWW Document]. 2016. URL https://clinicaltrials.gov/study/NCT02746744.

Russo, C.V., Saccà, F., Frau, J., Annovazzi, P., Signoriello, E., Bonavita, S., Grasso, R., Clerico, M., Cordioli, C., Laroni, A., Capobianco, M., Torri Clerici, V., Sartori, A., Cavalla, P., Maniscalco, G.T., La Gioia, S., Caleri, F., Giugno, A., Iodice, R., Carotenuto, A., Cocco, E., Fenu, G., Zaffaroni, M., Baroncini, D., Lus, G., Gallo, A., De Mercanti, S.F., Lapucci, C., Di Francescantonio, V., Brambilla, L., Sormani, M.P., Signori, A., 2022. A real-world study of alemtuzumab in a cohort of Italian patients. Eur. J. Neurol. 29, 257–266. https://doi.org/10.1111/ene.15121.

Saccà, F., Lanzillo, R., Signori, A., Maniscalco, G.T., Signoriello, E., Lo Fermo, S., Repice, A., Annovazzi, P., Baroncini, D., Clerico, M., Binello, E., Cerqua, R., Mataluni, G., Bonavita, S., Lavorgna, L., Zarbo, I.R., Laroni, A., Rossi, S., Pareja Gutierrez, L., La Gioia, S., Frigeni, B., Barcella, V., Frau, J., Cocco, E., Fenu, G., Torri Clerici, V., Sartori, A., Rasia, S., Cordioli, C., Di Sapio, A., Pontecorvo, S., Grasso, R., Barrilà, C., Russo, C.V., Esposito, S., Ippolito, D., Bovis, F., Gallo, F., Sormani, M.P., 2019. Determinants of therapy switch in multiple sclerosis treatment-naïve patients: a real-life study. Mult. Scler. J. 25, 1263–1272. https://doi.org/10.1177/1352458518790390.

Saraste, M., Bezukladova, S., Matilainen, M., Tuisku, J., Rissanen, E., Sucksdorff, M., Laaksonen, S., Vuorimaa, A., Kuhle, J., Leppert, D., Airas, L., 2021. High serum neurofilament associates with diffuse white matter damage in MS. Neurol. Neuroimmunol. neuroinflammation 8, 1–11. https://doi.org/10.1212/NXI.0000000000000926.

Sejbaek, T., Nielsen, H.H., Penner, N., Plavina, T., Mendoza, J.P., Martin, N.A., Elkjaer, M.L., Ravnborg, M.H., Illes, Z., 2019. Dimethyl fumarate decreases neurofilament light chain in CSF and blood of treatment naïve relapsing MS patients. J. Neurol. Neurosurg. Psychiatry 90, 1324–1330. https://doi.org/10.1136/jnnp-2019-321321.

Sen, M.K., Hossain, M.J., Mahns, D.A., Brew, B.J., 2023. Validity of serum neurofilament light chain as a prognostic biomarker of disease activity in multiple sclerosis. J. Neurol. 270, 1908–1930. https://doi.org/10.1007/s00415-022-11507-y.

Signori, A., Saccà, F., Lanzillo, R., Maniscalco, G.T., Signoriello, E., Repice, A.M., Annovazzi, P., Baroncini, D., Clerico, M., Binello, E., Cerqua, R., Mataluni, G., Perini, P., Bonavita, S., Lavorgna, L., Zarbo, I.R., Laroni, A., Pareja-Gutierrez, L., La Gioia, S., Frigeni, B., Barcella, V., Frau, J., Cocco, E., Fenu, G., Clerici, V.T., Sartori, A., Rasia, S., Cordioli, C., Stromillo, M.L., Di Sapio, A., Pontecorvo, S., Grasso, R., Barone, S., Barrilà, C., Russo, C.V., Esposito, S., Ippolito, D., Landi, D., Visconti, A., Sormani, M.P., 2020. Cladribine vs other drugs in MS: merging randomized trial with real-life data. Neurol. Neuroimmunol. neuroinflammation 7. https://doi.org/10.1212/NXI.0000000000000878.

Singer, B.A., Keith, S., Howerter, A., Doll, H., Pham, T., Mehta, R., 2021. A study comparing patient and clinician perspectives of treatments for multiple sclerosis via group concept mapping. Patient Prefer. Adherence 15, 975–987. https://doi.org/10.2147/PPA.S297052.

Sotirchos, E.S., Fitzgerald, K.C., Singh, C.M., Smith, M.D., Reyes-Mantilla, M., Hersh, C. M., Hyland, M.H., Canissario, R., Simmons, S.B., Arrambide, G., Montalban, X., Comabella, M., Naismith, R.T., Qiao, M., Krupp, L.B., Nicholas, J.A., Akgün, K., Ziemssen, T., Rudick, R., Fisher, E., Bermel, R.A., Mowry, E.M., Calabresi, P.A., 2023. Associations of sNfL with clinico-radiological measures in a large MS population. Ann. Clin. Transl. Neurol. 10, 84–97. https://doi.org/10.1002/acn3.51704.

TENERE, n.d. A Study Comparing the Effectiveness and Safety of Teriflunomide and Interferon Beta-1a in Patients With Relapsing Multiple Sclerosis (TENERE) [WWW Document]. 2009. URL https://clinicaltrials.gov/study/NCT00883337.

Teunissen, C.E., Tumani, H.T., Bennett, J.L., Berven, F.S., Brundin, L., Comabella, M., Franciotta, D., Federiksen, J.L., Fleming, J.O., Furlan, R., Hintzen, R.Q., Hughes, S. G., Johnson, M.H., Krasulova, E., Kuhle, J., Magnone, M.C., Petzold, A., Rajda, C., Rejdak, K., Schmidt, H.K., Van Pesch, V., Waubant, E., Wolf, C., Hemmer, B., Deisenhammer, F., Giovannoni, G., 2010. Short commentary on ?a consensus protocol for the standardization of cerebrospinal fluid collection and biobanking? Mult. Scler. 16, 129–132. https://doi.org/10.1177/1352458509356368.

Thebault, S., Booth, R.A., Freedman, M.S., 2020. Blood neurofilament light chain: the neurologist's troponin? Biomedicines 8, 1–11. https://doi.org/10.3390/biomedicines8110523.

Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., Correale, J., Fazekas, F., Filippi, M., Freedman, M.S., Fujihara, K., Galetta, S.L., Hartung, H.P., Kappos, L., Lublin, F.D., Marrie, R.A., Miller, A.E., Miller, D.H., Montalban, X., Mowry, E.M., Sorensen, P.S., Tintoré, M., Traboulsee, A.L., Trojano, M., Uitdehaag, B.M.J., Vukusic, S., Waubant, E., Weinshenker, B.G., Reingold, S.C., Cohen, J.A., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 17, 162–173. https://doi.org/10.1016/S1474-4422(17)30470-2.

Valentino, P., Marnetto, F., Martire, S., Malucchi, S., Bava, C.I., Popovic, M., Bertolotto, A., 2021. Serum neurofilament light chain levels in healthy individuals: a proposal of cut-off values for use in multiple sclerosis clinical practice. Mult. Scler. Relat. Disord. 54 https://doi.org/10.1016/j.msard.2021.103090.

Varhaug, K.N., Torkildsen, Ø., Myhr, K.M., Vedeler, C.A., 2019. Neurofilament light chain as a biomarker in multiple sclerosis. Front. Neurol. 10, 1–6. https://doi.org/10.3389/fneur.2019.00338.

Vermunt, L., Otte, M., Verberk, I.M.W., Killestein, J., Lemstra, A.W., van der Flier, W.M., Pijnenburg, Y.A.L., Vijverberg, E.G.B., Bouwman, F.H., Gravesteijn, G., van de Berg, W.D.J., Scheltens, P., van Harten, A.C., Willemse, E.A.J., Teunissen, C.E., 2022. Age- and disease-specific reference values for neurofilament light presented in an online interactive support interface. Ann. Clin. Transl. Neurol. 9, 1832–1837. https://doi.org/10.1002/acn3.51676.

Williams, T., Zetterberg, H., Chataway, J., 2021. Neurofilaments in progressive multiple sclerosis: a systematic review. J. Neurol. 268, 3212–3222. https://doi.org/10.1007/s00415-020-09917-x.

Yamamura, T., 2023. Time to reconsider the classification of multiple sclerosis. Lancet Neurol. 22, 6–8. https://doi.org/10.1016/S1474-4422(22)00469-0.