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ORIGINAL COMMUNICATION

In multiple sclerosis patients a single serum neurofilament light chain (sNFL) dosage is strongly associated with 12 months outcome: data from a real-life clinical setting

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Abstract

Background Neurofilament light chain (NFL) is a neuroaxonal cytoskeletal protein released into cerebrospinal fluid (CSF) and eventually into blood upon neuronal injury. Its detection in serum (sNFL) makes it a promising marker in multiple sclerosis (MS).

Objective To evaluate the usefulness of a single dosage of sNFL in clinical practice.

Methods 626 consecutive relapsing–remitting (RR) MS patients treated with disease modifying treatments (DMTs) for at least 12 months underwent a single sNFL dosage. 553 patients had NEDA-3 status (no relapses, no disability progression, no new/enlarging or contrast-enhancing lesions on brain magnetic resonance imaging) in the 12 months prior blood sampling. sNFL levels were measured by single molecule array (Simoa™). Association between sNFL levels and NEDA-3 status at 12, 24, and 36 months was evaluated with logistic regression models adjusted for sex, EDSS, disease duration, and type of DMTs.

Results 469 out of the 553 NEDA-3 patients had normal sNFL level, whereas 42 had elevated level. The two groups did not differ regarding baseline characteristics. A very strong association between elevated sNFL levels and loss of NEDA-3 status within 12 months was found, with an odds ratio [OR] of 10.74 (95% CI 4.34–26.57); 15 and 10 patients with normal and elevated sNFL, respectively lost NEDA-3 ($p < 0.001$). The effect was not detected during the subsequent 13–24 and 25–36 months.

Conclusions A single elevated sNFL is strongly associated with NEDA-3 loss within 1 year. Elevated sNFL in apparently stable patients suggests an ongoing disease activity below the detection threshold of standard parameters.

Keywords SNFL · Fluid biomarkers · NEDA-3 · Patients' monitoring

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Introduction

Multiple sclerosis (MS) is a central nervous system (CNS) disorder characterized by acute inflammatory damage, chronic compartmentalized inflammation, and neurodegeneration [1].

Early and continuous use of disease-modifying therapies (DMTs) is associated with improved long-term outcomes. This emphasizes the importance of early diagnosis and intervention, as well as rapid identification of non-responder patients that should switch to other more efficacious therapies [2, 3]. In this context, there is a strong need of a sensitive indicator of treatment efficacy to be applied in clinical practice for patients monitoring.

With the development of high efficacious therapies, the concept of "no evidence of disease activity" (NEDA) has been introduced in MS [4]. This acronymous has been adopted from other diseases such as cancer where treatment is intended to free the patient from the disease.

NEDA-3, referring to no relapses, no increase in EDSS (Expanded Disability Status Scale), no new or enlarging T2-weighted lesions, and no T1-weighted contrast-enhancing lesions on brain magnetic resonance imaging (MRI), has become the treatment goal for MS and an outcome measure in clinical trials [5–9].

Recently, the concept has been even more explored as an ever-increasing body of evidence has suggested that both focal and chronic diffuse inflammation and neurodegeneration are already present at disease onset [10, 11]. Chronic active white matter lesions (slowly expanding lesions, SEL), meningeal lymphoid aggregates and more in general compartmentalized inflammation are related to whole brain and gray matter atrophy [12], are strongly predictive of disability progression [13–15] and observed since early phase of the disease [16]. For these reasons, brain atrophy has lately been added as a fourth component of NEDA (NEDA-4).

Therefore, in clinical practice there is a need of biological parameters able to detect, measure and monitor pathological process that underlie and predict irreversible disability and better define treatment response to DMTs. The inclusion of fluid biomarkers to NEDA-3 has been suggested to improve measurement of disability progression [17]. Literature reports the association between chronic inflammation within the CNS and increased levels of sNFL [18, 19]. Furthermore, several studies have shown that sNFL correlates with inflammatory disease activity and therapy response and may predict disability worsening [20–26]. However, these important pieces of information are at group level, whereas in everyday clinical practice is necessary to establish if and when the quantification of sNFL in a single patient with MS (pwMS) is informative. In particular, if the quantification of sNFL added to the routinary blood test performed in pwMS during DMTs can help in the management of patients.

In a previous study, we established age-dependent reference values for sNFL to enable the interpretation of sNFL dosing at a single-patient level [27] and in clinical practice [28, 29].

We quantified sNFL levels in 626 consecutives unselected patients with RRMS according to revised 2017 Mc Donald criteria [30], in treatment with different DMTs whose blood samples had been collected in occasion of treatment monitoring and stored at CReSM (Regional Referral MS Centre)—BioBank. We evaluated the usefulness and applicability of sNFL quantification as a "routinary test" in a MS Clinic and explored its correlation with subsequent clinical outcome.

Methods

Patients and methods

This is a real-life retrospective monocentric study. 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 informed consent.

MS patients

Patients' inclusion criteria were age between 18 and 59 years old; diagnosis of RRMS according to revised McDonald criteria [30]; availability of at least one sNFL dosage; an ongoing DMT treatment for at least 12 months before blood sampling for the sNFL dosage; availability of clinical and MRI data for at least 12 months before and after blood sampling; written informed consent. Twelve months of therapy is a generally accepted period after which we are confident that DMTs have become effective [31, 32]. Exclusion criteria were pregnancy and concomitant neurological diseases.

626 patients with RRMS met the inclusion criteria and were selected for the study.

Among them, 553 patients had a NEDA-3 status in the 12 months prior blood sampling for sNFL dosage.

NEDA-3 status was defined, according to literature definition [33], as the composite of three related measures, namely no clinical relapses, no increase in EDSS score, and no activity seen on MRI (new or enlarging T2 hyperintense lesions or gadolinium-enhancing lesions) for 12 months prior sNFL dosage. The NEDA-3 status was retrospectively assessed by a neurologist blind for sNFL values.

Methods

sNFL dosage

Blood samples were collected in serum tubes (BD Vacutainer, Becton, Dickinson and Company) and processed within 2 h from collection according to CRESM Biobank standard procedures [34] and international guidelines [35].

Blood samples were centrifuged at 3000 × 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 [36] 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). The 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%) [27]. Particularly, as defined in our previous work, we considered as "normal" those samples for which sNFL quantification range comprising [value ± 10%value] was all below the specific age-dependent cut-off level; "borderline" those samples for which sNFL quantification range comprising [value ± 10%value] included the specific age-dependent cut-off level; "elevated" those samples for which sNFL quantification range comprising [value ± 10%value] was entirely above the specific age-dependent cut-off level.

Statistical analysis

Statistical analysis was performed using Python version 3.11.5.

Descriptive statistical analysis of clinical, demographic, and biological data was carried out using χ^2 and Mann–Whitney tests accordingly.

Association between sNFL levels and NEDA-3 status at 12, 24, and 36 months was evaluated with logistic regression models adjusted for sex, EDSS, disease duration and type of DMTs. Type of treatment was categorized as moderate including platform therapies, teriflunomide, dimethyl fumarate or high efficacious therapy including fingolimod, natalizumab, cladribine, alemtuzumab, and rituximab [37].

Logistic regression analysis has been repeated at three-time intervals stratifying patients according to the cause of NEDA-3 loss ("inflammation" when clinical relapse and /or radiological activity was registered, or "progression" when EDSS increase was reported; p value < 0.05 was considered statistically significant.

Results

553 out of 626 included patients were in NEDA-3 status. 469 out of 553 had normal levels of sNFL, and 42 had elevated sNFL; the remaining 42 were excluded from the analysis because sNFL dosage was borderline, that is within the inter-assay coefficient of variation of 10% described in our previous work [27]. Among 42 patients with elevated sNFLs, 1 was lost at follow up. Overall, 510 NEDA-3 patients were evaluated, of whom 469 with normal sNFL (92%) and 41 with elevated sNFL (8%).

Clinical and demographic characteristics of patients with normal and elevated sNFL at baseline and during follow up are described in Table 1. The group of patients with normal sNFL did not differ significantly from the group with elevated sNFL regarding sex distribution, age, disease duration, EDSS, and type of therapy at baseline. At 12 months after blood sampling, 25 out of 510 patients lost NEDA-3 status: the percentage of EDA (Evidence of Disease Activity) patients with elevated sNFL was significantly higher compared to the percentage of EDA patients with normal sNFL (24.4% and 3.2%, respectively, $p < 0.001$). More in details, loss of NEDA-3 was due to signs of inflammation (both clinical relapse and new or contrast enhancing lesions) in 18/25 patients or due to progression (EDSS increase without relapse or MRI change) in 7/25 patients.

On the contrary, at 24 and 36 months after blood sampling, the percentage of EDA patients with normal or elevated sNFL did not differ. Clinical, biological, and demographic patients' characteristics according to NEDA or EDA status at the three time points are described in Table 2. Patients in NEDA or in EDA status did not differ according to sex distribution, age, EDSS, disease duration and type of treatment at 12 months after blood sample, the only significant difference being the percentage of patients with elevated sNFL in the two groups. At 24 and 36 months after blood sample, NEDA and EDA patients only differed for EDSS, that was significantly higher in EDA group compared to the NEDA one. After adjusting for sex, age, disease duration, EDSS and type of DMTs, we found a very strong association between elevated sNFL levels and loss of NEDA-3 status within 12 months; more in details, patients with elevated sNFL had a risk of losing NEDA-3 status 11 time greater than patients with normal sNFL (odds ratio [OR] = 10.74, 95% CI 4.34–26.57). The effect was not detected during the subsequent 13–24 months (OR = 1.4, 95% CI 0.39–4.94) and for 25–36 months (OR = 1.8, 95% CI 0.48–6.68). Figure 1 shows in detail the transition from NEDA to EDA status during the follow up. At any time points, logistic regression analysis adjusted for the same variables (sex, age, disease duration, EDSS and type of treatment), was

Table 1 Patients' clinical and demographic characteristics according to sNFL level, at baseline and during follow up

	Normal level of sNFL	Elevated level of sNFL	*p* value
N	469	42	
Female /male, *n*	319/150	31/11	0,548
Age at sample, years, median (range)	42 (18–59)	43 (20–57)	0,973
Disease duration at sampling, years, median (range)	10 (1–38)	13 (1–32)	0,306
EDSS at sampling, median (range)	1.8 (0–7.5)	2.0 (0–7.0)	0,365
HET*, *n* (%)	246 (52.5)	20 (47.6)	0,773
EDA at 12 months, *n*° (%)	15 (3.2)	10 (24.4)	< 0.001
Inflammation, *n* (%)	13 (86.7)	5 (50)	0,122
Progression, *n* (%)	2 (13.3)	5 (50)	
EDA at 24 months, *n*° (%)	33 (7.0)	3 (7.3)	1,000
Inflammation, *n* (%)	22 (66.6)	0 (0)	0,099
Progression, *n* (%)	11 (33.3)	3 (100)	
EDA at 36 months, *n*° (%)	24 (5.1)	3 (7.3)	0,810
Inflammation, *n* (%)	17 (70.8)	1 (33.3)	0,516
Progression, *n* (%)	7 (29.2)	2 (66.6)	

**HET* (High Efficacy Therapy): fingolimod, natalizumab, cladribine, alemtuzumab, rituximab

Table 2 Clinical, biological and demographic characteristics according to NEDA-3 status at 12, 24 and 36 months

	NEDA-3 at 12 m	EDA-3 at 12 m	*P* valuve	NEDA-3 at 12 m	EDA-3 at 12 m	*P* valuve	NEDA-3 at 12 m	EDA-3 at 12 m	*P* valuve
N	485	25		449	36		422	27	
Elevated sNFL, *n*° (%)	31 (6.4)	10 (40)	< 0.001	28 (6.2)	3 (8.3)	0.888	25 (5.9)	3 (11.1)	0.503
Female, *n*°	330	19	0.539	303	27	0.456	286	17	0.760
Age at sample, years, median (range)	42	40	0.237	42	46	0.241	42	42	0.331
Disease duration at sampling, years, median (range)	10	10	0.687	10	7	0.513	10	12	0.473
EDSS at sampling, median (range)	1.5	1.5	0.362	1.5	2	0.031	1.5	2	0.010
HET, *n* (%)	254	11	0.541	239	15	0.245	228	11	0.253
Type of EDA									
Inflammation, *n* (%)	NA	18 (72)		NA	22 (61.1)	–	NA	18 (66.7)	–
Progression, *n* (%)	NA	7 (28)		NA	14 (389)	–	NA	9 (33.3)	–

**HET* (High Efficacy Therapy): fingolimod, natalizumab, cladribine, alemtuzumab, rituximab

performed in NEDA-3 patients and in the group of patients in EDA status for inflammatory activity (either clinical or radiological) or for progression. Within 12 months, a strong association was found between elevated sNFL and EDA status both for inflammation and for progression, with an OR greater for progression (OR = 6.6, 95% CI 2.13–20.53 and OR = 39.8, 95% CI 6.99–227.35, respectively).

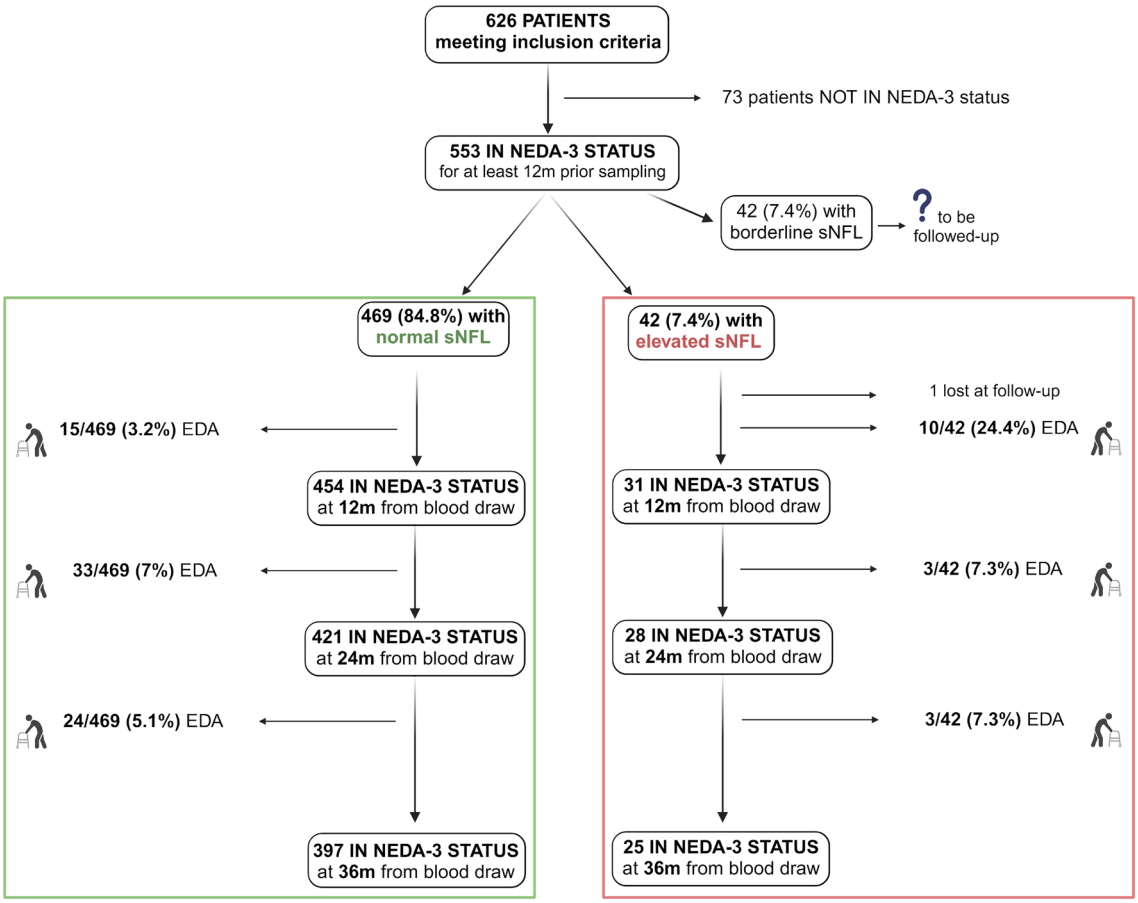

Fig. 1 Transition from NEDA to EDA status during the follow up (Created in BioRender. Bertolotto, a. (2024) BioRender.com/x28y635")

Discussion

This is a real life, retrospective single Centre study on a selected cohort of RRMS patients with no evidence of clinical and radiological disease activity according to NEDA-3 definition [31]. The aim of the study was to explore if a single sNFL dosage could be useful as a supplementary tool for the monitoring of MS patients in the clinical setting. Many changes have occurred in patients' management in recent years: the increase of treatment options, with the availability of high efficacious drugs, the acquisition of new imaging and biological fluid biomarkers, a better knowledge of pathogenetic mechanisms underlying disability and the subsequent change of treatment goal. With the almost complete suppression of relapses and MRI activity thanks to the availability of HET, the goal of any treatment aimed to reach freedom from any disease activity or NEDA. NEDA has first been used as an endpoint in clinical trials, but now it became a target in the clinical setting to navigate among clinical decisions. Neurologists feel the need to intercept promptly, measure and monitor the subclinical diffuse brain damage that manifests clinically as disease progression. The use of conventional brain MRI generally does not allow monitoring of smoldering lesions. Furthermore, in routinary clinical setting often no standardized image acquisition protocols are used, neither unconventional MRI or precise atrophy measurement are available and cognitive evaluation is not always performed. Due to these reasons, the identification of suboptimal responders is often retrospective, delayed and related on patients' report. In this complex scenario, we aimed at evaluating whether sNFL dosage is informative in detecting subclinical activity that can prelude both acute inflammation and progression in the real word. We focused the attention on RRMS patients on active treatment for at least 12 months and in NEDA-3 status for more than 1 year followed in our Centre. This study shows two interesting findings: (1) the strong association of a single elevated sNFL with the risk of losing NEDA-3 status in the following year and (2) the presence of elevated sNFL in a percentage of apparently stable patients. In our work, elevated sNFL was associated with an 11 times greater risk of acquiring EDA status both for disability progression and for clinical and/or radiological reactivation during the subsequent 12 months compared to having normal sNFL levels. It is known from

literature that higher sNFL levels correlate with patient's risk for developing Gd + lesions, new T2 lesions on MRI in the following year, and predict disease worsening and progression independent of relapse activity [24–38]. A recent work from Basel University [39] assessed the applicability of sNfL for identification of people at risk for future disease activity and showed that sNfL percentiles and Z scores indicate a gradually increased risk for future acute and chronic disease activity, and that they can be used to compare the long-term effectiveness of disease-modifying therapies.

On the basis of these data, a recent work by Freedman and Colleagues propose the use of serum and CSF NFL in conjunction with other measures in clinical decision-making for patients with multiple sclerosis [40]. This result has important implications; first of all neurologists may have an easily repeatable, minimally invasive and low-cost monitoring tools in clinical practice; second, sNFL may help MS clinicians with therapeutic decision-making, as elevated sNFL may indicate the need to perform closer monitoring and/or consider a change of therapy. In the present work we found that a single randomly performed elevated sNFL is strongly associated with NEDA-3 loss; but an open question is when to and how often measure sNFL.

According to Freedman et al. [40], it is suggested to measure sNFL at baseline, 3 to 6 months after starting DMTs (rebaseline), 3 to 6 months after clinical relapse o MRI activity. Future fields of application of sNFL can be the monitoring after immunoreconstitutive DMTs, and during de-escalation strategies. Regarding the presence of elevated sNFL in apparently stable patients, we suggest that elevated sNFL levels should represent a red flag for clinicians, as sNFL may identify ongoing disease activity that is below the detection threshold of standard clinical and MRI parameters. They could denote the presence of a cognitive relapse, or spinal radiological activity that is rarely monitored in routinary follow up, or modifications in brain MRI undetectable with conventional imaging. It is known that sNfL levels increase already at the presymptomatic stage of MS, capturing an ongoing neuroaxonal degeneration [41]. Therefore, detection of increased sNFL level suggests neurologists to strictly monitor those patients to early identify any transition to a progressive phenotype. Furthermore, we found that sNFL levels can be increased also in patients who had been clinically and radiologically stable for a long time. This result should lead to caution in suspending therapy in NEDA patients and should encourage neurologists to take advantage of the sNFL dosage to better characterize disease-free patients.

The study has some limitations due to its retrospective and monocentric nature; besides, clinical evaluation was based exclusively on EDSS increase and MRI data were obtained yearly but at different time interval from blood sampling; nevertheless, it represents the starting point for future studies aimed at personalizing patient's management. In conclusion we believe sNFL dosage is an informative instrument in the routinary management of patients and can offer the neurologist an additional simple and minimally invasive tool to perform a better patient profiling.

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Declarations

Conflicts of interest Malucchi Simona received compensation for speaking and consulting from Biogen, Merck, Novartis, Roche. Valentino Paola received speaker honoraria from Roche, research support from Merck, grant support from Quanterix. Bava Cecilia Irene: nothing to discose. Martire Serena: nothing to disclose. Di Sapio Alessia received compensation for speaking and consulting by Biogen, Novartis, Roche, Sanofi, Alexion, Sandoz and reimbursement by Merck, Biogen, Genzyme and Roche for attending conferences. Lo Re Marianna received compensation for consulting by Novartis. Bertolotto Antonio served on the scientific advisory board of Almirall, Bayer, Biogen, Genzyme; received speaker honoraria from Biogen, Novartis, Sanofi, grant support from Almiral, Biogen, Associazione San Luigi Gonzaga ONLUS, Fondazione per la Ricerca Biomedica ONLUS, Mylan, Novartis and the Italian Multiple sclerosis Society.

Ethical 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 informed consent.

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