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Multiple Sclerosis and Related Disorders 47 (2021) 102666

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

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

Original article

Variations in consecutive serum neurofilament light levels in healthy controls and multiple sclerosis patients

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A B S T R A C T

Background Neurofilament light is a neuronal protein detectable in serum (sNfL), with high potential as disease activity biomarker in multiple sclerosis (MS). To date, little is known about sNfL fluctuations between 2 consecutive measurements in healthy controls (HC) and MS patients. Yet this information is critical, as it will help define a clinically significant variation.

Methods sNfL was measured at 2 consecutive time points in a cohort of 90 MS patients (untreated relapsing remitting MS (uRRMS), n=35; treated relapsing remitting MS (tRRMS), n= 21; secondary progressive MS, SPMS, n=21; primary progressive MS, PPMS, n=13), and 90 age-matched HC, using the Simoa NfL light® assay.

Results Mean sNfL was elevated in all MS subtypes compared to HC ($p<0.0001$), and positively associated with age in HC ($r=0.70$, $p<0.001$), confirming previous reports. Mean sNfL was higher at follow-up compared to baseline in HC ($p<0.001$), and lower in uRRMS($p=0.036$) and tRRMS ($p=0.008$). At follow-up, a similar proportion of HC (50.0%), untreated RRMS (51.4%), treated RRMS (33.3%), SPMS (45.0%) and PPMS (46.2%) had variations in sNfL levels exceeding 20% of baseline levels.

Conclusions Our data suggest variations in sNfL occur both in HC and MS populations to a similar extent and magnitude. Variations between two consecutive sNfL measurements may reflect natural variations and not necessarily variations in inflammatory disease activity.

Introduction

Neurofilament light (NfL) is a cytoskeletal protein exclusively expressed by neurons. Its levels are elevated in blood of clinically isolated syndrome (CIS) and multiple sclerosis (MS) patients compared to healthy controls (HC), and correlate with clinical and MRI markers of focal inflammatory disease activity[1,2,3,4,5,6]. In relapsing-remitting MS (RRMS), serum NfL (sNfL) decreases after disease modifying therapy (DMT) initiation, and the magnitude of the decrease correlates with the effectiveness of the DMT in reducing focal acute inflammatory disease activity[3,7,8,9]. Together, these data indicate sNfL may be a useful tool to monitor MS inflammatory disease activity and biological response to treatment, both in clinical trials and routine practice. The distribution of sNfL in HC and MS is characterized by high inter-individual variability and significant overlap[2,10,4], indicating a clinically relevant cut-off value defining elevated sNfL may be difficult to determine. Consequently, on an individual patient level, the variation between 2 measurements, rather than absolute sNfL values, may be more informative of changes in acute inflammatory disease activity. To date, little is known about sNfL level fluctuations between 2 consecutive measurements in HC and MS patients. Yet this information is critical, as it will help define a clinically significant variation. Towards this goal, we compared sNfL levels between 2 measurements 6 months apart in HC (n=90) and a cohort of MS patients (n=90), including RRMS, primary progressive MS (PPMS), and secondary progressive MS (SPMS), using a high precision immunoassay. We evaluated variations of sNfL on an individual participant-level by calculating the ratio of 2 consecutive measurements of sNfL. This ratio, which gives an indication of the magnitude of sNfL change with respect to baseline value, was compared between diagnostic groups.

Methods

Cohort

90 HC and 90 MS patients were enrolled between July and September 2003. Consecutive patients with MS were recruited by sending a letter prior to their regular check-up visit at the MS centre of the Amsterdam University Medical Center. Patients were asked to bring a genetically unrelated neurologically healthy control along, in general their partner. Hospital personnel volunteered as controls when this was

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https://doi.org/10.1016/j.msard.2020.102666
Received 16 November 2020; Accepted 28 November 2020
Available online 1 December 2020

not possible. Patients and HC were enrolled simultaneously. All participants were recruited on a voluntary basis, and signed informed consent for the use of serum and diagnostic information. MS was diagnosed according to Mc Donald criteria[11]. Neurologically healthy controls were defined as patients without signs or symptoms of a neurological disease. The study was approved by the Medical Ethical Committee of the Amsterdam University Medical Center. Of the 90 MS patients, 56 had RRMS, 21 had secondary progressive (SP) MS and 13 had primary progressive (PP) MS (**Table**). 37.5 % of the RRMS patients were treated with injectable disease modifying therapies (DMT) at time of inclusion, consisting of interferon beta or glatiramer acetate. Treatment regimen was not modified during the duration of the study. The proportion of females was higher in the MS group compared to the HC group (p=0.012).

Serum sampling and serum neurofilament light chain measurement

Serum was collected at 2 time points, baseline (BL) and follow-up (FU), with a 6 months interval, and stored according to international guidelines for biobanking[12]. The BL sample was collected at the end of summer (September) and the FU sample at the end of winter (March) for all participants, and FU/BL ratios were calculated for each participants. sNfL was measured using the NfL light kit on a Simoa HD-1 analyser (Quanterix), according to manufacturer instructions. For each individual, both time points were measured on a same run. Prior to use, the commercial NfL assay kit was validated in-house according to international consensus protocols[13].

Statistical analysis

Three outliers with values above the 95th percentile (one SPMS patient and two HC) were excluded from the analysis. Statistical analysis was performed is SPSS (version 20.0.0.0) and R (version 3.2.3). Proportions were compared using the chi-square test. Correlations were estimated using the Spearman correlation coefficient. sNfL was log-transformed to achieve normality. Between-group comparisons of sNfL and FU/BL ratios of sNfL were performed using ANCOVA, correcting for age. The Bonferroni correction was used to adjust for multiple comparisons. Paired t-tests were used to compare sNfL at BL and FU. Cohen's d was used as a measure of effect size, with $0.2 < d < 0.5$ considered a 'small' effect size, $0.5 < d < 0.8$ a 'medium' effect size, and $d > 0.8$ a 'large' effect size. A p-value < 0.05 was considered significant.

table 1 and figure 1

Results

sNfL assay precision

High intra-assay precision is a prerequisite for the comparison of biomarker levels between 2 timepoints. We performed a complete validation of the NfL light kit on a Simoa HD-1 analyser prior to sNfL measurement in our samples. The mean intra-assay coefficient of variation (CV) and inter-assay CV calculated from three independent samples evaluated in six independent runs was 3.3% and 8.5% respectively (**Supplementary Figure A**). The lower limit of quantification was 0.71 pg/mL. The calibration curve with recombinant NfL had acceptable parallelism with sNfL in serum, with a mean parallelism of 87% (**Supplementary Figure B**). Dilution was linear (**Supplementary Figure C**). In conclusion, the validation of the assay was excellent, in particular its precision, which is an essential prerequisite when investigating between measurement fluctuations.

sNfL in healthy controls and multiple sclerosis

sNfL was measured in serum samples collected at BL and FU (**Table**). sNfL was positively associated with age in HC (r=0.70, p<0.001). There was no association with age in MS. At BL, sNfL was higher in all MS subtypes compared to HC (**Figure A**, $p<10^{-5}$), but was similar between MS subtypes. At FU, sNfL was higher in RRMS and SPMS compared to HC (**Figure A**, $p<10^{-5}$ and p=0.02 respectively), and similar between MS subtypes. BL and FU sNfL values were highly correlated in HC (r=0.80, $p<10^{-5}$) and in all MS subtypes (uRRMS, r=0.83, $p<10^{-5}$; tRRMS, r=0.92, $p<10^{-5}$; PPMS, r=0.63, p=0.02; SPMS, r=0.69, p=0.01). sNfL was significantly higher at FU compared to BL in HC, and the effect size was intermediate (**Table**, d=0.55, p<0.001). sNfL was lower at FU compared to BL in untreated RRMS (**Table**, d=0.37, p=0.036) and treated RRMS (**Table**, d=0.65, p=0.008), and the effect sizes were small and large, respectively, suggesting a treatment effect. In SPMS and PPMS, BL and FU sNfL levels did not differ significantly (**Table**).

sNfL FU/BL ratio as a measure of sNfL variation

In order to quantify sNfL variation between 2 measurements in an individual participant, we calculated the ratio of FU over BL sNfL (FU/BL ratio) for each participant, and investigated its distribution in HC and MS subtypes (**Figure B**). At FU, the proportion of individuals with sNfL fluctuations beyond 20% of BL values (ie FU/BL > or =1.2 or < or =0.8) was similar across all diagnostic categories (**Figure B**, p=0.70).

Table 1
Demographics of the cohort included in the analysis

Status	subtype	n	Age (SD)	% Female	Median EDSS (range)	Mean sNfL at baseline in pg/mL(SD)	Mean sNfL at follow-up in pg/mL (range)	Baseline versus follow-up sNfL Cohen's d and p-value
Healthy controls	NA	88	44.5 (11.4)	48.9	NA	7.1 (2.9)	8.1 (3.2)	d=0.55, p<0.001
Multiple sclerosis		89						
	uRRMS	35	43.5 (9.4)	80.0	4.0 (1-7)	14.4 (9.8)	12.4 (9.4)	d=0.37, p=0.036
	tRRMS	21	39.6 (10.6)	57.1	3.5 (2.0-7.0)	12.0 (8.5)	10.0 (7.3)	d=0.65, p=0.008
	SPMS	20	47.6 (13.2)	70.0	5.5 (2-8)	13.1 (7.6)	11.9 (5.9)	d=0.24, p=0.294
	PPMS	13	54.3 (9.5)	46.2	6.0 (4-9)	14.5 (5.8)	12.9 (4.4)	d=0.36, p=0.214

Legend uRRMS, untreated relapsing remitting multiple sclerosis; tRRMS, disease modifying treatment treated relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; EDSS, expanded disability status score; sNfL, serum neurofilament light; DMTs, disease modifying therapies; pg/mL, picogrammes per millilitre.

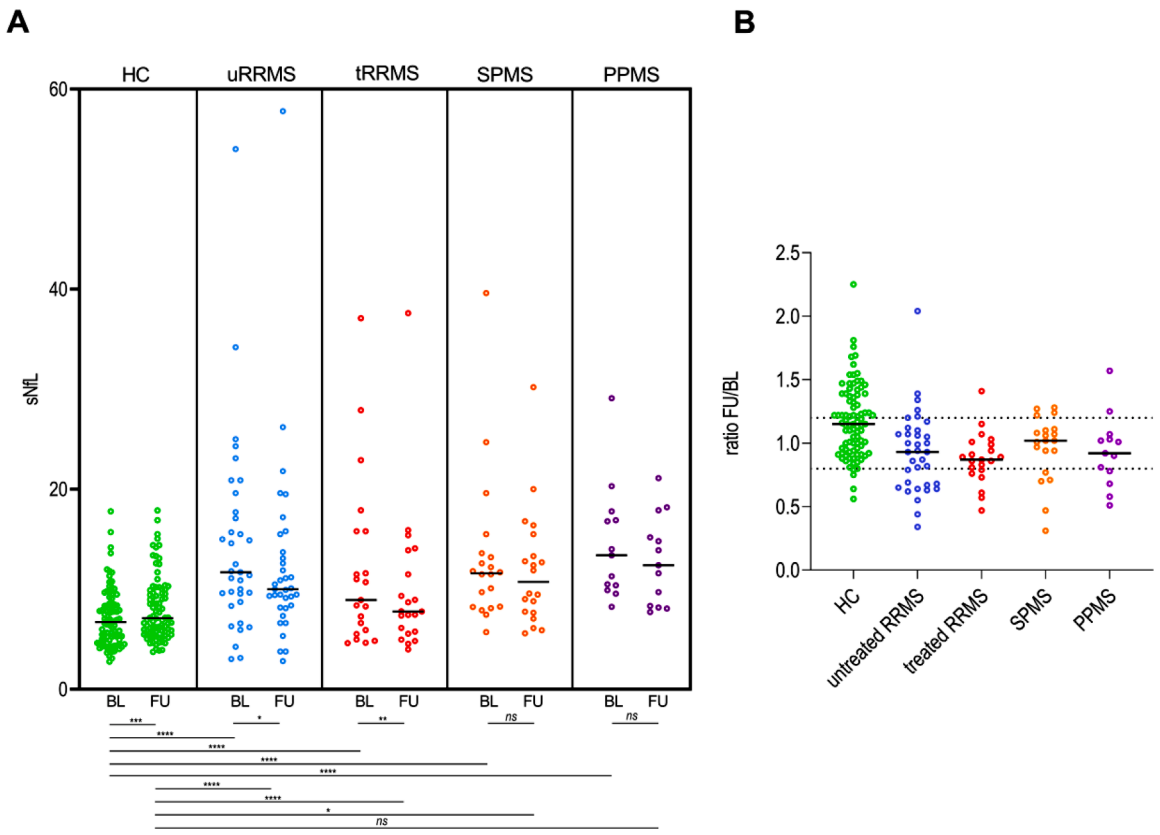

Figure 1. Legend A Median serum neurofilament light (sNfL) in picogramme per millilitre (pg/mL) at baseline (BL) and follow-up (FU) in healthy controls (HC), untreated relapsing-remitting multiple sclerosis (RRMS), treated RRMS, secondary progressive multiple sclerosis (SPMS), and primary progressive multiple sclerosis (PPMS); *=p<0.05; **=p< 0.01; ***=p<0.001; ****=p<0.0001; *ns*=non-significant. **B** Median FU over BL sNfL ratio (FU/BL ratio) in HC, untreated RRMS, treated RRMS, SPMS, and PPMS. Dotted lines demarcate FU/BL ratios of 0.8 and 1.2. 50.0% HC, 51.4% untreated RRMS, 33.3% treated RRMS, 45% SPMS, and 46.2% PPMS had FU/BL ratios equal or beyond these boundaries in both directions (FU/BL ratio < or = 0.8, and > or = to 1.2). These proportions were not significantly different between diagnostic groups (p=0.70).

Discussion

sNfL is a promising biomarker of acute inflammatory disease activity in MS. Prior to its use as an endpoint in clinical trials or as a biological disease response marker in routine clinical practice, characterizing its variations between two consecutive measurements in HC and MS populations is essential. This will help define a clinically meaningful variation, a variable that hasn't been determined so far. Here we investigated variations in sNfL between 2 consecutive measurements in HC, RRMS, SPMS and PPMS. We first demonstrate that the assay used to measure sNfL has high precision, indicating the variations in sNfL between two measurements identified in this study likely reflect biological fluctuations rather than fluctuations related to the measurement tool. We found that sNfL is elevated in RRMS, SPMS and PPMS compared to HC, and that sNfL is positively associated with age in HC but not in MS subtypes, confirming previous reports[14,15]. In HC, sNfL was higher at FU than at BL.

In both DMT-treated and untreated RRMS patients, mean sNfL and standard deviation decreased at FU measurement, and may correspond to a regression to the mean. Alternatively, for the DMT-treated patients, this could correspond to a treatment effect, as the effect size was larger than in the untreated group. In the present cohort, RRMS patients were treated with first-line injectable therapies exclusively, which have low efficiency in reducing acute focal inflammatory disease activity, compared to second and third- line DMTs. Residual focal inflammatory activity may be an explanation for the fluctuations observed in this disease group. However, lack of clinical information about the time from last relapse, from new MRI lesions and/or time from DMT initiation prevents us from relating these biological changes to modifications in disease activity, and additional studies are needed. In SPMS and PPMS, BL and FU sNfL did not differ significantly. A similar proportion of HC and MS patients had FU/BL ratios </= 0.2 or >/= 1.2, reflecting fluctuations in FU sNfL levels beyond 20% of BL levels. This finding is important, as it indicates that on an individual patient basis, similar variations in sNfL are observed in HC and MS, and variables independent of disease status can modify sNfL levels. Studies are currently ongoing to identify these variables. In conclusion, our data suggest that variations in sNfL occur both in HC and MS populations to a similar extent. Thus, variation between two consecutive sNfL measurements may reflect natural variation and not necessarily variations in inflammatory disease activity.

Disclosures

CB, IMWV, JJAH, KJ, CET have nothing to disclose

Funding

There are no specific funding related to this study

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2020.102666.

References

1 Kuhle, J, Barro, C, Disanto, G, et al., 2016. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler J 22 (12), 1550–1559. https://doi.org/10.1177/1352458515623365.

2 Disanto, G, Adiutori, R, Dobson, R, et al., 2015. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. J Neurol Neurosurg Psychiatry 87 (2). https://doi.org/10.1136/jnnp-2014-309690 jnnp-2014-309690.

3 Siller, N, Kuhle, J, Muthuraman, M, et al., March 2018. Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult Scler J, 135245851876566. https://doi.org/10.1177/1352458518765666.

4 Disanto, G, Barro, C, Benkert, P, et al., 2017. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol 81 (6), 857–870. https://doi.org/10.1002/ana.24954.

5 Novakova, L, Zetterberg, H, Sundström, P, et al., 2017. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 89 (22), 2230–2237. https://doi.org/10.1212/WNL.0000000000004683.

6 Håkansson, I, Tisell, A, Cassel, P, et al., 2018. Neurofilament levels, disease activity and brain volume during follow-up in multiple sclerosis. J Neuroinflammation 15 (1), 209. https://doi.org/10.1186/s12974-018-1249-7.

7 Disanto, G, Barro, C, Benkert, P, et al., 2017. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol 81 (6), 857–870. https://doi.org/10.1002/ana.24954.

8 Novakova, L, Zetterberg, H, Sundström, P, et al., 2017. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 89 (22), 2230–2237. https://doi.org/10.1212/WNL.0000000000004683.

9 Varhaug, KN, Barro, C, Bjørnevik, K, et al., 2018. Neurofilament light chain predicts disease activity in relapsing-remitting MS. Neurol - Neuroimmunol Neuroinflammation 5 (1), e422. https://doi.org/10.1212/NXI.0000000000000422.

10 Kuhle, J, Barro, C, Disanto, G, et al., 2016. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler J 22 (12), 1550–1559. https://doi.org/10.1177/1352458515623365.

11 Mcdonald, W, 2001. Compston a, Edan G, et al. Recommended diagnostic criteria for multiple scle ... [Ann Neurol . 2001] - PubMed - ... Recommended diagnostic criteria for multiple sclerosis : guidelines from the International Panel on the diagnosis of multiple sclerosis . Publication Types, MeSH T. Ann Neurol 59 (April), 11456302. https://doi.org/10.1002/ana.1032.

12 Teunissen, CE, Petzold, A, Bennett, JL, et al., 2009. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology 73 (22), 1914–1922. https://doi.org/10.1212/WNL.0b013e3181c47cc2.

13 Andreasson, U, Perret-Liaudet, A, van Waalwijk van Doorn, LJC, et al., 2015. A practical guide to immunoassay method validation. Front Neurol 6 (Aug), 1–8. https://doi.org/10.3389/fneur.2015.00179.

14 Siller, N, Kuhle, J, Muthuraman, M, et al., March 2018. Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult Scler J. https://doi.org/10.1177/1352458518765666, 135245851876566.

15 Barro, C, Benkert, P, Disanto, G, et al., May 2018. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain. https://doi.org/10.1093/brain/awy154.