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Serum neurofilament-light concentration and real-world outcome in MS

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ABSTRACT

Background: Prognostication in multiple sclerosis (MS) remains challenging. Biomarkers capable of providing this information at diagnosis would be valuable in shaping therapeutic decisions. Measurement of neurofilament light (NfL) has shown promise in predicting clinical outcomes in established MS, but its ability to predict outcomes in real-world cohorts at diagnosis requires further validation.

Methods: We used linear regression to evaluate the relationship between serum NfL (sNfL), measured at the time of diagnosis with short-term (1-year) and medium-term (5-year) clinical outcomes in 164 people with MS from a real-world, population-based cohort. Cox proportional hazards regression was used to analyse the association between sNfL and subsequent hazard of relapse or sustained accumulation of disability (SAD). Analyses were adjusted for age and disease-modifying treatment (DMT).

Results: sNfL concentration at diagnosis was modestly associated with baseline EDSS score ($\beta=0.272$, 95% CI 0.051 to 0.494, $p=0.016$). However, no significant associations were found between baseline sNfL and odds of relapse at 12-months, 5-year EDSS change, or the hazard of relapse or SAD over 5 years follow-up. Dichotomising baseline sNfL according to the median sNfL did not change these findings.

Conclusions: sNfL appears to be of limited clinical utility in predicting future irreversible neurological disability in a largely untreated real-world population, and remains insufficiently validated to shape treatment decisions at the time of diagnosis. Further studies may be needed for sNfL to be considered as a prognostic marker in the MS clinic. However the masking effect of DMTs on the natural disease trajectory will continue to pose challenges.

INTRODUCTION

Diagnosing multiple sclerosis (MS) can usually be accomplished with a high degree of accuracy (1), but predicting clinical outcome is considerably more challenging. Disease-modifying therapies (DMTs) hold promise for improving long-term outcomes in MS but can also carry risks. Individualisation of MS therapy to balance benefit and risk can be guided to some extent by clinical, demographic and imaging features, but these characteristics are only modestly predictive for an individual. Biomarkers capable of providing additional prognostic information in MS would be valuable to guide the management of patients and therapeutic decisions.

Neurofilament light (NfL), a protein that contributes to the structure and function of neurons, has become one of the most widely studied candidate prognostic biomarkers in MS. High levels of NfL in the cerebrospinal fluid (CSF) of people with MS (pwMS), especially at the time of relapse, has long been regarded as a marker of neuroaxonal damage (2, 3). Moreover, CSF-NfL has shown promise in predicting clinical outcome in pwMS (4-7). Previously, the low sensitivity of enzyme-linked immunosorbent assay (ELISA) techniques for measuring NfL was a major barrier to measuring serum NfL. However, a more sensitive antibody-based single-molecule array (Simoa) (8), has enabled quantification of NfL at very low concentrations in serum. Correlations have been demonstrated between CSF-NfL and serum-NfL (sNfL) concentrations quantified using this method, suggesting future promise for a less invasive prognostic test (2, 6, 9, 10).

sNfL has been shown by some authors to be moderately predictive of short-term clinical outcomes using group-wise comparisons (9, 11). Longer-term associations have also been demonstrated in clinical trial cohorts. Kuhle *et al.* found baseline sNfL to be predictive of reaching an EDSS score ≥ 6.0 after 11 years in people with relapsing MS (RMS) taking part in the long-term extension study of the pivotal trial of intramuscular interferon β -1a (12). Similarly, analysis in a group of 127 pwMS from a prospective longitudinal study showed that baseline sNfL was predictive of 5-year EDSS score (13).

Whilst these findings are encouraging, they were derived from cohorts recruited on the

basis of disease activity, and studied at a point several years after diagnosis. Participants in these cohorts also experienced high rates of disease-modification. It would be valuable to validate these findings in real-world MS cohorts, especially those recruited early in the disease course and unbiased towards selection for specific DMTs, in order to determine the utility of sNfL for therapeutic decision-making at the point of diagnosis. In this study we report on the relationship between sNfL measured at the time of diagnosis, and 5-year clinical outcomes in a real-world population-based cohort of pwMS.

METHODS

Participants

PwMS were recruited as part of a long running observational study in South East Wales, United Kingdom, which has been described previously (14). Patients were included who had a diagnosis of MS (1), had bio-archived serum taken within 12-months of diagnosis, and at least five years of clinical follow-up. All patients had given informed consent and the study has Research Ethics Committee approval (ref no. 05/WSE03/111).

Procedures

Participants taking part in the observational study are invited to annual clinic visits and data are collected at each clinical encounter, including measurement of EDSS, relapse history, and DMT review. Where patients cannot attend a clinic in person, a postal questionnaire is sent to patients, which includes an assessment of current disability using a validated self-reported EDSS tool (15). Blood samples are acquired at each clinical encounter and archived in a biorepository. Brain and spinal cord MRI scans are performed as part of standard clinical care for diagnostic and clinical evaluation. When obtained, these MR images were acquired on a 1.5T GE Signa HDx scanner using a T2-weighted CSE sequence (TR 580ms, TE 14ms, slice thickness 5mm).

Sample processing

Venous blood samples were collected in a BD vacutainer (Gold SST™ II Advance) between August 2006 and August 2013. The samples were centrifuged at 4500rpm for 10 minutes at 4°C within 2-3 hours of collection. The resulting serum supernatant was subsequently split into 300µl aliquots and stored at -80°C. Quantification of the sNfL concentration was carried out at Queen Mary University of London using a single-molecule array technique (8). Samples were thawed only once for analysis, which was performed using the Quanterix Simoa™ NF-light® Advantage Kit and Simoa HD-1 Analyzer. This highly sensitive immunoassay takes a 2-step approach; NfL molecules in the serum are captured by anti-NfL antibody-coated capture beads, labelled, and identified with fluorescence. The total fluorescent signal is then quantified by the Simoa optical system, and the concentration of NfL in the sample is interpolated from a standard curve. All samples were run on a single plate in one day in duplicate, and the two measurements averaged.

Statistics

A number of clinical outcomes were used as dependent variables in our analyses. The occurrence of a relapse in the 12-months post-sampling was recorded. In addition, time to next event over the follow-up period was defined as the time between serum sampling and the next clinical relapse. EDSS scores recorded at the time of serum sampling and at a time point five years after sampling (each ± 12 months) were used as an indicator of disability at baseline and to calculate 5-year change in EDSS. Time to sustained accumulation of disability (SAD) was also calculated. SAD was defined as an increase in EDSS score of 1.5 if baseline was 0, an increase of 1.0 if baseline was 1.0-5.0, or an increase of 0.5 if baseline EDSS was ≥ 5.5 , sustained for at least 6-months (16). sNfL concentrations were normalised (\log_2) and used as an independent variable in regression analyses. In addition, patients were dichotomised using the median sNfL as a cut-off, and this grouping was subsequently used as a binary variable in regression models.

The association of baseline sNfL concentration with baseline EDSS score and 5-year change in EDSS score was determined using linear regression, with age at disease onset and DMT use included as independent covariates. The time interval between serum sampling and 5-

year EDSS was also entered as an independent covariate to adjust for the potential effect of using a ± 12 -month time-window. Other independent covariates considered were: sex, disease course at onset, and annualised relapse rate from onset to sample collection, which if found to demonstrate a univariate statistically significant association with baseline EDSS/5-year EDSS change, were entered into the final multivariate regression models. The association between baseline sNfL and the odds of a relapse occurring during the 12-months after serum sampling was tested in those with RMS using logistic regression, including covariates as described for the previous analysis. The relationship between baseline sNfL and hazard of next event and SAD were analysed using Cox proportional hazards regression. We included age as a covariate and censored these analyses at the time of commencement of DMT in order to avoid masking of the natural disease trajectory. As with other analyses, only those additional covariates that demonstrated a statistically significant association in univariate analyses were retained in the final multivariate model.

We performed three sensitivity analyses: 1) excluding data from participants whose serum samples had been obtained within 1-month of experiencing a relapse, as sNfL levels are likely to be higher around this time (11, 17); 2) including baseline brain T2 lesion number as a covariate in models, as the number of T2 lesions has been shown to have prognostic value in MS (18). Baseline T2 lesion load was evaluated using the clinical MRI scan closest to serum sampling (within 12-months), and categorised into 0 lesions, 1-3 lesions, 4-9 lesions, and 10+ lesions (19); 3) performing Cox regression analyses without censoring patients when they commenced on DMT, but instead including DMT use during follow-up as a binary covariate. Analyses were carried out using IBM SPSS Statistics 25.

RESULTS

One hundred and sixty-four patients had a serum sample available from the time of MS diagnosis and at least five years of clinical follow-up (Table 1). Mean absolute duration from diagnosis to serum sampling was 3.5 months (SD 3.4) and mean duration from symptom onset to serum sampling was 1.9 years (SD 1.4). In this recently diagnosed cohort, only 10 patients (7%) had commenced DMT by the time of serum sampling (a mean of 6.7 months

post-diagnosis) and had been on treatment for a mean of 4.0 months (SD 3.2). Median sNfL concentration in the cohort was 13.7pg/ml (range 2.7-159.3pg/ml, interquartile range (IQR) 15.6pg/ml).

	Total cohort, n=164	In remission at baseline sampling, n=135	Relapse +/- 1-month of sampling, n=29
Females (n, %)	117 (71%)	95 (70%)	22 (76%)
Relapsing onset MS (n, %)	146 (89%)	118 (87%)	28 (97%)
Progressive onset MS (n, %)	18 (11%)	17 (13%)	1 (3%)
Age at disease onset (mean, SD)	35.1 years (10.8)	35.6 years (11.2)	34.4 years (9.0)
Absolute interval between diagnosis and serum sampling (median, range)	2.9 months (0 - 11.9, IQR 5.5)	3.0 months (0-11.9, IQR 5.4)	2.6 months (0-10.3, IQR 4.8)
EDSS at baseline (median, range)	2.5 (0-7.5, IQR 2.5)	2.5 (0-7.5, IQR 2.5)	2.5 (0-6.0, IQR 1.5)
sNfL at baseline (median, range)	13.7 (2.7-159.3, IQR 15.6)	11.0 (2.7-159.3, IQR 13.9)	21.3 (8.0-154.8, IQR 26.5)
DMT exposure (n, %)			
1. Before serum sampling	10 (6%) -6 IFN, 4 Az	10 (7%) -6 IFN, 4 Az	0
2. Within 1-year of serum sampling	59 (36%) -36 IFN, 2 GA, 21 Az	42 (31%) -25 IFN, 2 GA, 15 Az	17 (59%) -11 IFN, 6 Az
3. Within 2-years of serum sampling	75 (46%) -45 IFN, 3 GA, 1 DF, 26 Az	54 (40%) -32 IFN, 2 GA, 1 DF, 19 Az	21 (72%) -13 IFN, 1 GA, 7 Az
4. During total follow-up	94 (57%) -54 IFN, 5 GA, 3 DF, 30 Az, 2 Nz	69 (51%) -40 IFN, 3 GA, 2 DF, 22 Az, 2 Nz	25 (86%) -14 IFN, 2 GA, 1 DF, 8 Az

TABLE 1. Clinical and demographic features of the cohort. IQR = interquartile range, IFN= interferon beta, GA=glatiramer acetate, Az=alemtuzumab, DF=dimethyl fumarate, Nz=natalizumab.

Serum NfL in relation to baseline EDSS score

Baseline EDSS scores were derived from clinical examination in 148 patients (90%), with the remainder obtained via a validated postal questionnaire. The median absolute interval between serum sampling and baseline EDSS was 0.0 months (range 0.0 – 11.5, IQR 2.1).

Multivariate linear regression analysis showed that log-transformed sNfL concentration at

diagnosis was modestly associated with baseline EDSS score ($\beta=0.272$, 95% CI 0.051 to 0.494, $p=0.016$) (Figure 1). Age at onset ($\beta=0.031$ 95% CI 0.004 to 0.058, $p=0.026$), disease course ($\beta=1.912$ 95% CI 0.985 to 2.839, $p<0.001$) and previous DMT exposure ($\beta=0.386$ 95% CI -0.624 to 1.396, $p=0.452$) were retained in the final model as covariates. The association of baseline sNFL with baseline EDSS score was reproduced when dichotomising sNFL levels $</>13.7\text{pg/ml}$ ($\beta=0.826$, 95% CI 0.307 to 1.345, $p=0.002$) (Figure 2).

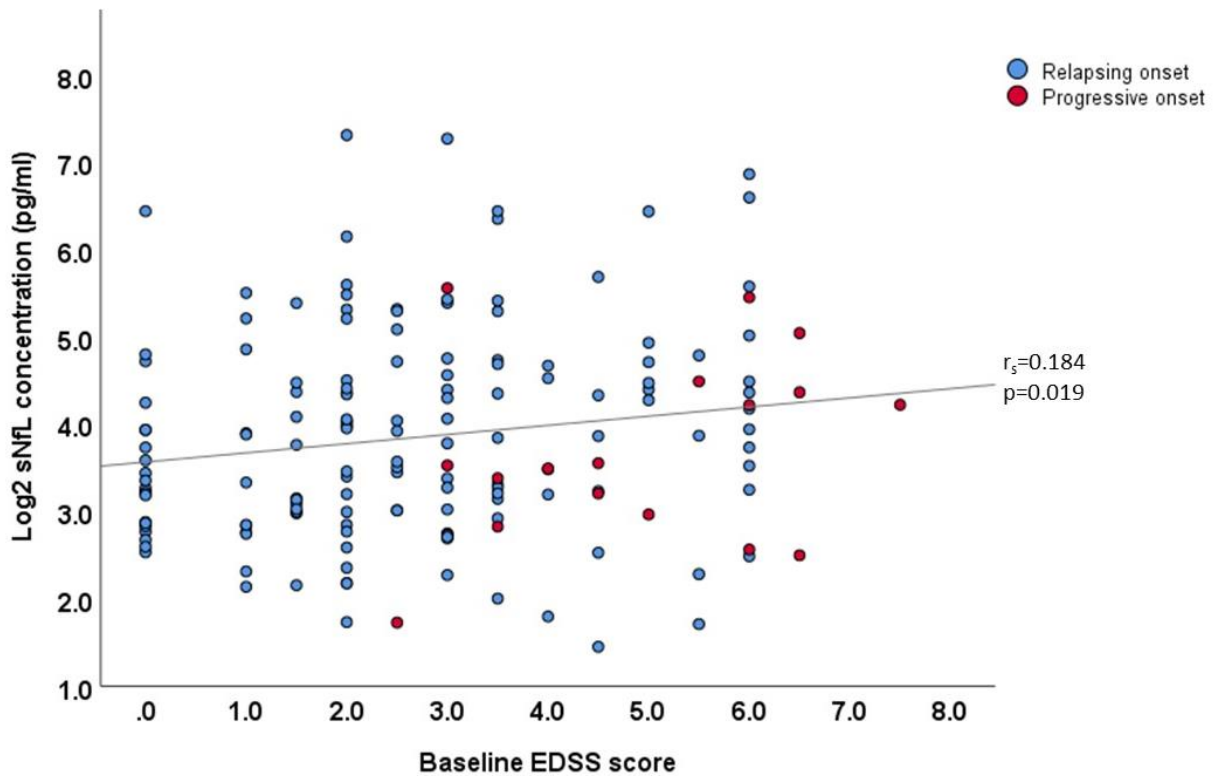


FIGURE 1

Scatter plot showing baseline EDSS score and baseline log2 sNFL mean concentration, by initial disease course

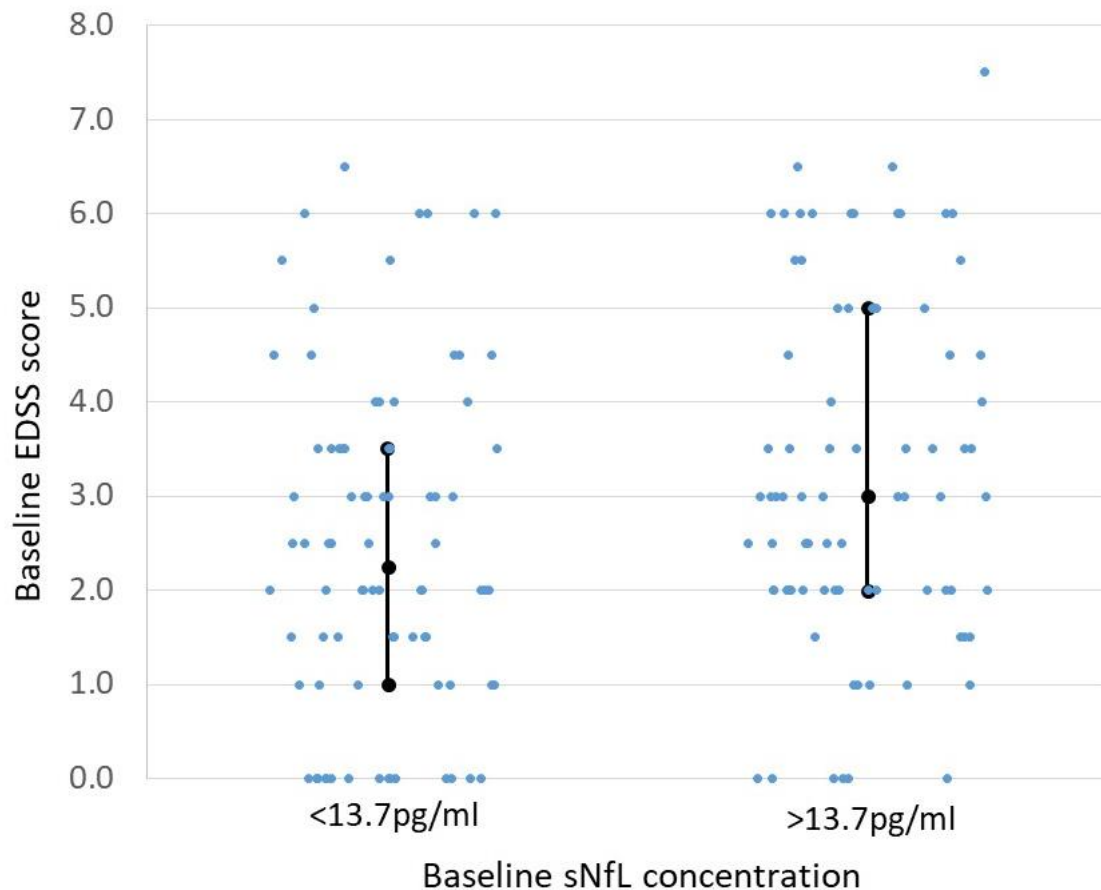


FIGURE 2

Scatter plot showing baseline EDSS score by baseline sNfL group (above or below median baseline sNfL concentration). Median EDSS and interquartile range for each group is also shown.

Serum NfL as a predictor of subsequent relapses

No association was found between log-transformed or dichotomised baseline sNfL concentration and odds of relapse during the 12 months following serum sampling in people with RMS when adjusted for age and DMT use (log-transformed sNfL odds ratio (OR) 1.15, 95% CI 0.86 to 1.53, $p=0.351$; sNfL \leq 13.7pg/ml, OR 1.93, 95% CI 0.95 to 3.92, $p=0.071$).

Eighty-eight patients experienced a clinical relapse before censoring. Neither log-transformed nor dichotomised baseline sNfL concentration showed an association with hazard of next relapse (censored at commencement of DMT and covaried for age; log-

transformed sNfL hazard ratio (HR) 1.00, 95% CI 0.85 to 1.18, $p=0.986$, sNfL ≤ 13.7 pg/ml HR 1.19, 95% CI 0.78 to 1.83, $p=0.427$).

Serum NfL as a predictor of 5-year change in EDSS score

132 pwMS had both baseline and 5-year EDSS measurements and were included in the linear regression modelling of 5-year change in EDSS score. Five year EDSS scores were derived from clinical examination in 115 patients (87%), with the remainder obtained via a validated postal questionnaire. Age at onset, disease course, DMT use, and interval between serum sampling and 5-year EDSS were retained as covariates in the final model. There was no association between log-transformed baseline sNfL concentration and 5-year change in EDSS score ($\beta=-0.180$, 95% CI -0.436 to 0.076, $p=0.167$), nor when categorising patients according to whether baseline sNfL was ≤ 13.7 pg/ml ($\beta=-0.26$, 95% CI -0.87 to 0.34, $p=0.389$).

Serum NfL as a predictor of time to sustained accumulation of disability (SAD)

Forty-two patients showed SAD prior to censoring. Baseline sNfL showed no association with hazard of SAD (censored at commencement of DMT) when adjusted for age at onset and initial disease course (HR 1.066, 95% CI 0.791 to 1.435, $p=0.675$). This finding was confirmed when sNfL levels were dichotomised into sNfL ≤ 13.7 pg/ml (HR 1.20, 95% CI 0.64 to 2.26, $p=0.573$) (Figure 3).

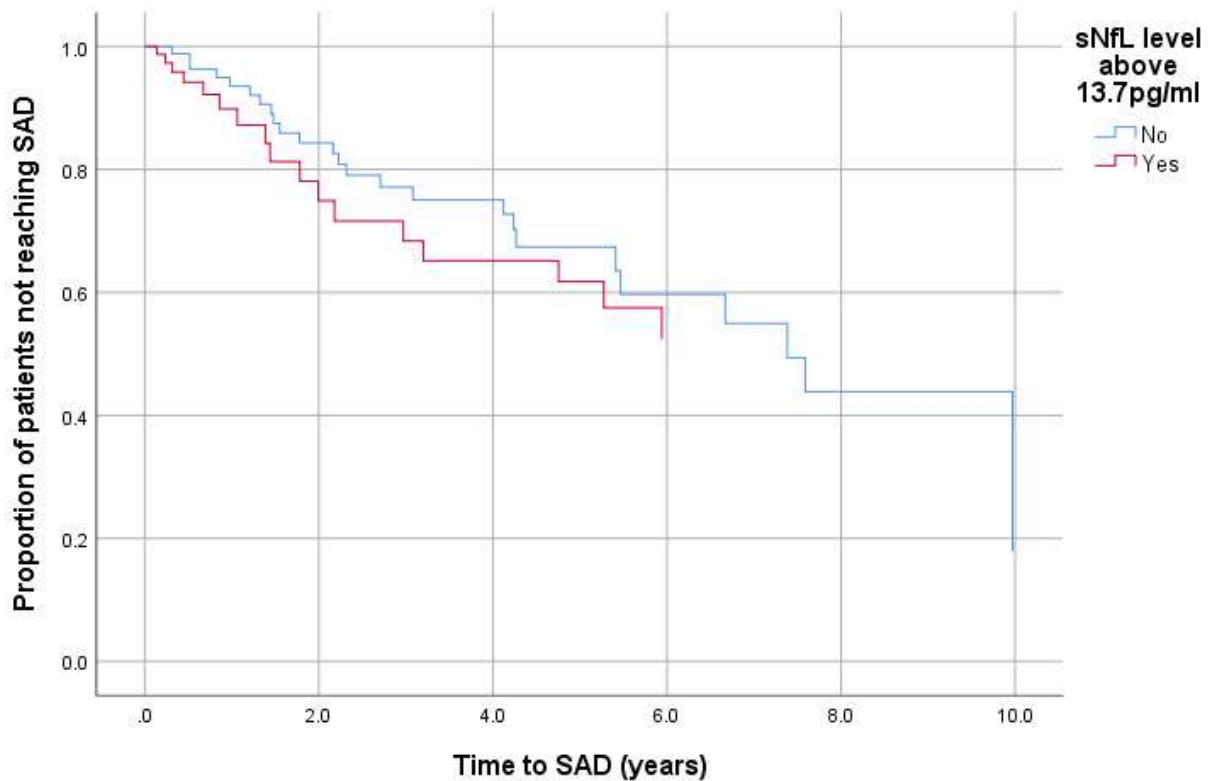


FIGURE 3
Kaplan-Meier survival curve, showing time to sustained accumulation of disability (SAD) by baseline sNfL level, dichotomised by whether sample was above or below the median value of the cohort (13.7pg/ml)

Sensitivity analyses

When excluding pwMS whose serum sample had been obtained within 1-month of relapse (n=29, Table 1), the relationship between baseline log-transformed sNfL and baseline EDSS was no longer significant ($\beta=0.23$, 95% CI -0.04 to 0.50, $p=0.088$), although the relationship between baseline sNfL \leq median (11.0pg/ml) remained significant. There were no other substantial changes in the results. Our results were also not substantially altered when Cox regression analyses for time to next event and SAD were performed without censoring patients at initiation of DMT (but including a binary covariate for DMT use during follow-up). The significance of associations between sNfL and future disability were similar in models that included baseline T2 lesion number as a covariate, with no relevant changes (n=125).

DISCUSSION

Reliable prognostic biomarkers are urgently needed in order to enable individualisation of treatment in MS. sNfL has shown promise as a minimally invasive tissue biomarker that is predictive of clinical outcomes (9, 11-13). However, studies to date have often been conducted in selected cohorts, including those enrolled into trials of DMTs (10, 20, 21), and data from population-based cohorts are scarce. Moreover, existing reports have mostly focused on clinical outcomes in individuals with established disease (9-11, 21), whereas key management decisions in the clinic are often made closer to the time of diagnosis, with the aim of improving longer-term prognosis. Numerous reports have cited associations of sNfL with disability using group-wise comparisons, categorising patients according to cut-off values for sNfL (6, 9, 11), but uncertainty remains over the most appropriate cut-off, and how predictive sNfL is of future disability at an individual level. We have attempted to address some of these issues in the current study, where we report the associations of sNfL, measured at the time of diagnosis, with short- (1 year) and medium-term (5 years) clinical outcomes in a real-world cohort of pwMS. In addition to the proximity to diagnosis and symptom onset, serum sampling predated DMT commencement in 93% of patients.

We have demonstrated a modest association of sNfL and existing disability, which is consistent with previously reported associations (9-11, 22). However, neither log-transformed nor dichotomised sNfL levels predicted odds of relapse in the following year, medium-term clinical outcomes (5-year EDSS change), time to next relapse, or time to SAD using adjusted regression analyses in this real-world cohort.

Two of the most convincing studies to date that have previously demonstrated a significant relationship between sNfL levels and short-term clinical outcomes dichotomised patients using cut-off values for sNfL. Disanto *et al.* demonstrated increased annualised relapse rates and EDSS worsening 1-2 years post-sampling in clinically isolated syndrome (CIS) and RMS patients with sNfL levels above the 80th percentile of healthy control values (9). The same authors subsequently reproduced these findings in a separate cohort of 257 pwMS and CIS with an analysis adjusted for other predictors of outcome including baseline T2 lesion load (11). However, even when dichotomising the cohort the predictive power was modest (estimated OR 2.79 for sNfL above the 90th percentile), which is consistent with our finding

that sNfL values are of limited predictive value for individuals. There is also lack of agreement on the most appropriate sNfL cut-off. In a study of 41 patients with CIS and RRMS, baseline sNfL concentration >14.2pg/ml was predictive of disease activity at four years, but sensitivity of 72% and specificity of 57% suggest doubtful clinical utility (6).

Previous studies demonstrating a relationship between sNfL and recent relapse and MRI activity (2, 9, 23, 24), suggest that isolated sNfL levels are most informative about contemporary inflammatory disease activity. This is likely to drive correlations with short-term clinical outcomes and may also explain the loss of association between log-transformed sNfL and contemporary disability observed in our study when patients in relapse were excluded. It may also explain the lack of association between isolated baseline sNfL measurements and medium to long-term clinical outcomes, found in our study and by other groups. Canto *et al.* concluded that sNfL was not associated with relapse activity or long-term (10 years) disability progression in a cohort of 607 patients, although mean disease duration in these patients was 8.6 years, and 61% were receiving DMTs at the time of sampling (25). Another cohort of 122 pwMS in whom serum sampling was performed closer to diagnosis, found no association between baseline sNfL and EDSS after ten years (20). Two studies that have demonstrated an association of sNfL with long-term clinical outcomes included cohorts with up to 16.3 years disease duration at baseline serum sampling (12, 13). This raises the possibility that sNfL has more prognostic utility for medium-long term outcomes in individuals with more established MS, and less concurrent relapse and inflammatory disease activity. Removing the confounding effect of recent relapses on sNfL levels is relatively impractical in people with early MS for whom the biomarker could inform a treatment decision. Several groups have shown that sNfL remains elevated for around 3-months after an inflammatory event (2, 3, 9, 11, 17). There is some evidence that sequential sampling of sNfL, with an area-under-the-curve analysis, may provide a more useful marker of future disability, by adjusting for short-term fluctuations of sNfL (11, 20). We were unable to investigate the utility of longitudinal measurements of sNfL in our study due to the absence of sufficient numbers of repeated samples. Nevertheless, the optimum sampling interval needs to be ascertained, and the utility of any

predictive marker at diagnosis for making therapeutic decisions would be undermined by the need to measure it over many months.

There are other possible explanations for a lack of association between baseline sNfL and medium- to long-term clinical outcomes in this and other studies. Firstly, clinical measurements such as EDSS are widely appreciated to have limitations in capturing the complexity of MS disability. The demonstration in other studies of significant associations between baseline sNfL and imaging markers of MS outcome such as T2 lesion load and brain atrophy indicates that sNfL may still be a relevant predictive biomarker (24, 26, 27). Higher sNfL levels have been shown to predict higher rates of brain atrophy over durations as long as 10 years (21). The second possible explanation for a lack of association between baseline sNfL and medium- to long-term clinical outcomes in disease-modified cohorts is the masking effect that DMT has on the natural trajectory of the disease. All our analyses were adjusted or censored for DMT exposure, but we accept that this does not address selection bias for people with the most active disease to be treated with the most potent DMTs. Our cohort consisted of a relatively high proportion of patients who commenced monoclonal antibody therapy, which may include individuals with less favourable prognostic features at baseline. Nevertheless, we also used several time-based analyses of clinical outcomes, and censored follow-up at the commencement of DMT, but were still unable to demonstrate a predictive association of sNfL. Lastly, whilst CSF-NfL and sNfL have been shown to correlate (2, 6, 9, 10), sNfL may not adequately reflect neuroaxonal damage, because of diurnal CSF production rates, CSF flow paths that vary with posture, and sites of CSF absorption. Meanwhile, sNfL levels reflect both peripheral nerve and central sources of production, and bioavailability may be affected by the presence of circulating autoantibodies to neurofilament that develop in the context of disease (28, 29).

The use of observational real-world data brings advantages to the validation of markers that have previously been tested in clinic-based or clinical trial cohorts. However it also has limitations, in particular the lack of a systematic approach to the timing and acquisition of clinical and imaging data. We have tried to adjust for this in our analyses but recognise that

it may have influenced the current findings. We also used a fixed cut-off that does not take into account any effects of age on sNfL levels.

In conclusion, whilst we demonstrate a significant relationship between sNfL at the time of diagnosis and contemporary disability in this real-world cohort, sNfL appeared to be of limited clinical utility in predicting future irreversible neurological disability. Our findings on real-world data including patients at the point of diagnosis align with existing studies suggesting that sNfL remains insufficiently validated to aid prognosis and shape early treatment decisions at the time of diagnosis. Further studies exploring sequential sNfL measurements and work to develop universally accepted cut-offs are needed before sNfL could be incorporated as a prognostic marker in a clinical setting. However, the masking effect of DMTs on the natural disease trajectory and fluctuations in sNfL due to relapse activity will continue to pose challenges in the validation of any predictive biomarker for MS.

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Drs. Anderson, Bentley, Bianchi, and Joseph have nothing to disclose.

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AUTHORSHIP CONTRIBUTION STATEMENT

VA, ECT, NPR and GG contributed to study design, analysis and interpretation of the data, and drafting of the final manuscript. EB, FJ and RW-T contributed to data collection. SL performed biosample processing and requisition. LB performed NfL laboratory analysis. KEH advised on statistical analysis. SG and MM contributed to interpretation of the data, and drafting of the manuscript. All authors approved the final manuscript for intellectual content.

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