

# Serum Neurofilament Light concentrations are not associated with renal function in secondary progressive multiple sclerosis

T. Williams<sup>a,\*</sup>, H. Zetterberg<sup>b</sup>, J. Chataway<sup>a</sup>

<sup>a</sup> Queen Square Multiple Sclerosis Centre, UCL Queen Square Institute of Neurology, United Kingdom

<sup>b</sup> UK Dementia Research Institute at UCL, UCL Queen Square Institute of Neurology, United Kingdom

## ARTICLE INFO

### Keywords:

Neurofilament Light  
Multiple sclerosis  
Renal function  
Biomarkers

## ABSTRACT

**Background:** Neurofilament Light (NFL) is a promising biomarker of neuroaxonal injury. Its utility may be improved by expression relative to age-matched controls and by adjusting for other covariates, such as body mass index. It has recently been suggested that renal function may modulate the rate of clearance of NFL from circulation, which if confirmed would make renal function an important additional covariate to take into account when interpreting NFL data in research or clinical settings. Here we explore the relationship between renal function and NFL in a cohort of patients with secondary progressive multiple sclerosis (SPMS).

**Methods:** We examined data from patients with SPMS who took part in the MS-STAT randomised controlled trial. We use multivariable linear regression to explore the relationship between serum NFL and renal function, and additionally to examine whether including renal function as a covariate improves the ability of NFL to predict the subsequent rate of whole brain atrophy.

**Results:** Data on renal function and serum NFL was available for 122 patients. Mean eGFR 88 ml/min/1.73 m<sup>2</sup> (range 38.2–121.9). We found no evidence to support a relationship between renal function and serum NFL in this cohort. Furthermore, the inclusion of eGFR as a covariate in models assessing the relationship between NFL and the rate of whole brain atrophy had no significant effect upon the relationships observed.

**Conclusions:** We find no evidence for a relationship between renal function and NFL in a cohort of patients with secondary progressive multiple sclerosis. We hypothesise that the previously observed relationships between NFL and renal function related to associations between renal function and subclinical neuropathology, rather than due to modulating clearance of NFL from the circulation, but further research would be required to confirm such mechanisms.

## Introduction

Neurofilament light (NFL), quantified in serum or plasma, is rapidly progressing towards clinical practice as an easily accessible biomarker of neuro-axonal injury (Leppert and Kuhle, 2019). Facilitated by improvements in assay sensitivity, elevated NFL can be detected across a range of neurological disorders (Fyfe, 2019). It is not specific to any particular aetiology, instead quantifying the current degree of central or peripheral neuroaxonal injury independent of the disease process.

The accuracy of NFL in predicting clinically useful outcomes may be improved through the identification of additional covariates that modulate NFL independent of disease activity. Patient age is an established modifier of NFL concentrations in healthy controls, and the utility of NFL in diseased cohorts may be improved when NFL concentrations

are expressed relative to age-matched controls (Khalil et al., 2018). Similarly, when quantifying central nervous system (CNS) pathology, adjustment for circulating volume or body mass index (BMI) may again improve accuracy, accounting for the variable degree of dilution that occurs when CNS-derived NFL is released into the systemic circulation (Manouchehrinia et al., 2020).

Renal function has recently been reported to additionally modulate NFL concentrations (Akamine et al., 2020). The authors suggest that the negative correlation between renal function and NFL may be due to NFL being removed from the systemic circulation via the kidneys. If confirmed, this would be an important additional covariate to include in analyses of NFL, both when it is used as an outcome measure in clinical trials, and when contributing to clinical decision making.

Patients with secondary progressive multiple sclerosis (SPMS) have

\* Corresponding author.

E-mail address: [Thomas.williams@nhs.net](mailto:Thomas.williams@nhs.net) (T. Williams).

<https://doi.org/10.1016/j.bionps.2021.100044>

Received 22 September 2021; Received in revised form 6 October 2021; Accepted 9 October 2021

Available online 12 October 2021

2666-1446/© 2021 The Authors.

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

elevated serum NFL concentrations due to ongoing neuroaxonal damage within the CNS. At a group level, serum NFL is positively associated with measures of future disease worsening, such as whole brain atrophy (Williams et al., 2020a, 2020b). Here we re-examine the MS-STAT clinical trial cohort of patients with SPMS in order to further explore the role of renal function in NFL concentrations.

## Methods

The MS-STAT clinical trial cohort has been previously described (Chataway et al., 2014). Briefly, 140 patients with SPMS, age 18–65 and with an expanded disability status score (EDSS) of 4.0–6.5 were recruited. Serum samples were analysed for safety parameters, including renal function, using standard laboratory techniques as part of the screening procedure. eGFR was calculated by the abbreviated MDRD equation. Patients were subsequently randomised to high dose simvastatin (80 mg) or placebo, and assessed clinically and by magnetic resonance imaging (MRI) for 2 years.

Serum was stored at  $-80^{\circ}\text{C}$  until analysis. NFL was quantified using the Simoa NF-Light Advantage kit (Quanterix, Billerica, MA) on a HD-1 analyser, according to the manufacturer's instructions. Duplicate measurements were taken of each sample. Sample concentrations were extrapolated from a standard curve, fitted using a 4-parameter logistic algorithm. The lower limit of quantification (LLoQ) for NFL is 0.174 pg/ml (Quanterix, 2017). NFL data was skewed, and hence all analyses were performed following  $\log_2$ -transformation.

MRI was performed at baseline, 12 and 25 months, and analysed as described previously (Chataway et al., 2014; Eshaghi et al., 2019). The rate of whole brain atrophy was calculated from 3D T1-weighted images using the boundary shift integral method, and expressed as yearly percentage change in whole brain volume.

Multivariable linear regression models were constructed to assess the relationship between NFL and renal function. In the first model, baseline eGFR was included as the predictor variable, with baseline NFL as the outcome. Patient age, EDSS, T2 lesion volume, and number of relapses in the prior 24 months were additionally included as a covariates due to their established relationship with NFL.

A second model was constructed in order to assess whether the inclusion of eGFR as a covariate improved the ability of NFL to predict the subsequent rate of whole brain atrophy. Baseline NFL was included as the predictor variable, and yearly percentage change in whole brain volume as the outcome. Age and trial treatment allocation (high dose simvastatin or placebo) were included as covariates, and models were compared with and without the additional inclusion of eGFR as a further covariate.

For all regression models, assumptions were assessed regarding normality of residuals and homoskedasticity. When interpreting the coefficients and confidence intervals of the relationship between eGFR and sNFL, we inverse the  $\log_2$ -transformation of sNFL and present the relationships for a 10 ml/min/1.73 m<sup>2</sup> change in eGFR, so that the units are readily interpretable in pg/ml.

## Results

122 patients had baseline NFL and baseline eGFR data available, and were included in this analysis. The characteristics of this cohort are described in Table 1. The mean age was 51 years, and mean eGFR 88 ml/min/1.73 m<sup>2</sup> (S.D. 13.0, min 38.2, max 121.9). Of these patients, 112 additionally had data available on the rate of whole brain atrophy.

There was no evidence for an association between serum NFL and eGFR in this cohort (Fig. 1, and Table 1). The confidence intervals of our model suggest that a 10 ml/min/1.73 m<sup>2</sup> increase in eGFR, whilst controlling for the covariates, would be expected to result in a change in sNFL within the range of a 1.09 pg/ml decrease to a 1.06 pg/ml increase. Results were unchanged when we limited analyses to patients with age > 60 years (data not shown).

**Table 1**

Characteristics of the MS-STAT cohort with NFL and eGFR data.

N	122
Age	51.3 (6.9)
Gender (female)	67%
Duration MS (y)	21.4 (8.4)
Duration SPMS (y)	7.2 (5.2)
EDSS (median, IQR)	6 (5.5–6.5)
T2 lesion volume (ml)	21.8 (15.3)
Whole brain atrophy (%/year)	0.42% (0.52%)
eGFR (ml/min/1.73 m <sup>2</sup> )	88.8 (12.9; range 38.1–121.9)

Data represent mean (standard deviation) unless otherwise specified.

Including eGFR in models assessing the relationship between NFL and whole brain atrophy had no material impact upon the predictions (Table 2). eGFR was not in itself significantly associated with whole brain atrophy, and its inclusion as a covariate had minimal impact upon the relationship between NFL and whole brain atrophy (a doubling of baseline NFL was associated with a 0.207% increase in the rate of whole brain atrophy when eGFR was excluded, and a 0.206% increase in the rate of whole brain atrophy when eGFR was included. Overall model fit was similarly not improved by including eGFR as a covariate (likelihood ratio test,  $p = 0.718$ , AIC and BIC both worsened when eGFR included).

The table presents data from 3 separate linear regression models. In the first model, baseline eGFR (ml/min/1.73 m<sup>2</sup>) is the predictor, with  $\log_2$ -sNFL (pg/ml) as the outcome. Patient age, EDSS, T2 lesion volume, and number of relapses in the prior 24 months were additionally included as a covariates. We report the coefficient, with its 95% CI and p-value, for the relationship between eGFR and  $\log_2$ -sNFL from this model.

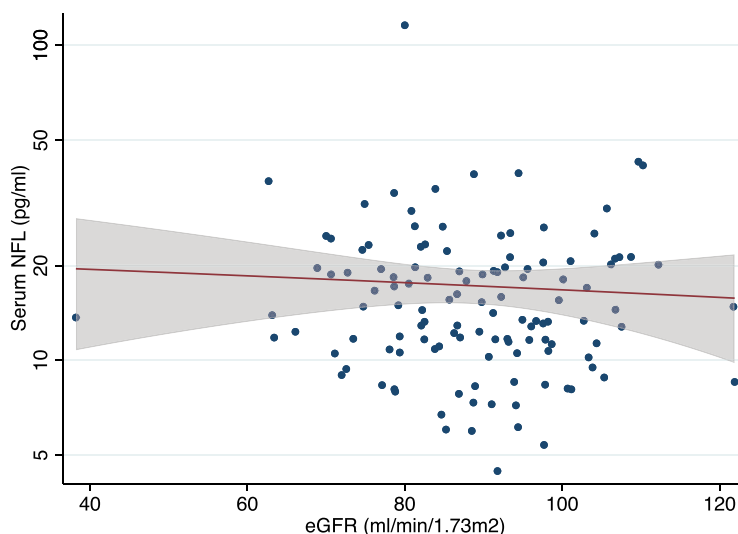
The second model assesses the relationship between baseline  $\log_2$ -sNFL (predictor), and the subsequent rate of whole brain atrophy over 2 years (outcome). Age and trial treatment allocation are included as covariates; eGFR is not included. We report the coefficient, with its 95% CI and p-value, for the relationship between baseline sNFL and the rate of whole brain atrophy.

The final model repeats this analysis, but additionally including baseline eGFR as a covariate, in order to show whether the inclusion of eGFR affects the relationship between sNFL and the rate of whole brain atrophy, and whether eGFR itself is associated with the outcome. eGFR, estimated glomerular filtration rate; sNFL, serum neurofilament light; CI, confidence interval; NA, not applicable; EDSS, expanded disability status scale.

## Discussion

In this cohort of patients with secondary progressive multiple sclerosis, we find no evidence for an association between renal function and serum NFL. Additionally, we find no evidence for renal function being an important covariate in the established relationship between serum NFL and the subsequent rate of whole brain atrophy (Williams et al., 2020a).

The previous report of a significant negative correlation between blood NFL and renal function was found in a cohort of healthy controls and patients with diabetes mellitus aged > 60 years. It is suggested that NFL may be cleared from the blood via the kidneys, which would make it vital to include renal function as a covariate in modelling of blood NFL data. It is important to note, however, that the molecular weight of NFL at 68 kDa is above the threshold at which free filtration at the glomerulus would be expected in the healthy kidney (Lawrence et al., 2017; Quanterix, 2017). Secondly, it is well established that declining renal function is associated with an increase in the incidence or severity of various neuropathologies (central or peripheral) in both those with and without diabetes (Kaewput et al., 2019; Seliger et al., 2004). These are often asymptomatic, and hence may be present in the cohorts previously examined (Akamine et al., 2020). A patient with worse renal function is



**Fig. 1.** Renal Function and Serum NFL in Multiple Sclerosis. Figure includes data from 122 patients with Secondary Progressive Multiple Sclerosis. Points represent raw data; line and shaded area represent a univariable linear regression slope and 95% confidence interval. There was no evidence of a relationship between serum NFL and eGFR in this cohort. NFL; Serum Neurofilament Light; eGFR, estimated Glomerular Filtration Rate.

**Table 2**  
Outputs of models assessing the relationship between eGFR, sNFL, and whole brain atrophy rate.

Model	Coefficient	95% CI of coefficient and p-value
<b>Baseline eGFR predicting Baseline log<sub>2</sub>-sNFL</b>	eGFR: -0.002	eGFR: -0.012 to 0.008 p = 0.677
<b>Baseline log<sub>2</sub>-sNFL predicting whole brain atrophy rate, without eGFR as covariate</b>	Log <sub>2</sub> NFL: 0.207 eGFR: NA	Log <sub>2</sub> NFL: 0.082-0.332 p = 0.001 eGFR: NA
<b>Baseline log<sub>2</sub>-sNFL predicting whole brain atrophy rate, with eGFR as covariate</b>	Log <sub>2</sub> NFL: 0.206 eGFR: -0.001	Log <sub>2</sub> NFL: 0.081-0.332 p = 0.002 eGFR: -0.008 to 0.006 p = 0.725

therefore more likely to have an asymptomatic peripheral neuropathy, or asymptomatic cerebrovascular disease, resulting in increased release of NFL through the resulting neuroaxonal damage.

If the previously observed negative relationship between NFL and renal function was due to renal function modulating the rate at which NFL is cleared from systemic circulation, one would expect to see this relationship in other disease cohorts. This would include patients with SPMS, where the on-going neuroaxonal damage drives release of NFL from the CNS. Renal function would also be expected to significantly modulate the relationship between NFL and other measures of disease severity, such as the rate of whole brain atrophy.

Whilst we conclude that there is no evidence to support a relationship between sNFL and renal function in this cohort of patients with SPMS, we acknowledge differences between our cohort and that presented by Akamine et al. Significant renal impairment was rare in the MS-STAT study, and hence our data should only be interpreted across the range of eGFR reported in Table 1. Additionally, whilst our results were unchanged when restricting analyses to those > 60 years, the majority of our patients were younger than 60. We also cannot exclude statistically significant relationships between eGFR and sNFL being found in larger cohorts, but the confidence intervals of our models suggest that this study is sufficiently powered to exclude an important influence of eGFR on sNFL in either direction. Based upon our conclusions, we therefore hypothesise that NFL may not be to be cleared from

the systemic circulation by a mechanism dependent upon renal function, and postulate that the previously observed relationship between blood NFL and renal function may be related to the established associations between worsening renal function and various, often asymptomatic, neuropathologies (Akamine et al., 2020). Such hypotheses, however, cannot be confirmed from our study, and required further research in cohorts that include the quantification of both renal function and NFL, together with measures of the subclinical neuropathologies (CNS small vessel disease, peripheral neuropathies) that we postulate may explain the previously observed relationship.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Dr. Thomas Williams is funded from the MS-STAT2 Clinical Trial Grant, United Kingdom (NIHR, UK MS Society, National MS Society).

**Author contribution statement**

**Thomas Williams:** study conception, data analysis, drafting of manuscript. **Henrik Zetterberg:** manuscript revisions. **Jeremy Chatway:** Chief Investigator of the MS-STAT clinical trial, manuscript revisions.

**Declaration of Competing Interest**

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: TW: is currently funded by the MS-STAT2 trial Grant, derived from the UK MS Society, the US MS Society, and the NIHR. I have received speaker honorarium for educational talks for Novartis and Merck. HZ: has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx, has given lectures in symposia sponsored by Cellctricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. JC: has received support from the Efficacy and Mechanism Evaluation Programme and Health Technology Assessment Programme (NIHR); UK Multiple Sclerosis Society and National Multiple Sclerosis Society. In the last three years, he has been a local principal investigator for trials in multiple sclerosis funded by: Receptos,

Novartis and Biogen Idec, and has received an investigator grant from Novartis outside this work. He has taken part in Advisory Boards/consultancy for Roche, Merck, MedDay, Biogen and Celgene.

## References

- Akamine, S., Marutani, N., Kanayama, D., Gotoh, S., Maruyama, R., Yanagida, K., Sakagami, Y., Mori, K., Adachi, H., Kozawa, J., Maeda, N., Otsuki, M., Matsuoka, T., Iwahashi, H., Shimomura, I., Ikeda, M., Kudo, T., 2020. Renal function is associated with blood neurofilament light chain level in older adults. *Sci. Rep.* 10 (1), 1–7. <https://doi.org/10.1038/s41598-020-76990-7>.
- Chataway, J., Schuerer, N., Alsanousi, A., Chan, D., MacManus, D., Hunter, K., Anderson, V., Bangham, C.R.M., Clegg, S., Nielsen, C., Fox, N.C., Wilkie, D., Nicholas, J.M., Calder, V.L., Greenwood, J., Frost, C., Nicholas, R., 2014. Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. *Lancet* 383 (9936), 2213–2221. [https://doi.org/10.1016/S0140-6736\(13\)62242-4](https://doi.org/10.1016/S0140-6736(13)62242-4).
- Eshaghi, A., Kievit, R.A., Prados, F., Sudre, C.H., Nicholas, J., Cardoso, M.J., Chan, D., Nicholas, R., Ourselin, S., Greenwood, J., Thompson, A.J., Alexander, D.C., Barkhof, F., Chataway, J., Ciccarelli, O., 2019. Applying causal models to explore the mechanism of action of simvastatin in progressive multiple sclerosis. *Proc. Natl. Acad. Sci.* 116 <https://doi.org/10.1073/pnas.1818978116>.
- Fyfe, I., 2019. Neurofilament light chain—new potential for prediction and prognosis. *Nat. Rev. Neurol.* 15 (10), 557. <https://doi.org/10.1038/s41582-019-0265-2>.
- Kaewput, W., Thongprayoon, C., Rangsri, R., Mao, M.A., Satirapoj, B., Cheungpasitporn, W., 2019. The association between renal function and neurological diseases in type 2 diabetes: a multicenter nationwide cross-sectional study. *Hosp. Pract.* 47 (1), 46–52. <https://doi.org/10.1080/21548331.2019.1549916>.
- Khalil, M., Pirpamer, L., Hofer, E., Voortman, M.M., Hallwirth, F., Barro, C., Fazekas, F., Schmidt, R., Kuhle, J., 2018. Normative values of serum neurofilament light levels. *ECTRIMS* 2018, 229010, P1170.
- Lawrence, M.G., Altenburg, M.K., Sanford, R., Willett, J.D., Bleasdale, B., Ballou, B., Wilder, J., Li, F., Miner, J.H., Berg, U.B., Smithies, O., 2017. Permeation of macromolecules into the renal glomerular basement membrane and capture by the tubules. *Proc. Natl. Acad. Sci. USA* 114 (11), 2958–2963. <https://doi.org/10.1073/pnas.1616457114>.
- Leppert, D., Kuhle, J., 2019. Blood neurofilament light chain at the doorstep of clinical application. *Neurol.: Neuroimmunol. NeuroInflamm.* 6 (5), 4–6. <https://doi.org/10.1212/NXI.0000000000000599>.
- 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 (1), 139–143. <https://doi.org/10.1002/acn3.50972>.
- Quanterix, 2017. Simoa™ NF-light® Advantage Kit Simoa™ NF-light® Advantage Kit SR-X Data Sheet. 1–2. (<https://www.quanterix.com/sites/default/files/assays/N4PA>) Data Sheet SR-X.pdf.
- Seliger, S.L., Siscovick, D.S., Stehman-Breen, C.O., Gillen, D.L., Fitzpatrick, A., Bleyer, A., Kuller, L.H., 2004. Moderate renal impairment and risk of dementia among older adults: the cardiovascular health cognition study. *J. Am. Soc. Nephrol.* 15 (7), 1904–1911. <https://doi.org/10.1097/01.ASN.0000131529.60019.FA>.
- Williams, T., Holdsworth, K., Nicholas, J., Katsanoulis, T., Wellington, H., Heslegrave, A., Zetterberg, H., Frost, C., Chataway, J., 2020a. Serum neurofilaments in Secondary Progressive Multiple Sclerosis: analysis from the MS-STAT trial. *ECTRIMS*.
- Williams, Thomas, Zetterberg, H., Chataway, J., 2020b. Neurofilaments in progressive multiple sclerosis: a systematic review. *J. Neurol.* <https://doi.org/10.1007/s00415-020-09917-x>.