ORIGINAL ARTICLE

The relationship of cerebrospinal fluid neurofilament levels with magnetic resonance imaging lesion location and disease activity in multiple sclerosis

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Abstract

Background and purpose: Neurofilament light chain (NFL) is an accepted biomarker of disease activity in multiple sclerosis (MS), but its relationship with magnetic resonance imaging (MRI) activity particularly in reference to lesion location and recurrent activity is not well understood.

Methods: In 139 MS patients who underwent lumbar punctures with follow-up in 25, the relationship between cerebrospinal fluid (CSF) NFL and cranial MRI based on lesion location and lesion number was evaluated. Spearman rank correlation was used to assess the association between CSF NFL and MRI lesion location and lesion counts at baseline and follow-up at 1 year. Multiple linear regression analysis was performed to assess which lesion location was most strongly associated with CSF NFL values.

Results: The associations between baseline CSF NFL and lesion location and follow-up lesions were modest, whilst those between baseline MRI and follow-up CSF NFL were greater: periventricular \((r = 0.31, p = 0.141)\), juxtacortical \((r = 0.47, p = 0.022)\), infratentorial \((r = 0.71, p \leq 0.001)\) and cord lesions \((r = 0.60, p = 0.002)\). All associations, however, improved following adjustment for disease duration and type of MS. Modelling revealed 53% of \((\log)\) CSF NFL could be explained by variance in baseline MRI lesion location.

Conclusions: Baseline CSF NFL did not correlate with current or future MRI activity and lesion location. However, baseline MRI activity explained around 53% of the variation in the follow-up CSF NFL, suggesting that the relationship between MRI and CSF NFL is mainly precedent rather than an association, that is one occurring before the other.

KEYWORDS
cerebrospinal fluid, MRI lesion count, MRI lesion location, neurodegeneration, neurofilament light chain

INTRODUCTION

The evaluation of disease activity in multiple sclerosis (MS) for treatment decisions until recently has relied upon the demonstration of magnetic resonance imaging (MRI) disease activity, namely T2 lesions, gadolinium enhancement and brain atrophy. The introduction of neurofilament light chain (NFL) levels into this equation as a biomarker, however, has led to an increasing understanding of the...
underlying neurodegenerative process with a greater sensitivity towards predicting long-term disease progression [1, 2], but the relationship between the two is not clear. First, the exact relationship between lesion location and NfL levels is not known. Secondly, there is uncertainty about the temporal relationship between MRI activity and NfL levels, that is, is it a precedent (an earlier event which serves as a guide) or an association (an event that simply precedes another). Rosso et al. [3] found that serum NfL levels were elevated within a 3-month window around new disease activity as evidenced by gadolinium enhancement and recent clinical relapse. In a sequential lumbar puncture study, cerebrospinal fluid (CSF) NfL was also found to be elevated in the first 2–3 months after a clinical relapse [4]. Location-wise, both brain and spinal cord gadolinium enhancing lesions rather than either individually were associated with elevated serum NfL levels, suggesting that serum NfL is a bulk biomarker of disease activity [5]. However, in a small study of 47 MS patients CSF NfL was found to correlate with cortical lesion volumes, although the authors do not discuss lesion location within the brain [6]. Therefore, the aims of this study were to evaluate the relationship between lumbar CSF NfL levels and lesion location, and the temporal relationship between MRI activity and NfL levels over two time points. The influence of age, gender, disease duration, MS disease sub-type, MS treatment and clinical relapses on this has also been factored in.

MATERIALS AND METHODS

Patient selection

A cohort of 139 MS patients who had undergone CSF sampling for NfL analysis at our centre with MRI scans within 6 months of the lumbar puncture were selected for this study. Of this cohort 25 MS patients (three to check their John Cunningham virus status on natalizumab; 22 to check treatment response) had a repeat lumbar puncture for NfL a year later with follow-up imaging. Clinical information on age, gender, the type of MS, disease duration, treatment and relapses over the past year was collected. This study was approved by the London—City and East Research Ethics Committee (20/LO/0023).

Cerebrospinal fluid neurofilament light chain analyses

Cerebrospinal fluid samples were collected in polypropylene tubes and centrifuged on the same day at 400 rpm for 10 min before aliquoting and storing at −80°C until use. CSF NfL measurements were performed at the Blizard Institute, Queen Mary University of London, London, UK, using the commercially available and validated solid-phase sandwich ELISA from Uman Diagnostics. The test uses an NfL-capturing antibody coated to the solid phase of a strip plate and a tracer antibody conjugated to horseradish peroxidase for the detection of captured NfL protein. All NfL analyses were performed in duplicate before averaging. CSF NfL measurements (pg/ml) were calculated using a standard curve according to the manufacturer’s instructions. The detection limit of the ELISA was 33 pg/ml. Intra- and inter-assay coefficients of variation were below 10%.

Magnetic resonance imaging

All patients were scanned on either a 1.5-T or 3-T Siemens Magnetom scanner with a standard protocol that included an axial T2-weighted scan and a three-dimensional volumetric fluid inversion recovery (FLAIR) sequence that were used for lesion assessment. Imaging parameters for the 1.5-T MR scanner axial T2 sequence were repetition time (TR) 4340 ms, echo time (TE) 93 ms, flip angle 150°, slice thickness 5 mm and 3D FLAIR TR 4800 ms, TE 402 ms, inversion time (TI) 1600 ms, fat suppression and 1 mm slice thickness. Imaging parameters for the 3-T MR scanner axial T2 sequence were TR 3500 ms, TE 106 ms, flip angle 150°, slice thickness 5 mm and 3D FLAIR TR 6000 ms, TE 395 ms, TI 2100 ms and 1 mm slice thickness. Imaging of the cervical spine included a sagittal T2-weighted sequence and axial T2-weighted imaging. Imaging parameters for the 1.5-T MR scanner sagittal T2 sequence were TR 3500 ms, TE 83 ms, flip angle 150°, slice thickness 3 mm and axial T2 sequence TR 5000 ms, TE 96 ms, flip angle 150° and 3 mm slice thickness. Imaging parameters for the 3-T MR scanner sagittal T2 sequence were TR 3200 ms, TE 115 ms, flip angle 124°, slice thickness 3 mm and axial T2 sequence TR 1070 ms, TE 17 ms, flip angle 25° and 3 mm slice thickness.

Lesion location was subdivided into periventricular, juxtacortical, infratentorial and spinal cord. Assessment of lesion load for both the initial and follow-up scans was recorded as 0, no lesion; 1, one lesion; 2, two or more lesions; 3, confluent lesion. Comparative assessment for disease activity was recorded as 0, no new lesion; 1, new lesion(s). Each MR scan was assessed independently by two radiologists (one neuroradiologist with 8 years of experience, one radiologist with 2 years’ experience of brain MR reporting) blinded to NfL data and clinical outcomes. Where there was a disagreement in values, an average of the two is given.

Statistical analysis

Owing to the exploratory nature of analyses there is no predefined hypothesis. Assessment of inter-observer agreement was done using the kappa statistic, with 95% confidence interval calculated using bootstrapping with 1000 replications. Correlation and linear regression was used to assess the strength of association between baseline NfL values and MRI lesions according to location and between baseline CSF NfL values and follow-up MRI lesions at year 1. Multiple linear regression was used to determine which MRI lesion location was most strongly associated with CSF NfL values (CSF NfL values log-transformed due to positive skew). Adjustments for multiple comparisons have not been made; however, adjustments for covariates disease duration, months between lumbar puncture and
MRI, age, sex, relapses, type of MS and treatment at baseline were performed and are presented in Table S1-S5. Statistical significance was taken as \( p < 0.05 \) (two-tailed) and all analyses were performed using Stata version 15 (StataCorp).

RESULTS

Descriptive statistics on baseline (\( n = 139 \)) and follow-up (\( n = 25 \)) CSF neurofilaments and MRI lesion location at baseline and follow-up can be found in Table 1. The median duration between the MRI and the lumbar puncture was a median 0.1 months (interquartile range -0.7, 0.8). The agreement between the two marking radiologists ranged from kappa 0.783 to 0.937 for the different lesion locations on MRI indicating substantial to near perfect agreement.

Association between baseline CSF neurofilament light chain and MRI lesion location

There were modest associations between baseline NfL values and MRI lesion location (Figure 1). Baseline CSF NfL was positively associated with baseline periventricular (\( r = 0.275, p = 0.001, n = 137 \)), juxtacortical (\( r = 0.251, p = 0.003, n = 137 \)), infratentorial (\( r = 0.244, p = 0.004, n = 137 \)) and less so baseline cervical cord lesions (\( r = 0.178, p = 0.039, n = 135 \)). Following adjustment for covariates, the slope is steeper when adjusted for disease duration and type of MS, in this case secondary progressive MS (SPMS), with borderline significance for all lesion locations (see Table S1: periventricular lesions \( r = 0.356, p = 0.006, \) juxtacortical lesions \( r = 0.341, p = 0.005, \) infratentorial lesions \( r = 0.348, p = 0.003, \) cord lesions \( r = 0.279, p = 0.012 \) for disease duration; and periventricular lesions \( r = 0.350, p = 0.031, \) juxtacortical lesions \( r = 0.338, p = 0.021, \) infratentorial lesions \( r = 0.324, p = 0.027, \) cord lesions \( r = 0.289, p = 0.035 \) for SPMS).

There were less strong associations between baseline CSF NfL values and follow-up MRI lesions at year 1, none of which was statistically significant. With adjustment for disease duration the association was again strengthened (see Table S2): periventricular lesions \( r = 0.324, p = 0.021, \) juxtacortical lesions \( r = 0.316, p = 0.019, \) infratentorial lesions \( r = 0.327, p = 0.014, \) and for type of MS, in this case primary progressive MS (PPMS) (periventricular lesions \( r = 0.442, p = 0.011, \) juxtacortical lesions \( r = 0.434, p = 0.010, \) infratentorial lesions \( r = 0.438, p = 0.009, \) cord lesions \( r = 0.494, p = 0.003 \)) and relapses with cord lesions only (\( r = 0.361, p = 0.006 \)). There were no significant associations between baseline CSF NfL values and change in the number of lesions (i.e., follow-up lesions – baseline).

### TABLE 1  Descriptive statistics

|                         | N     | Mean | SD   | Median | IQR       |
|-------------------------|-------|------|------|--------|-----------|
| Gender                  | 60 male; 79 female | –    | –    | –      | –         |
| Age                     | 139   | 43   | 13   | 44     | 32 to 52  |
| Relapse prior to baseline | 67 no; 72 yes | –    | –    | –      | –         |
| Relapse prior to year 1  | 24 no; 1 year | –    | –    | –      | –         |
| Disease duration (years) | 139   | 6    | 7    | 3      | 1 to 10   |
| Type of MS              | 59 RRMS; 32 PPMS; 24 SPMS; 24 CIS | –    | –    | –      | –         |
| On treatment at baseline | 114 no; 25 yes | –    | –    | –      | –         |
| On treatment at year 1   | 8 no; 17 yes | –    | –    | –      | –         |
| Months between lumbar puncture and MRI | 137 | 0.00 | 2.00 | 0.07 | -0.68 to 0.77 |
| CSF NfL (pg/ml)          |       |      |      |        |           |
| Baseline                 | 139   | 973  | 1906 | 381    | 221 to 723|
| Year 1                   | 25    | 420  | 216  | 401    | 235 to 602|
| Lesion location          |       |      |      |        |           |
| Baseline periventricular lesions | 137 | 1.91 | 0.96 | 2.0    | 2.0 to 2.5|
| Baseline juxtacortical lesions | 137 | 1.28 | 0.92 | 2.0    | 0.0 to 2.0|
| Baseline infratentorial lesions | 137 | 1.07 | 0.96 | 1.0    | 0.0 to 2.0|
| Baseline cervical cord lesions | 135 | 1.27 | 1.07 | 1.5    | 0.0 to 2.0|
| Follow-up periventricular lesions | 72 | 2.04 | 0.85 | 2.0    | 2.0 to 3.0|
| Follow-up juxtacortical lesions | 72 | 1.35 | 0.9  | 2.0    | 0.5 to 2.0|
| Follow-up infratentorial lesions | 72 | 1.17 | 0.96 | 1.3    | 0.0 to 2.0|
| Follow-up cord lesions    | 60    | 1.42 | 1.01 | 2.0    | 0.0 to 2.0|

Abbreviations: CIS, clinically isolated syndrome; CSF, cerebrospinal fluid; IQR, interquartile range; MRI, magnetic resonance imaging; NfL, neurofilament light chain; PPMS, primary progressive multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.
Association between follow-up CSF neurofilament light chain and MRI lesion location

Despite the small sample number, the magnitude of the association between follow-up CSF NfL and baseline MRI lesion location was uniformly higher—periventricular lesions ($r = 0.310$, $p = 0.141$, $n = 24$, Figure 2a), juxtacortical ($r = 0.466$, $p = 0.022$, $n = 24$, Figure 2b), infratentorial ($r = 0.708$, $p < 0.001$, $n = 24$, Figure 2c) and cervical cord ($r = 0.600$, $p = 0.002$, $n = 23$, Figure 2d). The adjusted figures for covariates were not statistically significant apart from the type of MS being PPMS with periventricular lesions (see Table S3); $r = 0.663$, $p = 0.020$, juxtacortical lesions ($r = 0.680$, $p = 0.017$) and cord lesions ($r = 0.735$, $p = 0.040$).

The association between follow-up CSF NfL and follow-up MRI lesion location 1 year later was still highly significant with infratentorial lesions ($r = 0.633$, $p = 0.0001$, $n = 16$) and less so with juxtacortical lesions ($r = 0.546$, $p = 0.029$, $n = 16$). The relationship between follow-up CSF NfL and follow-up occurrence of lesions in the infratentorial and juxtacortical locations was lost after adjustment for covariates (see Table S4). There was no significant association of follow-up NfL with changes in lesions, including in the infratentorial compartment ($r = 0.0141$, $p = 0.961$, $n = 16$).

Determination of which MRI lesion location is most strongly associated with CSF NfL values

It was also investigated whether starting disease-modifying treatments (DMTs) influenced change in CSF NfL levels compared to patients not on a DMT or remaining on the same DMT. The median CSF NfL (interquartile range) for those that started on a DMT was $-191$ ($-568$ to $-5$) and for the other group it was $-63$ ($-800$ to $+5$); $p = 0.664$ (Mann–Whitney U test; Figure S1).

Multiple linear regression of baseline CSF NfL values with baseline lesion locations demonstrated that only 9% ($R^2 0.09$) of the variation in baseline (log) CSF NfL values could be explained by variation in MRI lesion location. The surviving independent association was with periventricular lesion location, but this is inconclusive because of the very small $R^2$. However, with follow-up CSF NfL 53% ($R^2 0.53$) of the variation in follow-up (log) CSF NfL values could be predicted by baseline MRI lesion locations, with 50% of (log) CSF NfL variation explained by baseline infratentorial lesions.
DISCUSSION

The relationship between CSF NfL, a biomarker of neuronal damage in MS, and MRI lesion location and activity is not well understood. In this study these relationships were examined in a hypothesis generating study. A stronger relationship was found between baseline MRI and follow-up CSF NfL than contemporary CSF NfL; the previous MRI activity explained around 53% of the variation in follow-up CSF NfL, that is, CSF NfL levels are more indicative of past disease activity. It is also possible that this relationship is due to ongoing Wallerian degeneration that is occurring as a result of lesion activity and may be an important consideration in smouldering MS. Rosso et al. [3] found at least in the blood that NfL was a poor indicator of future clinical relapses, and levels were 35% higher in samples taken within 3 months of gadolinium enhancement, supporting this precedent hypothesis. This is similar to Lycke et al. who found that CSF NfL remained elevated 2–3 months after a clinical relapse [4]. Singh et al. in a neuropathological study demonstrated that ongoing Wallerian degeneration in MS was focused in perilesional white matter in actively demyelinating and chronically active lesions, supporting a role for focal pathology in neurodegeneration [7].

Secondly, regardless of MS being a multifocal disease process, CSF NfL levels were found to be best associated with infratentorial lesions, with 50% of (log) follow-up CSF NfL variation being due to baseline infratentorial lesions. Concurrent with our findings, infratentorial pathology has also been found in a number of imaging studies to be a strong predictor of secondary progression in MS [8–10]. It is therefore possible that there is a strong association between infratentorial pathology, degree of neuroaxonal damage and disability progression.

Potential covariates that might potentially influence our findings were adjusted for and strong influences from disease duration and the type of MS, for example progressive MS (SPMS or PPMS) versus relapsing–remitting MS, were found. The strength of association between CSF NfL and lesion location improved in most instances following adjustment for these. It can be speculated that NfL is a bulk marker of disease activity in MS and what is being captured here is the increase in this with longer disease duration and disease progression, similar to greater lesion loads visualized by MRI with chronic MS. Therefore the relationship between CSF NfL and MRI lesions strengthens when disease duration and progression are factored in. Other factors, such as age, relapse activity or whether on treatment at baseline, starting treatment versus no treatment/no change in treatment did not influence the association.

No association was found between baseline CSF NfL and the follow-up MRI activity, which has implications for the use of this

**FIGURE 2** Association between follow-up CSF NfL and baseline MRI lesion location: (a) periventricular, (b) juxtacortical, (c) infratentorial and (d) cervical cord. Number of baseline lesions are graded a 0 (no lesions), 1 (one lesion), 2 (two or more lesions) and 3 (confluent lesions)
test as a predictive marker for future activity. It should be noted that this may be due to the commencement of immunosuppressive treatments in the interim and the follow-up scans were only within a year of the baseline CSF sample. Although follow-up treatments were not found to influence the association much it is well known that immunosuppressive treatments particularly highly active ones lower CSF NfL values. There is also good evidence from longitudinal cohort studies supporting a prognostic role for CSF NfL in determining future MRI disease activity and, in particular, brain atrophy [11].

One of the main limitations of this study was the low availability of follow-up CSF in patients. Repeated lumbar puncture studies owing to their invasive nature are low in numbers but have in this instance provided invaluable pathological information. Of the 25 individuals who had a repeat CSF sampling, the majority were for checking treatment response. It is unlikely that the small number who underwent repeat CSF sampling to check their John Cunningham virus would bias the overall analysis, but it needs to be considered. It was not possible to study lesion change in this study either as the magnitude of change was small, and larger sampled studies are needed to look at change in MRI lesion load in more detail. Another potential limitation was that only white matter lesion load was assessed and cortical lesions were not investigated. Moreover, multiple comparisons were not adjusted for since it was pre-planned to investigate a number of different hypotheses, and in such context correction can be inappropriate [12, 13].

In summary, it is shown that CSF NfL in MS is reflective of more precedent MRI disease activity than current activity, or future activity, and it associates mostly with infratentorial lesion load. This dynamic between biology and imaging lesion burden will aid in deciphering the clinical-radiological paradox further, but analysis from large-scale, well-characterized prospective cohorts is needed to verify these findings.

AUTHOR CONTRIBUTIONS
Ashok Adams: Data curation (equal); formal analysis (equal); methodology (equal); project administration (equal); writing – original draft (equal); writing – review and editing (equal). William Tilden: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); validation (equal). Jonathan Bestwick: Formal analysis (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). David Holden: Data curation (equal); formal analysis (equal); methodology (equal). Lucia Bianchi: Data curation (equal); formal analysis (equal); methodology (equal). Ide Smets: Data curation (equal); methodology (equal); resources (equal). Gavin Giovannoni: Conceptualization (equal); writing – review and editing (equal). Sharmilee Gnanapavan: Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); project administration (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST
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DATA AVAILABILITY STATEMENT
All data included in these analyses will be shared as anonymized data via request from any qualified investigator.

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SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

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