Serum neurofilament light chain correlations in patients with a first clinical demyelinating event in the REFLEX study: a *post hoc* analysis

Jens Kuhle, David Leppert, Giancarlo Comi, Nicola de Stefano, Ludwig Kappos (ID)**, Mark S. Freedman, Andrea Seitzinger and Sanjeev Roy**

Abstract

Background: In REFLEX, subcutaneous interferon beta-1a (sc IFN β-1a) delayed the onset of multiple sclerosis (MS) in patients with a first clinical demyelinating event (FCDE).

Objectives: This *post hoc* analysis aimed to determine whether baseline serum neurofilament light (sNfL) chain can predict conversion to MS and whether correlations exist between baseline sNfL and magnetic resonance imaging (MRI) metrics.

Methods: sNfL was measured for 494 patients who received sc IFN β-1a 44 μg once weekly (qw; $n = 168$), three times weekly (tiw; $n = 161$), or placebo ($n = 165$) over 24 months. Median baseline sNfL (26.1 pg/mL) was used to define high/low sNfL subgroups. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox's proportional hazard model to determine factors influencing the risk of conversion to MS. Kaplan–Meier estimates calculated median time-to-conversion to MS (McDonald 2005 criteria) or clinically definite MS (CDMS; Poser criteria). Correlations between sNfL and MRI findings were assessed using Spearman's rank correlation coefficient (r).

Results: Multivariable models indicated that high baseline sNfL was associated with the likelihood of converting to MS and inversely to time-to-conversion (HR = 1.3, 95% CI: 1.03–1.64; $p = 0.024$). Significant additional factors affecting conversion to McDonald MS were on-study treatment (sc IFN β-1a/placebo; qw: HR = 0.59, 95% CI: 0.46–0.76; tiw: HR = 0.45, 95% CI: 0.34–0.59), classification of FCDE (monofocal/multifocal; HR = 0.69, 95% CI: 0.55–0.85), and most baseline imaging findings (T2 and T1 gadolinium-enhancing [Gd+] lesions; HR = 1.02, 95% CI: 1.01–1.03 and HR = 1.07, 95% CI: 1.03–1.11); all $p \leqslant 0.001$. Conversion to CDMS showed similar results. At month 24, sNfL was strongly correlated with a mean number of combined unique active ($r = 0.71$), new T2 ($r = 0.72$), and new T1 Gd+ ($r = 0.60$) lesions; weak correlations were observed between sNfL and clinical outcomes for all treatment groups.

Conclusion: Higher baseline sNfL was associated with an increased risk of MS conversion, a risk that was mitigated by treatment with sc IFN β-1a tiw.

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Keywords: interferon beta-1a, multiple sclerosis, serum neurofilament light chain

Correspondence to:
Jens Kuhle
Department of Neurology, University Hospital Basel, Petersgraben 4, Basel CH-4031, Switzerland

Multiple Sclerosis Centre and Research Center for Clinical Neuroimmunology and Neuroscience (RC2NB), Departments of Biomedicine and Clinical Research, University Hospital and University of Basel, Spitalstrasse 2, Basel CH-4031, Switzerland
Jens.Kuhle@usb.ch

David Leppert
Ludwig Kappos
Multiple Sclerosis Centre and Research Center for Clinical Neuroimmunology and Neuroscience (RC2NB), Departments of Biomedicine and Clinical Research, University Hospital and University of Basel, Basel, Switzerland

Giancarlo Comi
Casa di Cura Privata del Policlinico, Università Vita-Salute San Raffaele, Milan, Italy

Nicola de Stefano
Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy

Mark S. Freedman
Department of Medicine and the Ottawa Hospital Research Institute, University of Ottawa, Ottawa, ON, Canada

Andrea Seitzinger
Biostatistics, Merck Healthcare KGaA, Darmstadt, Germany

Sanjeev Roy
Global Clinical Development – Immunology, Ares Trading S.A. (an affiliate of Merck KGaA), Eysins, Switzerland

Introduction

Multiple sclerosis (MS) is a chronic, disabling disease that requires long-term treatment and regular monitoring while also being associated with negative effects on health-related quality of life due to reduced physical, cognitive, and psychosocial functioning.[1–3]

The current standard for measuring neuroaxonal damage associated with disease activity in patients with MS is magnetic resonance imaging (MRI)-assessed lesion burden and brain atrophy.[4] Alongside such imaging, various biomarkers have been explored to help identify patients at greater risk of aggressive disease phenotypes as well as aiding the selection of appropriate disease-modifying therapies (DMTs).[5–7] Serum neurofilament light (sNfL) chain is meanwhile a very promising and useful biomarker of neuroaxonal damage in MS as well as drug response. Six years before the onset of clinical MS, sNfL levels are higher than in matched controls, suggesting the biomarker is hugely relevant for prognosis and that neuroaxonal damage occurs for years before MS onset.[8] The baseline level of sNfL appears to predict disease course in early MS[9] and future disability progression in those with established disease.[10,11] The timely identification of patients with potentially more aggressive forms of MS or suboptimal treatment response could mean that the disease course may be favorably altered by providing early access to high-efficacy treatments.[9,12]

Subcutaneous interferon beta-1a (sc IFN β-1a) has proven efficacy in the treatment of patients with a first clinical demyelinating event (FCDE), in terms of significantly delaying the onset of MS (according to McDonald 2005 criteria) or clinically definite MS (CDMS; according to Poser criteria) in the REFLEX study.[13] Of note, a prior analysis of patients from this study found that those treated with sc IFN β-1a had reduced sNfL concentration as early as 6 months post-baseline.[14] Against this background, the current analysis was designed to further explore whether baseline sNfL concentration can predict conversion to MS in patients with an FCDE. Our primary research question was to determine whether baseline sNfL concentration can predict conversion to MS and whether any correlation exists between sNfL and MRI outcomes in such patients.

Methods

Study design and procedures
In this *post hoc* analysis, sNfL concentrations were measured in blood samples from patients who participated in the 2-year, randomized, double-blind REFLEX study (NCT00404352), in which eligible patients received sc IFN β-1a 44 μg (Rebif®, Merck Healthcare KGaA, Darmstadt, Germany) once weekly (qw), three times weekly (tiw), or placebo. Enrolment for REFLEX occurred at 67 study locations across 27 countries, beginning in November 2006, with study completion in July 2011.

As has been described previously,[13] patients were eligible for inclusion in the REFLEX study if they were aged 18–50 years, had an Expanded Disability Status Scale (EDSS) score of ≤5.0, and had experienced a first event suggestive of MS ≤60 days before study entry; and at least two clinically silent lesions of ≥3 mm on T2-weighted brain MRI scan (at least one of which was ovoid, periventricular, or infratentorial). Exclusion criteria included a diagnosis of MS according to McDonald 2005 criteria, other diseases that could explain the signs and symptoms a patient was experiencing at the index event, and previous use of any other immunomodulatory or immunosuppressive therapy, or use of any corticosteroids within 30 days prior to initiating treatment in REFLEX.[13]

Study endpoints and statistical analysis
The *post hoc* analyses described here were performed using data from the double-blind period of the REFLEX study up to CDMS conversion and included patients who had serum samples available from baseline and at least one other time point during the study. The sNfL concentration of samples taken at baseline, month 6, month 12, and month 24 was analyzed using a sensitive single molecule array assay (as described by Disanto *et al.*[15]) and were run on a Simoa HD-1 instrument (Quanterix, Billerica, MA, USA) using a two-step Assay Neat 2.0 protocol.

Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated from univariable Cox's proportional hazard models used to determine the factors influencing time to conversion to MS. A stepwise multivariable Cox's proportional hazard model was used with factors or covariates selected from the univariable models (threshold $p < 0.15$) that were seen to have an influence on conversion to MS. These factors were on-study treatment, age at baseline, FCDE classification (monofocal/multifocal), and number of T1 gadolinium-enhancing (Gd+) and T2 lesions. Baseline sNfL concentration was also included as a factor, being categorically defined in relation to the median value of 26.1 pg/mL as either low (baseline sNfL ≤ median) or high (baseline sNfL > median).

For both models, variable selection was based on a two-sided Wald test.

The median time to conversion to MS (McDonald 2005 criteria) or CDMS in each treatment group by month 24 was calculated using Kaplan-Meier curve estimates.

Correlations between sNfL and MRI findings were assessed using Spearman's rank correlation coefficient (*r*), according to two approaches: baseline sNfL with baseline lesion count and normalized brain volume; and sNfL at month 24 with (1) number of new MRI lesions between baseline and month 24 and (2) mean number of combined unique active (CUA), new T2, T1 Gd+, and T1 hypointense lesions per patient per scan during the 24-month double-blind period.

Correlation analyses were performed on observed data and the strength of correlation was defined as follows: very strong (*r* = 0.80–1.00), strong (*r* = 0.60–0.79), moderate (*r* = 0.40–0.59), weak (*r* = 0.20–0.39), or very weak (*r* = 0.00–0.19).

Sample size and statistical power considerations

In REFLEX, the primary endpoint was the time to conversion to MS (McDonald 2005 criteria) where a total of 450 patients were required to achieve 165 events for the comparison sc IFN β-1a 44 µg tiw *versus* placebo. This sample size was required to achieve 90% power with a two-sided 0.05 alpha error for detecting a HR of 0.6 for the primary comparison. The time to conversion was assumed to be exponentially distributed, and no adjustment for the multiplicity of the alpha error was applied.

Patients were randomly and equally allocated to each of the three treatment arms at enrolment to REFLEX and were included in this *post hoc* analysis based on the criteria mentioned above.

Results

Overall, 517 patients were randomly assigned to treatment in REFLEX, of whom 494 patients were included in this analysis (sc IFN β-1a qw, *n* = 168; sc IFN β-1a tiw, *n* = 161; placebo, *n* = 165). The mean ± standard deviation (SD) age for these 494 patients was 30.8 (±8.1) years, and 318 (64.4%) were female (Table 1).

In the overall sNfL analysis set, the interquartile range for sNfL concentrations at baseline was 15.4–61.8 pg/mL, with a median of 26.1 pg/mL. Patients with low sNfL at baseline (i.e. ≤26.1 pg/mL) had numerically fewer T1 Gd+, T1 hypointense, and T2 lesions compared with the high baseline sNfL cohort. Lesion volumes at baseline were also lower in the low sNfL subgroup compared with the high sNfL subgroup, but there were no observed differences in normalized brain volume.

Over the 24-month study period, concentrations of sNfL were reduced in all three groups; these reductions occurred as early as 6 months post-baseline and were more visibly reduced in patients with high baseline sNfL and for those treated with sc IFN β-1a (Supplemental Figure S1).

sNfL at baseline and conversion to MS

The multivariable stepwise analysis determined that high sNfL at baseline was correlated with the likelihood of conversion to MS (McDonald 2005 criteria); significant factors in this multivariable model were baseline sNfL subgroup (high/low: HR = 1.3, 95% CI: 1.03–1.64; *p* = 0.024), on-study treatment *versus* placebo (sc IFN β-1a qw: HR = 0.59, 95% CI: 0.46–0.76; sc IFN β-1a tiw: HR = 0.45, 95% CI: 0.34–0.59; both *p* < 0.001), classification of FCDE (monofocal/multifocal: HR = 0.69, 95% CI: 0.55–0.85; *p* < 0.001), baseline number of T1 Gd+ (HR = 1.07, 95% CI: 1.03–1.11; *p* = 0.001) and T2 lesions (HR = 1.02, 95% CI: 1.01–1.03; *p* < 0.001), and age at baseline (<30/≥30 years: HR = 1.47, 95% CI: 1.19–1.82; *p* < 0.001) (Table 2).

Regarding multivariable models of conversion to CDMS (Poser criteria), the only remaining significant factors were on-study treatment *versus* placebo (sc IFN β-1a qw: HR = 0.45, 95% CI: 0.30–0.68; sc IFN β-1a tiw: HR = 0.46, 95% CI: 0.30–0.71; both *p* < 0.001), and number of T1 Gd+ (HR = 1.10, 95% CI: 1.04–1.16; *p* < 0.001) and T2 lesions (HR = 1.01, 95% CI: 1.00–1.02; *p* = 0.053) at baseline. Results for the other factors, for example, age at baseline and baseline sNfL subgroup, were significant in the univariable models but were not significant in the stepwise multivariable Cox's proportional hazard model and, as such, were not subsequently included in the multivariable model.

Table 1. Patient demographics and disease characteristics at baseline.

Full sNfL analysis set (*n*=494)				
Characteristic	**Placebo (*n*=165)**	**sc IFN β-1a qw (*n*=168)**	**sc IFN β-1a tiw (*n*=161)**	**Overall (*n*=494)**
Age, years	30.7 (7.7)	30.9 (8.2)	30.8 (8.6)	30.8 (8.1)
Female, *n* (%)	108 (65.5)	104 (61.9)	106 (65.8)	318 (64.4)
Time since FCDE, days				
Median (Q1–Q3)	59.0 (56.0–60.0)	59.0 (57.0–60.0)	59.0 (56.0–60.0)	59.0 (56.0–60.0)
EDSS score mean (median)	1.53 (1.5)	1.52 (1.5)	1.57 (1.5)	1.54 (1.5)
sNfL value, pg/mL				
Mean (SD)	59.3 (90.8)	54.6 (106.2)	45.7 (62.4)	53.3 (88.6)
Median (Q1–Q3)	24.6 (15.6–61.8)	26.7 (16.5–51.9)	25.3 (15.4–48.4)	26.1 (15.8–53.1)
Number of T1 Gd+ lesions	1.2 (2.8)	1.5 (3.5)	1.3 (2.5)	1.3 (3.0)
T1 Gd+ lesion volume, mm^3	193.4 (594.2)	199.8 (604.6)	148.3 (409.1)	108.9 (544.2)
Number of T1 hypointense lesions	5.5 (7.7)	6.0 (7.6)	5.8 (6.9)	5.8 (7.4)
T1 hypointense lesion volume, mm^3	660.9 (1049.3)	790.7 (1304.7)	691.2 (1074.1)	714.9 (1149.1)
Number of T2 lesions	20.9 (19.9)	24.0 (21.3)	22.3 (19.0)	22.4 (20.1)
T2 lesion volume, mm^3	3300.6 (3980.4)	3931.8 (4762.7)	3093.0 (3434.4)	3447.6 (4112.2)
Normalized brain volume, cm^3	1545.9 (63.7)	1536.0 (66.9)	1536.2 (74.2)	1539.4 (68.4)
Low baseline sNfL (⩽ median baseline value; *n*=247)				
Characteristic	**Placebo (*n*=85)**	**sc IFN β-1a qw (*n*=80)**	**sc IFN β-1a tiw (*n*=82)**	**Overall (*n*=247)**
Age, years	31.2 (7.5)	31.2 (8.1)	32.1 (7.7)	31.5 (7.7)
Female, *n* (%)	56 (65.9)	55 (68.8)	56 (68.3)	167 (67.6)
Time since FCDE, days				
Median (Q1–Q3)	59.0 (56.0–60.0)	59.0 (57.0–60.0)	59.0 (57.0–60.0)	59.0 (57.0–60.0)
EDSS score, mean (median)	1.52 (1.5)	1.52 (1.5)	1.49 (1.5)	1.51 (1.5)
sNfL value, pg/mL				
Mean (SD)	15.7 (5.7)	15.8 (5.6)	15.2 (5.3)	15.6 (5.5)
Median (Q1–Q3)	15.7 (12.2–20.0)	16.1 (11.5–20.9)	15.5 (11.7–18.5)	15.7 (11.7–20.1)
Number of T1 Gd+ lesions	0.3 (0.7)	0.5 (1.0)	0.4 (0.9)	0.4 (0.9)
T1 Gd+ lesion volume, mm^3	25.8 (72.0)	53.9 (183.0)	18.1 (66.4)	32.4 (119.2)
Number of T1 hypointense lesions	3.4 (4.9)	5.1 (7.7)	4.6 (5.7)	4.3 (6.2)
T1 hypointense lesion volume, mm^3	381.3 (794.0)	566.4 (1217.6)	596.1 (1186.6)	512.6 (1079.0)
Number of T2 lesions	15.1 (14.2)	19.6 (18.3)	16.9 (13.8)	17.2 (15.6)
T2 lesion volume, mm^3	1754.2 (2291.9)	2261.6 (3150.7)	2081.9 (2567.4)	2027.3 (2682.7)
Normalized brain volume, cm^3	1544.6 (64.6)	1535.5 (63.5)	1529.3 (72.3)	1536.6 (67.0)

(Continued)

Table 1. (Continued)

High baseline sNfL (>median baseline value; n = 247)				
Characteristic	Placebo (n = 80)	sc IFN β-1a qw (n = 88)	sc IFN β-1a tiw (n = 79)	Overall (n = 247)
Age, years	30.2 (8.0)	30.7 (8.2)	29.4 (9.3)	30.1 (8.5)
Female, n (%)	52 (65.0)	49 (55.7)	50 (63.3)	151 (61.1)
Time since FCDE, days				
Median (Q1–Q3)	59.0 (56.0–60.0)	59.0 (56.0–60.0)	59.0 (55.0–60.0)	59.0 (56.0–60.0)
EDSS score, mean (median)	1.54 (1.5)	1.52 (1.5)	1.64 (1.5)	1.57 (1.5)
sNfL value, pg/mL				
Mean (SD)	105.7 (113.4)	89.9 (137.8)	77.3 (77.3)	91.0 (113.4)
Median (Q1–Q3)	63.1 (40.3–123.5)	50.9 (35.7–92.8)	48.9 (35.8–85.8)	53.4 (36.2–98.7)
Number of T1 Gd+ lesions	2.2 (3.7)	2.4 (4.6)	2.2 (3.2)	2.3 (3.9)
T1 Gd+ lesion volume, mm^3	371.4 (815.6)	332.5 (796.2)	283.4 (550.0)	329.4 (731.4)
Number of T1 hypointense lesions	7.7 (9.4)	6.8 (7.4)	7.1 (7.7)	7.2 (8.2)
T1 hypointense lesion volume, mm^3	958.0 (1200.8)	994.6 (1353.9)	790.0 (940.7)	917.3 (1183.1)
Number of T2 lesions	27.0 (23.1)	28.0 (23.1)	27.8 (22.0)	27.6 (22.7)
T2 lesion volume, mm^3	4943.7 (4690.2)	5450.3 (5443.3)	4142.5 (3893.6)	4867.9 (4758.4)
Normalized brain volume, cm^3	1547.2 (63.1)	1536.5 (70.1)	1543.5 (75.9)	1542.2 (69.7)

Mean (SD) unless stated otherwise.
EDSS, Expanded Disability Status Scale; FCDE, first clinical demyelinating event; Gd+, gadolinium enhancing; IFN, interferon; Q, quartile; qw, once weekly; sc, subcutaneous; sc IFN β-1a, subcutaneous interferon beta-1a; SD, standard deviation; sNfL, serum neurofilament light chain; tiw, three times weekly.

Low versus high sNfL at baseline and time to conversion to MS

The median time to conversion to MS (McDonald 2005 criteria) was longer in patients in the low sNfL subgroup (128, 272, and 801 days for placebo, sc IFN β-1a qw, and tiw, respectively) than in the high sNfL subgroup (93, 97, and 142 days for placebo, sc IFN β-1a qw, and tiw, respectively). Corresponding median time to conversion to CDMS could not be reported since the proportion of patients who converted was below 50%; however, at the 25% level, the time to conversion to CDMS was longer in the low sNfL subgroup (445 days for placebo, and not estimable for sc IFN β-1a since this level was not reached for either therapeutic dose) than in the high sNfL subgroup (345, 699, and 651 days for placebo, sc IFN β-1a qw, and tiw, respectively) [Figure 1(a) and (b) and Supplemental Table S1).

Correlation between sNfL at baseline and MRI findings/clinical outcomes

Moderate, positive correlations were observed between baseline sNfL and baseline MRI findings, including the volume of T2 lesions and the volume and number of T1 Gd+ lesions (r=0.55, 0.49, and 0.46, respectively; all p < 0.0001) [Table 3 and Supplemental Figure S2(a)]. There were weak correlations between baseline sNfL and other baseline MRI findings, including the volume and number of T1 hypointense lesions and number of T2 lesions (r=0.36, 0.35, and 0.30, respectively; all p < 0.0001). Very weak correlations were seen between baseline sNfL and normalized brain volume (r=0.01, p=0.8180) (Table 3).

During the 24-month double-blind treatment period, the mean number of MRI lesions was significantly lower in the sc IFN β-1a tiw group compared with placebo (Supplemental Table

Table 2. Multivariable analysis[a] of time to conversion to MS or clinically definite MS.

Factor	Parameter estimate	SE	HR (95% CI)	*p* Value[b]
Time to conversion to MS (McDonald 2005 criteria)				
Treatment				
(sc IFN β-1a qw/placebo)	−0.53	0.13	0.59 (0.46–0.76)	<0.001
(sc IFN β-1a tiw/placebo)	−0.80	0.14	0.45 (0.34–0.59)	<0.001
Age at baseline (<30/≥30 years)	0.39	0.11	1.47 (1.19–1.82)	<0.001
FCDE classification (monofocal/multifocal)	−0.38	0.11	0.69 (0.55–0.85)	<0.001
Number of T1 Gd+ lesions at baseline	0.07	0.02	1.07 (1.03–1.11)	0.001
Number of T2 lesions at baseline	0.02	0.00	1.02 (1.01–1.03)	<0.001
Baseline median sNfL subgroup (high/low)[c]	0.26	0.12	1.3 (1.03–1.64)	0.024
Time to conversion to clinically definite MS (Poser criteria)				
Treatment				
(sc IFN β-1a qw/placebo)	−0.80	0.21	0.45 (0.30–0.68)	<0.001
(sc IFN β-1a tiw/placebo)	−0.77	0.22	0.46 (0.30–0.71)	<0.001
Number of T1 Gd+ lesions at baseline	0.09	0.03	1.10 (1.04–1.16)	<0.001

[a]A stepwise multivariable Cox's proportional hazard model was performed using factors selected from the univariable model (threshold $p < 0.15$). Only statistically significant factors in the multivariable model are shown.
[b]Two-sided Wald test.
[c]Low baseline sNfL: baseline sNfL ≤ median; high baseline sNfL: baseline sNfL > median.
CI, confidence interval; FCDE, first clinical demyelinating event; Gd+, gadolinium enhancing; HR, hazard ratio; IFN, interferon; MS, multiple sclerosis; qw, once weekly; sc, subcutaneous; sc IFN β-1a, subcutaneous interferon beta-1a; SE, standard error; sNfL, serum neurofilament light chain; tiw, three times weekly.

S2). In the placebo group, for the same time-frame, strong correlations were observed between sNfL at month 24 and the mean number of CUA, new T2 lesions, and new T1 Gd+ lesions [Table 4 and Supplemental Figure S2(b)]. The lower Spearman's rank correlation coefficients observed in the sc IFN β-1a tiw treatment group indicate a lower number and volume of T1 and T2 lesions compared with sc IFN β-1a qw and placebo. Very weak correlations were observed between sNfL at month 24 and clinical outcomes (EDSS, brain volume, and qualifying relapses) in the placebo and sc IFN β-1a-treated groups (Table 4).

Discussion

Recently, sNfL has received growing interest around how this biomarker may predict the future disease course in early MS, as well as how sNfL concentrations are affected in response to DMTs. In this analysis of REFLEX, sNfL concentrations of patients experiencing a first event suggestive of early MS were correlated with current and future MRI outcomes along with conversion to MS, as defined by McDonald 2005 and Poser criteria. Using a multivariable analysis, results from the study show that treatment with sc IFN β-1a delayed the time to conversion and reduced the proportion of patients who converted to MS over the 24-month study period.

Conversion to MS (McDonald 2005 criteria) or CDMS (Poser criteria)

Several studies have reported higher sNfL concentrations in patients with clinically isolated
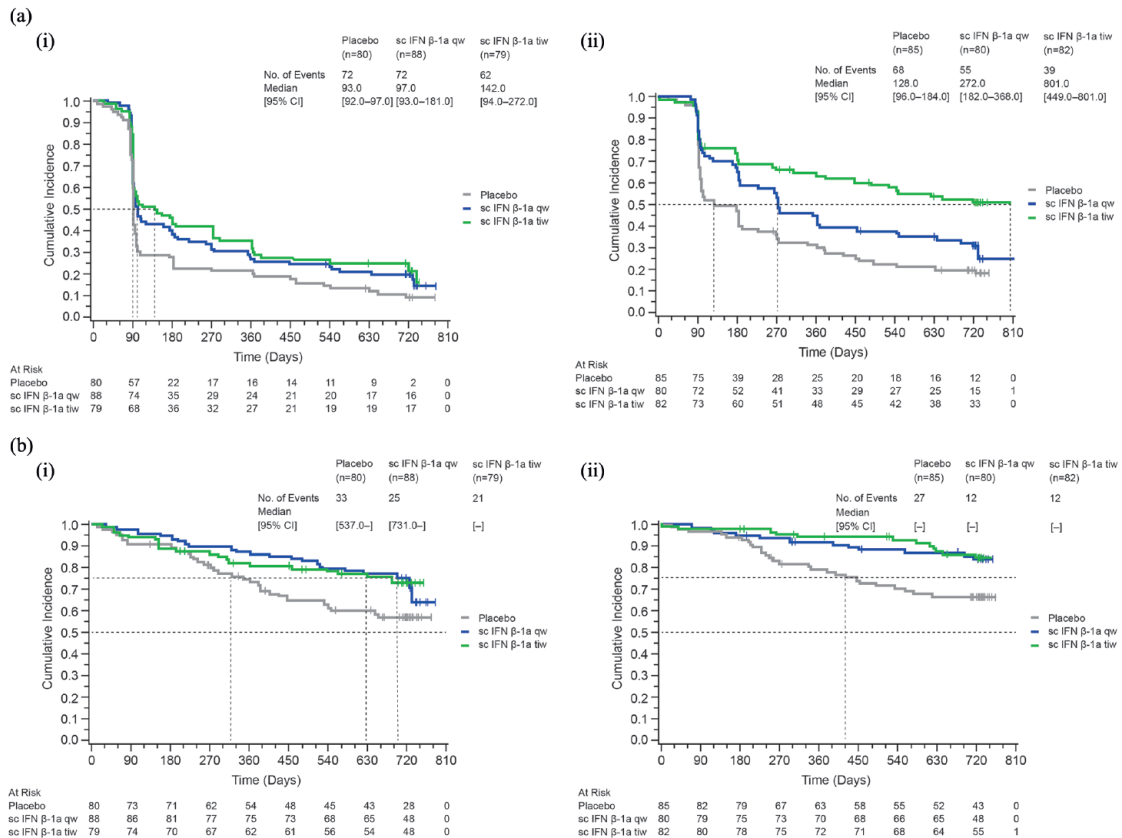
Figure 1. Kaplan–Meier cumulative incidence curves for time to conversion to MS (McDonald 2005 criteria) or clinically definite MS (Poser criteria) by treatment group, for each baseline sNfL subgroup. (a) Time to conversion to MS (McDonald 2005 criteria). (i) Low baseline sNfL (⩽ median baseline value). (ii) High baseline sNfL (> median baseline value). (b) Time to conversion to clinically definite MS (Poser criteria). (i) Low baseline sNfL (⩽ median baseline value). (ii) High baseline sNfL (> median baseline value). CI, confidence interval; IFN, interferon; MS, multiple sclerosis; qw, once weekly; sc, subcutaneous; sc IFN β-1a, subcutaneous interferon beta-1a; sNfL, serum neurofilament light chain; tiw, three times weekly.

Table 3. Correlation between sNfL concentration and MRI findings at baseline.

Characteristic	Overall Spearman's rank correlation coefficient (*r*) (*n* = 494)	*p* Value
Volume of T2 lesions, mm³	***0.545***	<0.0001
Volume of T1 Gd+ lesions, mm³	***0.490***	<0.0001
Number of T1 Gd+ lesions	***0.464***	<0.0001
Volume of T1 hypointense lesions, mm³	0.358	<0.0001
Number of T2 lesions	0.345	<0.0001
Number of T1 hypointense lesions	0.298	<0.0001
Normalized brain volume*, cm³	0.010	0.8180

Bold, italicized values indicate moderate correlations.
*Based on *n* = 488.
Gd+, gadolinium enhancing; MRI, magnetic resonance imaging; sNfL, serum neurofilament light chain.

Table 4. Correlation between month 24 sNfL and MRI and clinical findings at month 24, according to treatment group.

Characteristic	Spearman's rank correlation coefficient (*r*)		
	Placebo	sc IFN β-1a qw	sc IFN β-1a tiw
Lesion count per patient per scan[a] during the 24-month double-blind period	*n* = 91	*n* = 113	*n* = 115
Mean number of CUA lesions	**0.708*****	***0.485*****	0.133
Mean number of new T2 lesions	**0.715*****	***0.458*****	0.105
Mean number of new T1 Gd+ lesions	**0.598*****	***0.400*****	0.308**
Mean number of new T1 hypointense lesions	*0.494****	*0.477****	0.182
Lesion count at month 24	*n* = 133	*n* = 139	*n* = 138
Number of new T2 lesions	*0.564****	0.372***	0.274**
Volume of total T2 lesions, mm^3	*0.453****	0.286**	0.215*
Number of new T1 Gd+ lesions	0.344***	0.360***	0.231*
Volume of total T1 Gd+ lesions, mm^3	0.379***	0.355***	0.232*
Number of new T1 hypointense lesions	0.319**	0.259*	0.292**
Volume of total T1 hypointense lesions, mm^3	*0.430****	0.267*	0.194*
EDSS (change from baseline) at month 24	*n* = 138 0.014	*n* = 140 −0.148	*n* = 145 −0.011
Brain volume (% change from baseline) at month 24	*n* = 127 −0.135	*n* = 136 −0.081	*n* = 135 −0.218
Qualifying relapses during a 24-month, double-blind period	*n* = 141 0.012	*n* = 141 −0.031	*n* = 148 0.080

Bold, italicized values indicate moderate correlations; bolded blue values indicate strong correlations.
[a]MRI metrics collected at several time points during a 24-month period were used for the correlation analysis; data reported for patients with sNfL and MRI/clinical data were available for analysis.
*$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$.
CUA, combined unique active; EDSS, Expanded Disability Status Scale; Gd+, gadolinium enhancing; IFN, interferon; MRI, magnetic resonance imaging; qw, once weekly; sc, subcutaneous; sc IFN β-1a, subcutaneous interferon beta-1a; sNfL, serum neurofilament light chain; tiw, three times weekly.

syndrome or a FCDE. However, there have been few published examples of how sNfL may be used prognostically to determine the risk of conversion to MS.[12,16,17] It should be noted that these studies explored the risk of conversion to MS according to McDonald 2010[12,17] or 2017 criteria,[16] whereas our analysis of REFLEX investigated conversion according to the earlier 2005 criteria that were in place at the time of study initiation. We found that *higher* baseline sNfL concentration was associated with an *increased* risk of conversion to MS, thus indicating that sNfL may be a prognostic

biomarker of disease in patients with a FCDE. One such study found a 1-point increase in the HR for the risk of conversion to CDMS for every 100 pg/mL increase in sNfL using a univariable model and found the adjusted HR for CDMS conversion remained significant in a multivariable model.[17]

Factors such as receiving treatment with sc IFN β-1a, the classification of FCDE (mono- or multifocal), and low numbers of MRI lesions at baseline were also found to be independently

associated with a reduced risk of conversion to MS. Indeed, the respective multivariable models both showed significance for on-study treatment (*versus* placebo) and for the number of T1 Gd+ and T2 lesions for the conversion to both MS (McDonald 2005 criteria) and CDMS (Poser criteria), respectively.

Correlations between sNfL and MRI findings/clinical outcomes

The presence of MRI lesions is commonly associated with a more active disease course, and as such it would be expected that higher sNfL concentrations would be present. Indeed, there was a consistent association between sNfL concentration and both MRI and clinical outcomes during the study. At baseline, for example, higher sNfL concentrations were observed in patients who had higher numbers of T1 Gd+, T1 hypointense, and T2 MRI lesions, possibly reflecting recent/ongoing neuroaxonal damage[18] and/or greater disease activity.[19] Moderate, albeit significant, correlations were observed between baseline sNfL concentration and baseline T2 lesion volumes, T1 Gd+ lesion volumes, and T1 Gd+ lesion numbers, findings that are consistent with previous observations[15] and studies of plasma NfL.[18] These findings were also similar to those of patients treated with intramuscular IFN β-1a, which showed moderate correlations in the number of T1 Gd+ lesions and T2 lesion volumes.[5]

During the 24-month, double-blind study period and at the month 24 timepoint, strong correlations were observed between sNfL and CUA, T1 Gd+, and T2 lesions in the placebo group. It should be noted that patients in the placebo group had a similar number of lesions as compared to the treated subgroups at baseline.

Studies have found increased sNfL close to the onset of clinical relapse,[15,16,20] with the appearance of T1 Gd+ and new and/or enlarging T2 lesions,[15,16,19,20] and EDSS progression.[12,20,21] sNfL concentration at the time of diagnosis was also found to predict disease activity over a succeeding 2-year period.[22] Although some studies have found correlations between sNfL and EDSS worsening, these observations were not made in this *post hoc* analysis of REFLEX, which may be due to the study population. In early MS, there are very few changes to disability and any changes that are present can be difficult to detect. Other

studies have also found no correlations between sNfL and EDSS,[16,17,19] with some studies indicating a correlation using univariable and multivariable models.[11,15]

Taken together, the results from this analysis suggest that patients with a FCDE and high baseline sNfL have a higher probability of disease worsening. Indeed, since the REFLEX study was conducted, treatment practices have changed, and early treatment may be used in patients with high levels of sNfL to maintain neurological function.[23–25]

Study limitations

This analysis is limited by the use of the McDonald 2005 criteria, which were the current means for determining conversion to MS at the time the REFLEX study commenced (November 2006). It should also be noted that the sNfL data in this analysis were collected over a short 2-year timeframe, during which patients may not have converted to MS based on their disease phenotype. However, longer-term studies have found that at 10 years, there was no statistically significant difference in the risk of CDMS between patients with low *versus* high sNfL concentrations.[5] It should also be considered that the present analysis of REFLEX focuses on a patient population that had experienced a FCDE. For this population, the time-to-conversion analysis was deemed to be important and therefore the multivariable analyses explored the primary and secondary endpoints of time-to-conversion to McDonald MS (2005 criteria) and CDMS (Poser criteria). For this analysis, the multivariable models were not expanded to other variables, thus raising an interesting question about the interactions with lesion volume and brain atrophy, which needs further exploration. Lastly, not using percentiles/*Z*-scores – adjusting for age and BMI based on healthy controls – was a shortcoming of the work. However, large reference databases are not available for the homebrew assay used to measure sNfL in this study; consequently, we were not able to use percentiles/*Z*-scores.[11] However, all models were adjusted for age.

In conclusion, we found that higher baseline sNfL concentration was associated with an increased risk of conversion to MS (McDonald 2005 criteria) or CDMS (Poser criteria) in patients with a FCDE. Age, multifocal disease, and number of

T1 or T2 lesions at baseline were also confirmed as significant determinants for risk of conversion. Conversion was delayed in all patients treated with sc IFN β-1a tiw regardless of baseline sNfL concentrations; yet, this delay in conversion was particularly noticeable for patients who had baseline sNfL values below the median of 26.1 pg/mL. Concerning MRI parameters, a higher sNfL concentration at baseline was moderately correlated with lesion load and T1 Gd+ lesion count in such patients, and this association was also present for placebo recipients at month 24. However, these correlations were weak in the sc IFN β-1a tiw group, consistent with a significant reduction in lesion counts compared with placebo. Together, these exploratory findings highlight the complex interplay between sNfL concentration, MRI outcomes, and risk of conversion to MS in patients with a FCDE, and the benefits of treatment with sc IFN β-1a in such patients.

Declarations

Ethics approval and consent to participate
The REFLEX study was undertaken in compliance with the Declaration of Helsinki and standards of Good Clinical Practice according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Before initiation of the study at each center, the relevant institutional review board or independent ethics committee reviewed and approved the study protocol, patient information leaflet, informed consent forms, and investigator brochure. All participants provided written informed consent at the screening visit of REFLEX, and additional approvals were sought from the Swiss Ethics Committee for the current *post hoc* analysis of sNfL (Swiss EC, approval number: 2018-01394).

Consent for publication
Not applicable.

Author contributions
Jens Kuhle: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing – review & editing.

David Leppert: Funding acquisition; Methodology; Writing – review & editing.

Giancarlo Comi: Conceptualization; Investigation; Writing – review & editing.

Nicola de Stefano: Conceptualization; Investigation; Writing – review & editing.

Ludwig Kappos: Conceptualization; Supervision; Writing – review & editing.

Mark S. Freedman: Conceptualization; Formal analysis; Methodology; Visualization.

Andrea Seitzinger: Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing – review & editing.

Sanjeev Roy: Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing – review & editing.

Competing interests
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Availability of data and materials
Any requests for data by qualified scientific and medical researchers for legitimate research purposes will be subject to Merck's Data Sharing Policy. All requests should be submitted in writing to Merck's data-sharing portal https://www.merckgroup.com/en/research/our-approach-to- research-and-development/healthcare/clinical-trials/commitment-responsible-data-sharing.html. When Merck has a co-research, co-development, or co-marketing or co-promotion agreement, or when the product has been out-licensed, the responsibility for disclosure might be dependent on the agreement between parties. Under these circumstances, Merck will endeavor to gain agreement to share data in response to requests.

ORCID iD
Ludwig Kappos 🔟 https://orcid.org/0000-0003-4175-5509

Supplemental material
Supplemental material for this article is available online.

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