RESEARCH ARTICLE

Development of an age-adjusted model for blood neurofilament light chain

Christopher Harp[1,†], Gian-Andrea Thanei[2,†], Xiaoming Jia[1], Jens Kuhle[3], David Leppert[3], Sabine Schaedelin[4], Pascal Benkert[4], H-Christian von Büdingen[2], Robert Hendricks[1] & Ann Herman[1]

[1]Genentech, Inc., South San Francisco, California, USA
[2]F. Hoffmann-La Roche Ltd, Basel, Switzerland
[3]Departments of Medicine, Biomedicine and Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland
[4]Clinical Trial Unit, Department of Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland

Correspondence
Christopher Harp, OMNI Biomarker Development, Genentech, Inc. 1 DNA Way Mailstop 258A, South San Francisco, CA 94080, USA. Tel: (650)467-8855; E-mail: harp.christopher@gene.com

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†Co-first authors.

Abstract

Objective: To develop an age-adjustment model for neurofilament light chain (NfL), an emerging injury marker in patients with a range of neurologic conditions including multiple sclerosis (MS). **Methods**: Serum and plasma samples were collected from a healthy donor (HD) cohort of 118 individuals aged 24 to 66 years, 90 patients with relapsing MS (RMS) and 22 patients with progressive MS (PMS). Serum and plasma samples were assessed for NfL using the SIMOA assay (Quanterix NfL Advantage Kit™). A log-linear model was used to evaluate the relationship between NfL and age and to calculate age-adjusted NfL levels. **Results**: Higher serum and plasma NfL levels were significantly associated with increasing HD age. Log-transformation of blood NfL levels reduced heteroscedasticity and skewness. A log-linear model enabled adjustment for age-related increase in serum and plasma NfL levels (2.3% [95% CI, 1.6–2.9] and 2.6% [95% CI, 1.3–3.3] per year, respectively). Following age adjustment, NfL did not show significant association with HD sex or ethnicity. While unadjusted serum NfL levels were elevated in patients with PMS (mean age 56 years) compared with those with RMS (mean age 37 years), age-adjusted NfL levels did not differ. **Interpretation**: A log-linear, age adjustment model was developed to enable comparison of NfL levels across populations with different ages. While additional data and evidence are needed for patient-level adoption, this could be a valuable tool for interpreting NfL levels across a range of patient groups with neurologic conditions.

Introduction

Neurofilament light chain (NfL) is expressed in neuronal axons and shed into the extracellular space and peripheral blood following neuroaxonal injury in patients with a range of neurologic conditions.[1] The potential use of NfL as a biomarker has been proposed for several neurodegenerative conditions including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, other forms of dementia, multiple sclerosis (MS), traumatic brain injury, and HIV infection.[2–9] The development of single molecule array (SIMOA) technology, an ultra-sensitive enzyme-linked immunosorbent assay (ELISA) technique, allows for minimally invasive detection of NfL in peripheral blood samples with sensitivity in the picomolar range.[9,10] NfL levels partially distinguish individuals with disease from healthy controls and decrease with effective disease management.[1] Recently, several studies suggest that NfL may also be useful as a predictor of future disease activity.[11–13]

Importantly, demographic factors such as age, sex, and ethnicity can all have considerable effects on measures used in studies of neurological disease.[14–22] Age, in particular, appears to influence NfL in both healthy and diseased populations,[1,3,4,7,10,23,24] with increased age correlating with greater and more variable NfL levels.[23,25] The evidence to support sex effects on NfL in healthy donors and in patients with neurological disease, including PD and AD is limited.[26–28] Correlations between ethnicity and NfL have also been observed in patients with

AD and diabetes.[29,30] These effects have the potential to confound comparisons of NfL both within and between various patient cohorts. To avoid biases when using NfL as a biomarker, considerations are needed to both identify and control for these factors.

In MS, NfL is elevated with acute inflammatory disease activity (gadolinium [Gd+] lesions and/or clinical relapses) and measures of disease severity including the expanded disability status scale (EDSS);[5,31] however, whether NfL levels differ across patients with relapsing MS (RMS) versus patients with progressive MS (PMS) remains unclear, especially given that age differences may confound a direct comparison between these populations. To fully characterize NfL in MS and other neurological disorders, it is important to first understand the non-disease related behavior of NfL in healthy individuals. In this analysis, the influence of age (as well as sex and ethnicity) on blood NfL levels was examined in a healthy donor population. A log-linear model based on these findings was developed to separate disease-related effects from effects associated with different age distributions. Finally, the model was applied to a cohort of patients with MS to examine whether a difference exists in the NfL distribution between patients with RMS versus patients with PMS.[32,33]

Methodology

Participants

Healthy donor serum and EDTA-plasma samples were acquired from 118 individuals aged 24 to 66 years as part of a Genentech employee donor program or purchased from PrecisionMed Inc. (Solano Beach, CA, USA). The Genentech employee donor program screens for individuals in general good health without known neurologic conditions or a history of neurologic injury, bloodborne infections (HIV, HCV, HBV) or anemia. Of 83 individuals with medical comorbidity data, 65 (79%) reported no known conditions. Other participants reported hypothyroidism (5), diabetes mellitus (4), hypertension (4), asthma (2), dyslipidemia (2), and rheumatoid arthritis (1). Blood draws occurred during business hours, primarily in the morning (median 10:30 AM, range 7:15 AM–2:00 PM). For healthy donor samples obtained from PrecisionMed Inc., these cognitively normal donors had no neurological disease and no history or family history of significant neuropsychiatric disease. Further, they required a Mini Mental State Examination (MMSE) of >28 to qualify. Human serum and EDTA-plasma from 90 patients with RMS and 22 patients with PMS (17 with primary progressive MS [PPMS], five with secondary progressive MS [SPMS]) were obtained through a research collaboration agreement between Genentech, Inc. and the University of California San Francisco (UCSF, San Francisco, USA). Patients with MS were recruited at UCSF between 2012 and 2017. International Panel (McDonald) Criteria was used for diagnosis of RMS and PPMS.[34] Lublin et al. 2014 criteria (retrospective diagnosis by a history of gradual worsening after an initial relapsing disease course, with or without acute exacerbations during the progressive course) was used for diagnosis of SPMS.[35]

NfL measurements

Frozen serum and plasma samples were assessed for NfL using a SIMOA assay (Quanterix NfL Advantage Kit™) that has demonstrated adequate sensitivity and precision to measure in both matrices. Complete methodological details have been previously described.[36] In brief, a calibrator curve (rhuman) was freshly prepared from concentrate in SIMOA NfL Advantage calibrator diluent (Quanterix). Individual paired human matrix samples (serum, EDTA-plasma) were diluted 1:4 in the SIMOA NfL advantage sample diluent according to the kit protocol. All samples were transferred to a SIMOA microtiter plate and two replicates from each microtiter well were measured for the analysis.

Statistical analyses

Associations between NfL and patient demographic factors (age, sex, and ethnicity) were determined using Spearman's r analysis, *t*-test, and one-way ANOVA, respectively. A log-linear model was used to calculate age-adjusted NfL levels. Theoretical age-adjusted NfL percentiles based on the assumption of a log-normal distribution of NfL conditional on age with fixed variance were calculated. Unadjusted and age-adjusted NfL distributions in the MS cohort were compared using the Kolmogorov–Smirnov test (K–S test). All tests were two-sided and had a significance level of 0.05. Computation of the percentiles was performed with distribution tables or standard statistical software. Univariate and multiple linear regression was used to assess the relationship between demographic, clinical, and MRI features (independent variables) with NfL levels (dependent variable).

Results

Relationship between age and blood serum NfL in healthy donors

The healthy donor cohort age distribution is shown in Figure 1A. In this cohort, a significant correlation was observed between age and serum NfL (Spearman's

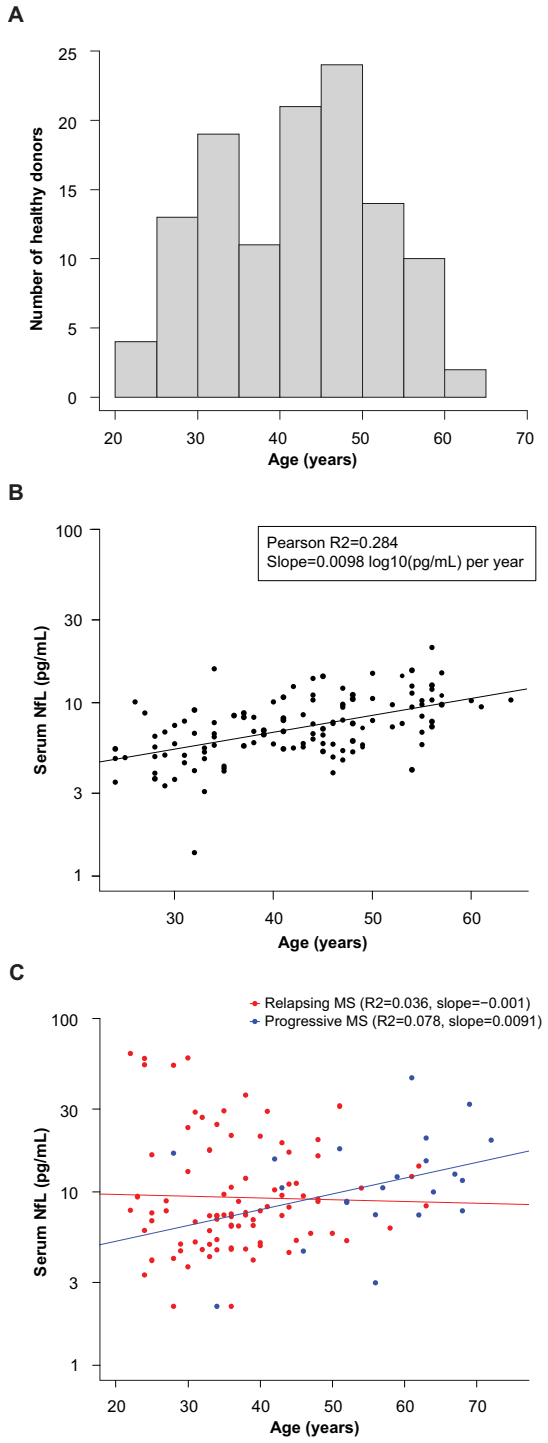

Figure 1. Age distribution of healthy donor cohort and relationship between serum NfL and age before age adjustment in the MS cohort. MS, multiple sclerosis; NfL, neurofilament light chain. (A) Age distribution of the healthy donor cohort. (B) Log axis NfL age relationship in the MS cohort. (C) NfL-age relationship in the MS cohort.

r = 0.54; $p < 0.001$). A similar relationship was observed between age and plasma NfL (Spearman's r = 0.53; $p < 0.001$) (Figure S1A), and a significant correlation was found between serum and plasma NfL (Spearman's r = 0.95; $p < 0.001$) (Figure S1B). Further inspection of serum NfL versus age in the healthy donor population showed a heteroscedastic relationship (increased variability with higher age) with a positively skewed distribution. Application of $\log_{10}$ transformation to serum NfL reduced the heteroscedasticity and skew compared with the untransformed data (Figure 1B). Therefore, subsequent development of the model for interpreting patient NfL levels included $\log_{10}$ transformation prior to adjustments for age.

Derivation of the age-adjustment model

The relationship between $\log_{10}$NfL and age was determined using linear regression analysis (Table 1), which identified an age-related increase in serum and plasma NfL of 2.3% (95% CI, 1.6%–2.9%) and 2.6% (95% CI, 1.8%–3.3%) per year, respectively. The resulting model derived from this analysis enabled calculation of age-adjusted NfL levels and percentiles relative to a healthy donor population (Figure 2).

Formula for age-adjusted NfL levels

To account for the impact of age on NfL, a log-linear model was used to adjust the NfL level to a reference age of 18 years:

$$NfL_{adj} = 10^{log10(NfL) - Slope(Age - 18)} \quad (1)$$

Applying the model coefficients derived from the healthy donor cohort, this translated to:

$$serum\ NfL_{adj} = 10^{log10(serum\ NfL) - 0.009767 * (Age - 18)} \quad (2)$$

$$plasma\ NfL_{adj} = 10^{log10(plasma\ NfL) - 0.01103 * (Age - 18)} \quad (3)$$

The resulting NfL_{adj} values reflect what the NfL levels would be at an age of 18 years, facilitating like-for-like comparisons between populations with different age distributions (Table 2).

Relationship between NfL and sex and ethnicity in the HD cohort following age adjustment

Following adjustment for age, no significant association was observed between NfL levels and sex (Figure 3A), and

Table 1. Linear regression analysis of log_{10}NfL (serum and plasma) with age.

Component/Compartment	Intercept	Slope (Age)	SD Residuals
Serum	0.439271	0.009767	0.1524622
Plasma	0.27973	0.01103	0.1715229

NfL, neurofilament light chain.

no significant differences were observed in NfL levels between patients of different ethnicities (Figure 3B) in the healthy donor cohort.

Application of the NfL age-adjustment model to a MS cohort

The impact of age adjustment on NfL levels was assessed in a cohort of 90 patients with RMS (mean age, 37.0; SD, 9.1 years) and 22 patients with PMS (mean age, 56.0; SD, 11.7 years). Additional clinical characteristics of this cohort are shown in Table 3. Before adjusting for age, a right-shift (elevation) in the distribution of NfL levels was observed among patients with PMS versus RMS (K–S test; $p = 0.055$; Figure 4A), which might initially be attributed to differences between MS subtypes. However, after applying this model to these data, this effect was largely removed and considerable overlap was observed between the two cohorts (K–S test; $p = 0.6$; Figure 4B), suggesting that the degree of currently occurring neuroaxonal injury detected in blood is not necessarily higher in patients with PMS compared with RMS. Interestingly, following age-adjustment, NfL levels were observed to decrease with age (Spearman rank correlation; $r = -0.236$, $p = 0.012$), especially in patients with RMS ($r = -0.244$, $p = 0.020$) (Figure 4C). By contrast, no significant relationship between age and unadjusted NfL was observed in this population (Figure 1C). Clinical relapses and inflammatory disease activity are known to associate with a shorter disease course,[37] which may partially explain the NfL elevation observed in younger patients. While this cohort was underpowered to evaluate the relationship between age and NfL stratified by acute disease activity, the observed inverse relationship between age and NfL appeared to be more evident in patients with RMS with recent inflammatory activity on MRI (Figure S1). Lastly, analysis of demographic, clinical, and MRI variables replicated previous reports that a key driver of NfL elevation is recent disease activity detectable on MRI, and this relationship was more pronounced when examining adjusted NfL levels (Table 4). These findings demonstrated that age adjustment of NfL levels may help reduce confounding factors and/or uncover relationships more relevant to the underlying disease biology.

Discussion

NfL is emerging as a useful injury marker in patients with a range of nervous system conditions. In healthy individuals, NfL is reported to increase nonlinearly with age.[23] This model describing an exponential increase in serum NfL with age at approximately 2.3% increase per year in this healthy donor cohort (age 24–66 years) is generally consistent with published results, and may have better evidence in the middle age ranges compared with published studies. The nonlinear increase in NfL may be due to a combination of natural brain volume loss associated

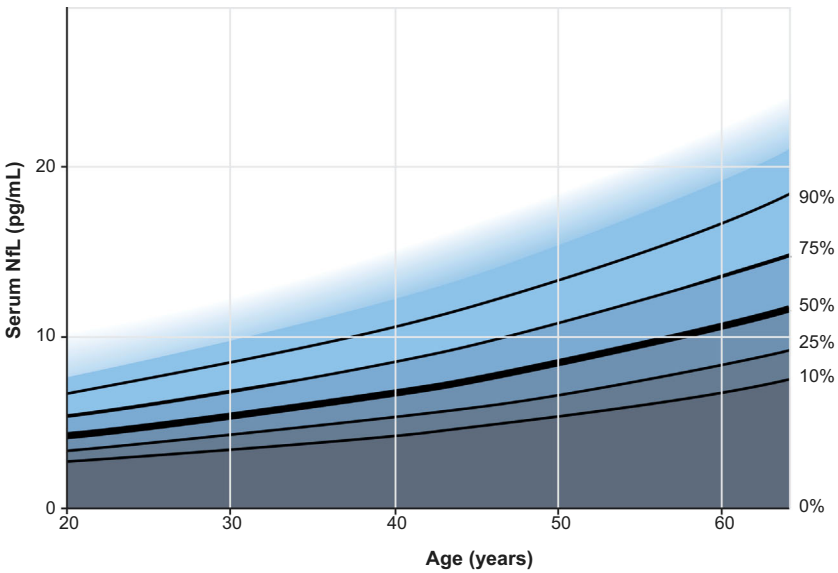

Figure 2. NfL age-adjusted healthy donor percentile curves. The modelled median (50th percentile) NfL level with age is shown with the bolded black line. NfL, neurofilament light chain.

Table 2. Theoretical serum NfL values (pg/mL) corresponding to age-adjusted percentiles.

	Age (years)							
Percentile	25	30	35	40	45	50	55	60
10th	2.75	3.08	3.44	3.85	4.31	4.82	5.40	6.04
25th	3.40	3.81	4.26	4.77	5.33	5.97	6.68	7.48
50th	4.31	4.82	5.40	6.04	6.76	7.56	8.46	e
75th	5.46	6.11	6.84	7.66	8.57	9.59	10.73	12.00
90th	6.76	7.57	8.47	9.47	10.60	11.86	13.27	14.85

NfL, neurofilament light chain.

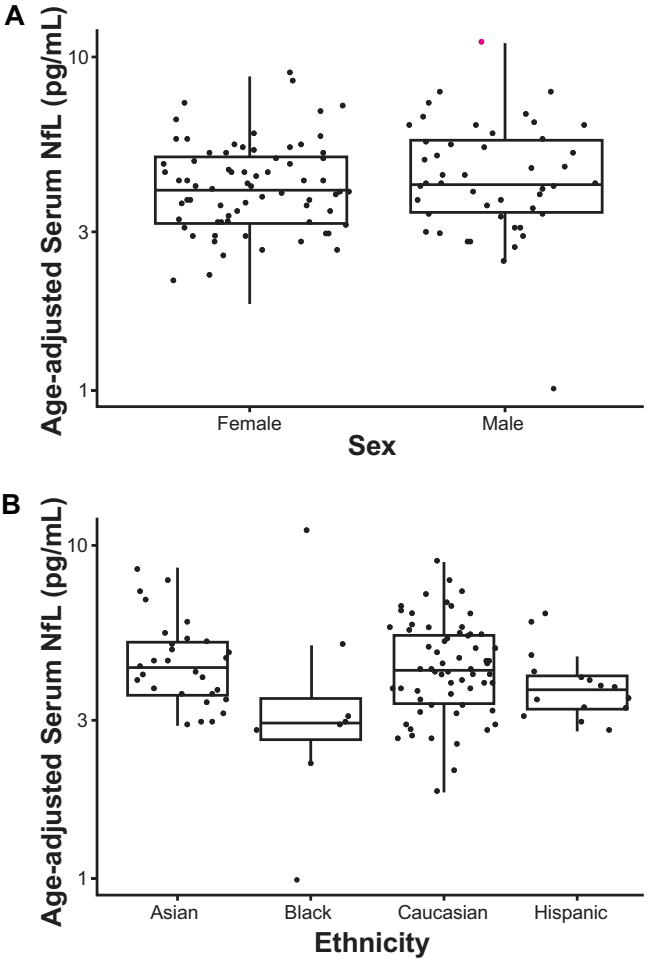

Figure 3. NfL relationship with sex and ethnicity following age adjustment. NfL, neurofilament light chain. (A) Log-transformed age-adjusted healthy donor serum NfL relationship with sex. (B) Log-transformed age-adjusted healthy donor serum NfL relationship with ethnicity.

with aging and/or decreased cerebrospinal fluid turnover commonly found in older individuals.[23,38]

This study established a straightforward log-transform model to calculate age-adjusted NfL values, enabling a more informed comparison of NfL distributions across different patient cohorts. This model is suited for cohort investigations of NfL and may be most applicable in populations within the age range established by this healthy donor cohort. Moreover, this study contributed an important healthy donor reference for examining NfL in

Table 3. Healthy donor and MS cohort characteristics.

	Healthy Donor (n = 118)	RMS (n = 90)	PPMS (n = 17)	SPMS (n = 5)	Progressive MS (PPMS + SPMS) (n = 22)
Age, mean (SD), years	42.5 (9.8)	37.0 (9.1)	53.2 (11.5)	65.8 (5.9)	56.0 (11.7)
Female, n (%)	71 (60.2)	61 (67.8)	8 (47.1)	2 (40.0)	10 (45.5)
Ethnicity, n (%)[a]					
White	66 (55.9)	55 (61.1)	12 (70.6)	5 (100.0)	17 (77.3)
Black	8 (6.8)	4 (4.4)	1 (5.9)	0	1 (4.5)
Hispanic	15 (12.7)	7 (7.8)	0	0	0
Asian	29 (24.6)	2 (2.2)	0	0	0
Serum NfL, median (IQR), pg/mL					
Unadjusted	7.13 (1.37–20.8)	7.64 (5.42–12.90)	9.99 (7.38–16.10)	12.80 (11.70–14.00)	11.11 (8.02–16.37)
Adjusted	4.09 (1.00–11.0)	4.95 (3.58–8.06)	4.12 (2.94–7.93)	4.95 (4.67–5.22)	4.30 (3.24–7.06)
EDSS, n (%)[b]					
<4	-	68 (75.6)	6 (35.4)	0	6 (27.3)
≥4	-	10 (11.1)	9 (52.9)	2 (40.0)	11 (50.0)
Patients receiving steroids, n (%)	-	7 (7.7)	1 (5.8)	0	1 (4.5)
Patients receiving DMT, n (%)[c]	-	15 (16.7)	1 (5.8)	3 (60.0)	4 (18.2)
Subcutaneous injection (interferon beta-1a, glatiramer acetate)	-	6 (6.7)	1 (5.8)	0	1 (4.5)
Oral (fingolimod, dimethyl fumarate, teriflunomide)	-	6 (6.7)	0	2 (40.0)	2 (9.1)
Intravenous infusion (rituximab, natalizumab)	-	3 (3.3)	0	1 (20.0)	1 (4.5)
Patients with brain MRI within prior 3 months, n (%)[d]	-	67 (74.4)	10 (58.8)	3 (60)	13 (59.1)
Patients with T1 Gd+ lesions, n (%)	-	30 (33.3)	2 (11.8)	0	2 (9.1)
Time from brain MRI to NfL measurement in patients with Gd + lesion, median (range), days	-	0 (0–84)	0 (0–0)	-	0 (0–74)
Patients receiving lumbar puncture, n (%)	-	86 (95.6)	16 (94.1)	3 (60)	19 (86.4)
Patients with OCB positivity, n (%)	-	72 (80)	14 (82.3)	3 (60)	17 (77.3)

DMT, disease-modifying therapy; EDSS, extended disability status scale; Gd, gadolinium; IQR, interquartile range; N/A, not available; MS, multiple sclerosis; NfL, neurofilament light chain; OCB, oligoclonal band; PPMS, primary progressive multiple sclerosis; RMS, relapsing multiple sclerosis.
[a]Ethnicity data were not available for 22 people with RMS and four people with PPMS.
[b]EDSS scores missing for 12 people with RMS, two people with PPMS and three people with SPMS.
[c]Treatment information missing for one person with PPMS.
[d]T1 Gd+ lesions data were unavailable for 36 people with RMS, nine people with PPMS, and five people with SPMS.

a variety of neurologic conditions, especially those that impact patients in early to middle adulthood, including MS. Importantly, use of a healthy donor-derived adjustment helped to distinguish the impact of natural aging on NfL levels, which may be distinct from disease-specific age-related changes and otherwise indistinguishable through analysis of a disease cohort alone.

Application of this NfL age adjustment to a MS cohort demonstrated the relevance of this method for understanding the role of NfL in MS. While NfL is elevated with MS disease activity and disease burden,[10,39] it remains unclear whether NfL is a useful marker for distinguishing between patients with a predominantly relapsing versus progressive disease course. While initial analysis showed an elevation in unadjusted serum NfL levels in patients with PMS versus those with RMS, this observation did not hold following age adjustment, suggesting that the observed difference was driven by age

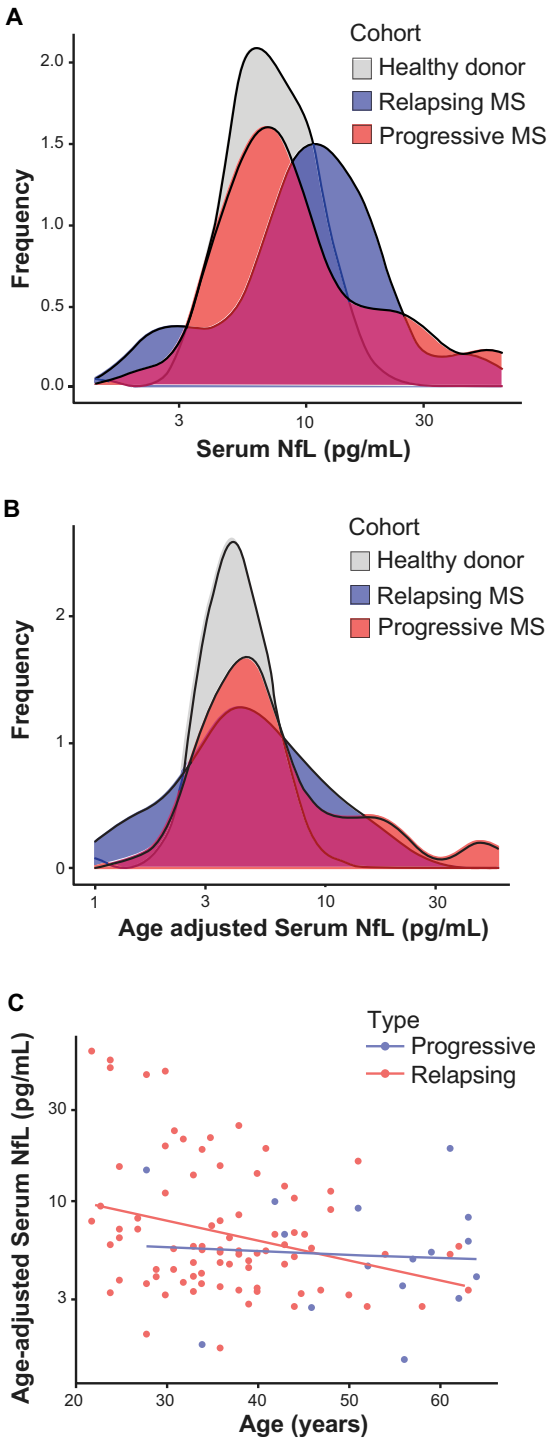

Figure 4. Application of the age-adjustment model to the MS cohort. MS, multiple sclerosis; NfL, neurofilament light chain; PMS, progressive multiple sclerosis; RMS, relapsing multiple sclerosis. (A) Distribution of NfL in patients with PMS or RMS before age adjustment. (B) Distribution of NfL in patients with PMS or RMS after age adjustment. (C) Relationship between age and age-adjusted serum NfL in the PMS and RMS populations.

difference between these groups. While elevated NfL levels may reflect injury due to acute disease activity and/or insidious progressive biology, the lack of clear difference in adjusted NfL between patients with RMS or PMS supports the notion that neuroaxonal injury, including insidious progressive injury, occurs throughout the disease spectrum. Additional investigation is needed to understand the clinical utility of NfL given the dual contribution of relapse and progressive MS injury on NfL levels.[40] Nonetheless, this model, derived from an independent healthy donor cohort, helps to advance understanding of NfL by adjusting for an important confounder in interpreting results.

Despite the accessibility and relevance of the developed model, additional investigation is needed to understand model performance at the individual level and implications for clinical practice. The limited sample size and age range of healthy donor participants were important limitations, as age-related NfL elevations may be disproportionally greater in older individuals (60+ years) compared with a younger cohort.[23] The limited sample size does not allow for reliable estimation of the amount of deviation from normal individuals, particularly beyond the 90th percentile. This model did not account for potential comorbidities associated with older age (e.g., cardiovascular disease,[41] diabetes[42], and cognitive impairment) or other factors that could impact NfL levels (e.g., body weight, normalized brain volume). This model was developed using NfL data analyzed on the SIMOA platform, and additional investigation is needed to understand generalizability to data collected on other platforms. Nonetheless, these methods provided here may be useful for deriving appropriate healthy donor models based on other platforms. Despite these limitations, this study provided important data to contextualize NfL levels in relation to age and provided a simple model and reference that may be useful across a range of neurologic conditions on the group level.

In conclusion, a log-linear, age adjustment model was developed to enable comparison of NfL levels across populations with different ages. While additional evidence is needed for patient-level adoption, this could be a valuable tool for interpreting NfL levels across a range of neurologic conditions.

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Table 4. Association of demographic, clinical, and MRI variables with serum NfL in the MS cohort using univariate and multiple linear regression.

		Unadjusted NfL		Age-adjusted NfL			
		Univariate		Univariate		Multivariate	
Variable	1 SD or unit of change	Effect on log10 NfL	*p* value	Effect on log10 NfL	*p* value	Effect on log10 NfL	*p* value
Age (years)	12.2	0.032 (−0.027, 0.091)	0.29	−0.087 (−0.146, −0.087)	0.0044	−0.11 (−0.213, −0.005)	0.043
Sex	Male vs Female	0.239 (0.126, 0.354)	7.4e-5	0.221 (0.102, 0.341)	4.3e-4	0.166 (0.025, 0.307)	0.025
Disease type	PMS vs RMS	0.074 (−0.074, 0.222)	0.33	−0.112 (−0.264, 0.040)	0.15	−0.016 (−0.305, 0.274)	0.92
Brain MRI Gd + lesions (within 3 months)	4.34	0.120 (0.050, 0.190)	0.0012	0.145 (0.075, 0.215)	1.2e-4	0.112 (0.040, 0.183)	0.0032
Disease- modifying treatment	Treatment vs no treatment	0.063 (−0.074, 0.201)	0.37	0.052 (−0.090, 0.195)	0.47	0.111 (−0.061, 0.282)	0.21
EDSS	1.66	0.089 (0.029, 0.149)	0.0046	0.039 (−0.026, 0.104)	0.24	0.074 (−0.014, 0.163)	0.11

EDSS, expanded disability status scale Gd, gadolinium; NfL, neurofilament light chain; PMS, primary multiple sclerosis; RMS, relapsing multiple sclerosis.

Conflict of Interest

The authors also report the following conflict of interest: Christopher Harp: is an employee of Genentech, Inc. and a shareholder of F. Hoffmann-La Roche Ltd. Gian-Andrea Thanei: is an employee and shareholder of F. Hoffmann-La Roche Ltd. Xiaoming Jia: is an employee of Genentech, Inc. and a shareholder of F. Hoffmann-La Roche Ltd. Jens Kuhle: received speaker fees, research support and travel support and/or served on advisory boards for ECTRIMS, Swiss MS Society, Swiss National Research Foundation (grant no. 320030_160221), University of Basel, Bayer, Biogen, Genzyme, Merck, Novartis, Protagen AG, Roche and Teva. David Leppert: is currently employed at University Hospital Basel and has previously been an employee and stockholder of F. Hoffmann-La Roche Ltd. and Novartis AG. Sabine Schaedelin: report no conflict of interest. Pascal Benkert: report no conflicts of interest. H-Christian von Büdingen: is an employee and shareholder of F. Hoffmann-La Roche Ltd. Robert Hendricks: is an employee of Genentech, Inc. and a shareholder of F. Hoffmann-La Roche Ltd. Ann Herman: is an employee of Genentech, Inc. and a shareholder of F. Hoffmann-La Roche Ltd.

Author Contributions

Christopher Harp: Conceptualization, methodology, formal data analysis and validation, writing (reviewing and editing), final approval. Gian-Andrea Thanei: Conceptualization, methodology, formal data analysis and validation, writing (reviewing and editing), final approval. Xiaoming Jia: Conceptualization, methodology, formal data analysis and validation, writing (reviewing and editing), final approval. Jens Kuhle: Conceptualization, writing (reviewing and editing), final approval. David Leppert: Conceptualization, writing (reviewing and editing), final approval. Sabine Schaedelin: Conceptualization, writing (reviewing and editing), final approval. Pascal Benkert: Conceptualization, writing (reviewing and editing), final approval. H-Christian von Büdingen: Conceptualization, writing (reviewing and editing), final approval. Robert Hendricks: Conceptualization, writing (reviewing and editing), final approval. Ann Herman: Conceptualization, investigation, writing (reviewing and editing), final approval.

References

1. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol. 2018;14(10):577-589.
2. Zetterberg H, Skillback T, Mattsson N, et al. Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. JAMA Neurol. 2016;73(1):60-67.
3. Backstrom D, Linder J, Jakobson Mo S, et al. NfL as a biomarker for neurodegeneration and survival in Parkinson disease. Neurology. 2020;95(7):e827-e838.
4. Byrne LM, Rodrigues FB, Blennow K, et al. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. Lancet Neurol. 2017;16(8):601-609.
5. Manouchehrinia A, Stridh P, Khademi M, et al. Plasma neurofilament light levels are associated with risk of

disability in multiple sclerosis. Neurology. 2020;94(23): e2457-e2467.
6. Skillback T, Farahmand B, Bartlett JW, et al. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology. 2014;83 (21):1945-1953.
7. Shinomoto M, Kasai T, Tatebe H, et al. Plasma neurofilament light chain: a potential prognostic biomarker of dementia in adult down syndrome patients. PLoS One. 2019;14(4):e0211575.
8. Shahim P, Gren M, Liman V, et al. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Sci Rep. 2016;7(6):36791.
9. Gisslén M, Price RW, Andreasson U, et al. Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. EBioMedicine. 2015;3:135-140.
10. Disanto G, Barro C, Benkert P, et al. Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2017;81(6):857-870.
11. Bjornevik K, Munger KL, Cortese M, et al. Serum neurofilament light chain levels in patients with Presymptomatic multiple sclerosis. JAMA Neurol. 2020;77 (1):58-64.
12. Anderson AM, Easley KA, Kasher N, et al. Neurofilament light chain in blood is negatively associated with neuropsychological performance in HIV-infected adults and declines with initiation of antiretroviral therapy. J Neurovirol. 2018;24(6):695-701.
13. Forgrave LM, Ma M, Best JR, DeMarco ML. The diagnostic performance of neurofilament light chain in CSF and blood for Alzheimer's disease, frontotemporal dementia, and amyotrophic lateral sclerosis: a systematic review and meta-analysis. Alzheimers Dement (Amst). 2019;11:730-743.
14. Mehta KM, Yaffe K, Pérez-Stable EJ, et al. Race/ethnic differences in AD survival in US Alzheimer's disease centers. Neurology. 2008;70(14):1163-1170.
15. Morris JC, Schindler SE, McCue LM, et al. Assessment of racial disparities in biomarkers for Alzheimer disease. JAMA Neurol. 2019;76(3):264-273.
16. Amezcua L, McCauley JL. Race and ethnicity on MS presentation and disease course. Multiple Sclerosis (Houndmills, Basingstoke, England). 2020;26(5):561-567.
17. Harbo HF, Gold R, Tintore M. Sex and gender issues in multiple sclerosis. Ther Adv Neurol Disord. 2013 Jul;6 (4):237-248.
18. Członkowska A, Ciesielska A, Gromadzka G, Kurkowska-Jastrzębska I. Gender differences in neurological disease. Endocrine. 2006;29(2):243-256.
19. Nebel RA, Aggarwal NT, Barnes LL, et al. Understanding the impact of sex and gender in Alzheimer's disease: a call to action. Alzheimers Dement. 2018;14(9):1171-1183.
20. Turner RC, Seminerio MJ, Naser ZJ, et al. Effects of aging on behavioral assessment performance: implications for clinically relevant models of neurological disease. J Neurosurg. 2012;117(3):629.
21. Boyle PA, Yu L, Leurgans SE, et al. Attributable risk of Alzheimer's dementia attributed to age-related neuropathologies. Ann Neurol. 2019;85(1):114-124.
22. Ruano L, Portaccio E, Goretti B, et al. Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes. Mult Scler J. 2017;23(9):1258-1267.
23. Khalil M, Pirpamer L, Hofer E, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nat Commun. 2020;11(1):812.
24. Walsh P, Sudre CH, Fiford CM, et al. The age-dependent associations of white matter hyperintensities and neurofilament light in early- and late-stage Alzheimer's disease. Neurobiol Aging. 2021;97:10-17.
25. Hviid CVB, Knudsen CS, Parkner T. Reference interval and preanalytical properties of serum neurofilament light chain in Scandinavian adults. Scand J Clin Lab Invest. 2020;80(4):291-295.
26. Hansson O, Janelidze S, Hall S, et al. Blood-based NfL: a biomarker for differential diagnosis of parkinsonian disorder. Neurology. 2017;88(10):930-937.
27. Lin Y-S, Lee W-J, Wang S-J, Fuh J-L. Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. Sci Rep. 2018;8(1):17368.
28. Bridel C, van Wieringen WN, Zetterberg H, Tijms BM, Teunissen CE, Group atN. Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. JAMA Neurology. 2019;76(9):1035-1048.
29. Howell JC, Watts KD, Parker MW, et al. Race modifies the relationship between cognition and Alzheimer's disease cerebrospinal fluid biomarkers. Alzheimers Res Ther. 2017;9(1):88.
30. Korley FK, Goldstick J, Mastali M, et al. Serum NfL (neurofilament light chain) levels and incident stroke in adults with diabetes mellitus. Stroke. 2019;50(7):1669-1675.
31. Kuhle J, Kropshofer H, Haering DA, et al. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology. 2019;92(10): e1007-e1015.
32. Filippi M, Bar-Or A, Piehl F, et al. Multiple sclerosis. Nat Rev Dis Primers. 2018;4(1):43.
33. Miclea A, Salmen A, Zoehner G, et al. Age-dependent variation of female preponderance across different phenotypes of multiple sclerosis: a retrospective cross-sectional study. CNS Neurosci Ther. 2019;25(4):527-531.
34. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018;17(2):162-173.

35. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 2014;83(3):278-286.
36. Hendricks R, Baker D, Brumm J, et al. Establishment of neurofilament light chain Simoa assay in cerebrospinal fluid and blood. Bioanalysis. 2019;11(15):1405-1418.
37. Cree BAC, Hollenbach JA, Bove R, et al. Silent progression in disease activity-free relapsing multiple sclerosis. Ann Neurol. 2019;85(5):653-666.
38. Reiber H. Flow rate of cerebrospinal fluid (CSF)--a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. J Neurol Sci. 1994;122(2):189-203.
39. Barro C, Benkert P, Disanto G, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain. 2018;141 (8):2382-2391.
40. Rosso M, Healy BC, Saxena S, et al. MRI lesion state modulates the relationship between serum neurofilament light and age in multiple sclerosis. J Neuroimaging. 2021;31(2):388-393.
41. Barro C, Chitnis T, Weiner HL. Blood neurofilament light: a critical review of its application to neurologic disease. Ann Clin Transl Neurol. 2020;7(12):2508-2523.
42. Celikbilek A, Tanik N, Sabah S, et al. Elevated neurofilament light chain (NFL) mRNA levels in prediabetic peripheral neuropathy. Mol Biol Rep. 2014;41 (6):4017-4022.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Scatterplots of plasma NfL relationship with age and serum NfL

Figure S2. Relationship between age and age-adjusted serum NfL in patients with RMS with brain and spine MRI data within 3 months prior to same acquisition (n = 52).

