Serum neurofilament light chain reference database for individual application in paediatric care: a retrospective modelling and validation study

Ahmed Abdelhak, Franziska Petermeier*, Pascal Benkert*, Sabine Schädelin, Johanna Oechtering, Aleksandra Maleska Maceski, Michael Kabesch, Tobias Geis, Otto Laub, Georg Leipold, Claudio Gobbi, Chiara Zecca, Ari Green, Hayrettin Tumani, Eline Willemse, Heinz Wiendl, Cristina Granziera, Ludwig Kappos, David Leppert, Emmanuelle Waubant, Sven Wellmann‡, Jens Kuhle‡*

Summary

Background Neurological conditions represent an important driver of paediatric disability burden worldwide. Measurement of serum neurofilament light chain (sNfL) concentrations, a specific marker of neuroaxonal injury, has the potential to contribute to the management of children with such conditions. In this context, the European Medicines Agency recently declared age-adjusted reference values for sNfL a top research priority. We aimed to establish an age-adjusted sNfL reference range database in a population of healthy children and adolescents, and to validate this database in paediatric patients with neurological conditions to affirm its clinical applicability.

Methods To generate a paediatric sNfL reference dataset, sNfL values were measured in a population of healthy children and adolescents (aged 0–22 years) from two large cohorts in Europe (the Coronavirus Antibodies in Kids from Bavaria study, Germany) and North America (a US Network of Paediatric Multiple Sclerosis Centers paediatric case-control cohort). Children with active or previous COVID-19 infection or SARS-CoV-2 antibody positivity at the time of sampling, or a history of primary systemic or neurological conditions were excluded. Linear models were used to restrospectively study the effect of age and weight on sNfL concentrations. We modelled the distribution of sNfL concentrations as a function of age-related physiological changes to derive reference percentile and Z score values via a generalised additive model for location, scale, and shape. The clinical utility of the new reference dataset was assessed in children and adolescents (aged 1–19 years) with neurological diseases (epilepsy, traumatic brain injury, bacterial CNS infections, paediatric-onset multiple sclerosis, and myelin oligodendrocyte glycoprotein antibody-associated disease) from the paediatric neuroimmunology clinic at the University of California San Francisco (San Francisco, CA, USA) and the Children's Hospital of the University of Regensburg (Regensburg, Germany).

Findings Samples from 2667 healthy children and adolescents (1336 [50·1%] girls and 1331 [49·9%] boys; median age 8·0 years [IQR 4·0–12·0]) were used to generate the reference database covering neonatal age to adolescence (target age range 0–20 years). In the healthy population, sNfL concentrations decreased with age by an estimated 6·8% per year until age 10·3 years (estimated multiplicative effect per 1 year increase 0·93 [95% CI 0·93–0·94], p<0·0001) and was mostly stable thereafter up to age 22 years (1·00 [0·52–1·94], p>0·99). Independent of age, the magnitude of the effect of weight on sNfL concentrations was marginal. Samples from 220 children with neurological conditions (134 [60·9%] girls and 86 [39·1%] boys; median age 14·7 years [IQR 10·8–16·5]) were used to validate the clinical utility of the reference Z scores. In this population, age-adjusted sNfL Z scores were higher than in the reference population of healthy children and adolescents (p<0·0001) with higher effect size metrics (Cohen's *d*=1·56) compared with the application of raw sNfL concentrations (*d*=1·28).

Interpretation The established normative sNfL values in children and adolescents provide a foundation for the clinical application of sNfL in the paediatric population. Compared with absolute sNfL values, the use of sNfL Z score was associated with higher effect size metrics and allowed for more accurate estimation of the extent of ongoing neuroaxonal damage in individual patients.

Funding Swiss National Science Foundation, US National Institutes of Health, and the National Multiple Sclerosis Society.

Introduction

Accurate monitoring of neuroaxonal injury in neurological and systemic diseases is of pivotal importance at both the population and individual patient level. For instance, methods that measure neuroaxonal injury in children with high specificity could facilitate early and accurate detection of conditions associated with short-term and long-term neurological disabilities, which would have considerable socioeconomic effects. Such methods could also augment clinical trials that are evaluating an

Lancet Neurol 2023

Published Online July 28, 2023 https://doi.org/10.1016/S1474-4422(23)00210-7

See Online/Articles https://doi.org/10.1016/S1474-4422(23)00284-3

*Contributed equally as co-first authors

‡Contributed equally as co-last authors

UCSF Weill Institute for Neurosciences, Department of Neurology, University of California San Francisco, San Francisco, CA, USA (A Abdelhak MD, Prof A Green MD, Prof E Waubant MD); **University Children's Hospital Regensburg, Hospital St Hedwig of the Order of St John, University of Regensburg, Regensburg, Germany** (F Petermeier MD, Prof M Kabesch MD, T Geis MD, Prof S Wellmann MD); **Department of Neurology** (P Benkert PhD, S Schädelin MSc, J Oechtering MD, A Maleska Maceski MSc, E Willemse PhD, Prof C Granziera PhD, Prof L Kappos MD, Prof D Leppert MD, Prof J Kuhle MD), **Multiple Sclerosis Center and Research Center for Clinical Neuroimmunology and Neuroscience Basel, Departments of Biomedicine and Clinical Research** (P Benkert, S Schädelin, J Oechtering, A Maleska Maceski, E Willemse, Prof C Granziera, Prof L Kappos, Prof D Leppert, Prof J Kuhle), **and Translational Imaging in Neurology Basel, Department of Biomedical Engineering, Faculty of Medicine** (Prof C Granziera), **University Hospital Basel and University of Basel, Basel, Switzerland; Paediatric Office Laub, Rosenheim, Germany** (O Laub MD);

Paediatric Office Dr Leipold, Regensburg, Germany (G Leipold MD)**; Multiple Sclerosis Center, Department of Neurology, Neurocenter of Southern Switzerland, Lugano, Switzerland** (Prof C Gobbi MD, C Zecca MD)**; Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano, Switzerland** (Prof C Gobbi, C Zecca)**; Department of Neurology, University of Ulm, Ulm, Germany** (Prof H Tumani MD)**; German Center for Neurodegenerative Diseases, Ulm, Germany** (Prof H Tumani)**; Department of Neurology with Institute of Translational Neurology, University of Muenster, Muenster, Germany** (Prof H Wiendl MD)

Correspondence to: Prof Sven Wellmann, Department of Neonatology, University Children's Hospital Regensburg, Hospital St Hedwig of the Order of St John, University of Regensburg, 93053 Regensburg, Germany
sven.wellmann@barmherzige-regensburg.de

or

Prof Jens Kuhle, Multiple Sclerosis Centre and Research Center for Clinical Neuroimmunology and Neuroscience, Departments of Neurology, Biomedicine, and Clinical Research, University Hospital Basel and University of Basel, 4031 Basel, Switzerland
jens.kuhle@usb.ch

Research in context

Evidence before this study

Relevant literature was identified by searching PubMed from database inception up to March 20, 2023, for articles published in English, using the search terms: "neurofilament" and "pediatrics" or "paediatrics" or "children". Serum neurofilament light chain (sNfL) is elevated in numerous paediatric neurological and systemic conditions, with lowering of concentrations under effective treatments. Despite differences in individuals with disease versus control groups, the effect of age on sNfL has been poorly defined. Furthermore, no age-adjusted reference range exists for individuals younger than 18 years.

Added value of this study

In this work, we have developed age-adjusted sNfL reference curves to help diagnose and monitor neurological conditions up to age 20 years. Using data from two large cohorts from centres in Europe and North America, we established sNfL percentiles and Z scores, adjusted for age, and integrated them into a new public web-based application. In a well defined clinical use case, we showed the superior potential of the newly developed sNfL Z scores, compared with absolute sNfL values, in reflecting ongoing neuroaxonal injury in organic neurological conditions.

Implications of all the available evidence

Similar to in the adult population, sNfL is likely to have a relevant contribution to the counselling of children with paediatric neurological conditions. Paediatric neurological diseases contribute substantially to paediatric disability burden worldwide. The normative values established for sNfL for the paediatric population in this study represent a crucial advance to overcome existing constraints in interpreting raw values, and provides the foundation for the clinical use of blood NfL measurements in this population.

expanding range of targeted causal and disease-modifying treatments.

Neurofilament light chain (NfL) is a neuron-specific protein, and the release of NfL into CSF and blood has become a first-in-class biomarker of neuronal damage.[1] However, physiological factors and various pathological conditions can affect the concentrations of NfL in serum (sNfL), and the interpretation of sNfL concentrations can be complicated if based on raw values.[2,3] In 2022, the development of a large, age-adjusted reference dataset of NfL values from healthy adults was an important step towards a contextualised understanding of NfL concentrations in blood.[4] Use of age-adjusted sNfL concentrations in adults has shown potential in predicting disease activity and capturing treatment response in multiple sclerosis.[4] In line with these findings, in 2022 the US Food and Drug Administration granted breakthrough designation status for an sNfL assay (the Quanterix Simoa NfL plasma test) for accelerated consideration as a method for multiple sclerosis activity monitoring in individuals older than 18 years.[5]

Evidence supports the value of sNfL as a biomarker in diseases of children and adolescents, and testing for this biomarker might contribute substantially to the management of paediatric neurological conditions. Indeed, neonatal prematurity[6] and diseases such as multiple sclerosis,[7,8] Huntington's disease,[9] and spinal muscle atrophy[10,11] in children are associated with increased sNfL concentrations. Furthermore, reduction of elevated sNfL as a consequence of therapeutic interventions can be used to assess treatment effects, as reported for disease-modifying treatments in multiple sclerosis[12] and for nusinersen in spinal muscle atrophy.[10] These studies in paediatric populations indicate utility in rare disabling paediatric conditions.

Without normative values of sNfL in children, wide-scale application of sNfL as a biomarker for paediatric populations in clinical and research settings is hindered. In 2022, the European Medicines Agency acknowledged the absence of reference values for sNfL as a crucial shortcoming for implementation of this biomarker in the paediatric neurology setting.[13] Thus, efforts to establish both a reference dataset for sNfL in the paediatric population and age-adjusted normalised scores are considered top research priorities. In this international collaboration study, we aimed to establish an age-adjusted sNfL reference database using immunoassay data in a large population of healthy children and adolescents. We subsequently assessed the clinical application of the reference dataset in children and adolescents with various neurological diseases.

Methods

Study design and participants

For retrospective modelling and validation, we used prospectively collected individual serum samples (one sample per person). To generate a paediatric sNfL reference dataset, we used serum samples collected from a population of healthy children and adolescents from two large cohorts in Europe and North America. The first cohort was from the Coronavirus Antibodies in Kids from Bavaria study (CoKiBa) in southern Germany; samples for this cohort were collected cross-sectionally between May 22 and July 22, 2020, to assess the incidence of SARS-CoV2 antibodies. CoKiBa recruited two groups of healthy individuals who were either part of an ongoing public health prevention programme, or the children of families who actively wanted to participate.[14] The samples used in this study were from individuals aged 0–16 years. Exclusion criteria for this sNfL reference dataset were active COVID-19 infection, history of COVID-19 infection,

or SARS-CoV-2 antibody positivity at the time of sampling.[14] The second cohort was the US Network of Paediatric Multiple Sclerosis Centers (US_{pedHC}), from which we included samples from children and adolescents (aged 2–22 years) without multiple sclerosis. Samples were obtained cross-sectionally from 16 paediatric clinics in the USA, between January, 2008, and February, 2014, as part of an ongoing multicentre case-control study.[15] In both cohorts, children and adolescents with a history of primary systemic or neurological conditions were excluded from our reference population. Overall sample selection for the reference database is presented in the appendix (p 6).

To confirm the clinical utility and value of the proposed age-adjusted sNfL reference values, we evaluated sNfL values in outpatients (aged 1–19 years) with neurological diseases (epilepsy, traumatic brain injury, bacterial CNS infections, paediatric-onset multiple sclerosis, and myelin oligodendrocyte glycoprotein antibody-associated disease) from the paediatric neuroimmunology clinic at the University of California San Francisco (San Francisco, CA, USA).[16] Samples were collected cross-sectionally between October, 2006, and March, 2019. The patients with paediatric-onset multiple sclerosis (n=142) and myelin oligodendrocyte glycoprotein antibody-associated disease (n=20) have been reported on previously.[16] Additional samples were collected from inpatients and outpatients (aged <18 years) with the specified neurological conditions at the Children's Hospital of the University of Regensburg (Regensburg, Germany) between October, 2017, and July, 2018.

Institutional review boards at respective centres approved this study. Written informed consent was obtained from participants or corresponding legal guardians.

Procedures

Basic demographic data were collected at the time of sampling, including age, sex, race, and body weight. We measured sNfL concentrations with the Simoa NF-light Advantage kit (version 1) on an HD-X Analyzer (both from Quanterix, Lexington, MA, USA).[4] sNfL was measured according to the protocol provided by the company. Samples were measured in duplicate. Inter-assay variability was evaluated with three native quality-control serum samples in each measurement run. All samples produced signals greater than the analytical sensitivity of the assay. Mean intra-assay variability and inter-assay variability were lower than 10%.

Statistical analysis

Participant characteristics are presented by cohort as median and IQR. Visual inspection of the association between sNfL and age in healthy children showed a distinct pattern in early childhood versus late childhood. Therefore, we first modelled the association between sNfL (dependent variable; log-transformed) and age separately in these two groups using the Akaike information criterion as a goodness-of-fit measure. A linear term for age (as compared to spline terms) best described the data. Thereafter, a segmented regression approach was used to model the association over the entire age range. Consequently, the association was assumed to be piecewise linear (after log-transformation of sNfL) and the unknown inflexion point was estimated as an actual parameter of the model, with use of a linearisation technique described previously.[17] The proportion of variability accounted for by the segmented regression model was expressed by adjusted R square (R^2_{adj}). R^2_{adj} was also determined for separate regression models with a linear term for age, for individuals older than the inflexion point age and for individuals younger than the inflexion point age to quantify the importance of age in explaining the variation of sNfL in these two age periods. Estimates from models with log-transformed sNfL as the dependent variable were back-transformed (exponentiated) and therefore represent multiplicative effects on the geometric mean of sNfL.

See Online for appendix

Following estimation of the effect of age, we fitted two separate linear models to assess the effect of weight on sNfL concentrations in healthy children and adolescents, in the group who were younger and the group who were older than the inflexion point detected by the segmented regression model. Using log-transformed sNfL as a dependent variable, we compared R^2_{adj} when including weight and age as compared with age alone. In these models, the variance inflation factor was used as a measure of the magnitude of collinearity between age and weight.

To generate sNfL reference curves, we used a generalised additive model for location, scale, and shape (GAMLSS), which was based on a Box-Cox *t* distribution, to accurately model the association between sNfL and age. We tested several distributions and compared goodness-of-fit statistics using the Bayesian information criterion to find the most appropriate fit for the data. Skewness and kurtosis were found to not be age-dependent and were estimated as constant parameters of the model. sNfL was the dependent variable and the final model used spline terms with three degrees of freedom for the explanatory variable (age). The model fit was assessed visually with detrended Q–Q plots. This statistical approach and the underlying distribution used in the model allowed for precise estimation of outer percentiles and Z scores, as described previously.[4,18] Percentiles express the percentage of children and adolescents from the general population who are expected to have sNfL values lower than the given value, taking into account age. Z scores are interchangeable with percentiles and express the deviation of an age-adjusted sNfL value in terms of the number of standard deviations from the mean in a reference population.[4] The clinical utility of the Z scores was assessed by comparing sNfL raw values (log-transformed) and sNfL Z scores

	CoKiBa (n=2235)	US_{pedHC} (n=432)	Total (n=2667)
Age, years	7·0 (4·0–10·0)	15·4 (12·3–17·7)	8·0 (4·0–12·0)
Sex			
Female	1090 (48·8%)	246 (56·9%)	1336 (50·1%)
Male	1145 (51·2%)	186 (43·1%)	1331 (49·9%)
Race			
White	NA	265 (61·3%)	NA
Black or African American	NA	72 (16·7%)	NA
Asian	NA	42 (9·7%)	NA
Multiracial	NA	35 (8·1%)	NA
Native American or Alaskan Native	NA	5 (1·2%)	NA
Not reported	NA	13 (3·0%)	NA
Bodyweight, kg	NA	57·0 (47·2–69·8)*	NA
sNfL concentration, pg/mL	4·9 (3·9–6·4)	3·9 (2·9–5·1)	4·8 (3·7–6·2)

Data are median (IQR) or n (%). CoKiBa=Coronavirus Antibodies in Kids from Bavaria study. NA=not available. sNfL=serum neurofilament light chain. US_{pedHC}=US Network of Paediatric Multiple Sclerosis Centers (healthy controls). *Bodyweight available for 378 individuals.

Table 1: **Demographic characteristics of healthy children and adolescents**

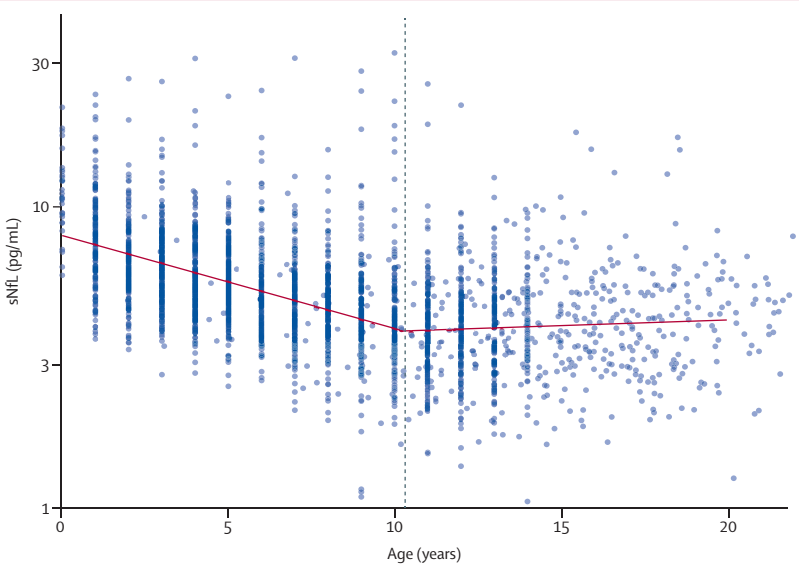


Figure 1: **Association between age and sNfL concentrations in healthy children and adolescents**
The segmented regression model indicated that the association between sNfL and age could be approximated by two separate linear models with log(sNfL) as a dependent variable and the optimal inflexion point at age 10·3 years (vertical dashed line). Dots represent individual samples (one per person). In healthy children younger than 10·3 years, increasing age up to 10·3 years was associated with lower sNfL concentrations (6·8% decrease per year). In healthy children and adolescents aged 10·3 years or older, age was not significantly associated with sNfL concentration. sNfL=serum neurofilament light chain.

between the healthy population and those with neurological conditions by quantifying the gain in precision after accounting for the non-linear effect of age on sNfL. This quantification was done by numerically describing the effect sizes in these group comparisons with use of Cohen's *d* and supported by Wilcoxon-rank sum test.[19] We had a high number of samples from individuals in the reference population in the age range 0–20 years (n=2638, 98·9%) and we found good agreement of the reference curves between older adolescents and the recently published references for adults,[4] allowing for a transition between the reference curves. Thus, we defined the target age range of the reference values to be 0–20 years.

In all analyses, p values lower than 0·05 were considered to indicate statistical significance. All analyses were performed in R (version 4.3.1).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

sNfL values from 2667 healthy children and adolescents from the CoKiBa (n=2235) and US_{pedHC} (n=432) cohorts were used to generate the reference database. Demographic data and measured sNfL concentrations are reported in table 1 and the appendix (p 7). The total population of healthy children and adolescents comprised 1336 (50·1%) girls and 1331 (49·9%) boys, and median age was 8·0 years (IQR 4·0–12·0). Median sNfL concentration was 4·9 pg/mL (IQR 3·9–6·4) for the CoKiBa samples and 3·9 pg/mL (2·9–5·1) for the US_{pedHC} samples.

sNfL concentrations were not independent of age, and different patterns of association were identified in different age ranges (figure 1). The segmented regression model indicated that the association could be approximated by two separate linear associations with an optimal inflexion point at 10·3 years (9·6–10·9). In children younger than 10·3 years, sNfL concentrations decreased by an estimated 6·8% with each 1 year increase in age (estimated multiplicative effect per 1 year increase 0·93 [95% CI 0·93–0·94], p<0·0001). In children and adolescents aged 10·3 years or older, up to the upper age bound of 22 years, age was not associated with sNfL concentrations (1·00 [0·52–1·94], p>0·99). Overall, age accounted for 26·0% of the variation in sNfL (R^2_{adj} 0·260) in the segmented regression model, which was driven by the correlation in age younger than 10·3 years (R^2_{adj} 0·237; older than 10·3 years, R^2_{adj} 0·002). Independently of the effect of age, sex did not affect sNfL concentration (p=0·95 in a separate GAMLSS model including sex as a covariate in addition to age).

Bodyweight was only available in the US_{pedHC} cohort, in 378 of 432 individuals. In healthy children younger than 10·3 years with available bodyweight (n=46; appendix p 3), age alone accounted for 13·8% of the variability in sNfL concentrations (R^2_{adj} 0·138; estimated multiplicative effect per 1 year increase, 0·93 [0·89–0·98], p=0·0064) and bodyweight alone only accounted for 7·9% of the variability (R^2_{adj} 0·079), with a 10 kg increase in weight associated with an estimated 8·8% decrease in sNfL concentration (estimated multiplicative effect 0·91 [0·84–0·99], p=0·033; appendix p 3). Combining the age and bodyweight variables led to a decrease in adjusted

variance compared with age alone (R^2_{adj} 0·125), because the two variables were moderately correlated (variance inflation factor of 1·7). In children and adolescents aged 10·3 years or older (n=332), bodyweight alone correlated with sNfL concentrations. However, the magnitude of the effect appeared to be small, with a 10 kg increase in bodyweight associated with an estimated 3·7% decrease in sNfL concentration (estimated multiplicative effect 0·96 [0·94–0·99], p=0·0015), and the effect accounted for only 2·7% of sNfL variation (R^2_{adj} 0·027; appendix p 3). When combining age and weight in the statistical model for children and adolescents aged 10·3 years or older, the model explained 4·8% of the sNfL variation (R^2_{adj} 0·048; appendix p 3).

The distribution of sNfL concentrations in the function of age-related physiological changes was modelled to derive percentile and Z score values via a GAMLSS model. The age-adjusted sNfL measures (percentiles and Z scores) can be retrieved in a numerical format and as a graphical illustration (figure 2; appendix pp 4–5, 8–9) online.

The clinical utility of the reference database was validated in 220 children and adolescents with neurological conditions, comprising 134 (60·9%) girls and 86 (39·1%) boys (median age 14·7 [IQR 10·8–16·5]; table 2). In this population, both raw sNfL concentrations and sNfL Z scores were higher than in the reference population (both p<0·0001), but sNfL Z scores were associated with larger effect size metrics than the raw concentrations (sNfL Z scores, Cohen's *d*=1·56; and absolute sNfL values, *d*=1·28; figure 3). In this clinical use case, Z scores showed greater distinctive power between various neurological diseases and healthy children and adolescents when compared with raw values, as shown by consistently higher effect size measures expressed by Cohen's *d* and lower p values across the different disease subgroups (appendix p 10).

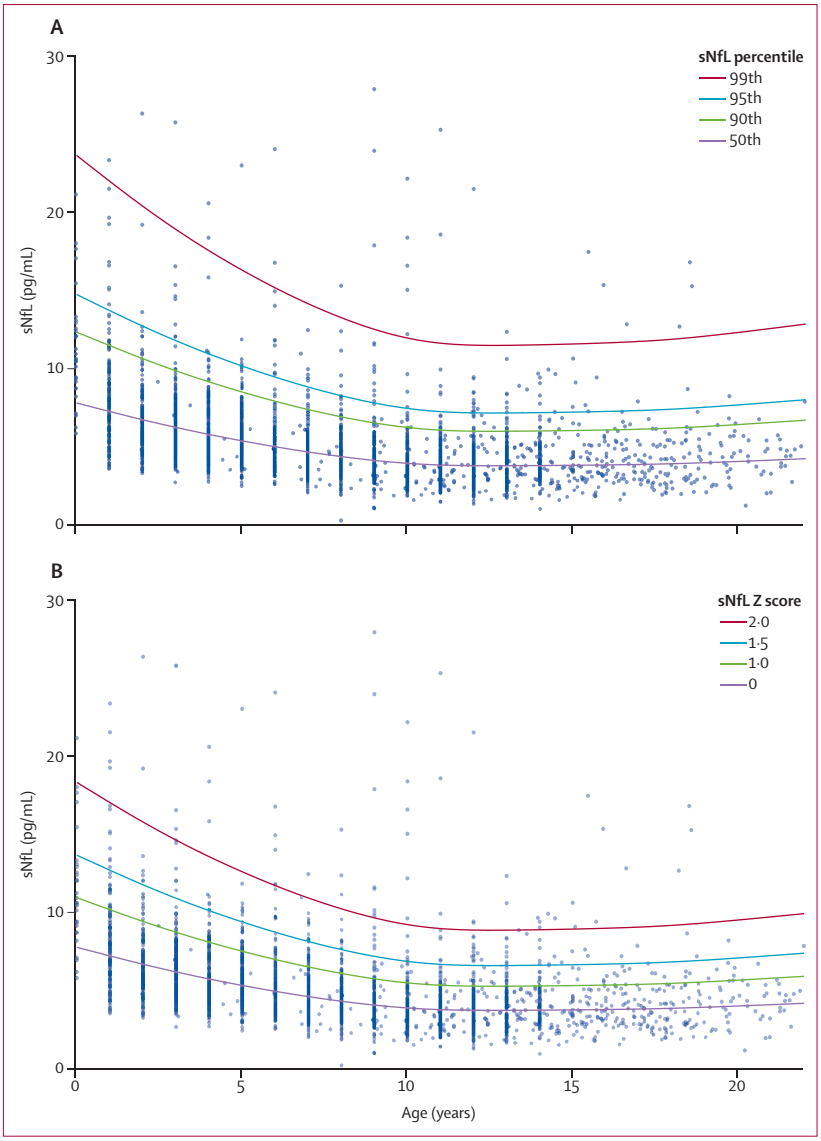


Figure 2: **sNfL percentile and Z score reference curves in healthy children and adolescents**
A generalised additive model for location, scale, and shape with a spline term for age was used to model the non-linear association of sNfL concentration (pg/mL) with age in healthy children. Dots represent individual samples (one per person). From this model, percentile values (A) and Z scores (B) were derived, defining the deviation from reference while adjusting for age. sNfL=serum neurofilament light chain.

Discussion

In this study, we used a highly sensitive immunoassay to measure sNfL concentrations in a large international cohort, and established a paediatric reference dataset to interpret sNfL values in clinical practice and research settings. To allow for broad use by the scientific community, we have implemented an online application to generate Z scores for individuals aged 20 years or younger (appendix p 9), which complements the adult reference application.[4]

Similar to in adults, age is a predominant and disease-independent modifier of sNfL concentrations in children and adolescents. However, in the current study, the directionality of sNfL correlation with age varied depending on age group, in contrast with the adult population, who showed an exponential increase in sNfL concentrations with age.[4] We found that sNfL concentrations were highest after birth, with a steep yearly decrease until stabilisation at around 10 years of age. In early and late adolescence, there was a trend towards increasing sNfL concentration with increasing age, which is known to continue in adulthood before accelerating in elderly individuals.[4] High sNfL concentrations in the early years of life are unlikely to be related to accelerated neuroaxonal injury. A more likely primary explanation for the strong negative correlation between sNfL and age in early years might be the physiological change of distribution volume. Total blood volume has been shown to expand substantially after birth, and it stabilises in early adulthood (appendix p 11).[20,21] It is also noteworthy that CNS maturation accelerates within the first few years after birth.[22,23] Whether the

For the **online application** see https://shiny.dkfbasel.ch/baselnflreference-for-kids

	Paediatric-onset multiple sclerosis (n=142)	Epilepsy (n=36)	Myelin oligodendrocyte glycoprotein antibody-associated disease (n=20)	Bacterial CNS infections (n=12)	Traumatic brain injury (n=10)
Sex					
Female	95 (66·9%)	15 (41·7%)	15 (75·0%)	5 (41·7%)	4 (40·0%)
Male	47 (33·1%)	21 (58·3%)	5 (25·0%)	7 (58·3%)	6 (60·0%)
Age, years	15·0 (12·9–16·8)	11·4 (6·0–15·0)	8·8 (7·4–11·4)	8·8 (8·0–12·3)	11·3 (6·8–17·2)
sNfL concentration, pg/mL	21·2 (9·5–42·3)	5·5 (3·7–7·0)	32·7 (11·4–69·7)	6·8 (4·6–10·3)	5·8 (4·6–7·1)
sNfL Z score	2·8 (2·1–3·2)	0·6 (0·3–1·5)	3·0 (2·2–3·3)	0·9 (0·4–2·0)	0·8 (0·4–1·4)

Data are n (%) or median (IQR). sNfL=serum neurofilament light chain.

Table 2: **Children and adolescents included in the clinical use case, by neurological disorder**

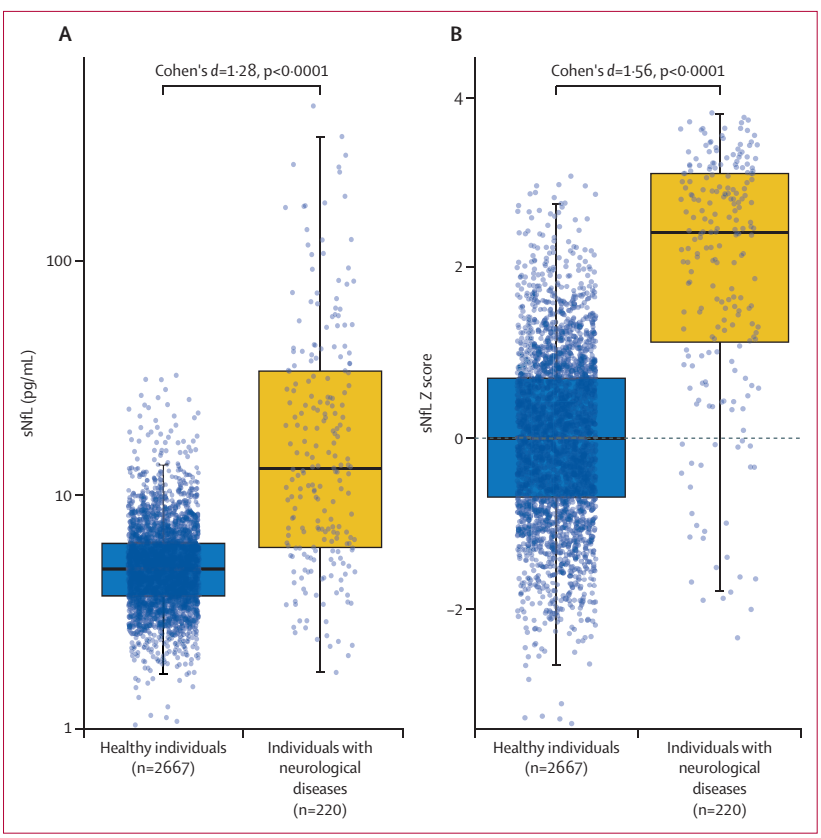


Figure 3: **Absolute sNfL values and sNfL Z scores**

Absolute sNfL values (A) and sNfL Z scores (B) were from healthy children and adolescents, who were used to create the sNfL reference database, and children and adolescents with neurological conditions. Application of sNfL Z scores versus absolute sNfL concentrations increased the magnitude of difference (as expressed by the effect size measure Cohen's *d*) between healthy children and adolescents (reference population) and patients with neurological diseases. The horizontal line at Z=0 in part B represents the expected mean value in the reference population. Box-plots represent the median, IRQ, and 1·5 times the IQR from the first and third quartile. Dots represent individual samples (one per person). p values refer to Wilcoxon rank-sum tests. sNfL=serum neurofilament light chain.

inherent structural changes in the CNS could also contribute to higher sNfL concentrations in early years or reduction of sNfL concentration with age is unknown.

The reported age-adjusted reference ranges in our paediatric population do not correct for bodyweight or body-mass index (BMI), by contrast with the largest adult reference database to date.[4] Despite limited available data, we found that adding bodyweight to the function with age did not improve the statistical models in children younger than 10·3 years, and while age older than 10·3 years had no association with sNfL (minimal slope in the reference curves), the effect of bodyweight became visible with older age and accounted for a minor fraction of sNfL variability. In children, age, height, and bodyweight are expected to have high collinearity. After height stabilises in late adolescence and early adulthood, bodyweight change (and thus BMI) could affect sNfL concentration.[3,24] In our cohort, the comparison of the magnitude of these effects across the two identified age ranges highlights the importance of accounting for age differences in the paediatric population: a small age change of half a year in younger children (age <10·3 years) had approximately the same magnitude of effect on sNfL concentrations as did a 10 kg change in bodyweight in older children and adolescents. Therefore, correcting for the confounding effect of age can be considered more important than bodyweight in this population, in whom neurological diseases can span age ranges of one to two decades, and for whom a simple linear age-adjustment is not appropriate. Considering bodyweight or BMI in generating reference sNfL range is substantially more important in adults than in the paediatric population, to potentially avoid misinterpretation of sNfL concentrations due to abnormally high or low bodyweight. Conversely, sNfL Z scores express the deviation from a reference while accounting for the unique, non-linear, and non-monotonic form of the association between sNfL and age, allowing for the detection of subtle, abnormal sNfL elevations that might otherwise remain obscured by the variability due to age (eg, in patients in this study with epilepsy or traumatic brain injury, appendix p 10).

In addition to defining unique physiological variations, we have shown the applicability of age-adjusted Z scores in a well defined clinical use case of various paediatric neurological conditions. Use of the sNfL Z score was associated with higher effect size metrics than absolute sNfL concentrations, indicating a superior statistical power in differentiating healthy children and adolescents

from those with the evaluated neurological diseases. This outcome is especially relevant for studies investigating novel treatments for rare hereditary or acquired paediatric neurological disorders and individual application.

Our study has some limitations. The effect of bodyweight on sNfL concentrations was only evaluated in a subset of participants. Nevertheless, bodyweight was only weakly associated with sNfL in this population. The smooth continuity between the paediatric reference range and adult reference range (with additional BMI adjustment for adult values) over the evaluated percentiles supports our approach (appendix p 8). The dataset also did not enable us to evaluate other comorbidities, such as renal function or diabetes, which are known to affect blood NfL concentrations in adults.[4,25] White participants represented the majority of included participants from the US_{pedHC} cohort, and data on race were not available for the CoKiBa cohort. Therefore, the generalisability of our findings to diverse populations needs to be confirmed in future cohorts.[26]

In summary, the presently established reference values for sNfL on the basis of Z scores in the paediatric population represent a crucial step to overcoming the constraints of interpreting raw values. The reference values also provide a foundation for the clinical use of blood NfL measurements in the paediatric population and for individual counselling.

Contributors

AA, PB, SS, HW, LK, EWa, DL, SW, AG, HT, and JK conceptualised the study. AA, FP, PB, SS, JO, AMM, MK, TG, OL, GL, CGo, CZ, EWi, CGr, DL, EWa, SW, and JK collected and curated the data. AA, PB, SS, EW, DL, SW, and JK analysed the data. DL and JK acquired funding. AA, PB, SS, EWa, AMM, DL, SW, and JK designed the methods. PB, AMM, SS, SW, EWa, and JK contributed to project administration. DL, SW and JK supervised the study. PB, SS, AA, SW, EWa, and JK accessed and verified the data. AA, PB, EWi, SS, and JK created the figures. AA, PB, SS, and JK wrote the original draft. All authors reviewed and substantially edited the final manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

AA has received research funding from DMSG, AMSEL, the Bavarian MS Trust, and UCSF Weill Institute for Neurosciences. JO has received research support from the Swiss MS Society and served on advisory boards for Roche and Merck. CGo's employer (Ente Ospedaliero Cantonale) has received compensation for CG's speaking activities, consulting fees, or research grants from Almirall, Biogen Idec, Bristol Myers Squibb, Lundbeck, Merck, Novartis, Sanofi, Teva Pharma, and Roche. CZ is recipient of a grant for senior researchers provided by AFRI (l'Area Formazione Accademica, Ricerca e Innovazione). HT has received consulting and/or speaker honoraria from Alexion, Bayer, Biogen, Celgene, GlaxoSmithKline, Janssen, Merck, Novartis, Roche, Sanofi Genzyme, and Teva. HW has received honoraria and consultation fees from Bayer Healthcare, Biogen, Fresenius Medical Care, GlaxoSmithKline, GW Pharmaceuticals, Merck Serono, Novartis, Sanofi Genzyme, and Teva. CGr's employer (University Hospital Basel) has received the following fees which were used exclusively for research support: advisory boards and consultancy fees from Actelion, Novartis, Genzyme-Sanofi, GeNeuro, Hoffmann La Roche, and Siemens Healthineers; speaker fees from Biogen, Hoffmann La Roche, Teva, Novartis, Janssen, and Genzyme-Sanofi; and research grants from Hoffmann La Roche, GeNeuro, Genzyme, and Biogen. LK's institutions (University Hospital Basel and the Research Center for Clinical Neuroimmunology and Neuroscience Basel) have received and dedicated to research support: payments for steering committee and advisory board participation, consultancy services, and participation in educational activities from Actelion, Bayer, Bristol Myers Squibb, df-mp (Dörries Frank Molnia & Pohlman), Celgene, Eli Lilly, EMD Serono, Genentech, GlaxoSmithKline, Janssen, Japan Tabacco, Merck, MH Consulting, Minoryx, Novartis, Hoffmann-La Roche, Senda Biosciences, Sanofi, Santhera, Shionogi, TG Therapeutics, and Wellmera; license fees for Neurostatus-UHB products; and grants from Novartis, Innosuisse and Roche. DL has received grants from Progressive MS Alliance and is Chief Medical Officer of GeNeuro. SW is Chief Medical Officer and co-founder of Neopredix, a spin-off company of the University of Basel. JK has received speaker fees, research support and/or travel support from, and/or served on advisory boards for, the Swiss MS Society, the Swiss National Research Foundation (grant number 320030_189140/1), the University of Basel, the Progressive MS Alliance, Alnylam, Bayer, Biogen, Bristol Myers Squibb, Celgene, Immunic, Merck, Neurogenesis, Novartis, Octave Bioscience, Quanterix, Roche, Sanofi, and Stata DX. All other authors declare no competing interests.

Data sharing

Following publication, written requests for access to the data reported in this paper will be considered by the corresponding authors (jens.kuhle@usb.ch and sven.wellmann@barmherzige-regensburg.de) and principal investigators of the included cohorts, and a decision will be made about the appropriateness of the use of the data. If the use is appropriate, a data sharing agreement will be put in place before a fully de-identified version of the dataset used for the analysis with individual participant data is made available. The exact data, documents, and related items that are shared will be decided during the request process.

Acknowledgments

This study was funded by the Swiss National Science Foundation (grant number 320030_189140/1). Acquisition of samples from the healthy paediatric cohort from the US Network of Paediatric Multiple Sclerosis Centers was supported by a US National Institutes of Health grant (R01NS071463) and a National Multiple Sclerosis Society grant (HC-1509-06233). We are thankful to all participants, study nurses, technicians, and physicians for their engagement in the study. We also thank the centres of the Coronavirus Antibodies in Kids from Bavaria study and the US Network of Pediatric Multiple Sclerosis Centers for the collection of high-quality samples and for allowing the inclusion of samples in the current analysis.

References

1 Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol* 2018; **14:** 577–89.

2 Abu-Rumeileh S, Abdelhak A, Foschi M, et al. The multifaceted role of neurofilament light chain protein in non-primary neurological diseases. *Brain* 2023; **146:** 421–37.

3 Koini M, Pirpamer L, Hofer E, et al. Factors influencing serum neurofilament light chain levels in normal aging. *Aging (Albany NY)* 2021; **13:** 25729–38.

4 Benkert P, Meier S, Schaedelin S, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *Lancet Neurol* 2022; **21:** 246–57.

5 Quanterix. Quanterix granted breakthrough device designation from US FDA for NfL test for multiple sclerosis. April 22, 2022. https://www.quanterix.com/press-releases/quanterix-granted-breakthrough-device-designation-from-us-fda-for-nfl-test-for-multiple-sclerosis/ (accessed Oct 18, 2022).

6 Depoorter A, Neumann RP, Barro C, et al. Neurofilament light chain: blood biomarker of neonatal neuronal injury. *Front Neurol* 2018; **9:** 984.

7 Wong YYM, Bruijstens AL, Barro C, et al. Serum neurofilament light chain in pediatric MS and other acquired demyelinating syndromes. *Neurology* 2019; **93:** e968–74.

8 van der Vuurst de Vries RM, Wong YYM, Mescheriakova JY, et al. High neurofilament levels are associated with clinically definite multiple sclerosis in children and adults with clinically isolated syndrome. *Mult Scler* 2019; **25:** 958–67.

9 Byrne LM, Schultz JL, Rodrigues FB, et al. Neurofilament light protein as a potential blood biomarker for Huntington's disease in children. *Mov Disord* 2022; **37:** 1526–31.

10 Olsson B, Alberg L, Cullen NC, et al. NFL is a marker of treatment response in children with SMA treated with nusinersen. *J Neurol* 2019; **266:** 2129–36.

11 Wurster CD, Steinacker P, Günther R, et al. Neurofilament light chain in serum of adolescent and adult SMA patients under treatment with nusinersen. *J Neurol* 2020; **267:** 36–44.

12 Reinert MC, Benkert P, Wuerfel J, et al. Serum neurofilament light chain is a useful biomarker in pediatric multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* 2020; **7:** e749.

13 European Medicines Agency. Letter of support of neurofilament light in childhood neurological diseases. Nov 10, 2022. https://www.ema.europa.eu/en/documents/other/letter-support-neurofilament-light-childhood-neurological-diseases_en.pdf (accessed March 4, 2023).

14 Laub O, Leipold G, Toncheva AA, et al. Symptoms, SARS-CoV-2 antibodies, and neutralization capacity in a cross sectional-population of German children. *Front Pediatr* 2021; **9:** 678937.

15 Casper TC, Rose JW, Roalstad S, et al. The US Network of Pediatric Multiple Sclerosis Centers: development, progress, and next steps. *J Child Neurol* 2015; **30:** 1381–87.

16 Ziaei A, Nasr Z, Hart J, et al. High serum neurofilament levels are observed close to disease activity events in pediatric-onset MS and MOG antibody-associated diseases. *Mult Scler Relat Disord* 2023; **74:** 104704.

17 Muggeo VM. Estimating regression models with unknown break-points. *Stat Med* 2003; **22:** 3055–71.

18 Borghi E, de Onis M, Garza C, et al. Construction of the World Health Organization child growth standards: selection of methods for attained growth curves. *Stat Med* 2006; **25:** 247–65.

19 Thompson B. Effect sizes, confidence intervals, and confidence intervals for effect sizes. *Psychol Sch* 2007; **44:** 423–32.

20 Howie SR. Blood sample volumes in child health research: review of safe limits. *Bull World Health Organ* 2011; **89:** 46–53.

21 Rudolph C, Rudolph A, Hostetter M, Lister G, Siegel N. Rudolph's pediatrics, 21st edn. New York: McGraw-Hill, 2002.

22 Zhang Y, Shi J, Wei H, Han V, Zhu WZ, Liu C. Neonate and infant brain development from birth to 2 years assessed using MRI-based quantitative susceptibility mapping. *Neuroimage* 2019; **185:** 349–60.

23 Yeung MS, Zdunek S, Bergmann O, et al. Dynamics of oligodendrocyte generation and myelination in the human brain. *Cell* 2014; **159:** 766–74.

24 Manouchehrinia A, Piehl F, Hillert J, et al. Confounding effect of blood volume and body mass index on blood neurofilament light chain levels. *Ann Clin Transl Neurol* 2020; **7:** 139–43.

25 Fitzgerald KC, Sotirchos ES, Smith MD, et al. Contributors to serum NfL levels in people without neurologic disease. *Ann Neurol* 2022; **92:** 688–98.

26 Abdelhak A, Kuhle J, Green AJ. Challenges and opportunities for the promising biomarker blood neurofilament light chain. *JAMA Neurol* 2023; **80:** 542–43.

THE LANCET
Neurology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Abdelhak A, Petermeier F, Benkert P, et al. Serum neurofilament light chain reference database for individual application in paediatric care: a retrospective modelling and validation study. *Lancet Neurol* 2023; published online July 28. https://doi.org/10.1016/S1474-4422(23)00210-7.

Serum neurofilament light chain reference database for individual application in paediatric care

Ahmed Abdelhak*, Franziska Petermeier*, Pascal Benkert* *et al.*

Supplementary material

Table of Contents

Supplementary Table 1. Comparison of demographic characteristics between subjects with and without available data on body weight in the US_{pedHC} dataset.

	Subjects with available body weight (*n*= 378)	Subjects without available body weight (*n*= 54)	All subjects (*n*= 432)
Female sex (n, %)	214, 56·6%	32, 59·3%	246, 56·9%
Age at sampling	15·4 [12·4–17·8]	15·4 [11·2–17·2]	15·4 [12·3–17·7]
Body weight (kg)	57·0 [47·2–69·8]	NA	57·0 [47·2–69·8]
sNfL (pg/ml)	3·9 [2·9–5·1]	3·9 [2·9–5·1]	3·9 [3·1–5·1]

Values are reported as median and interquartile range if not mentioned otherwise.

Abbreviations: US_{pedHC}: US Paediatric Multiple Sclerosis Network (healthy controls); sNfL: serum neurofilament light chain; NA: not available.

Supplementary Table 2. The statistical association between age, weight and sNfL.

Age group	Adjusted R^2	Term	Estimate [95% confidence interval]	p-value
Age < 10·3 years old (*n*= 46)	0·138	Age	0·93 [0·89–0·98]	0·006
	0·079	Weight	0·91 [0·84–0·99]	0·033
	0·125	Age	0·95 [0·89–1·01]	0·075
		Weight	0·97 [0·87–1·01]	0·557
Age ≥ 10·3 years old (*n*= 332)	0·005	Age	1·01 [1·00–1·03]	0·097
	0·027	Weight	0·96 [0·94–0·99]	0·002
	0·048	Age	1·02 [1·0 –1·04]	0·005
		Weight	0·95 [0·95–0·98]	<0·001

Estimates (multiplicative effects) from individual linear regression models with log(NfL) as dependent variable. Reading example: In children younger than 10·3 years, sNfL decreases by approximately 7% per year (model 1; 6·8% in the segmented regression model on all samples, **Figure 1**) and adding weight did not further improve the model as indicated by the adjusted R^2. In children older than 10·3 years, weight explained 2·7% of the variance in sNfL and the effect was relatively small: on average sNfL was 4% lower per 10kg increase in weight (i.e. an effect comparable to half a year in age in the younger).

Age: per year; weight: per 10kg.

Abbreviations: sNfL: serum neurofilament light chain.

Supplementary Table 3. Estimated sNfL concentrations (pg/ml) at a given age and percentile value.

	Percentiles					
Age (years)	50th	80th	90th	95th	97·5th	99th
1	7·3	9·6	11·5	13·7	16·6	22
2	6·7	8·9	10·6	12·7	15·4	20·4
3	6·2	8·3	9·9	11·8	14·3	18·9
4	5·8	7·7	9·2	10·9	13·2	17·5
5	5·4	7·1	8·5	10·2	12·3	16·3
6	5	6·6	7·9	9·5	11·4	15·2
7	4·7	6·2	7·4	8·8	10·7	14·2
8	4·4	5·8	6·9	8·3	10	13·3
9	4·1	5·5	6·5	7·8	9·4	12·5
10	4	5·2	6·2	7·5	9	12
11	3·8	5·1	6·1	7·3	8·8	11·6
12	3·8	5	6	7·2	8·7	11·5
13	3·8	5	6	7·2	8·7	11·5
14	3·8	5	6	7·2	8·7	11·5
15	3·8	5·1	6·1	7·2	8·7	11·6
16	3·9	5·1	6·1	7·3	8·8	11·7
17	3·9	5·1	6·1	7·3	8·9	11·8
18	3·9	5·2	6·2	7·4	9	11·9
19	4	5·3	6·3	7·5	9·1	12·1
20	4·1	5·4	6·4	7·7	9·3	12·3

Reading example: A five-year-old with sNfL of 10·2 pg/ml has a value on the 95th percentile, i.e. only 5% of healthy children of the same age have values as high or higher.

Abbreviations: sNfL: serum neurofilament light chain.

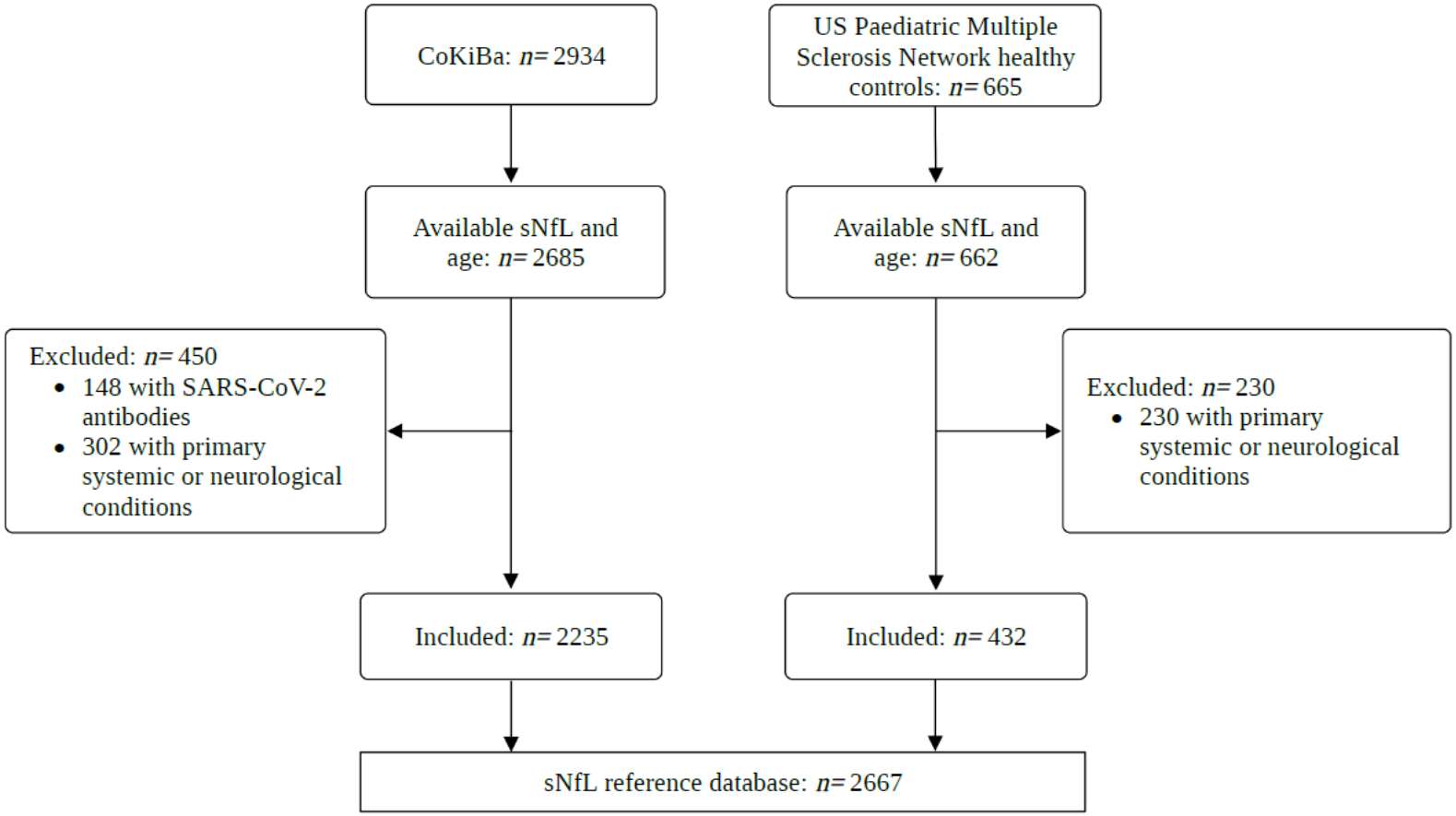
Supplementary Table 4. Estimated sNfL concentrations (pg/ml) at a given age and Z score.

	Z score (Z)							
Age	Z = -2	Z = -1	Z = 0	Z = 1	Z = 1·5	Z = 2	Z = 2·5	Z = 3
1	3·8	5·4	7·3	10·2	12·7	17	26	51·9
2	3·5	5	6·7	9·5	11·8	15·8	24·1	48·1
3	3·3	4·6	6·2	8·8	10·9	14·6	22·4	44·6
4	3	4·3	5·8	8·1	10·2	13·6	20·8	41·4
5	2·8	4	5·4	7·6	9·4	12·6	19·3	38·5
6	2·6	3·7	5	7	8·8	11·8	17·9	35·8
7	2·5	3·5	4·7	6·6	8·2	11	16·7	33·4
8	2·3	3·2	4·4	6·2	7·7	10·3	15·7	31·3
9	2·2	3·1	4·1	5·8	7·2	9·7	14·8	29·5
10	2·1	2·9	4	5·6	6·9	9·3	14·1	28·2
11	2	2·8	3·8	5·4	6·7	9	13·8	27·4
12	2	2·8	3·8	5·3	6·7	8·9	13·6	27·1
13	2	2·8	3·8	5·3	6·7	8·9	13·6	27·1
14	2	2·8	3·8	5·4	6·7	8·9	13·6	27·2
15	2	2·8	3·8	5·4	6·7	9	13·7	27·3
16	2	2·8	3·9	5·4	6·7	9	13·8	27·5
17	2	2·9	3·9	5·5	6·8	9·1	13·9	27·7
18	2·1	2·9	3·9	5·5	6·9	9·2	14·1	28·1
19	2·1	3	4	5·6	7	9·4	14·3	28·5
20	2·1	3	4·1	5·7	7·1	9·6	14·6	29·1

Reading example: Whereas a sNfL concentration of 6·7 pg/ml represents an average value for a 2-year-old child (mean in healthy controls is Z score 0), this concentration (6·7-6·9 pg/ml) observed in children between 11 and 18 years represents an elevated sNfL level (Z score of 1·5: sNfL concentration 1·5 standard deviations above the mean in healthy controls; equivalent to 87th percentile).

Abbreviations: sNfL: serum neurofilament light chain.

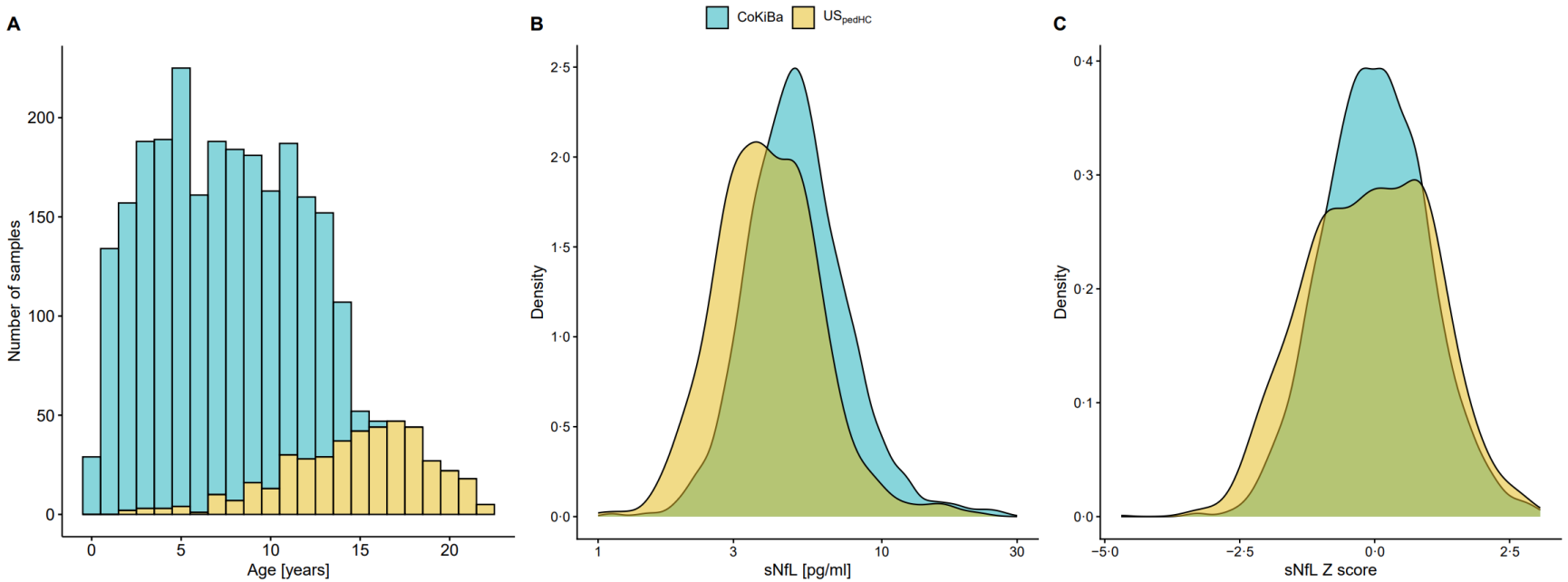
Supplementary Figure 1. Flowchart representing sample selection for sNfL reference database.



Legend: Sample selection process for inclusion in the sNfL reference database.

Abbreviations: CoKiBa: The Coronavirus antibodies in Kids from Bavaria study; sNfL: serum neurofilament light chain.

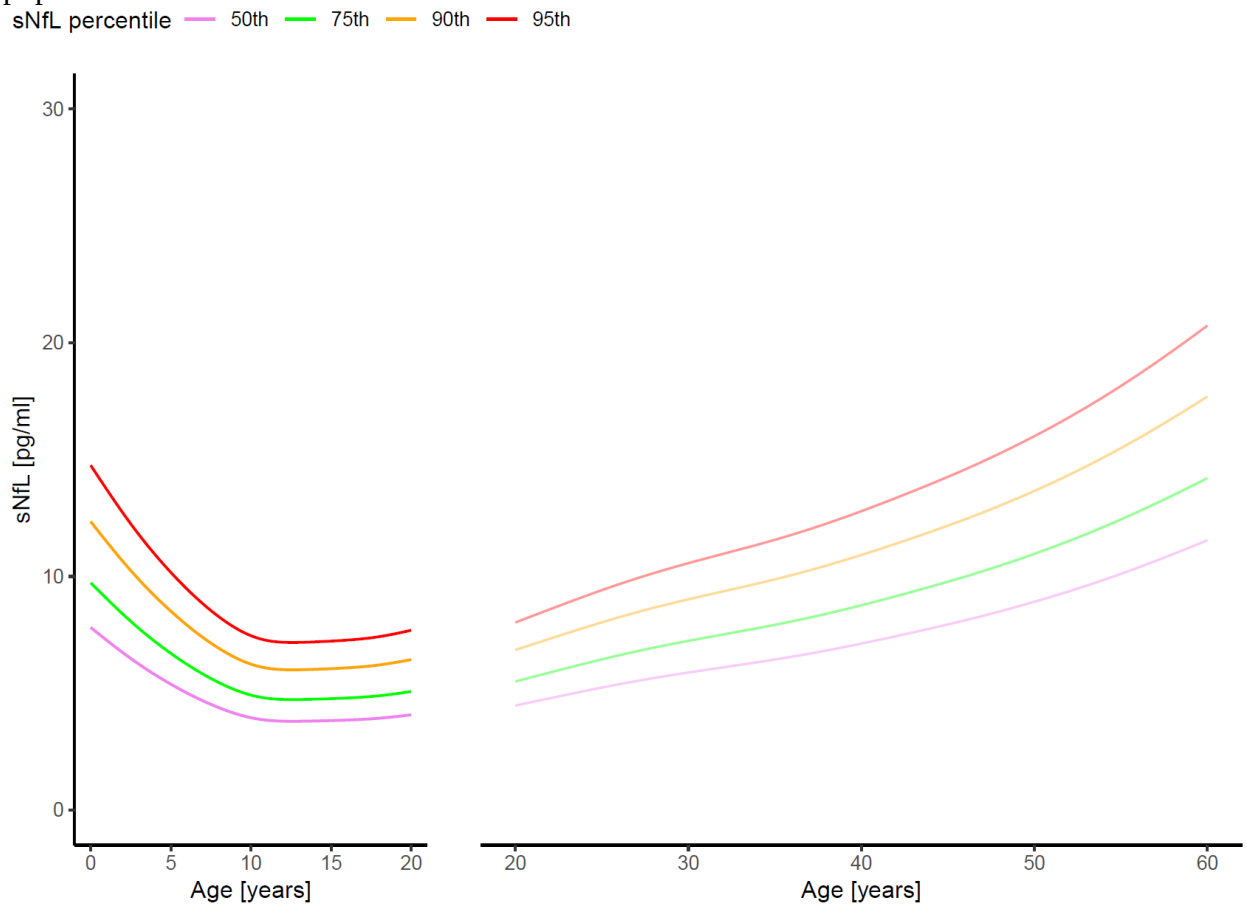
Supplementary Figure 2. Distribution of age (A), sNfL (B) and sNfL Z scores (C) in the included control samples from CoKiBa and US_{pedHC}.



Legend: Age at sampling overlapped between CoKiBa and US_{pedHC} mainly between 10 and 14 years of age (**A**). Differences in age explained differences in absolute sNfL levels between CoKiBa and US_{pedHC} (B), while sNfL Z scores completely overlapped between CoKiBa and US_{pedHC} (**C**; n= 2667 in **A**, **B** and **C**).

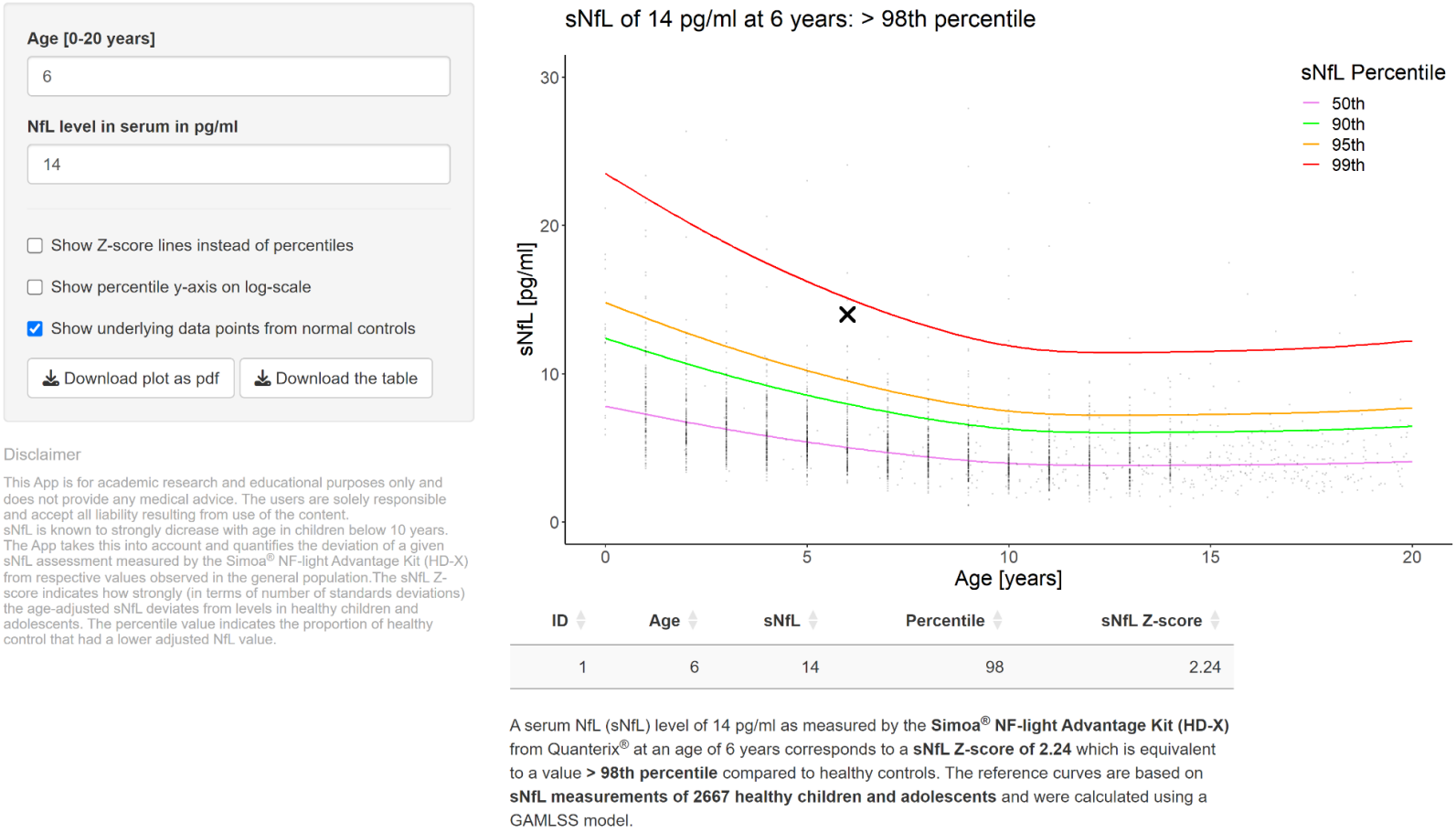
Abbreviations: CoKiBa: The Coronavirus antibodies in Kids from Bavaria study; US_{pedHC}: US Paediatric Multiple Sclerosis Network (healthy controls).

Supplementary Figure 3. sNfL percentiles reference curves in the paediatric and adult population.



Legend: Combined sNfL percentiles reference curves in paediatric and adult populations[1] show smooth continuity between the proposed cut-offs for sNfL percentiles in the two independent reference populations. A generalized additive model for location, scale, and shape (GAMLSS) was used to model the non-linear association of sNfL concentration (pg/mL) in controls and age (from birth to 20 years of age (left) and until the age of 65 (right, published previously[1]).
Abbreviations: sNfL: serum neurofilament light chain.

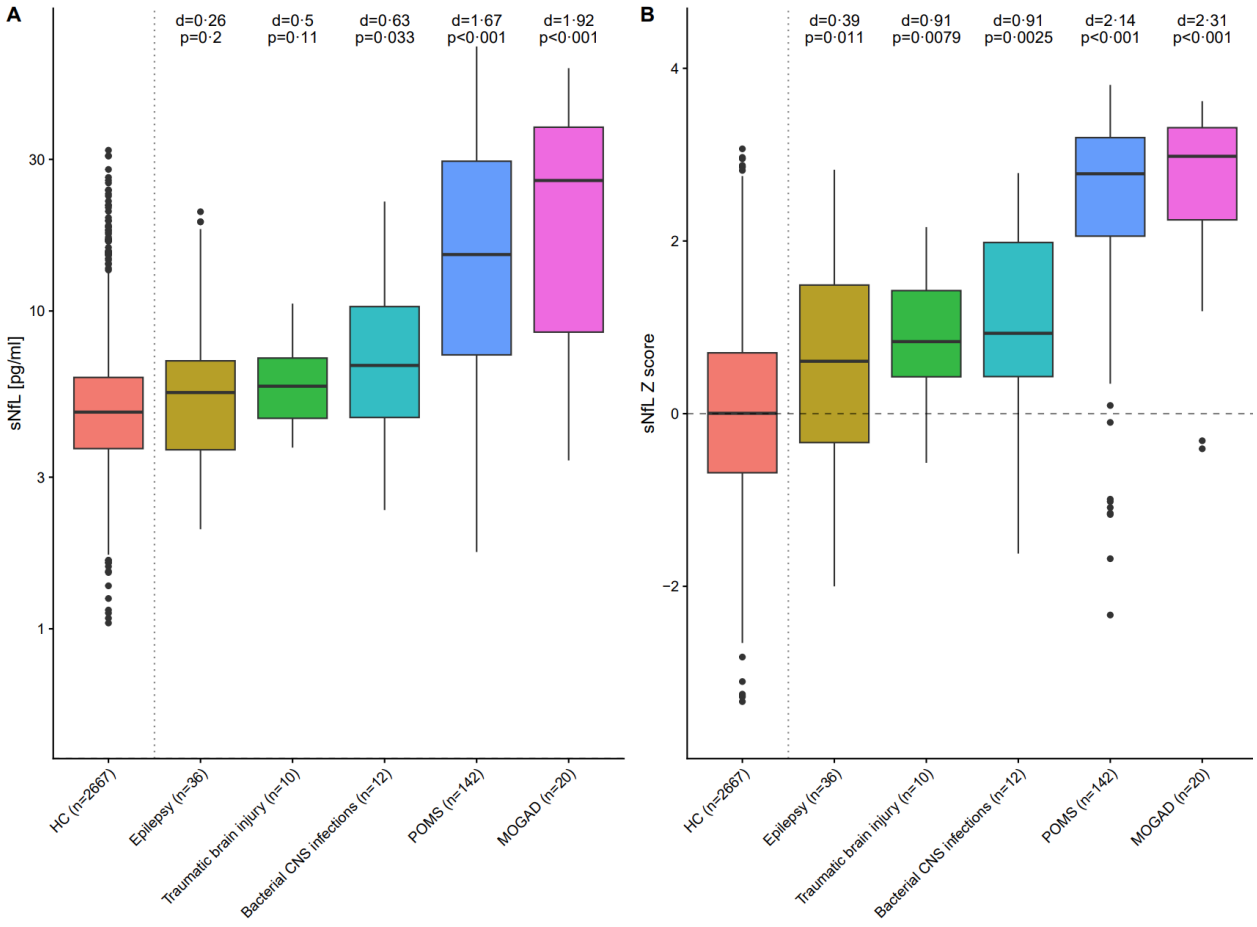
Supplementary Figure 4. Screenshot of the sNfL Shiny App for the calculation of age adjusted sNfL percentiles and Z scores in the paediatric population.



Legend: A web application for calculating age-corrected sNfL percentiles and Z scores for the age range 0-20 years and providing a graphical representation of the individual measurement is available at: https://shiny.dkfbasel.ch/baselnflreference-for-kids.

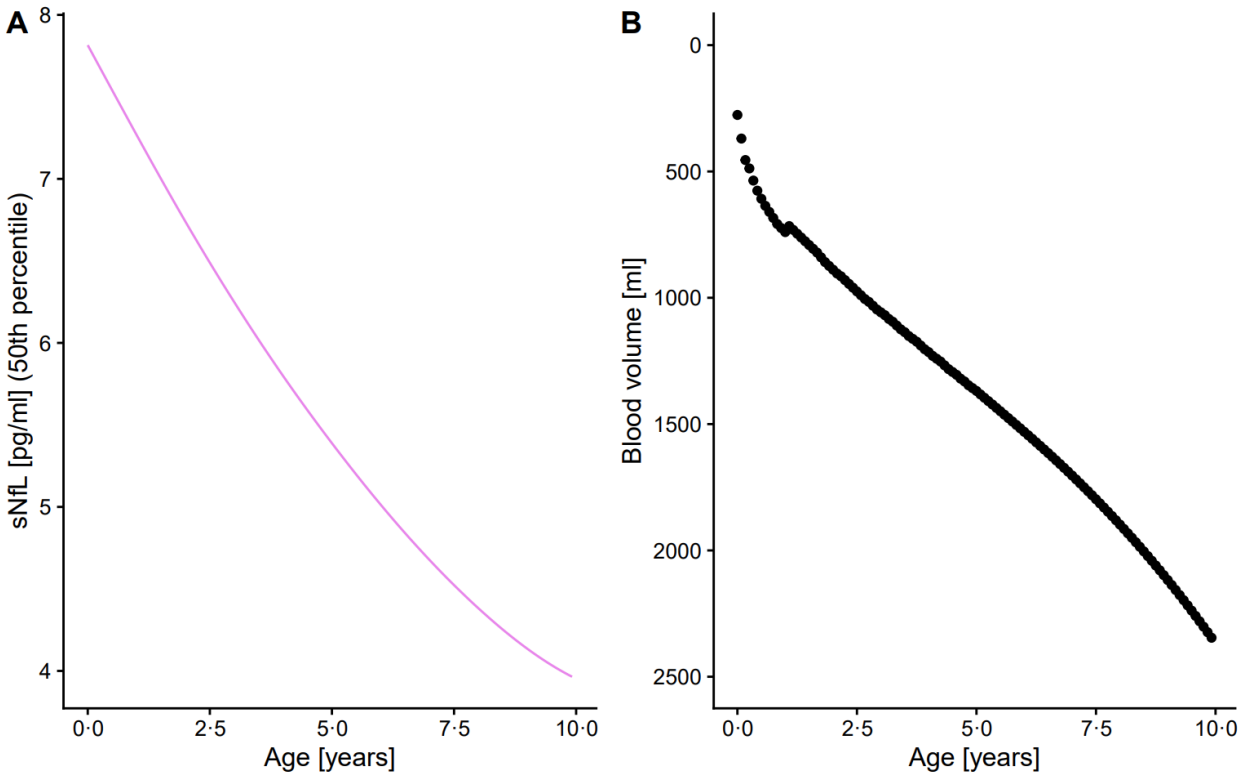
Abbreviation: sNfL: serum neurofilament light chain.

Supplementary Figure 5. Comparison of raw sNfL and sNfL Z scores between healthy children and subgroups of paediatric cases.



Legend: Application of sNfL Z scores (B) versus absolute sNfL concentrations (A) improved the detection of abnormally elevated sNfL concentrations in paediatric cases with central nervous system diseases compared to healthy controls. The effect size of the difference is expressed by Cohen's d and p-values refer to individual Wilcoxon rank sum test comparisons. The dashed horizontal line at Z=0 in 5B represents the expected mean value in the reference population. Abbreviation: CNS: Central nervous system; HC: healthy controls, MOGAD: Myelin oligodendrocyte glycoprotein antibody associated disease; POMS: Paediatric onset multiple sclerosis; sNfL: serum neurofilament light chain.

Supplementary Figure 6. Evolution of measured sNfL concentration in the reference population and the estimated average blood volume until the age of 10 years.



Legend: sNfL concentration (**A**) decreases with age and shows a strong alignment with the evolution of calculated average blood volume (**B**) in healthy children. Estimated blood volume in ml = weight in kg x age group factor. Average normal weight was extracted from the World Health Organization reference dataset of weight, height and body mass index[2] and correcting for established age factors[3,4]; 0-2 months: 85, 3-12 months: 80, 13-120 months: 75).

References

1. Benkert P, Meier S, Schaedelin S, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *Lancet Neurol* 2022; **21**(3): 246-57.

2. WHO. Child growth standards. 2006. https://www.who.int/tools/child-growth-standards/standards (accessed 8. December 2022).

3. Morgan and Mikhail's Clinical Anesthesiology. 5th Edition ed: McGraw-Hill; 2013.

4. Nadler SB, Hidalgo JH, Bloch T. Prediction of blood volume in normal human adults. *Surgery* 1962; **51**(2): 224-32.