Neurofilament light chain in blood as a diagnostic and predictive biomarker for multiple sclerosis: A systematic review and meta-analysis

Liangxia Ning, Bin Wang*

Department of Neurology, Yuncheng Central Hospital, The Eighth Shanxi Medical University, Yuncheng, China

* binwang0411@163.com

Abstract

Background

Neurofilament light chain (NfL) in cerebrospinal fluid (CSF) is a biomarker of multiple sclerosis (MS). However, CSF sampling is invasive and has limited the clinical application. With the development of highly sensitive single-molecule assay, the accurate quantification of the very low NfL levels in blood become feasible. As evidence being accumulated, we performed a meta-analysis to evaluate the diagnostic and predictive value of blood NfL in MS patients.

Methods

We performed literature search on PubMed, EMBASE, Web of Science and Cochrane Library from inception to May 31, 2022. The blood NfL differences between MS vs. controls, MS vs. clinically isolated syndrome (CIS), progressive MS (PMS) vs. relapsing-remitting MS (RRMS), and MS in relapse vs. MS in remission were estimated by standard mean difference (SMD) and corresponding 95% confidence interval (CI). Pooled hazard ratio (HR) and 95%CI were calculated to predict time to reach Expanded Disability Status Scale (EDSS) score ≥4.0 and to relapse.

Results

A total of 28 studies comprising 6545 MS patients and 2477 controls were eligible for meta-analysis of diagnosis value, and 5 studies with 4444 patients were synthesized in analysis of predictive value. Blood NfL levels were significantly higher in MS patients vs. controls (SMD = 0.64, 95%CI 0.44–0.85, P<0.001), vs. non-matched controls (SMD = 0.76, 95%CI 0.56–0.96, P<0.001) and vs. CIS patients (SMD = 0.30, 95%CI 0.18–0.42, P<0.001), in PMS vs. RRMS (SMD = 0.56, 95%CI 0.27–0.85, P<0.001), and in relapsed patients vs. remitted patients (SMD = 0.54, 95%CI 0.16–0.92, P = 0.005). Patients with high blood NfL levels had shorter time to reach EDSS score ≥4.0 (HR = 2.36, 95%CI 1.32–4.21, P = 0.004) but similar time to relapse (HR = 1.32, 95%CI 0.90–1.93, P = 0.155) compared to those with low NfL levels.
Conclusion
As far as we know, this is the first meta-analysis evaluating the diagnosis and predictive value of blood NfL in MS. The present study indicates blood NfL may be a useful biomarker in diagnosing MS, distinguishing MS subtypes and predicting disease worsening in the future.

Introduction
Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease affecting over two million people around the world [1]. The clinical courses and manifestations of MS are highly variable encompassing mild or benign forms that may not need treatment and progressive stage that develops irreversible clinical and cognitive deficits with limited response to standard treatment [2]. Highly effective treatments have been developed and become widely available in recent years [3]. Reliable markers for disease detection, staging and prognosis prediction are warranted for the decision-making of best therapy to improve prognosis.

Neurofilament light chain (NfL) in cerebrospinal fluid (CSF) is an emerging biomarker for MS. NfL is a subunit of neurofilaments constituting neuronal and axonal cytoskeleton in central nervous system (CNS) as well as part of the peripheral nervous system, which is released to CSF and blood when neuronal and axonal damage occur [4]. It directly reflects the neuroaxonal injury in many inflammatory, neurodegenerative, traumatic and ischemic diseases of CNS [5, 6]. Previous studies have found more abundant CSF NfL in MS patients than in sex- and age-matched controls and suggested that CSF NfL may help distinguish MS subtypes [7]. It has also reported as a biomarker for frontotemporal dementia (FTD), Alzheimer’s disease (AD), amyotrophic lateral sclerosis (ALS), and atypical parkinsonian disorder (APD) [8]. However, CSF acquisition is a relatively invasive procedure that limits the clinical application, especially longitudinal and repetitive sampling for disease monitoring, of CSF NfL.

In patients with neurological disorders, NfL is released in a large amount to CSF when neural cells are damaged and eventually into the bloodstream [9]. Previous studies mostly focused on CSF levels since the conventional detection methods, such as enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence (ECL)-based assay, had low sensitivity in quantifying the low blood levels [10, 11]. Recently, the development of highly sensitive single-molecule assay (SIMOA) has allowed the accurate quantification of low blood concentrations of NfL and now been widely used [12]. The blood levels of NfL by SIMOA are nearly 40-fold lower than CSF levels but highly correlated with CSF levels, magnetic resonance imaging (MRI) lesions and clinical symptoms [13, 14]. Serum NfL is now widely accepted to monitor disease activity and response to disease-modifying therapy (DMT) [14, 15], and becomes more and more refined as a biomarker in MS [16].

With the increasing evidence of blood NfL measurements in MS patients, we performed the present systematic review and meta-analysis to evaluate the value of blood NfL in diagnosing MS, distinguishing MS subtypes and severity, and predicting disease worsening.

Methods
Literature search strategy
The present systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [17]. Candidate articles investigating the diagnostic or predictive value of blood NfL levels in MS were systematically searched in electronic literature databases including PubMed, EMBASE, Web of
Science and Cochrane Library from inception to May 31, 2022. The following keywords were used for literature search: (“neurofilament light chain” OR “neurofilament-light chain” OR “neurofilament” OR NfL OR sNfL OR pNfL) AND “multiple sclerosis”. Additional relevant articles were obtained by manually searching the reference lists of eligible studies.

Inclusion and exclusion criteria
All eligible studies should meet the following criteria: (1) measured serum or plasma NfL concentrations in adult MS patients; (2) investigated the diagnostic or predictive value of blood NfL levels; (3) provided sufficient data for meta-analysis. MS was diagnosed according to Poser [18] or McDonald criteria [19–21]. NfL was measured by SIMOA, electrochemiluminescence method (ECL) or enzyme linked immunosorbent assay (ELISA). In details, for diagnostic value, the blood NfL levels were compared between MS vs. controls which included healthy control (HC) and non-inflammatory neurological disease control (NINDC), MS vs. clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS) vs. progressive MS (PMS), and MS in relapse vs. MS in remission. The mean value and standard deviation (SD) of blood NfL levels, or the other statistics that can be converted to mean and SD, in both groups should be provided. For predictive value, hazard ratio (HR) estimate and corresponding 95%CI for high blood NfL levels predicting the time to Expanded Disability Status Scale (EDSS) score ≥4.0 or relapse should be provided. Cases series, meeting abstracts, reviews, meta-analyses and studies with pediatrics patients were excluded. For articles with overlapped samples, only the one with largest sample size was included.

Quality assessment
For studies comparing the blood NfL levels in two group, the quality was assessed by using Newcastle-Ottawa scale (NOS) for case-control studies, which comprised selection, comparability and exposure domains. For studies investigating the predictive value, the quality was assessed by using NOS for cohort studies, which contained selection, comparability and outcome domains. The total stars assigned to all items were 9. Studies with 5 or 6 stars were considered as moderate-quality studies and those with 7 or more stars were of high quality.

Data extraction
We extracted the following information from all eligible studies: first author, year of publication, country, diagnostic criteria of MS, sample source (serum or plasma), method of blood NfL measurement, baseline characteristics (sample size, age, gender, EDSS score, disease duration, DMT use). For diagnostic value, the mean value and SD of NfL levels in both groups were extracted. If the studies only provided median value with interquartile (IQR) or range of NfL levels, we converted these values to mean and SD statistics by using methods introduced by Wan et al [22] and Luo et al [23]. Similarly, the median with IQR or range of baseline age, EDSS score and disease duration were converted to mean with SD when we performed meta-regression analysis. For predictive value, the cutoffs of high NfL levels and the HR estimates for EDSS score ≥4.0 or relapse were extracted.

The literature search and selection, quality assessment and data extraction were performed by two independent researchers. Discrepancies were resolved by further discussion of these two researchers.

Statistical analysis
Between-study heterogeneity was evaluated by I^2 statistic and Q test. I^2 <25%, between 25% and 50%, and >50% indicated low, medium and high levels of heterogeneity, respectively. For
meta-analysis with $I^2 < 50\%$ and P value of Q test $> 0.10$, the fixed-effect model was used; otherwise, the random-effect model was applied. The effect sizes were estimated by standard mean difference (SMD) and 95%CI with Cohen’s d [24] for diagnostic value and calculated by HR and 95%CI for predictive value. We considered the SMD of $\leq 0.2$, between 0.2 and 0.8, and $\geq 0.8$ as small, moderate and large effect size, respectively [24]. For MS vs. Control, subgroup analyses regarding control type (HC, NINDC), sample source (serum, plasma), NfL detection method (SIMOA, ECL or ELISA) and DMT use (no, mixed or missing) were performed. Specifically, only if the authors declared enrollment of age-matched controls, the study was classified as age-matched; otherwise it was not age-matched, even though there was no statistical difference by baseline age comparison. Since studies have shown blood NfL was highly correlated with age, we analyzed age-matched studies and non-age-matched studies separately. Meta-regression analyses for mean age, percent of female, mean disease duration, mean EDSS score and sample size were also performed to identify potential source of heterogeneity for meta-analyses including 10 or more eligible studies. Sensitivity analysis was also performed with Leave-One-Out method, i.e. omitting one study and recalculating the pooled effect size each time. Publication bias was assessed by viewing the symmetry of funnel plot and by Egger’s test. All analyses were performed by using STATA 16 (StataCorp, TX, USA).

Results
Baseline characteristics of eligible studies
A total of 31 studies fulfilling the inclusion and exclusion criteria were finally included in quantitative analysis (Fig 1) [10, 11, 13, 14, 25–51]. Among them, 28 studies comprising 6545 MS patients and 2477 controls were eligible for meta-analysis of diagnosis value (Table 1), and 5 studies with 4444 MS patients were synthesized in meta-analysis of predictive value (S1 Table).

For diagnosis value analysis, 4 studies detected plasma NfL (pNfL) concentrations [26, 34, 36, 43] and the others measured serum NfL (sNfL) levels. Two studies applied ECL method [10, 11], one used ELISA [40], and the others adopted the highly sensitive SIMOA method for NfL measurements in blood. Ten studies enrolled age-matched controls with MS patients [13, 28, 32, 34–37, 40, 44, 45] and 7 recruited sex-matched controls [28, 32, 34–36, 40, 43]. The other 18 studies that did not declare whether controls were age-matched were then considered as not age-matched studies, even though there was no statistical difference of age at baseline comparison in some studies. As to DMT use, 7 recruited treatment-naïve patients [10, 28, 32, 35, 36, 42, 46], while the other studies reported a proportion of patients treated with DMT or missing information of DMT use. Quality assessment using NOS for case-control studies identified 19 moderate-quality studies that had 5 or 6 stars and 9 high-quality studies with 7–9 stars (S2 Table). The characteristics of the included studies were summarized in Table 1.

Among studies exploring the predictive value of blood NfL concentrations, 3 measured sNfL and 2 detected pNfL [32, 34, 41, 47, 48]. The cutoffs for high NfL levels were 80th percentile of age-corrected reference values in two studies but differed in the other studies. Two studies investigated the association of high blood NfL level with time to relapse and 3 with time to reaching ESS score $\geq 4.0$. All studies were awarded with 7 stars according to NOS for cohort studies (S3 Table). The characteristics of these studies were summarized in S1 Table.

MS vs. control
Twenty-three studies compared blood NfL between MS patients and controls. Age-matched and non-age-matched studies were analyzed in separate. In analysis of age-matched studies, 3683 MS patients and 1304 age-matched healthy controls were included (Table 2). There was
obvious between-study heterogeneity ($I^2 = 65.0\%$) and the random-effect model was used. The blood NfL levels in MS were significantly higher than those in age-matched controls with a moderate effect size (SMD = 0.64, 95%CI 0.44–0.85, P < 0.001, Fig 2). We observed large effect size in studies recruiting treatment-naïve MS patients (SMD = 0.91, 95%CI 0.39–1.43) and moderate effect size in studies with mixed use or missing data of DMT (SMD = 0.56, 95%CI 0.32–0.80; between-subgroup comparison P = 0.236). Blood NfL difference between MS and non-matched controls was analyzed in 14 studies comprising 1414 MS patients and 1375 controls (Table 2). MS patients had significantly higher NfL levels than non-matched controls...
Table 1. Characteristics of studies included in meta-analysis for diagnosis value of blood NfL concentrations.

| Author               | Year | Country          | Diagnosis   | Patient group | Control group | Comparison                  |
|----------------------|------|------------------|-------------|---------------|---------------|----------------------------|
|                      |      |                  | N | Age, y | % Female | Disease duration, y | EDSS score | DMT use (%) | Condition | N | Age, y | % Female |
| Disanto 2015         | Various | MS | 100 | 31.2 | 67 | NA | 2.18 | NA | | HC | 92 | 36.4 | 63 | MS vs. HC, MS vs. CIS |
| Kuhle 2016           | Switzerland | MS | 31 | 31.6 | 64.5 | 1.32 | 2 | 0 | | HC | 18 | 30.8 | 55.6 | MS vs. HC, Relapse vs. Remission |
| Disanto 2017         | Switzerland | MS, SMSC cohort | 246 | 42.4 | 65.9 | 8.21 | 2.82 | 50.8 | | HC | 254 | 44.4 | 68.1 | MS vs. HC |
|                      |       | MS, LUGANO cohort | 142 | 38.5 | 64.9 | NA | NA | NA | | |
| Piehl 2017           | Sweden | MS | 39 | 39.6 | 61.5 | NA | 2.4 | NA | | NINDC | 27 | 35.2 | 55.6 | MS vs. NINDC |
| Novakova 2017        | Sweden | PMS | 82 | 48 | 54.9 | NA | 5.4 | NA | | HC | 42 | 28 | 40.5 | PMS vs. RRMS, Relapse vs. Remission |
| Barro 2018           | Switzerland | MS | 257 | 44.5 | 69.6 | 11.05 | 3 | 64.6 | | HC | 258 | 44.3 | 68.6 | MS vs. HC, PMS vs. RRMS |
| Hakansson 2018       | Sweden | MS | 41 | 30.29 | 78 | 11.8 | 1.68 | 0 | | HC | 22 | 33.1 | 77.3 | MS vs. HC |
| Abdelhak 2018        | Germany | MS in relapse | 18 | 31.8 | NA | 0.62 | 1.82 | 11.1 | | NA | NA | NA | NA | Relapse vs. Remission |
|                      |       | MS in remission | 24 | 37.4 | NA | 4.19 | 2.88 | 16.7 | | |
| Hogel 2018           | Finland | MS | 79 | 50.2 | 70.9 | 15.48 | 3.7 | 64.6 | | HC | 13 | 47 | 69.2 | MS vs. HC, PMS vs. RRMS |
| Ferraro 2019         | Italy | PMS | 70 | 58.9 | 30 | 20 | 6.32 | 0 | | HC | 10 | 56.9 | 40 | PMS vs. RRMS |
|                      |       | RRMS | 21 | 42.9 | 28.6 | 9.56 | 1.32 | 0 | | |
| Watanabe 2019        | Japan | MS | 49 | 39 | 73.5 | 8.16 | 4.03 | 55.1 | | HC | 49 | 46.2 | 85.7 | MS vs. HC, PMS vs. RRMS |
| Thebault 2019        | Canada | MS | 23 | 27 | 51.9 | 7.42 | 4.82 | 100 | | NINDC | 33 | 37.5 | 72.7 | MS vs. NINDC |
| Jakimovski 2019      | US | MS | 127 | 48.4 | 70.1 | 16.3 | 3.2 | 78.7 | | HC | 52 | 43.8 | 86.8 | MS vs. HC, MS vs. CIS, PMS vs. RRMS |
| Sejbaek 2019         | Denmark | MS | 52 | 34.1 | 86.5 | NA | 1.77 | 0 | | HC | 23 | 38.2 | 87 | MS vs. HC |
| Baldassari 2019      | US | MS | 22 | 46.4 | 68.2 | 12.4 | 5.5 | 0 | | HC | 10 | 47.1 | 60 | MS vs. HC |
| Manouchehrinia 2020  | Sweden | MS | 3092 | 38.4 | 70.3 | 4.23 | NA | NA | | HC | 1026 | 39.8 | 73.2 | MS vs. HC |
| Bittner 2020         | Germany | MS | 445 | 32.4 | 67.2 | 2 | 1.5 | 0 | | NA | NA | NA | NA | MS vs. CIS |
|                      |       | CIS | 369 | 33.4 | 69.4 | 0.14 | 1.5 | 0 | | |
| Thebault 2020        | Canada | MS | 67 | 38 | 70.1 | NA | 1.5 | 3.0 | | NINDC | 37 | 38 | 81.1 | MS vs. NINDC, Relapse vs. Remission |
| Ayrignac 2020        | France | PMS | 18 | 50.8 | 77.8 | 3.5 | 3.86 | 0 | | NA | NA | NA | NA | PMS vs. RRMS, Relapse vs. Remission |
|                      |       | RRMS | 111 | 39.9 | 74.8 | 7.17 | 1.35 | 48.7 | | |
| Huss 2020            | Germany | PMS | 39 | 53 | 53.8 | NA | 5.65 | 7.7 | | NA | NA | NA | NA | PMS vs. RRMS, Relapse vs. Remission |
|                      |       | RRMS | 47 | 36.1 | 61.7 | NA | 2.53 | 14.9 | | |
| Olsson 2020          | Denmark | MS, cohort 1 | 49 | 36.1 | 65.3 | 2.94 | 1.68 | 0 | | HC | 58 | 38.1 | 48.3 | MS vs. HC |
|                      |       | MS, cohort 2 | 68 | 35.3 | 76.5 | 1.18 | 2 | 0 | | HC | 50 | 33 | 68 | MS vs. HC |
| Bridel 2020          | Netherlands | MS | 89 | 45.1 | 71.9 | NA | NA | 23.6 | | HC | 88 | 44.5 | 44.3 | MS vs. HC, PMS vs. RRMS |
| Saraste 2020         | Finland | MS | 79 | 48.1 | 75.9 | 14.27 | 2.91 | 68.4 | | HC | 10 | 48.3 | 70 | MS vs. HC, PMS vs. RRMS |

(Continued)
Between-subgroup comparison showed a significantly larger effect size of treatment-naïve subgroup than treatment subgroup (SMD = 1.20 vs. 0.65, P = 0.007).

We further compared the blood NfL levels in patients at different MS stages (RRMS and PMS) with those in HC. A total of 1239 RRMS vs. 858 HC from 16 studies and 362 PMS vs. 522 HC from 8 studies were included. RRMS patients had significantly higher levels of blood NfL (SMD = 0.58, 95%CI 0.36–0.80, P < 0.0001, Fig 3) compared with HC, which showed a moderate effect size. Moreover, a large effect size of the blood NfL difference between PMS patients and HC was observed (SMD = 1.01, 95%CI 0.65–1.36, P < 0.001, Fig 3).

**MS vs. CIS**

Three studies involving 672 MS and 487 CIS compared blood NfL levels between both groups. Among them, Disanto et al defined CIS according to the criteria proposed by Miller et al [52], and the other two according to 2010 revised McDonald criteria [20]. There was no between-study heterogeneity. Meta-analysis using the fixed-effect model was used showed significantly higher blood NfL levels in MS than in CIS (SMD = 0.30, 95%CI 0.18–0.42, P < 0.001, S2 Fig).

**PMS vs. RRMS**

A total of 842 PMS and 419 RRMS were included, and the random-effect model was used due to substantial heterogeneity (I² = 79.8%). We found that PMS patients had significantly higher levels of blood NfL than RRMS patients (SMD = 0.56, 95%CI 0.27–0.85, P < 0.001, Fig 4).

**MS in relapse vs. MS in remission**

Six studies compared blood NfL levels of MS in relapse vs. MS in remission (181 cases vs. 600 cases) and were included in synthesis analysis. Random-effect model analysis demonstrated higher NfL levels in relapsed patients than in remitted patients (SMD = 0.54, 95%CI 0.16–0.92, P = 0.005, Fig 5).

**Predictive value of high blood NfL level**

We investigated whether high blood NfL level at baseline could predict the hazard of reaching EDSS score ≥4.0 and relapse. Patients with higher blood NfL levels were earlier to reach EDSS score ≥4.0 compared with those with lower levels (HR = 2.36, 95%CI 1.32–4.21, P = 0.004, S3 Table 1. (Continued)).
Fig). However, no difference of time to relapse was observed between both groups (HR = 1.32, 95%CI 0.90–1.93, P = 0.155, S4 Fig).

Meta-regression analysis, sensitivity analysis and publication bias

We explored the potential source of heterogeneity by meta-regression analysis in “MS vs. Control” comparison (Table 3). Mean age was significantly correlated with SMD estimates in not-age-matched subgroup (P = 0.021, S5 Fig), indicating that mean age could partly explain the source of heterogeneity. However, the correlation was not found in age-matched subgroup (P = 0.488, S6 Fig). The association of SMD with percent of female, mean EDSS score, mean disease duration and sample size were not evident according to meta-regression analysis.

Sensitivity analysis using Leave-One-Out method demonstrated that omitting one single study did not significantly influence the pooled effect size of the rest of studies. There was no
obvious asymmetry in funnel plots of meta-analyses, and Egger’s test indicated no evident publication bias (S4 Table).

Discussion

As far as we know, this is the first meta-analysis investigating the diagnostic and predictive value of blood NfL concentrations in MS patients. In line with previous meta-analyses finding elevated CSF NfL concentration in MS patients [7, 53–55], the present study demonstrates NfL levels in blood, which are strongly correlated with those in CSF, are also significantly higher in MS patients compared with controls. Our study indicates that blood NfL may serve as a biomarker for MS diagnosis.

However, some influential factors, such as age, BMI and quantification process, should be noted upon the clinical utility of blood NfL [56]. Blood NfL levels are highly age-dependent. Among healthy controls, young individuals have low and relatively stable sNfL concentrations while people older than 60 years have annually increased sNfL levels associated with age-
related neurodegeneration [14, 57]. Besides, sNfL decreases with BMI in age stratified sub-
groups [58, 59]. Therefore, age and BMI are confounding factors for sNfL as a biomarker,
which may influence the clinical implementation. The comparison between MS and
unmatched controls may introduce some bias to the meta-analysis. This is supported by our
meta-regression analysis revealing a negative correlation between mean age and blood NfL dif-
fERENCE in not-age-matched studies (P = 0.021). On the contrary, among studies recruiting
age-matched controls, mean age was not associated with blood NfL difference (P = 0.488).
These results indicate that age-specific reference of blood NfL should be established. Recently,

### Table 1. Study summary of blood NfL concentrations between MS vs. HC and RRMS vs. HC

| Study                  | MS N | MS Mean | MS SD | Control N | Control Mean | Control SD | SMD with 95% CI | Weight (%) |
|------------------------|------|---------|-------|-----------|--------------|------------|-----------------|------------|
| **PMS vs. HC**         |      |         |       |           |              |            |                 |            |
| Novakova (2017)        | 82   | 40.25   | 62.69 | 42        | 14.84        | 13.77      | 0.49 [ 0.11, 0.87] | 4.62       |
| Barro (2018)           | 68   | 43.24   | 18.03 | 258       | 24.48        | 9.62       | 1.58 [ 1.29, 1.88] | 4.93       |
| Hogel (2018)           | 33   | 35.26   | 20.23 | 13        | 24.89        | 7.37       | 0.59 [ -0.07, 1.24] | 3.50       |
| Ferraro (2019)         | 70   | 12.94   | 4.54  | 10        | 9.57         | 3.96       | 0.75 [ 0.08, 1.43] | 3.42       |
| Watanabe (2019)        | 11   | 32.17   | 14.67 | 49        | 18.9         | 8.71       | 1.33 [ 0.63, 2.02] | 3.34       |
| Jakimovski (2019)      | 42   | 28.82   | 14.89 | 52        | 15.38        | 11.59      | 1.02 [ 0.59, 1.45] | 4.40       |
| Bridel (2020)          | 33   | 13.65   | 6.98  | 88        | 7.1          | 2.9        | 1.49 [ 1.05, 1.93] | 4.36       |
| Saraste (2020)         | 23   | 32.55   | 17.38 | 10        | 24.13        | 6.02       | 0.56 [ -0.19, 1.32] | 3.12       |
| **RRMS vs. HC**        |      |         |       |           |              |            |                 |            |
| Kuhle (2016)           | 31   | 12.88   | 18.26 | 18        | 2.27         | 4.26       | 0.72 [ 0.12, 1.31] | 3.72       |
| Novakova (2017)        | 204  | 66.84   | 269.65| 42        | 14.84        | 13.77      | 0.21 [ -0.12, 0.54] | 4.78       |
| Barro (2018)           | 178  | 31.1    | 15.7  | 258       | 24.48        | 9.62       | 0.53 [ 0.34, 0.73] | 5.23       |
| Hogel (2018)           | 46   | 20.62   | 10.6  | 13        | 24.89        | 7.37       | -0.43 [ -1.05, 0.19] | 3.63       |
| Ferraro (2019)         | 21   | 9.74    | 2.31  | 10        | 9.57         | 3.96       | 0.06 [ -0.70, 0.81] | 3.12       |
| Watanabe (2019)        | 38   | 26.8    | 17.57 | 49        | 18.9         | 8.71       | 0.59 [ 0.16, 1.03] | 4.39       |
| Jakimovski (2019)      | 85   | 19.13   | 10.56 | 52        | 15.38        | 11.59      | 0.34 [ -0.01, 0.69] | 4.73       |
| Sejback (2019)         | 52   | 16.4    | 14.44 | 23        | 7.3          | 3          | 0.75 [ 0.24, 1.25] | 4.10       |
| Olsson (2020) cohort 1 | 49   | 9.33    | 6.49  | 58        | 5.08         | 2.66       | 0.88 [ 0.49, 1.28] | 4.53       |
| Olsson (2020) cohort 2 | 68   | 11.82   | 4.92  | 50        | 6            | 2.14       | 1.46 [ 1.05, 1.87] | 4.49       |
| Bridel (2020)          | 56   | 13.5    | 9.41  | 88        | 7.1          | 2.9        | 1.02 [ 0.66, 1.37] | 4.70       |
| Saraste (2020)         | 56   | 19.65   | 9.13  | 10        | 24.13        | 6.02       | -0.51 [ -1.19, 0.17] | 3.40       |
| Szilasova (2021)       | 159  | 8.58    | 4.34  | 66        | 6.49         | 2.52       | 0.54 [ 0.24, 0.83] | 4.93       |
| Liu (2021)             | 98   | 18.15   | 14.48 | 84        | 4.78         | 2.07       | 1.25 [ 0.93, 1.57] | 4.84       |
| Cruz-Gonzalez (2021)   | 35   | 44.48   | 27.7  | 23        | 33.28        | 16.47      | 0.47 [ -0.06, 0.00] | 3.98       |
| Niranne (2021)         | 63   | 15.77   | 7.3   | 14        | 11.3         | 5.58       | 0.64 [ 0.05, 1.22] | 3.76       |

**Random-effects DerSimonian-Laird model**

Fig 3. Forest plot of blood NfL concentrations between PMS vs. HC and RRMS vs. HC. PMS: progressive MS; RRMS: relapsing-
remitting MS.

https://doi.org/10.1371/journal.pone.0274565.g003

PLOS ONE | https://doi.org/10.1371/journal.pone.0274565 | September 14, 2022
### Fig 4. Forest plot of blood NfL levels between PMS vs. RRMS.

![Forest plot image](https://doi.org/10.1371/journal.pone.0274565.g004)

| Study        | PMS N | PMS Mean | PMS SD | RRMS N | RRMS Mean | RRMS SD | SMD with 95% CI | Weight (%) |
|--------------|-------|----------|--------|--------|-----------|---------|----------------|------------|
| Novakova (2017) | 82    | 40.25    | 62.69  | 204    | 66.84     | 269.65  | -0.12 [-0.37, 0.14] | 11.87      |
| Barro (2018)   | 68    | 43.24    | 18.03  | 178    | 31.1      | 15.7    | 0.74 [0.45, 1.03]  | 11.60      |
| Hogel (2018)   | 33    | 35.26    | 20.23  | 46     | 20.62     | 10.6    | 0.95 [0.48, 1.42]  | 9.74       |
| Ferraro (2019) | 70    | 12.94    | 4.54   | 21     | 9.74      | 2.31    | 0.77 [0.27, 1.27]  | 9.43       |
| Watanabe (2019)| 11    | 32.17    | 14.67  | 38     | 26.8      | 17.57   | 0.32 [-0.36, 0.99]  | 7.69       |
| Jakimovski (2019)| 42   | 28.82    | 14.89  | 85     | 19.13     | 10.56   | 0.80 [0.41, 1.18]  | 10.67      |
| Ayriignac (2020)| 18   | 13.63    | 6.66   | 111    | 9.33      | 4.52    | 0.88 [0.37, 1.39]  | 9.34       |
| Huss (2020)    | 39    | 20.79    | 11.16  | 47     | 17.04     | 13.38   | 0.30 [-0.13, 0.73]  | 10.20      |
| Bridel (2020)  | 33    | 13.65    | 6.98   | 56     | 13.5      | 9.41    | 0.02 [-0.41, 0.45]  | 10.17      |
| Saraste (2020) | 23    | 32.55    | 17.38  | 56     | 19.65     | 9.13    | 1.07 [0.55, 1.58]  | 9.30       |

**Overall**

Heterogeneity: \( \tau^2 = 0.17 \), \( I^2 = 79.79\% \), \( H^2 = 4.95 \)

Test of \( \theta_i = \theta \); \( Q(9) = 44.52, p = 0.00 \)

Test of \( \theta = 0 \); \( z = 3.78, p = 0.00 \)

---

### Fig 5. Forest plot of blood NfL levels between MS in relapse vs. MS in remission.

![Forest plot image](https://doi.org/10.1371/journal.pone.0274565.g005)

| Study        | Relapse N | Relapse Mean | Relapse SD | Remission N | Remission Mean | Remission SD | SMD with 95% CI | Weight (%) |
|--------------|------------|--------------|------------|-------------|----------------|--------------|----------------|------------|
| Kuhle (2016) | 26         | 12.72        | 19.14      | 5           | 13.32          | 26.95        | -0.03 [-0.99, 0.93] | 9.87       |
| Novakova (2017)| 86       | 23.5         | 19.53      | 346         | 18.04          | 10.42        | 0.43 [0.19, 0.67]  | 23.81      |
| Abdelhak (2018)| 18        | 16.8         | 7.24       | 24          | 6.55           | 15.29        | 0.82 [0.18, 1.45]  | 15.16      |
| Thebault (2020)| 18        | 13.24        | 6.84       | 49          | 9.36           | 4.62         | 0.73 [0.18, 1.29]  | 16.87      |
| Ayriignac (2020)| 18        | 8.95         | 5.09       | 93          | 9.43           | 4.43         | -0.10 [-0.60, 0.41] | 17.96      |
| Liu (2021)    | 15         | 52.92        | 66.97      | 83          | 15.84          | 13.04        | 1.31 [0.73, 1.89]  | 16.32      |

**Overall**

Heterogeneity: \( \tau^2 = 0.14 \), \( I^2 = 68.95\% \), \( H^2 = 3.22 \)

Test of \( \theta_i = \theta \); \( Q(5) = 16.10, p = 0.01 \)

Test of \( \theta = 0 \); \( z = 2.80, p = 0.01 \)
several studies have tried to construct an age- and/or BMI-adjusted model for sNfL [16, 51]. Using multiple large datasets, Benkert et al established an age- and BMI-corrected reference database of sNfL values, and further showed the merit of sNfL percentiles and Z scores in predicting disease course and response to DMT [16]. Thus, age-corrected sNfL value or a composite index may be more reliable and can be used in future researches.

Besides of age, DMT use is another influential factor of blood NfL. DMT-treated patients had significantly lower sNfL levels in untreated patients, and the treatment effect was independent of all the other baseline variables as suggested by multivariate analysis [14]. In our meta-analysis, several studies only recruited patients who had not previously been treated with DMT. Subgroup analyses, in both age-matched and non-matched studies, demonstrated a larger SMD effect size in treatment-naïve subgroup than treatment subgroup, suggesting a potential role of DMT in reducing blood NfL. Follow-up of DMT-treated patients showed significantly reduced sNfL levels than baseline, which were not observed in untreated patients [30]. These observations also suggest that longitudinal sampling of blood NfL may help monitor DMT treatment effect in MS patients. However, the impact of DMT on blood NfL may vary among disease subtypes. Teriflunomide reduced sNfL in relapsing MS patients [60] and dimethyl fumarate decreased blood NfL in RRMS patients [36]. Whereas, no significant changes were observed in PMS patients treated with ibudilast [61] and SPMS patients with simvastatin treatment [62].

NfL is not a biomarker specific to MS. It reflects neuro-axonal damage and can be detected in elevated levels in the other inflammatory neurologic disorders. Despite higher blood NfL levels in MS than in NINDCs, no significant difference is observed between MS and inflammatory neurological disease controls (INDCs) [25, 30]. This phenomenon is also found in CSF measurements [8]. Both CSF and blood NfL cannot replace conventional MRI for differential diagnosis between MS and the other inflammatory neurologic disorders.

Apart from disease diagnosis, blood NfL may help differentiate MS from CIS and distinguish MS subtypes. CSF NfL can be used to distinguish CIS from healthy controls with high accuracy [63], whereas a recent meta-analysis showed no significant difference of CSF NfL levels between MS and CIS [7]. In present study, we found blood NfL levels were significantly higher in MS patients than in CIS patients. Bittner et al validated the application of sNfL in reclassifying CIS under McDonald diagnostic criteria 2010 (i.e. CIS[2010]) as CIS or RRMS under McDonald diagnostic criteria 2017 (i.e. CIS[2017] and RRMS[2017]), and found the

### Table 3. Results of meta-regression for blood NfL difference between MS and controls.

| Covariate                  | Coefficient | SE   | t     | P      |
|----------------------------|-------------|------|-------|--------|
| Age-matched                |             |      |       |        |
| Mean age                   | -0.014      | 0.02 | -0.65 | 0.488  |
| Percent of female          | -1.01       | 1.28 | -0.79 | 0.430  |
| Mean disease duration      | -0.015      | 0.035| -0.43 | 0.670  |
| Mean EDSS score            | 0.148       | 0.131| 1.13  | 0.259  |
| Sample size*               | -0.0013     | 0.0032| -0.39 | 0.696  |
| Not age-matched            |             |      |       |        |
| Mean age                   | -0.041      | 0.018| -2.30 | 0.021  |
| Percent of female          | 1.25        | 1.85 | 0.68  | 0.498  |
| Mean disease duration      | -0.033      | 0.02 | -1.63 | 0.103  |
| Mean EDSS score            | -0.090      | 0.098| -0.92 | 0.358  |
| Sample size                | -0.0006     | 0.0007| -0.86 | 0.387  |

* Excluding Manouchehrinia et al’s study that had a very large sample size.

https://doi.org/10.1371/journal.pone.0274565.t003
inclusion of sNfL to McDonald diagnostic criteria significantly increased the area under the curve [33]. Blood NfL may be a useful biomarker for differential diagnosis between CIS and MS.

We observed higher blood NfL concentrations in PMS patients than in RRMS patients with moderate effect size. This may be attributed to greater inflammatory activity in this group of patients, especially in secondary PMS (SPMS) [56], as well as older age of PMS patients than RRMS patients. Several included studies comparing PMS and RRMS showed significantly older age and higher NfL levels of PMS patients [26, 37, 49, 50]. After correction for age, PMS still had higher sNfL levels than RRMS patients [29]. Several studies revealed that RRMS patients with higher serum NfL levels had greater risk of conversion to SPMS [32, 34, 64]. However, there is no such difference in CSF samples, and even opposite results were observed in some meta-analyses [7, 54]. In addition, we found blood NfL levels were higher in relapsed MS patients than in remitted patients, which was similar to what has been found in CSF samples [7, 54].

Blood NfL is associated with future disease activity and progression [65]. Patients with baseline higher sNfL levels had higher risk of experiencing relapse, accelerated brain and spinal cord volume loss, and EDSS worsening post blood sampling [14, 29]. Upper tertile of longitudinal measures of sNfL predicted higher risk of EDSS worsening in a long term as far as 15 years [66]. We further assessed whether blood NfL could predict time to relapse and EDSS worsening through meta-analysis. Patients with high NfL levels were earlier to reach EDSS score ≥4.0 but had comparable time to relapse compared with those with low NfL levels. Thus, blood NfL can be used to predict disease progression of MS patients.

Several limitations in our study should be noted. Firstly, there was substantial between-study heterogeneity, which may be caused by cofounders such as age, gender, disease activity, and DMT usage. Secondly, the sample size of some subgroups, including PMS vs. RRMS, MS in relapse vs. MS in remission and the predictive value, was small. Thirdly, NfL levels in most studies were not in normal distribution and shown as median with IQR or range. We had to convert them into mean with SD, which did not accurately reflect the difference. Patient-level data may be warranted.

In conclusion, the present meta-analysis demonstrates that blood NfL is a potential biomarker for MS diagnosis, MS subtype differentiation, and the prediction of disease worsening.

**Supporting information**

S1 Checklist.
(DOCX)

S1 Table. Characteristics of studies included in meta-analysis for predictive value of blood NfL concentration.
(DOCX)

S2 Table. Quality assessment for studies included in meta-analysis of diagnosis value of blood NfL concentration according to NOS (case-control studies).
(DOCX)

S3 Table. Quality assessment for studies included in meta-analysis of predictive value of blood NfL concentration according to NOS (cohort studies).
(DOCX)

S4 Table. Egger’s test for publication bias.
(DOCX)
S1 Fig. Forest plot of blood NfL concentrations between MS patients vs. non-matched controls. (TIF)

S2 Fig. Forest plot of blood NfL levels between MS patients vs. CIS patients. CIS: clinically isolated syndrome. (TIF)

S3 Fig. Forest plot of high blood NfL levels predicting time to reach EDSS score ≥4.0. EDSS: Expanded Disability Status Scale; HR: hazard ratio. (TIF)

S4 Fig. Forest plot of high blood NfL levels predicting time to relapse. (TIF)

S5 Fig. Meta-regression analysis of mean age in correlation with NfL difference between MS and non-matched controls. (TIF)

S6 Fig. Meta-regression analysis of mean age in correlation with NfL difference between MS and age-matched controls. (TIF)

Author Contributions
Conceptualization: Bin Wang.
Data curation: Liangxia Ning, Bin Wang.
Formal analysis: Liangxia Ning.
Supervision: Bin Wang.
Writing – original draft: Liangxia Ning.
Writing – review & editing: Liangxia Ning, Bin Wang.

References
1. Group GBDNDC. Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 2017; 16(11):877–97. https://doi.org/10.1016/S1474-4422(17)30299-5 PMID: 28931491

2. Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, et al. Multiple sclerosis. Nat Rev Dis Primers. 2018; 4(1):43.

3. Rae-Grant A, Day GS, Marrie RA, Rabinstein A, Cree BAC, Gronseth GS, et al. Practice guideline recommendations summary: Disease-modifying therapies for adults with multiple sclerosis: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. Neurology. 2018; 90(17):777–88. https://doi.org/10.1212/WNL.0000000000005347 PMID: 29686116

4. Ferreira-Atuesta C, Reyes S, Giovanonni G, Gnanapavan S. The Evolution of Neurofilament Light Chain in Multiple Sclerosis. Front Neurosci. 2021; 15:642384. https://doi.org/10.3389/fnins.2021.642384 PMID: 33899088

5. Uphaus T, Bittner S, Grosche S, Steffen F, Muthuraman M, Wasser K, et al. NfL (Neurofilament Light Chain) Levels as a Predictive Marker for Long-Term Outcome After Ischemic Stroke. Stroke. 2019; 50(11):3077–84. https://doi.org/10.1161/STROKEAHA.119.026410 PMID: 31537188

6. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol. 2018; 14(10):577–89. https://doi.org/10.1038/s41582-018-0038-2 PMID: 30171200
Neurofilament light chain in blood as a biomarker for multiple sclerosis

7. Mottazmanesh S, Shobeiri P, Saghazadeh A, Teunissen CE, Burman J, Szalardy L, et al. Neuronal and glial CSF biomarkers in multiple sclerosis: a systematic review and meta-analysis. Rev Neurosci. 2021; 32(6):573–95.

8. Gaetani L, Blienkov K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019; 90(8):870–81. https://doi.org/10.1136/jnnp-2018-320106 PMID: 30967444

9. Carare RO, Bernardes-Silva M, Newman TA, Page AM, Nicoll JA, Perry VH, et al. Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. Neuropathol Appl Neurobiol. 2008; 34(2):131–44. https://doi.org/10.1111/j.1365-2990.2007.00926.x PMID: 18208483

10. Kuhle J, Barro C, Disanto G, Mathias A, Soneson C, Bonnier G, et al. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler. 2016; 22(12):1550–9. https://doi.org/10.1177/1352458515623365 PMID: 26754800

11. Disanto G, Adiutori R, Dobson R, Martinelli V, Dalla Costa G, Runia T, et al. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. J Neurol Neurosurg Psychiatry. 2016; 87(2):126–9. https://doi.org/10.1136/jnnp-2014-309690 PMID: 25716934

12. Kuhle J, Barro C, Andreassson U, Derfuss T, Lindberg R, Sandelius A, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin Chem Lab Med. 2016; 54(10):1655–61. https://doi.org/10.1515/cclm-2015-1195 PMID: 27071153

13. Hogel H, Rissane D, Barro C, Matilainen M, Nylund M, Kuhle J, et al. Serum glial fibrillary acidic protein correlates with multiple sclerosis disease severity. Mult Scler. 2020; 26(2):210–9. https://doi.org/10.1177/1352458518813937 PMID: 30570436

14. Disanto G, Barro C, Benkert P, Naegelin Y, Schadelin S, Giardiello A, et al. Serum neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2017; 81(6):857–70. https://doi.org/10.1002/ana.24954 PMID: 28312753

15. Kuhle J, Kropshofer H, Haering DA, Kundu U, Meinert R, Barro C, et al. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology. 2019; 92(10):e1007–e115. https://doi.org/10.1212/wnl.0000000000007032 PMID: 30737333

16. Benkert P, Meier S, Schadelin S, Manouchehrinia A, Yaldizli O, Maceski A, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. Lancet Neuro. 2022; 21(3):246–57. https://doi.org/10.1016/S1474-4422(22)00009-6 PMID: 35182510

17. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021; 372:n71. https://doi.org/10.1136/bmj.n71 PMID: 33782057

18. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol. 1983; 13(3):227–31. https://doi.org/10.1002/ana.410130302 PMID: 6847134

19. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018; 17(2):162–73. https://doi.org/10.1016/S1474-4422(17)30470-2 PMID: 29275977

20. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011; 69(2):292–302. https://doi.org/10.1002/ana.22366 PMID: 21387374

21. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol. 2001; 50(1):121–7. https://doi.org/10.1002/ana.1032 PMID: 11456302

22. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol. 2014; 14:135. https://doi.org/10.1186/1471-2288-14-135 PMID: 25524443

23. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. Stat Methods Med Res. 2018; 27(6):1785–805. https://doi.org/10.1177/0962280216669183 PMID: 27683581

24. Cohen J. Statistical power analysis for the behavioral sciences. Stat Power Anal Behav Sci. 1988; 2:567.

25. Watanabe M, Nakamura Y, Michalak Z, Isobe N, Barro C, Leppert D, et al. Serum GFAP and neurofilament light as biomarkers of disease activity and disability in NMOSD. Neurology. 2019; 93(13):e1299–e311. https://doi.org/10.1212/WNL.0000000000008160 PMID: 31471502
26. Ferraro D, Guicciardi C, De Biasi S, Pinti M, Bedin R, Camera V, et al. Plasma neurofilaments correlate with disability in progressive multiple sclerosis patients. Acta Neurol Scand. 2020; 141(1):16–21. https://doi.org/10.1111/ane.13152 PMID: 31350854

27. Abdelhak A, Huss A, Kassubek J, Tumani H, Otto M. Serum GFAP as a biomarker for disease severity in multiple sclerosis. Sci Rep. 2018; 8(1):14798. Epub 2018/10/06. https://doi.org/10.1038/s41598-018-33158-8 PMID: 30287780

28. Hakansson I, Tisell A, Cassel P, Blennow K, Zetterberg H, Lundberg P, et al. Neurofilament levels, disease activity and brain volume during follow-up in multiple sclerosis. J Neuroinflammation. 2018; 15(1):209. https://doi.org/10.1186/s12974-018-1249-7 PMID: 30021640

29. Barro C, Benkert P, Disanto G, Tsagkas C, Amann M, Naegelin Y, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain. 2018; 141(8):2382–91. https://doi.org/10.1093/brain/awy154 PMID: 29860296

30. Novakova L, Zetterberg H, Sundstrom P, Axelsson M, Khademi M, Gunnarsson M, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. 2017; 89(22):2230–7. https://doi.org/10.1212/WNL.0000000000004683 PMID: 29079686

31. Piehl F, Kockum I, Khademi M, Blennow K, Lycke J, Zetterberg H, et al. Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. Mult Scler. 2018; 24(8):1046–54. https://doi.org/10.1177/13524585171771532 PMID: 28627962

32. Thebault S, Abdoli M, Fereshtehnejad SM, Tessier D, Tabard-Cossa V, Freedman MS. Serum neurofilament light chain predicts long term clinical outcomes in multiple sclerosis. Sci Rep. 2020; 10(1):10381. https://doi.org/10.1038/s41598-020-67504-6 PMID: 32587320

33. Bittner S, Steffen F, Uphaus T, Muthuraman M, Fleischer V, Salmen A, et al. Clinical implications of serum neurofilament in newly diagnosed MS patients: A longitudinal multicentre cohort study. EBiomedicine. 2020; 56:102807. https://doi.org/10.1016/j.ebiom.2020.102807 PMID: 32460167

34. Manouchehrinia A, Stridh P, Khademi M, Leppert D, Barro C, Michalak Z, et al. Plasma neurofilament light levels are associated with risk of disability in multiple sclerosis. Neurology. 2020; 94(23):e2457–e67. https://doi.org/10.1212/WNL.0000000000009571 PMID: 32438467

35. Baldaassari LE, Planchon SM, Bemmel RA, Nakamura K, Fisher E, Feng J, et al. Serum neurofilament light chain concentration in a phase 1/2 trial of autologous mesenchymal stem cell transplantation. Mult Scler J Exp Transl Clin. 2019; 5(4):205521731987198. https://doi.org/10.1177/205521731987198 PMID: 31723439

36. Sejbaek T, Nielsen HH, Penner N, Plavina T, Mendoza JP, Martin NA, et al. Dimethyl fumarate decreases neurofilament light chain in CSF and blood of treatment naive relapsing MS patients. J Neurol Neurosurg Psychiatry. 2019; 90(12):1324–30.

37. Jakimovski D, Zivadinov R, Ramantnan M, Hagemeier J, Weinstock-Guttman B, Tomic D, et al. Serum neurofilament light chain level associations with clinical and cognitive performance in multiple sclerosis: A longitudinal retrospective 5-year study. Mult Scler. 2020; 26(13):1670–81. https://doi.org/10.1177/1352458519887198 PMID: 31516913

38. Thebault S, DRT, Lee H, Bowman M, Bar-Or A, Arnold DL, et al. High serum neurofilament light chain normalizes after hematopoietic stem cell transplantation for MS. Neuronal Neuroimmunolog Neuroinflamm. 2019; 6(6):e598. https://doi.org/10.1212/NXI.0000000000005598 PMID: 31516913

39. Niiranen M, Kontkanen A, Jaaskelainen O, Tertsunen HM, Selander T, Hartikainen P, et al. Serum GFAP and NfL levels in benign relapsing-remitting multiple sclerosis. Mult Scler Relat Disord. 2021; 56:103280. https://doi.org/10.1016/j.msard.2021.103280 PMID: 34627002

40. Cruz-Gomez AJ, Forero L, Lozano-Soto E, Cano-Cano F, Sammartino F, Rashid-Lopez R, et al. Cortical Thickness and Serum NFL Explain Cognitive Dysfunction in Newly Diagnosed Patients With Multiple Sclerosis. Neurology Neuroimmunolog Neuroinflamm. 2021; 8(6):e1074. https://doi.org/10.1212/NXI.000000000001074 PMID: 34465616

41. Lin TY, Vitkova V, Asseyer S, Martorell Serra I, Motamed S, Chien C, et al. Increased Serum Neurofilament Light and Thin Ganglion Cell-inner Plexiform Layer Are Additive Risk Factors for Disease Activity in Early Multiple Sclerosis. Neuronal Neuroimmunolog Neuroinflamm. 2021; 8(5):e1051. https://doi.org/10.1212/NXI.000000000001051 PMID: 34348969

42. Liu C, Lu Y, Wang J, Chang Y, Wang Y, Chen C, et al. Serum neurofilament light chain and glial fibrillary acidic protein in AQP4-IgG-seropositive neuromyelitis optica spectrum disorders and multiple sclerosis: A cohort study. J Neurochem. 2021; 159(5):913–22. https://doi.org/10.1111/jnc.15478 PMID: 34034261
44. Saraste M, Beuzukadova S, Matilainen M, Tuisku J, Rissanen E, Sucksdorff M, et al. High serum neurofilament associates with diffuse white matter damage in MS. Neurol Neuroimmunol Neuroinflamm. 2021; 8(1):e926. https://doi.org/10.1212/NXI.000000000000926 PMID: 33293460

45. Bridel C, Verberk IMW, Heijst JJAJ, Killestein J, Teunissen CE. Variations in consecutive serum neurofilament light levels in healthy controls and multiple sclerosis patients. Mult Scler Relat Disord. 2021; 47:102666. https://doi.org/10.1016/j.msard.2020.102666 PMID: 33291033

46. Olsson A, Gustavsen S, Hasselbalch IC, Langkilde AR, Sellebjerg F, Oturai AB, et al. Biomarkers of inflammation and epithelial barrier function in multiple sclerosis. Mult Scler Relat Disord. 2020; 46:102520. https://doi.org/10.1016/j.msard.2020.102520 PMID: 32980645

47. Haring DA, Kropshofer H, Kappos L, Cohen JA, Shah A, Meinert R, et al. Long-term prognostic value of longitudinal measurements of blood neurofilament light levels. Neurology Neuroimmunol Neuroinflamm. 2020; 7(5):e856. https://doi.org/10.1212/NXI.000000000000856 PMID: 32817406

48. Anderson V, Bentley E, Loveless S, Bianchi L, Harding KE, Wynford-Thomas RA, et al. Serum neurofilament-light concentration and real-world outcome in MS. J Neurol Sci. 2020; 417:117079. https://doi.org/10.1016/j.jns.2020.117079 PMID: 32781395

49. Huss A, Otto M, Senel M, Ludolph AC, Abdelhak A, Tumani H. A Score Based on NfL and Glial Markers May Differentiate Between Relapsing-Remitting and Progressive MS Course. From Front Neurol. 2020; 11:608. https://doi.org/10.3389/fneur.2020.00608 PMID: 32765393

50. Ayriognac X, Le Bars E, Duflot C, Hirtz C, Maleska Macesi A, Carrà-Dalliere C, et al. Serum GFAP in multiple sclerosis: correlation with disease type and MRI markers of disease severity. Sci Rep. 2020; 10(1):10923. https://doi.org/10.1038/s41598-020-67934-2 PMID: 32619616

51. Harp C, Thanei GA, Jia X, Kuhle J, Leppert D, Schaedelin S, et al. Development of an age-adjusted model for blood neurofilament light chain. Ann Clin Transl Neurol. 2022; 9(4):44–53. https://doi.org/10.1002/acn3.51524 PMID: 35229997

52. Miller DH, Weishenker BG, Filippi M, Banwell BL, Cohen JA, Freedman MS, et al. Differential diagnosis of suspected multiple sclerosis: a consensus approach. Mult Scler. 2008; 14(9):1157–74. https://doi.org/10.1177/1352458508096878 PMID: 18805839

53. Bridel C, van Wieringen WN, Zetterberg H, Tijms BM, Teunissen CE, and the NFLG, et al. Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. JAMA Neurol. 2019; 76(9):1035–48. https://doi.org/10.1001/jamaneurol.2019.1534 PMID: 31206160

54. Martin SJ, McGlasson S, Hunt D, Overell J. Cerebrospinal fluid neurofilament light chain in multiple sclerosis and its subtypes: a meta-analysis of case-control studies. J Neurol Neurosurg Psychiatry. 2019; 90(9):1059–67. https://doi.org/10.1136/jnnp-2018-319190 PMID: 31123141

55. Cai L, Huang J. Neurofilament light chain as a biological marker for multiple sclerosis: a meta-analysis study. Neuropsychiatr Dis Treat. 2018; 14:2241–54. https://doi.org/10.2147/NDT.S173280 PMID: 30214214

56. Jakimovski D, Dwyer MG, Bergslund N, Weinstock-Guttman B, Zivadinov R. Disease biomarkers in multiple sclerosis: current serum neurofilament light chain perspectives. Neurodegener Dis Manag. 2021; 11(4):39–40. https://doi.org/10.22217/ntf-2020-0058 PMID: 34196596

57. Khalil M, Pirpamer L, Hofer E, Voortman MM, Barro C, Leppert D, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nat Commun. 2020; 11(1):812. https://doi.org/10.1038/s41467-020-14612-6 PMID: 32041951

58. Koini M, Pirpamer L, Hofer E, Buchmann A, Pinter D, Ropele S, et al. Factors influencing serum neurofilament light chain levels in normal aging. Aging (Albany NY). 2021; 13(24):25729–38. https://doi.org/10.18632/aging.203790 PMID: 34923481

59. Manouchehrinia A, Plehl F, Hillert J, Kuhle J, Alfredsson L, Olsson T, et al. Con founding effect of blood volume and body mass index on blood neurofilament light chain levels. Ann Clin Transl Neurol. 2020; 7(1):139–43. https://doi.org/10.1002/acn3.50972 PMID: 31893583

60. Zhou R, Li H, Yang H, Jiang F, Cai H, Li J, et al. Serological markers exploration and real-world effectiveness and safety of teriflunomide in south Chinese patients with multiple sclerosis. Mult Scler Relat Disord. 2022; 58:103446. https://doi.org/10.1016/j.msard.2021.103446 PMID: 34929454

61. Fox RJ, Raska P, Barro C, Karafa M, Konig V, Bermel RA, et al. Neurofilament light chain in a phase 2 clinical trial of ibudilast in progressive multiple sclerosis. Mult Scler. 2021; 27(13):2014–22. https://doi.org/10.1177/1352458520986956 PMID: 33635141

62. Williams TE, Holdsworth KP, Nicholas JM, Eshaghii A, Katsanouli T, Wellington H, et al. Assessing Neurofilaments as Biomarkers of Neur oprotection in Progressive Multiple Sclerosis: From the MS-STAT Randomized Controlled Trial. Neurol Neuroimmunol Neuroinflamm. 2022; 9(2):e1130. https://doi.org/10.1212/NXI.0000000000001130 PMID: 35031587
63. Kuhle J, Plattner K, Bestwick JP, Lindberg RL, Ramagopalan SV, Norgren N, et al. A comparative study of CSF neurofilament light and heavy chain protein in MS. Mult Scler. 2013; 19(12):1597–603. https://doi.org/10.1177/1352458513482374 PMID: 23529999

64. Jakimovski D, Kuhle J, Ramanathan M, Barro C, Tomic D, Hagemeier J, et al. Serum neurofilament light chain levels associations with gray matter pathology: a 5-year longitudinal study. Ann Clin Transl Neurol. 2019; 6(9):1757–70. https://doi.org/10.1002/acn3.50872 PMID: 31437387

65. Kouchaki E, Dashti F, Mirazimi SMA, Alirezaei Z, Jafari SH, Hamblin MR, et al. Neurofilament light chain as a biomarker for diagnosis of multiple sclerosis. EXCLI J. 2021; 20:1308–25. https://doi.org/10.17179/excli2021-3973 PMID: 34602928

66. Kuhle J, Plavina T, Barro C, Disanto G, Sangurdekar D, Singh CM, et al. Neurofilament light levels are associated with long-term outcomes in multiple sclerosis. Mult Scler. 2020; 26(13):1691–9. https://doi.org/10.1177/1352458519885613 PMID: 31680621