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REVIEW

Neurofilament light chain as a biological marker for multiple sclerosis: a meta-analysis study

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Purpose: There is a need for biomarkers in multiple sclerosis (MS) to make an early diagnosis and monitor its progression. This study was designed to evaluate the value of neurofilament light (NFL) chain levels as cerebrospinal fluid (CSF) or blood biomarker in patients with MS by using a quantitative meta-analysis.

Methods: The PubMed, Embase, and Web of Science databases were systematically searched for relevant studies. Articles in English that evaluated the utility of NFL in CSF and blood in the diagnosis of MS were included. Data were extracted by two independent researchers. Mean ($\pm$ SD) NFL concentration for MS patients and control subjects were extracted. Review Manager version 5.3 software with a continuous-variable random-effects model was used to summarize the diagnostic indexes from eligible studies. The Newcastle–Ottawa Scale was used for assessing the quality and risk of bias of included studies. In addition, subgroup analysis and meta-regression were performed to assess potential heterogeneity sources.

Results: The meta-analysis included 13 articles containing results from 15 studies. A total of 10 studies measured NFL levels in CSF and five studies measured NFL levels in blood. Data were available on 795 participants in CSF and 1,856 participants in blood. Moreover, CSF NFL in MS patients was higher than that in healthy control groups (pooled standard mean difference [Std.MD]=0.88, 95% CI [0.50, 1.26], $P<0.00001$) and serum NFL in MS patients was higher than that in control subjects (pooled Std.MD=0.47, 95% CI [0.24, 0.71], $P<0.0001$).

Conclusion: NFL chain has significantly increased in MS patients, which substantially strengthens the clinical evidence of the NFL in MS. The NFL may be used as a prognostic biomarker to monitor disease progression, disease activity, and treatment efficacy in the future.

Keywords: multiple sclerosis, neurofilament light chain, meta-analysis

Introduction

Multiple sclerosis (MS) is a disease of the central nervous system (CNS) usually characterized by relapsing episodes of neurological dysfunction, often followed some years later by progressive and irreversible decline. It is one of the common causes of acquired neurological disability in young people in Northern Europe and the USA.[1] Once thought of as the model for inflammatory CNS disorder, evidence now suggests that the pathophysiology is complex and possibly occurs via multiple mechanisms, including axonal damage and neurodegeneration.[2] The extent of axonal damage and neurodegeneration reflects as a main determinant of patients' physical disability.[3,4] The complexity of the disease is derived by the inadequacy of current treatments and the progressive phase of the disease, during which patients gradually become more disabled, for which there is no effective therapy.[1]

Axonal loss and neurodegeneration are main elements of MS pathology, so an objective biomarker to detect and quantify them should be of great value. Neurofilaments

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(NFs) belong to the intermediate filament family of proteins and are the major components of the cytoskeleton of neurons.[5] NF can be divided according to the observed molecular weight into neurofilament light (NFL) chain (68 kDa), neurofilament intermediate (NFM) chain (160 kDa), and neurofilament heavy (NFH) chain (205 kDa).[6] Due to damage to axons of the CNS or peripheral nervous system, NFs would then occur in the cerebrospinal fluid (CSF) and the blood stream, where NF can be detected.[7,8] Evidence for increased CSF NF levels in MS mainly exists for NFH and NFL, whereas NFM has not been extensively studied so far.[9] Several test systems exist to determine NFH and NFL and a commercially available enzyme-linked immunosorbent assay (ELISA) to detect NFL is advantageous in discriminating patients with MS from healthy controls.[10]

The aim of this article is to investigate whether NFL levels in blood and CSF could be a credible marker for MS, either in differentiating patients from healthy controls or as biomarkers monitoring disease progression or predicting prognosis.[11]

Methods

This meta-analysis was performed according to the guidelines that are recommended by the PRISMA statement (Preferred Reporting Items for Systematic reviews and Meta-Analysis).[12]

Search strategy

Two researchers performed a systematic review of peer-reviewed English language articles from the databases of PubMed, Embase, and Web of Science with no year limitation from articles published up to October 1, 2017. The database search keywords were Neurofilament AND Multiple Sclerosis. We used Medical Subject Heading (MeSH) terms to retrieve literature in PubMed, and Emtree terms were used in Embase. Original clinical studies that reported data on NFL concentrations in patients with MS and control subjects were included. We also searched the reference lists of included studies. The literature search, title/abstract screening, the final decision on eligibility after full-text review, and data extraction were independently performed by two investigators.

Inclusion and exclusion criteria

The following inclusion criteria were adopted: 1) performed in adult patients who were >18 years of age; 2) the original article not in reviews, posters, or abstracts; 3) containing retrospective or prospective case–control studies; 4) including a group of patients who fulfilled the revised McDonald criteria from 2010[13] for MS; 5) detection of the CSF and blood NFL levels in MS patients and healthy control groups. Furthermore, exclusion criteria were listed as follows: 1) studies concerning juveniles (aged <18 years) and pregnant women; 2) animals or cell line studies, commentaries, meta-analysis, case reports or series, reviews, meetings, and editorials or manuscripts unrelated to the research topic; 3) used nonquantitative methods such as Western blot; 4) unsuitable data that the mean levels and SD of NFL cannot be appropriate; 5) the MS subjects with other neurological diseases such as brain infarction, amyotrophic lateral sclerosis, or cerebral hemorrhage; 6) studies without control groups or no healthy individuals; 7) had overlapped sample, or the sample size is less than five or the NF analyzed was assessed in fewer than three studies. We used the EndNote to remove duplicate data. Titles and abstracts were screened for eligible studies and full-text reviewed for potentially qualified studies subsequently. If multiple studies were derived from the same hospital or research center, authors were contacted for excluding overlapped samples.

Data extraction and quality assessment

Two of us (LC and JH) extracted the data. Data on sample size, mean, and SD of NFL chain concentration were extracted as primary outcomes. Data for potential analysis of gender, age, disease duration, sampling source, and type of sample (CSF or serum) were also extracted. If data were presented in a format from which means and SDs were not extractable or only presented graphically, then these measures were requested from the corresponding author of the publication and where a response was not received, we measured the data from the graphs by using digital ruler software (Engauge Digitizer). If the standard error of mean (SEM) was only reported, SD was estimated using the following formula: SD=SEM×Sqrt (sample size). The Newcastle–Ottawa scale (NOS) criteria,[14] which included the selection (0–4 scores), comparability (0–2 scores), and exposure (0–3 scores) categories (0 denoted noncompliance with any criteria, nine denoted fulfillment of all criteria), were used to evaluate the quality of the included original articles. Studies were of low-quality methodology in accordance with NOS score being lower than 6 scores.[15]

Statistical analysis

Heterogeneity was quantified with I^2 statistics.[16] Standard mean difference (Std.MD) and 95% CI from individual studies were calculated by weighted fixed-effect model

when the heterogeneity tests were $P \geq 0.05$. Accordingly, the random-effect model was used when the heterogeneity test was $P < 0.05$.[15] The data of CSF and blood NFL levels in MS patients compared with that in control subjects were extracted and pooled for separate meta-analysis. Sensitivity analysis and subgroup analysis were done to explore potential heterogeneity sources. Publication bias was assessed by funnel plots. Additionally, heterogeneity, pooled Std.MD, subgroup analysis, and funnel plots were calculated by Review Manager version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).[15] Also, separate regression of age, gender, sample size, and disease duration on Std.MD of the CSF and blood NFL levels in MS patients were calculated by the method-of-moments technique. Meta-regression was performed by STATA software (version 12.0, StataCorp, College Station, TX, USA).

Results
Search results and characteristics of the included studies

The initial search identified 177 records from PubMed, 389 records from Embase, and 754 records from the Web of Science, and two records from other sources. Nine hundred studies were identified totally, after removing duplicate papers.

Scanning of titles and abstracts resulted in identification of 93 articles for full-text scrutiny. Eighty studies were excluded because they lacked necessary data (n=22),[5,17–37] lacked control subjects (n=11),[38–48] were disease control groups (n=18),[49–66] were meeting abstracts (n=27),[67–93] and reported NFH chain data in less than three studies (n=2).[94,95] Two papers were included in two different studies.[96,97] Therefore, a total of 13 articles including results from 15 studies with 1,665 MS patients and 986 healthy volunteers were included in this meta-analysis (Figure 1).

The general characteristics of studies and participants included in the present meta-analysis and meta-regression are shown in Table 1. Thirteen groups were pooled, which comprises of 2,651 participants, including 795 participants in CSF and 1,856 participants in blood. The mean age of MS patients ranged from 31 to 44 years, whereas that of healthy volunteers ranged from 28 to 44.3 years. Furthermore, the mean disease duration of MS patients ranged from 0.31 to 8.4 years. Electrochemiluminescence (ECL)-based assay and ELISA were used to detect CSF and serum NFL level in all included studies. In accordance with NOS criteria,[14] more scores were given to the selection category when compared with other studies. The factors that were selected to evaluate the comparability were age and gender.
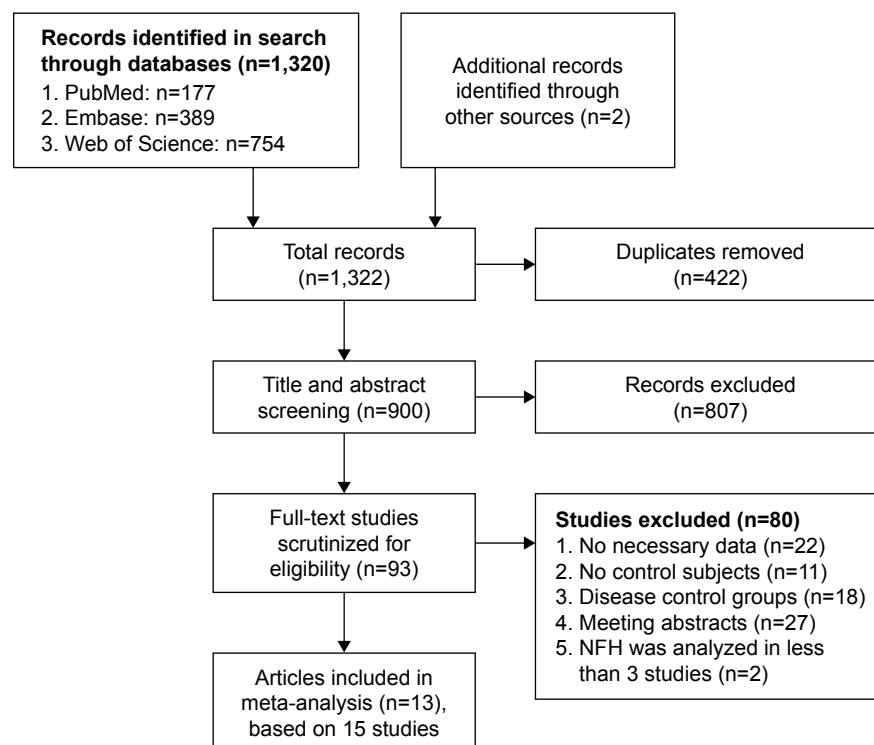

Figure 1 Flowchart depicting literature search and study selection.
Notes: Flow diagram of systematic search in the three databases. After removal of duplicates, reviews, and quality control, 13 articles were suitable for analysis.
Abbreviations: NFH, neurofilament heavy; NFL, neurofilament light.

Table 1 Characteristics of studies involved in the meta-analysis

Author, year	Country	Gender (% male)		Mean age (years)		Disease duration (year)	Sample origin	N		MS (NFL ng/L)		HC (NFL ng/L)	
		MS	HC	MS	HC			MS	HC	Mean	SD	Mean	SD
Novakova et al, 2017[96]	Sweden	29.9	59.5	37	28	NA	CSF	204	42	730	82,807	205	903.7
Haghighi et al, 2004[98]	Sweden	NA	NA	44	33	NA	CSF	47	50	258.7	186.7	128.3	15.8
Norgren et al, 2003[99]	Sweden	NA	NA	NA	NA	NA	CSF	5	11	2,500	3,354	31	76.3
Rosengren et al, 1996[100]	Sweden	NA	NA	35.6	53	8	CSF	5	11	463	402	128	6
Novakova et al, 2017[101]	Sweden	71.4	64.1	37	34	6.6	CSF	7	39	1,900	1,722	364	302.3
Novakova et al, 2017[102]	Sweden	37.2	64.1	39.7	33.6	NA	CSF	43	39	1,183	2,135	364	254
Håkansson et al, 2017[103]	Sweden	22	23	31	32	0.98	CSF	41	22	895	1,304	212	102.2
Novakova et al, 2015[104]	Sweden	35.5	68.8	36	41	8.4	CSF	31	16	2,391	5,274	308	95
Zhang et al, 2007[105]	China	36.5	43.5	39	29	8	CSF	63	46	26	33	10	7
Malmeström et al, 2003[106]	Sweden	47.8	70	32.4	35.4	7.9	CSF	23	50	1,727	1,711	125	152.5
Novakova et al, 2017[96]	Sweden	29.9	59.5	37	28	NA	Serum	204	42	16.9	1,095	10.5	44.4
Disanto et al, 2017[97]	Switzerland	35.2	31.9	37.9	44.3	NA	Serum	142	254	35.9	29.3	22.9	10.8
Disanto et al, 2017[97]	Switzerland	34.1	31.9	42.2	44.3	7.4	Serum	719	254	29.4	18.6	22.9	10.8
Kuhle et al, 2016[107]	Switzerland	35.5	44	32	31	3.6	Serum	31	18	9	17.4	1.3	3.9
Disanto et al, 2016[108]	Switzerland	33	37	30.6	35	0.31	Serum	100	92	24.1	28.4	7.9	8.6

Abbreviations: CSF, cerebrospinal fluid; HC, healthy control; MS, multiple sclerosis; N, number; NA, not available; NFL, neurofilament light chain.

In the studies by Haghighi et al,[98] Norgren et al,[99] and Rosengren et al,[100] gender difference was not mentioned. Moreover, age difference was also not mentioned in the study by Norgren et al.[99] Therefore, zero was given to the comparability category of that study (Table S1).[99] In the current study, the year of studies included ranged from 1996 to 2017.

NFL levels in CSF

The NFL meta-analysis between MS patients and healthy controls' involvement in CSF was based on 10 studies,[96,98–106] containing 469 MS patients and 326 healthy individuals. Random-effects meta-analysis demonstrated that patients with MS had significantly higher CSF NFL levels compared

with control subjects (pooled Std.MD=0.88, 95% CI [0.50, 1.26], $P<0.00001$) (Figure 2). All papers used ELISA to detect CSF NFL level.

NFL levels in blood

In blood, the NFL meta-analysis between MS patients and healthy controls' involvement was based on four studies,[96,97,107,108] containing 660 healthy volunteers and 1,196 MS patients. Random-effects meta-analysis demonstrated that patients with MS had significantly higher CSF NFL levels compared with control subjects (pooled Std.MD=0.47, 95% CI [0.24, 0.71], $P<0.0001$) (Figure 3). The original papers used ECL-based immune assay except for one that used ELISA.[96]

Study or subgroup	MS Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, random (95% CI)
Novakova et al, 2017[96]	730	27,942	204	205	305	42	12.7	0.02 (–0.31, 0.35)
Haghighi et al, 2004[98]	258.7	186.7	47	128.3	15.8	50	12.0	0.99 (0.57, 1.41)
Norgren et al, 2003[99]	2,500	3,354	5	31	76.3	11	5.9	1.30 (0.12, 2.48)
Rosengren et al,1996[100]	463	402	5	128	6	11	5.8	1.47 (0.26, 2.68)
Novakova et al, 2017[101]	1,900	1,722	7	364	302.3	39	7.6	2.17 (1.24, 3.10)
Novakova et al, 2017[102]	1,183	2,135	43	364	254	39	11.8	0.52 (0.08, 0.96)
Håkansson et al, 2017[103]	895	1,304	41	212	102.2	22	11.0	0.64 (0.11, 1.17)
Novakova et al, 2015[104]	2,391	5,274	31	308	95	16	10.3	0.48 (–0.14, 1.09)
Zhang et al, 2007[105]	26	33	63	10	7	46	12.2	0.62 (0.23, 1.01)
Malmeström et al, 2003[106]	1,727	1,711	23	125	152.5	50	10.7	1.65 (1.08, 2.21)
Total (95% CI)			**469**			**326**	**100**	**0.88 (0.50, 1.26)**

Heterogeneity: τ^2=0.26; χ^2=42.19, df=9 ($P<0.00001$); I^2=79%
Test for overall effect: Z=4.57 ($P<0.00001$)
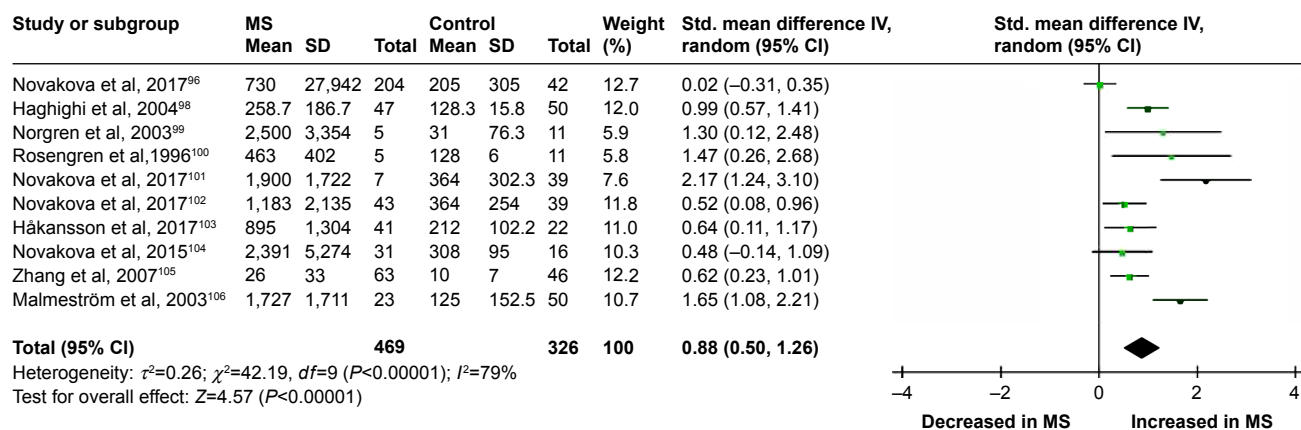

Figure 2 Meta-analysis of neurofilament light chain levels in CSF between MS patients and healthy controls.
Notes: The random-effect model was used. Unit of CSF neurofilament light chain level was ng/L. There was a highly significant difference between the two groups ($P<0.00001$).
Abbreviations: CSF, cerebrospinal fluid; IV, inverse variance; MS, multiple sclerosis.

Investigation of heterogeneity

This meta-analysis existed significantly heterogeneity in CSF (I^2=79%, P<0.00001) and blood (I^2=74%, P=0.004). Next, we attempted to explore the heterogeneity source among studies in the meta-analysis. These potential moderators include age, gender, sample size, disease duration, publication year, and publication bias. So, we carried out sensitivity analysis, subgroup, meta-regression analysis, and publication bias.

Sensitivity analysis

Sensitivity analysis demonstrated that no individual study significantly influenced the statistically significant differences in CSF and blood between MS patients and control subjects. Therefore, no individual study accounted for the significant heterogeneity (Table S2).

Subgroup

To study the causes of heterogeneity, we next performed subgroup analysis based on age-matched design, NFL concentration, and publication year in CSF (Table S3). The impact of heterogeneity was slightly increased (I^2=84%, P<0.00001), and the statistical significance was stable for age-matched studies. But for non-age-matched studies, no significant heterogeneity was found (I^2=27%, P=0.25), and the significance of the association between elevated NFL levels and MS was retained (Figure 4). The impact of heterogeneity was slightly decreased for the cutoff <1,000 ng/L of NFL concentration (I^2=75%, P=0.003), and there was no change in heterogeneity for the cutoff >1,000 ng/L of NFL concentration (I^2=79%, P=0.0009). Furthermore, the significance of the association between elevated NFL levels and MS was retained (Figure 5). Finally, for the publication year before 2010, the impact of heterogeneity was reduced to 21% (I^2=58%, P=0.05) and the statistical significance was

stable, but the impact of heterogeneity was slightly increased (I^2=80%, P=0.0005) and the statistical significance was also retained for the publication year after 2010 (Figure 6).

In addition, we carried out subgroup analysis based on sample size of MS in blood (Table S3). The heterogeneity disappeared when studies with sample size of MS patients <200 (I^2=0%, P=0.78) and >200 (I^2=74%, P=0.004) were pooled in turn. Even more, the significance of the association between elevated NFL levels and MS was stable for the sample <200 but not for the sample >200 (Figure 7).

Meta-regression

Because the number of studies was limited to NFL in blood are limited, the meta-regression analysis was performed for the NFL in CSF. In the meta-regression model, age, gender, sample size, and disease duration in MS patients were pooled. More importantly, Std.MD of CSF NFL was correlated with the sample size of MS patients (P=0.022) and the gender of MS patients (P=0.020). In contrast, there was no significant correlation between Std.MD of CSF NFL and age (P=0.709) and disease duration (P=0.698) in MS patients (Figure 8A–D). Meta-regression analysis suggested that the sample size and gender had moderate effects on the outcome of CSF NFL meta-analysis.

Publication bias

In this article, publication bias was evaluated by visual inspection of funnel plot. Results from the funnel plot suggested that there was a low risk for publication bias in CSF (Figure 9A) and blood (Figure 9B).

Discussion

To our knowledge, this is the first study to provide a comprehensive meta-analysis of the NFL chain as neuronal markers in MS. The meta-analysis included 13 case–control studies

Study or subgroup	MS Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, random (95% CI)	Std. mean difference IV, random (95% CI)
Novakova et al, 2017[96]	16.9	369.6	204	10.5	15	42	18.6	0.02 (−0.31, 0.35)	
Disanto et al, 2017[97]	35.9	29.3	142	22.9	10.8	254	24.0	0.66 (0.45, 0.87)	
Disanto et al, 2017[97]	29.4	18.6	719	22.9	10.8	254	26.8	0.38 (0.24, 0.53)	
Kuhle et al, 2016[107]	9	17.4	31	1.3	3.9	18	10.3	0.54 (−0.05, 1.13)	
Disanto et al, 2016[108]	24.1	28.4	100	7.9	8.6	92	20.3	0.76 (0.46, 1.05)	
Total (95% CI)			1,196			660	100	0.47 (0.24, 0.71)	

Heterogeneity: τ^2=0.05; χ^2=15.41, df=4 (P=0.004); I^2=74%
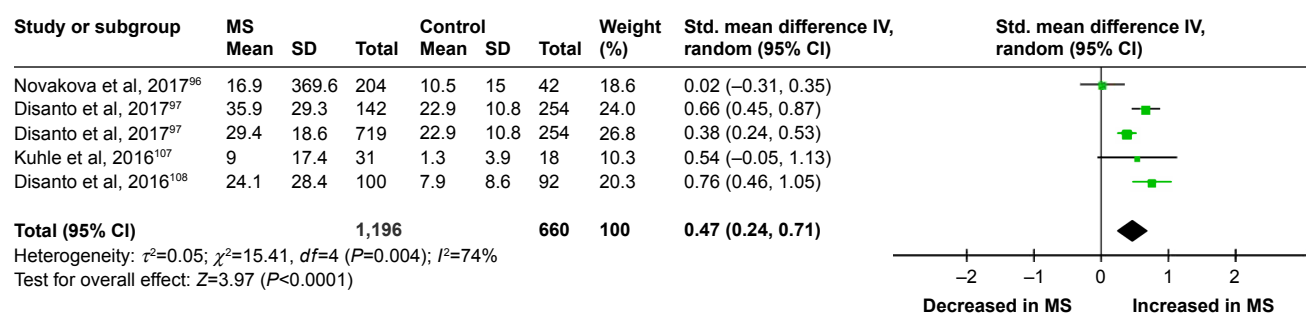Test for overall effect: Z=3.97 (P<0.0001)

Figure 3 Meta-analysis of neurofilament light chain levels in blood between MS patients and healthy controls.
Notes: The random-effect model was applied. Unit of blood neurofilament light chain level was ng/L. There was a highly significant difference between the two groups (P<0.0001).
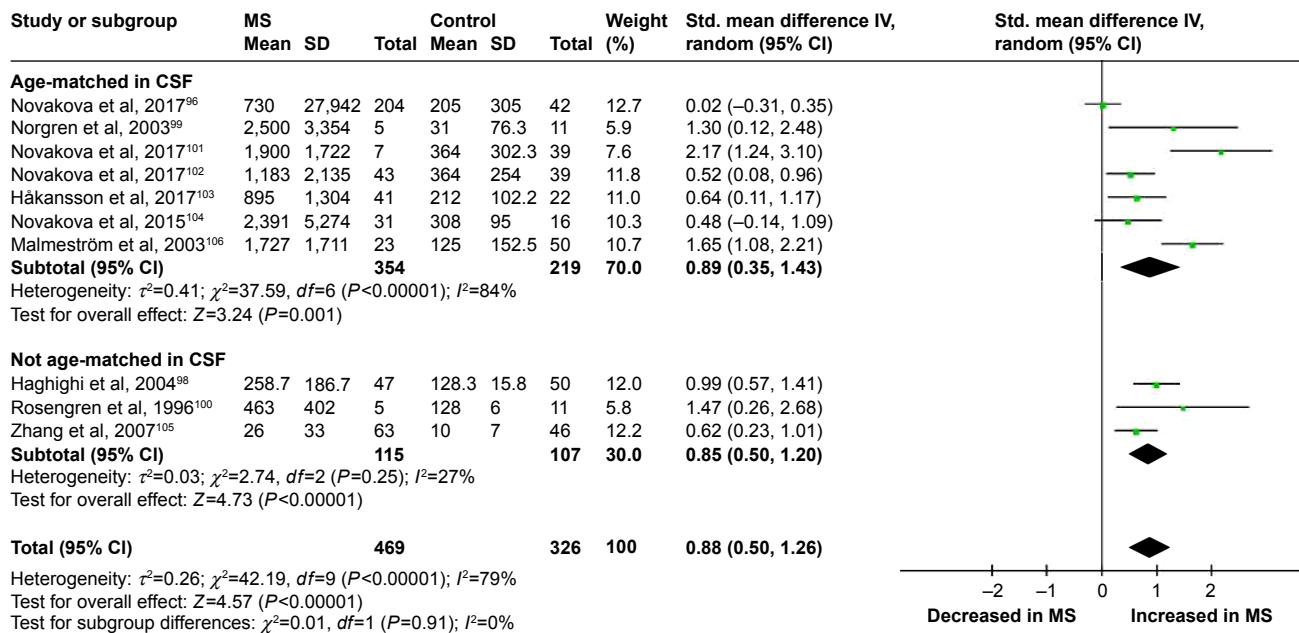**Abbreviations:** IV, inverse variance; MS, multiple sclerosis.

Study or subgroup	MS Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, random (95% CI)	Std. mean difference IV, random (95% CI)
Age-matched in CSF									
Novakova et al, 2017[96]	730	27,942	204	205	305	42	12.7	0.02 (−0.31, 0.35)	
Norgren et al, 2003[99]	2,500	3,354	5	31	76.3	11	5.9	1.30 (0.12, 2.48)	
Novakova et al, 2017[101]	1,900	1,722	7	364	302.3	39	7.6	2.17 (1.24, 3.10)	
Novakova et al, 2017[102]	1,183	2,135	43	364	254	39	11.8	0.52 (0.08, 0.96)	
Håkansson et al, 2017[103]	895	1,304	41	212	102.2	22	11.0	0.64 (0.11, 1.17)	
Novakova et al, 2015[104]	2,391	5,274	31	308	95	16	10.3	0.48 (−0.14, 1.09)	
Malmeström et al, 2003[106]	1,727	1,711	23	125	152.5	50	10.7	1.65 (1.08, 2.21)	
Subtotal (95% CI)			354			219	70.0	**0.89 (0.35, 1.43)**	

Heterogeneity: $\tau^2=0.41$; $\chi^2=37.59$, $df=6$ ($P<0.00001$); $I^2=84\%$
Test for overall effect: $Z=3.24$ ($P=0.001$)

Not age-matched in CSF									
Haghighi et al, 2004[98]	258.7	186.7	47	128.3	15.8	50	12.0	0.99 (0.57, 1.41)	
Rosengren et al, 1996[100]	463	402	5	128	6	11	5.8	1.47 (0.26, 2.68)	
Zhang et al, 2007[105]	26	33	63	10	7	46	12.2	0.62 (0.23, 1.01)	
Subtotal (95% CI)			115			107	30.0	**0.85 (0.50, 1.20)**	

Heterogeneity: $\tau^2=0.03$; $\chi^2=2.74$, $df=2$ ($P=0.25$); $I^2=27\%$
Test for overall effect: $Z=4.73$ ($P<0.00001$)

Total (95% CI)			469			326	100	**0.88 (0.50, 1.26)**	

Heterogeneity: $\tau^2=0.26$; $\chi^2=42.19$, $df=9$ ($P<0.00001$); $I^2=79\%$
Test for overall effect: $Z=4.57$ ($P<0.00001$)
Test for subgroup differences: $\chi^2=0.01$, $df=1$ ($P=0.91$); $I^2=0\%$

−2 −1 0 1 2
Decreased in MS　　Increased in MS

Figure 4 Subgroup analysis stratified by age for MS in CSF.
Notes: The random-effect model was used. Unit of CSF neurofilament light chain level was ng/L.
Abbreviations: CSF, cerebrospinal fluid; IV, inverse variance; MS, multiple sclerosis.

assessing 1,665 MS patients and 986 healthy control subjects and found evidence of significant elevations of NFL levels in MS patients compared with controls. Sensitivity analyses suggested that the significant associations between NFL levels and MS were not influenced by an individual study, suggesting that the overall results were stable. Although inconsistent results have been reported for NFL between

studies in the article, our meta-analysis provides evidence of neurodegeneration in MS, strengthening the clinical evidence that patients with MS have axonal injury.

Accumulating evidence shows that axonal damage and neurodegeneration emerge already in early phases of MS.[109] Axonal degeneration is thought to be the pathologic basis of disease progression in MS,[32] and this damage appears to

Study or subgroup	MS Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, random (95% CI)	Std. mean difference IV, random (95% CI)
Cutoff <1,000 ng/L in CSF									
Novakova et al, 2017[96]	730	27,942	204	205	305	42	12.7	0.02 (−0.31, 0.35)	
Haghighi et al, 2004[98]	258.7	186.7	47	128.3	15.8	50	12.0	0.99 (0.57, 1.41)	
Rosengren et al, 1996[100]	463	402	5	128	6	11	5.8	1.47 (0.26, 2.68)	
Håkansson et al, 2017[103]	895	1,304	41	212	102.2	22	11.0	0.64 (0.11, 1.17)	
Zhang et al, 2007[105]	26	33	63	10	7	46	12.2	0.62 (0.23, 1.01)	
Subtotal (95% CI)			360			171	53.7	**0.64 (0.20, 1.07)**	

Heterogeneity: $\tau^2=0.17$; $\chi^2=16.31$, $df=4$ ($P=0.003$); $I^2=75\%$
Test for overall effect: $Z=2.87$ ($P=0.004$)

Cutoff >1,000 ng/L in CSF									
Norgren et al, 2003[99]	2,500	3,354	5	31	76.3	11	5.9	1.30 (0.12, 2.48)	
Novakova et al, 2017[101]	1,900	1,722	7	364	302.3	39	7.6	2.17 (1.24, 3.10)	
Novakova et al, 2017[102]	1,183	2,135	43	364	254	39	11.8	0.52 (0.08, 0.96)	
Novakova et al, 2015[104]	2,391	5,274	31	308	95	16	10.3	0.48 (−0.14, 1.09)	
Malmeström et al, 2003[106]	1,727	1,711	23	125	152.5	50	10.7	1.65 (1.08, 2.21)	
Subtotal (95% CI)			109			155	46.3	**1.16 (0.51, 1.82)**	

Heterogeneity: $\tau^2=0.41$; $\chi^2=18.77$, $df=4$ ($P=0.0009$); $I^2=79\%$
Test for overall effect: $Z=3.49$ ($P=0.0005$)

Total (95% CI)			469			326	100	**0.88 (0.50, 1.26)**	

Heterogeneity: $\tau^2=0.26$; $\chi^2=42.19$, $df=9$ ($P<0.00001$); $I^2=79\%$
Test for overall effect: $Z=4.57$ ($P<0.00001$)
Test for subgroup differences: $\chi^2=1.73$, $df=1$ ($P=0.19$); $I^2=42.3\%$
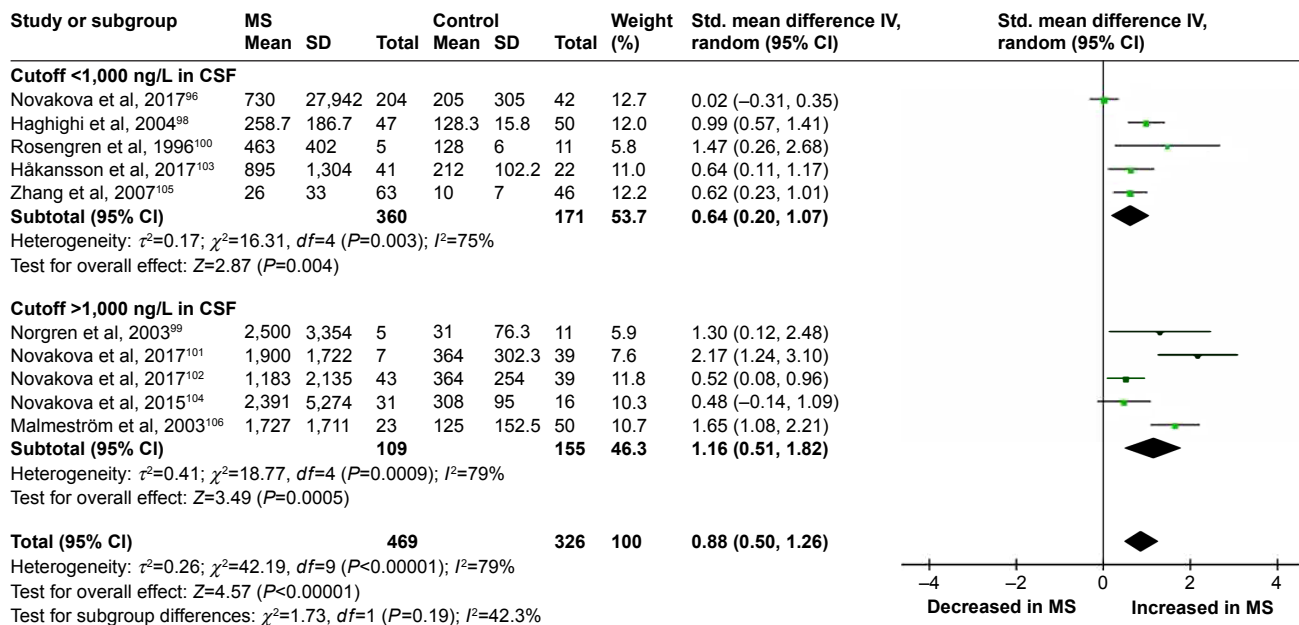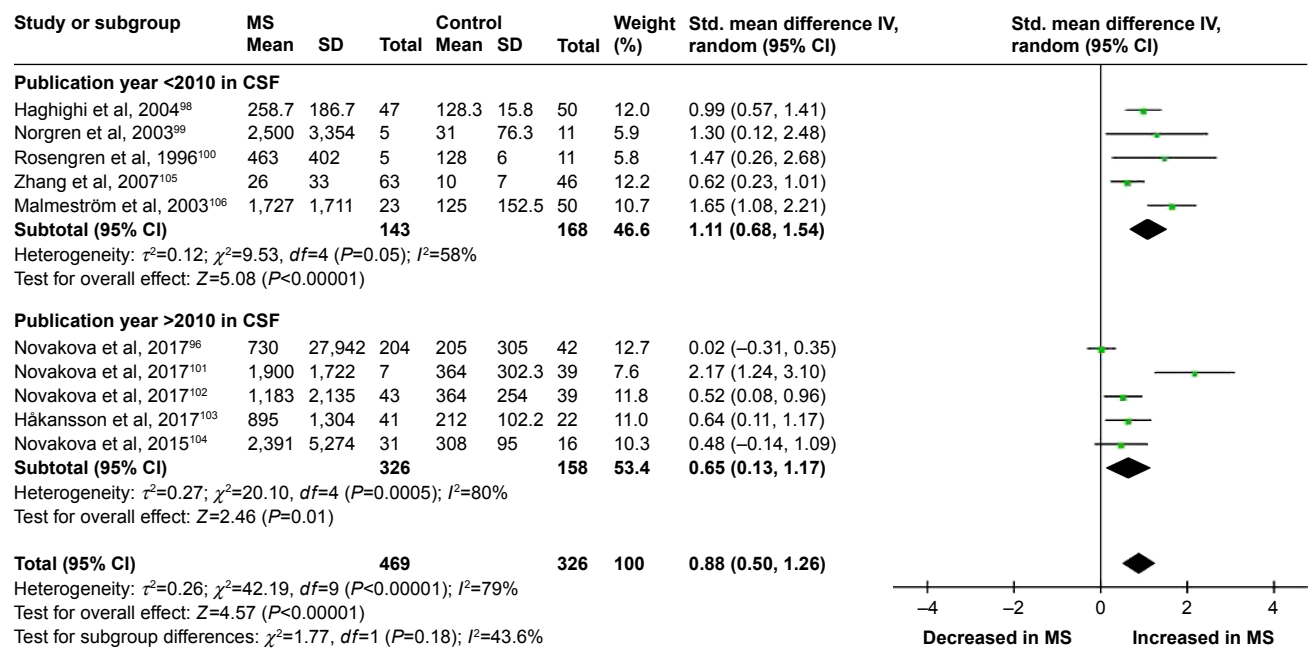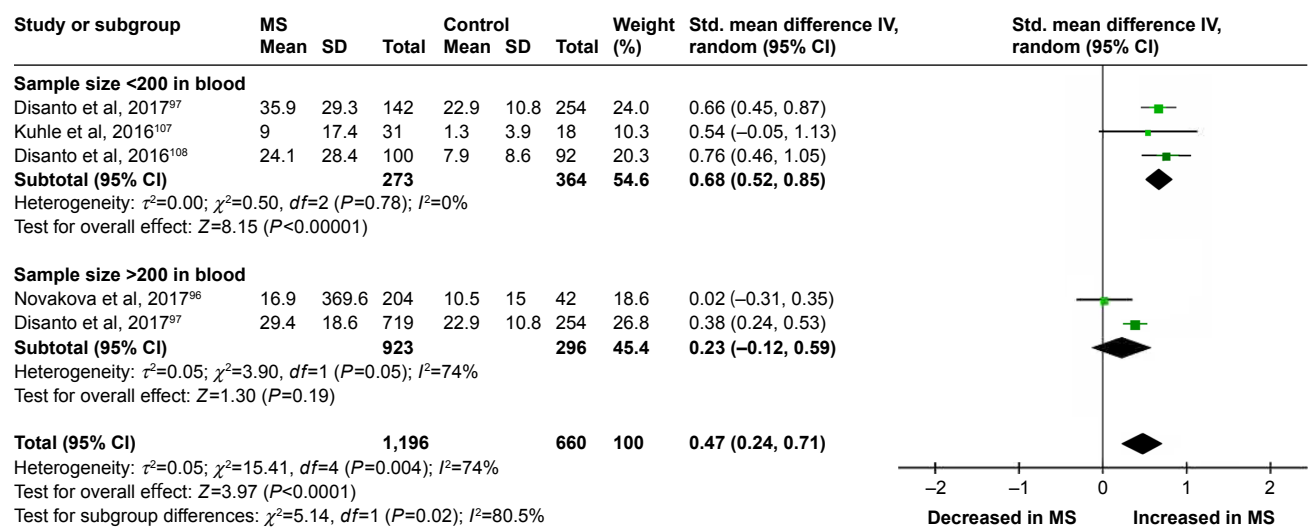
−4 −2 0 2 4
Decreased in MS　　Increased in MS

Figure 5 Subgroup analysis stratified by neurofilament light chain concentration for MS in CSF.
Notes: The random-effect model was used. Unit of CSF neurofilament light chain level was ng/L.
Abbreviations: CSF, cerebrospinal fluid; IV, inverse variance; MS, multiple sclerosis.

Study or subgroup	MS Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, random (95% CI)	Std. mean difference IV, random (95% CI)
Publication year <2010 in CSF									
Haghighi et al, 2004[98]	258.7	186.7	47	128.3	15.8	50	12.0	0.99 (0.57, 1.41)	
Norgren et al, 2003[99]	2,500	3,354	5	31	76.3	11	5.9	1.30 (0.12, 2.48)	
Rosengren et al, 1996[100]	463	402	5	128	6	11	5.8	1.47 (0.26, 2.68)	
Zhang et al, 2007[105]	26	33	63	10	7	46	12.2	0.62 (0.23, 1.01)	
Malmeström et al, 2003[106]	1,727	1,711	23	125	152.5	50	10.7	1.65 (1.08, 2.21)	
Subtotal (95% CI)			143			168	46.6	1.11 (0.68, 1.54)	
Heterogeneity: $\tau^2=0.12$; $\chi^2=9.53$, $df=4$ $(P=0.05)$; $I^2=58\%$									
Test for overall effect: $Z=5.08$ $(P<0.00001)$									
Publication year >2010 in CSF									
Novakova et al, 2017[96]	730	27,942	204	205	305	42	12.7	0.02 (−0.31, 0.35)	
Novakova et al, 2017[101]	1,900	1,722	7	364	302.3	39	7.6	2.17 (1.24, 3.10)	
Novakova et al, 2017[102]	1,183	2,135	43	364	254	39	11.8	0.52 (0.08, 0.96)	
Håkansson et al, 2017[103]	895	1,304	41	212	102.2	22	11.0	0.64 (0.11, 1.17)	
Novakova et al, 2015[104]	2,391	5,274	31	308	95	16	10.3	0.48 (−0.14, 1.09)	
Subtotal (95% CI)			326			158	53.4	0.65 (0.13, 1.17)	
Heterogeneity: $\tau^2=0.27$; $\chi^2=20.10$, $df=4$ $(P=0.0005)$; $I^2=80\%$									
Test for overall effect: $Z=2.46$ $(P=0.01)$									
Total (95% CI)			469			326	100	0.88 (0.50, 1.26)	
Heterogeneity: $\tau^2=0.26$; $\chi^2=42.19$, $df=9$ $(P<0.00001)$; $I^2=79\%$									
Test for overall effect: $Z=4.57$ $(P<0.00001)$									
Test for subgroup differences: $\chi^2=1.77$, $df=1$ $(P=0.18)$; $I^2=43.6\%$									

Figure 6 Subgroup analysis stratified by publication year for MS in CSF.
Notes: The random-effect model was used. Unit of CSF neurofilament light chain level was ng/L.
Abbreviations: CSF, cerebrospinal fluid; IV, inverse variance; MS, multiple sclerosis.

associate with the degree of clinical disability.[110] The cause of axonal loss in MS is still poorly known, probably there are both inflammation induced and neurodegenerative causes,[106] but it is likely that a destructive process directed against specific components of the axonal cytoskeleton may contribute to the accumulation of disability.[111]

NFL chain protein is a major part of the cytoskeletal protein of myelinated axons, and increased levels of NFL in MS patients indicate continuous axonal damage.[30,106] The high intraneuronal levels of NFs are of great significance both for diagnosis and for therapeutic effect in clinical medicine.[99] Based on our previous data, NFL levels are elevated during all stages of MS, particularly in relapsing-remitting MS[106] and in progressive MS,[96] whereas levels of NFL decrease toward normality during intervention with disease-modifying therapies,[40,112–116] suggesting that NFL related to different pathological processes involved in MS reflects disease activity, disease progression, and treatment efficacy.[101]

Study or subgroup	MS Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, random (95% CI)	Std. mean difference IV, random (95% CI)
Sample size <200 in blood									
Disanto et al, 2017[97]	35.9	29.3	142	22.9	10.8	254	24.0	0.66 (0.45, 0.87)	
Kuhle et al, 2016[107]	9	17.4	31	1.3	3.9	18	10.3	0.54 (−0.05, 1.13)	
Disanto et al, 2016[108]	24.1	28.4	100	7.9	8.6	92	20.3	0.76 (0.46, 1.05)	
Subtotal (95% CI)			273			364	54.6	0.68 (0.52, 0.85)	
Heterogeneity: $\tau^2=0.00$; $\chi^2=0.50$, $df=2$ $(P=0.78)$; $I^2=0\%$									
Test for overall effect: $Z=8.15$ $(P<0.00001)$									
Sample size >200 in blood									
Novakova et al, 2017[96]	16.9	369.6	204	10.5	15	42	18.6	0.02 (−0.31, 0.35)	
Disanto et al, 2017[97]	29.4	18.6	719	22.9	10.8	254	26.8	0.38 (0.24, 0.53)	
Subtotal (95% CI)			923			296	45.4	0.23 (−0.12, 0.59)	
Heterogeneity: $\tau^2=0.05$; $\chi^2=3.90$, $df=1$ $(P=0.05)$; $I^2=74\%$									
Test for overall effect: $Z=1.30$ $(P=0.19)$									
Total (95% CI)			1,196			660	100	0.47 (0.24, 0.71)	
Heterogeneity: $\tau^2=0.05$; $\chi^2=15.41$, $df=4$ $(P=0.004)$; $I^2=74\%$									
Test for overall effect: $Z=3.97$ $(P<0.0001)$									
Test for subgroup differences: $\chi^2=5.14$, $df=1$ $(P=0.02)$; $I^2=80.5\%$									

Figure 7 Subgroup analysis stratified by sample size for MS in blood.
Notes: The random-effect model was used. Unit of blood neurofilament light chain level was ng/L.
Abbreviations: IV, inverse variance; MS, multiple sclerosis.

Figure 8 Meta-regression of CSF NFL chain in MS patients relative to healthy controls.
Notes: Separate meta-regressions of age (**A**), sample size (**B**), gender (**C**), and disease duration (**D**) between MS patients and HCs on SMDs comparing the CSF NFL chain levels in MS patients with those in HC.
Abbreviations: CSF, cerebrospinal fluid; HC, healthy control; MS, multiple sclerosis; NFL, neurofilament light; SMD, standard mean difference.
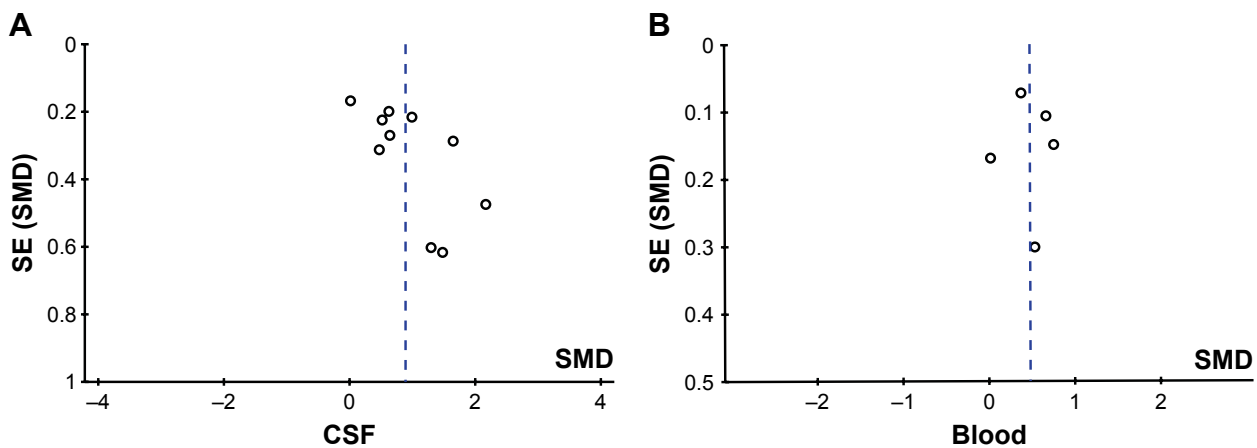

Figure 9 The publication bias of included studies when comparing the CSF (**A**) and blood (**B**) neurofilament light chain levels in MS patients with healthy controls.
Abbreviations: CSF, cerebrospinal fluid; MS, multiple sclerosis; SE, standard error; SMDs, standard mean differences.

The heterogeneity for the NFL in our present meta-analysis varied from zero to high. For the NFL significantly associated with MS, high levels of heterogeneity were found both in CSF and in blood studies. Therefore, subgroup analysis and meta-regression were used to explore the possible confounders that explained the high-level between-study heterogeneity. The subgroup analysis suggested that age designed (age-matched vs not age-matched), NFL concentration (cutoff <1,000 ng/L vs cutoff >1,000 ng/L), and publication year (before 2010 vs after 2010) partially explained the heterogeneity in CSF. First, there was low heterogeneity within the included studies except for age-matched studies, which indicated that age accounted for one of the heterogeneity sources (Figure 4). Second, subgroup analysis stratified by NFL concentration suggested that the heterogeneity was not significantly changed (Figure 5). Furthermore, there was reduced heterogeneity in studies for the publication year of MS patients before 2010 (Figure 6). Meanwhile, subgroup analysis was used to evaluate the influence of sample size (<200 vs >200) of MS patients in blood on heterogeneity. No heterogeneity appeared in studies with sample size of MS patients <200 (Figure 7). Thus, when comparing NFL levels in MS patients with controls, age designed and publication year in CSF, and sample size of MS patients in blood, it was recognized that they were heterogeneity sources.

In addition, when MS patients were compared with healthy volunteers, as shown by meta-regression, the result was significantly associated with the sample size (Figure 8B) and gender (Figure 8C) of MS patients in CSF. However, other potential confounders including age (Figure 8A) and disease duration (Figure 8D) of MS patients did not have to moderate influences on the outcome of the meta-analysis. Therefore, meta-regression analysis revealed that sample size and gender of MS patients in CSF are confounding factors for the outcome of the meta-analysis.

Following are several limitations in this meta-analysis: first, the meta-analysis of NFL levels in patients with MS compared with controls provided us pooled results from case–control (cross-sectional) studies. Furthermore, a great amount of studies included were from Western countries, while only one study was from China and there is no relevant report about other Asian populations or African populations. Undisputedly, selective bias was unavoidable. Second, we only included 13 studies, some of which had a relatively small sample size. However, the limited number of included studies was so few that the value of funnel plots was limited. Even more, the number of studies made it difficult to make

further analysis. Third, although we attempted to collect all the necessary data from the authors of studies there were still missing data, as some authors failed to respond to our queries. So we utilized a series of formulas to deduce and calculate the mean and SD from the published sample size, median, range, or interquartile range. These formulas were widely acknowledged and used in other meta-analysis.[117,118] Fourth, on the basis of NOS criteria, the differences between two factors, involving gender ratio and mean age, had reduced the comparability between MS patients and controls in a majority of included studies.[14] These limits highlight the need for continued investigations into the NFL levels in MS.

Conclusion

The findings of this meta-analysis have shown that there is elevated nervous system and peripheral blood concentrations of NFL in patients with MS. Therefore, NFL chain in CSF and blood sample can be used to discriminate the MS patients from healthy people. Furthermore, several studies suggest that the NFL in MS patients could predict disease activity and would decrease after treatment. There is promising evidence that NFL levels could be used as a useful prognostic biomarker to monitor disease progression, disease activity, and treatment efficacy in the future.

Author contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Quality scores of included studies

Study	Selection	Comparability	Exposure	Total
Novakova et al, 2017[1]	3	1	3	7
Disanto et al, 2017[2]	3	2	3	8
Haghighi et al, 2004[3]	3	1	3	7
Norgren et al, 2003[4]	3	0	3	6
Rosengren et al, 1996[5]	3	1	3	7
Novakova et al, 2017[6]	3	1	3	7
Novakova et al, 2017[7]	3	1	3	7
Håkansson et al, 2017[8]	3	2	3	8
Novakova et al, 2015[9]	3	1	3	7
Zhang et al, 2007[10]	3	2	3	8
Malmeström et al, 2003[11]	3	1	3	7
Kuhle et al, 2016[12]	3	1	3	7
Disanto et al, 2016[13]	3	2	3	8

Table S2 Results of sensitivity analysis

Including condition	Weighted standard mean difference			Heterogeneity	
	Effect size	**95% CI**	**P-value**	**I^2 (%)**	**P-value**
Sensitivity analysis in CSF					
Novakova et al, 2017[1]	0.98	[0.64, 1.32]	<0.00001	65	0.003
Haghighi et al, 2004[3]	0.88	[0.45, 1.30]	<0.0001	80	<0.00001
Norgren et al, 2003[4]	0.86	[0.46, 1.25]	<0.0001	81	<0.00001
Rosengren et al, 1996[5]	0.85	[0.46, 1.24]	<0.0001	80	<0.0001
Novakova et al, 2017[6]	0.76	[0.41, 1.12]	<0.0001	75	<0.0001
Novakova et al, 2017[7]	0.94	[0.51, 1.37]	<0.0001	81	<0.00001
Håkansson et al, 2017[8]	0.92	[0.50, 1.35]	<0.0001	81	<0.00001
Novakova et al, 2015[9]	0.94	[0.52, 1.35]	<0.0001	81	<0.00001
Zhang et al, 2007[10]	0.93	[0.49, 1.38]	<0.0001	81	<0.0001
Malmeström et al, 2003[11]	0.77	[0.41, 1.12]	<0.0001	73	0.0002
Sensitivity analysis in blood					
Novakova et al, 2017[1]	0.57	[0.37, 0.78]	<0.00001	61	0.05
Disanto et al, 2017[2]	0.42	[0.13, 0.70]	0.004	73	0.01
Disanto et al, 2017[2]	0.5	[0.17, 0.84]	0.003	77	0.005
Kuhle et al, 2016[12]	0.47	[0.20, 0.73]	0.0005	80	0.002
Disanto et al, 2016[13]	0.4	[0.15, 0.66]	0.002	73	0.01

Abbreviation: CSF, cerebrospinal fluid.

Table S3 Results of subgroup analysis

Including condition	Number of groups	Weighted standard mean difference			Heterogeneity	
		Effect size	**95% CI**	*P*-value	*I²* (%)	*P*-value
Subgroup analysis in CSF						
Age						
Age-matched	7	0.89	[0.35, 1.43]	0.001	84	<0.00001
Not age-matched	3	0.85	[0.50, 1.20]	<0.00001	27	0.25
NFL concentration						
Cutoff <1,000 ng/L	5	0.64	[0.20, 1.07]	0.004	75	0.003
Cutoff >1,000 ng/L	5	1.16	[0.51, 1.82]	0.0005	79	0.0009
Publication year						
Before 2010	5	1.11	[0.68, 1.54]	<0.00001	58	0.05
After 2010	5	0.65	[0.13, 1.17]	0.01	80	0.0005
Subgroup analysis in blood						
Sample size						
Less than 200	3	0.68	[0.52, 0.85]	<0.00001	0	0.78
More than 200	2	0.23	[−0.12, 0.59]	0.19	74	0.05

Note: Sub-group analysis stratified by sample size for multiple sclerosis in blood (less than 200 and more than 200).
Abbreviation: CSF, cerebrospinal fluid.

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