Systematic Review and Meta-Analysis

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# Neurofilament light chain in cerebrospinal fluid or blood as a biomarker for mild cognitive impairment

## A systematic review and meta-analysis

Jing Zhang, MM[a], Hongjiang Cheng, MM[b], Wei Liu, MM[b], Huimin Li, MM[b], Yi Song, MM[a], Longbin Jia, MM[b,*]

**Abstract**

**Background:** To allow early diagnosis and monitoring of disease progression, there is a need for biomarkers in mild cognitive impairment (MCI). Neurofilament light chain (NfL) is emerging protein biomarkers in neurodegenerative diseases and is of possible use in MCI. We aimed to assess the utility of NfL in blood and cerebrospinal fluid (CSF) as a biomarker in patients with MCI.

**Methods:** A systematic search with comparison of NfL level between individuals with MCI and healthy controls were retrieved from PubMed, Embase, and Web of Science. The standard mean difference and 95% confidence interval were calculated using the random-effect model to analyze the differentiation of NfL between patients and controls.

**Results:** A total of 7 studies were included. NfL was higher in 676 MCI than 504 healthy controls. Subgroup analysis according to sample type indicated that differentiation of NfL in CSF between patients with MCI and controls showed significant results but in blood. Moreover, the NfL increasing still existed when the NfL expression level was detected by enzyme-linked immunosorbent assay but single molecule array assay. However, no difference of NfL in MCI between Caucasian and Asian was found.

**Conclusions:** NfL expression level in CSF was increased in MCI individuals, which indicated that NfL in CSF could be a potential biomarker of MCI.

**Abbreviations:** CI = confidence interval, CSF = cerebrospinal fluid, ECL= electrochemiluminescence, ELISA = enzyme-linked immunosorbent assay, HCs = healthy controls, MCI = mild cognitive impairment, MMSE = Mini-Mental State Examination, NfL = neurofilament light chain, NOS = Newcastle-Ottawa Scale, SD = standard deviation, Simoa = single molecule array, SMD = standard mean difference, sNfL= Serum neurofilament light chain.

**Keywords:** biomarkers, blood, cerebrospinal fluid, meta-analysis, mild cognitive impairment, neurofilament light chain

## 1. Introduction

Dementia is a highly prevailing cognitive dysfunction, affecting patient's quality of life significantly. However, it is challenging and frustrating to develop effective treatment strategy for halting or even reversing the disease's progression.[1] As the symptomatic pre-dementia phase on the continuum of cognitive decline, characterized by objective impairment in cognition, which is not sufficiently severe to require help with daily living's normal activities, mild cognitive impairment (MCI) is defined.[2,3] In order to earlier diagnosis and interference, the bulk of MCI was performed in recent years hoping to avoid the progressing from MCI to dementia. An effective biomarker monitoring cognitive dysfunction would be described for the diagnosis, progression, and therapeutic effect of MCI.

Neurofilaments are neuronal-specific intermediate filaments determining the axonal caliber, which in turn partially decides the conduction velocity along the axon.[4] Neurofilaments are formed by the neurofilament light chain (NfL) apart from the heavy and medium protein counterparts,[4] which is one of the major cytoskeletal components in mature neurons.[5]

Under normal conditions,[6] low levels of NfL are constantly released from axons, probably in an age-dependent manner, with higher levels of NfL being released at older ages.[7] Nevertheless, in response to central nervous system axonal damage due to

Editor: Luis Manuel Castillo.

This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on August 25, 2021 and was last updated on August 25, 2021 (registration number INPLASY202180101).

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

[a] Changzhi Medical College, Changzhi, China, [b] Jincheng People's Hospital Affiliated to Shanxi Medical University, Jincheng, China.

[*] Correspondence: Longbin Jia, Jincheng People's Hospital Affiliated to Shanxi Medical University, Jincheng 048000, China (e-mail: sxjcjlb@163.com).



How to cite this article: Zhang J, Cheng H, Liu W, Li H, Song Y, Jia L. Neurofilament light chain in cerebrospinal fluid or blood as a biomarker for mild cognitive impairment: a systematic review and meta-analysis. Medicine 2022;101:9(e28932).

Received: 8 September 2021 / Received in final form: 6 February 2022 / Accepted: 8 February 2022

http://dx.doi.org/10.1097/MD.0000000000028932

inflammatory, neurodegenerative, traumatic, or vascular injury, NfL's release sharply rises.[7] Over the last 2 decades, cerebrospinal fluid (CSF) and blood NfL have been shown to be reliable biomarkers of axonal damage across a variety of neurological disorders.[7]

Conclusions had been drawn by previous research as to NfL may be not an indicator of MCI.[8] However, due to a limitation in the method of measurement, the sensitivity of NfL was unreliable.[8] Until the most recent 5-year, the number of studies on NfL of MCI has been risen owing to the more sensitive assay. A comprehensive meta-analysis is warranted to evaluate NfL performance in the diagnosis and intervention of MCI.

Therefore, the literature for studies on NfL in CSF and blood were searched, in which comparisons between patients with MCI and controls were conducted to analyze NfL performance as a biomarker for MCI.

## 2. Methods

### 2.1. Search strategy

PubMed, Web of Science, and Embase were searched by 2 investigators for studies published before May 1, 2021, with NfL level comparison between individuals with MCI and controls. MCI, NfL, CSF, and blood were the key words for searching the data. We also examined the references lists from relevant articles for additional sources.

### 2.2. Inclusion criteria and study selection

Two investigators independently evaluated the titles and abstracts of the identified articles to decide whether they met the study criteria. Differences were solved by consensus. The specific inclusion criteria were as follows:

1. study design: published case-control studies,
2. the definitions for MCI and controls were adequate,
3. NfL was detected in CSF, serum, or plasma in subjects with MCI and the control group,
4. published in English.

Articles were excluded if they met the following criteria:

1. duplicate articles,
2. case reports, meta-analysis, or review articles,
3. animal model or cell line research,
4. studies only had a neurodegenerative disease cohort or a control cohort, or did not have comparison,
5. the controls had other neurological, psychiatric, or physical diseases which would confound the results,
6. studies without sufficient data to allow for the extraction of NfL expression levels in MCI patients and controls,
7. two independent reviewers identified the titles and abstracts of literature, and the studies considered irrelevant were excluded (Fig. 1).

### 2.3. Data extraction and quality assessment

Information, including basic study information (first author and year of publication), ethnicity, numbers of patients and controls, age of participants, female ratio, sample type, detection method of NfL, and the concentration of NfL, was extracted from these articles. Any inconsistencies were solved by other researchers until a consensus was reached.

The study quality of the included studies was evaluated according to the Newcastle-Ottawa Scale scoring from 0 to 9, and a higher score stands for better quality. The assessment process was individually performed by 2 researchers, too.

### 2.4. Statistical analysis

Comprehensive meta-analysis software V 2.0 (Biostat) was adopted to perform the final data combination and meta-analysis. Standard mean deviation and 95% confidence intervals

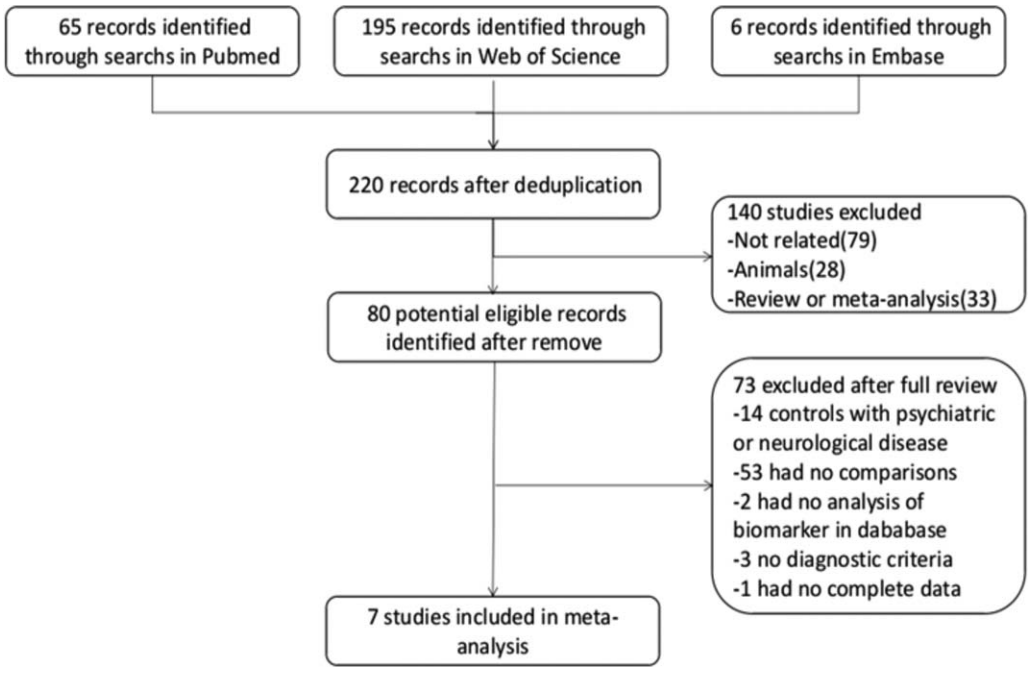


**Figure 1.** Flow diagram of studies search and selection process.

(CIs) were calculated for each group in each study. When the mean value, standard deviation (SD), correlation coefficient, and size of cohort were not available, a series of formulas, as described by Hozo et al and Wan et al,[9,10] was utilized to estimate the sample mean and SD from the published sample size, median, range, or inter-quartile range. If only subgroup values were available from the datasets provided, means were combined and the SDs were pooled to get the cohort mean and SD.[11]

The individual means and SD were analyzed in random effect models to estimate standard mean differences (SMDs) in NfL level between comparators (with 95% CI, and corresponding *P* value). Fisher Z test was used to combine the overall effect based on the correlation coefficient and sample size. *P* value less than .05 was considered as significant. If the $I^2$ statistics of the heterogeneity of the studies was less than 40%, the fixed effect meta-analysis model was chosen. If the $I^2$ statistics was more than 50%, the random effect model was applied.[12]

The results of the meta-analysis of NfL concentration difference between the MCI group and the control groups are illustrated as forest plots that show the SMD between the 2 groups. Subgroup analysis was conducted according to ethnicity, sample style, and NfL analysis methods.

### 2.5. Ethical statements

No ethical approval is required since this is a literature-based study.

## 3. Results

Sixty-five studies were retrieved from PubMed, 195 studies were from Web of Science, and 6 studies were from Embase. Then 140 of these studies were removed after reviewing the titles and abstracts. Finally, 80 full-text articles were assessed for eligibility, and 7 articles were included in the systematic review and meta-analyses (Fig. 1). The characteristics of all studies included in the meta-analysis were presented in Table 1. All articles were of medium quality.

A random-effects model was used because of the high heterogeneity between the studies ($I^2 = 84\%$). When compared with healthy controls (HCs) (Fig. 2), higher levels of NfL were noted in patients with MCI, which reached statistical significance (SMD = 0.36, 95%CI: [0.04,0.68], $P = .03$).

Subgroup analysis according to ethnicity demonstrated that NfL increasing was not significantly associated with MCI in Caucasian population (SMD = 0.37, 95%CI: [−0.02,0.75], $I^2 = 86\%$, $P = .06$), shown as Figure 3. In addition to ethnicity, the association between NfL and MCI was analyzed according to the sample types in this meta-analysis (Fig. 4). Four comparisons found that higher CSF NfL expression levels in MCI than HC (SMD = 0.61, 95%CI: [0.21,1.01], $I^2 = 85\%$, $P < .01$). However, 2 comparisons in plasma samples found that there was no association between NfL expression levels in MCI cases and HC subjects (SMD = 0.10, 95%CI: [−0.36,0.56], $I^2 = 73\%$, $P = .67$).

In the recent times, single molecule array (Simoa) technology, as a novel, highly effective detection method, had been employed in the detection of NfL concentration. Therefore, we analyzed the

**Table 1**

**Characteristics of the studies included in this meta-analysis.**

| First author | Year | Ethnicity | Cases subgroup | Age, yrs Cases | Sex, M/F Cases | Method of NfL analysis | Sample | Cases, NfL of mean | Cases, NfL of SD | Controls, NfL of mean | Controls, NfL of SD | NOS scores |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A. Wallin | 2011 | Caucasian | Not progressive MCI | 62.9 ± 7.6 | 71/90 | ELISA | CSF | 268.2 | 73 | 311 | 442.9 | 6 |
| | | | Progressive MCI | 60.9 ± 6.8 | 8/11 | ELISA | CSF | 252.1 | 7.1 | 311 | 442.9 | 6 |
| | | | Converting MCI | 65.7 ± 7.6 | 27/39 | ELISA | CSF | 584.4 | 1,133.00 | 311 | 442.9 | 6 |
| Harald Hampel | 2018 | Caucasian | — | 70.5 ± 7.6 | 27/14 | ELISA | CSF | 1212 | 748.2 | 634.5 | 204.3 | 7 |
| Yung-Shuan Lin | 2018 | Asian | — | 76.0 ± 5.6 | 27/56 | Simoa | Plasma | 20 | 7.3 | 17.8 | 6.4 | 8 |
| Petra Steinacker | 2018 | Caucasian | — | 63.1 ± 9.3 | 13/4 | Simoa | Serum | 16.6 | 8.1 | 21.7 | 20.6 | 8 |
| Bob Olsson | 2019 | Caucasian | — | 71.5 ± 9.1 | 56/58 | ELISA | CSF | 809.5 | 412.2 | 572.3 | 286.4 | 7 |
| Alberto Lleo | 2019 | Caucasian | — | 67 ± 8.4 | 79/49 | ELISA | CSF | 1019 | 736 | 584 | 314 | 7 |
| Elisabeth H. Thijssen | 2020 | Caucasian | — | 60.8 ± 14 | 26/21 | Simoa | Plasma | 14 | 8 | 15.2 | 8 | 7 |

CSF = cerebrospinal fluid, ELISA = enzyme-linked immunosorbent assay, MCI = mild cognitive impairment, NfL = neurofilament light chain, SD = standard deviation, Simoa = single molecule array.

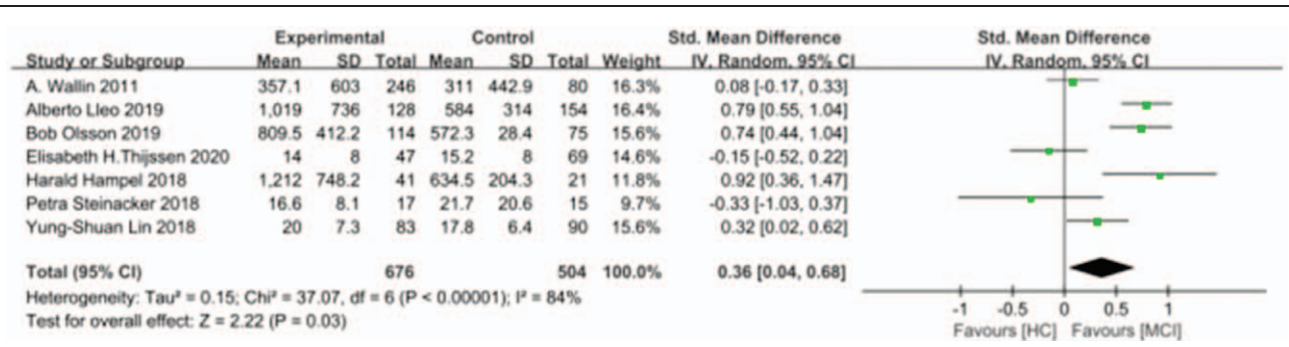


**Figure 2.** Forest plots of the association between NfL expression level and MCI individuals: overall analysis. For each study, the estimate of mean NfL level difference and its 95% confidence interval (95% CI) is plotted with a diamond. $Chi^2$ = chi-squared statistic, df = degrees of freedom, $I^2$ = I-squared heterogeneity statistic, IV = inverse variance, MCI = mild cognitive impairment, NfL = neurofilament light chain, SMD = standard mean difference, Z = Z-statistic.

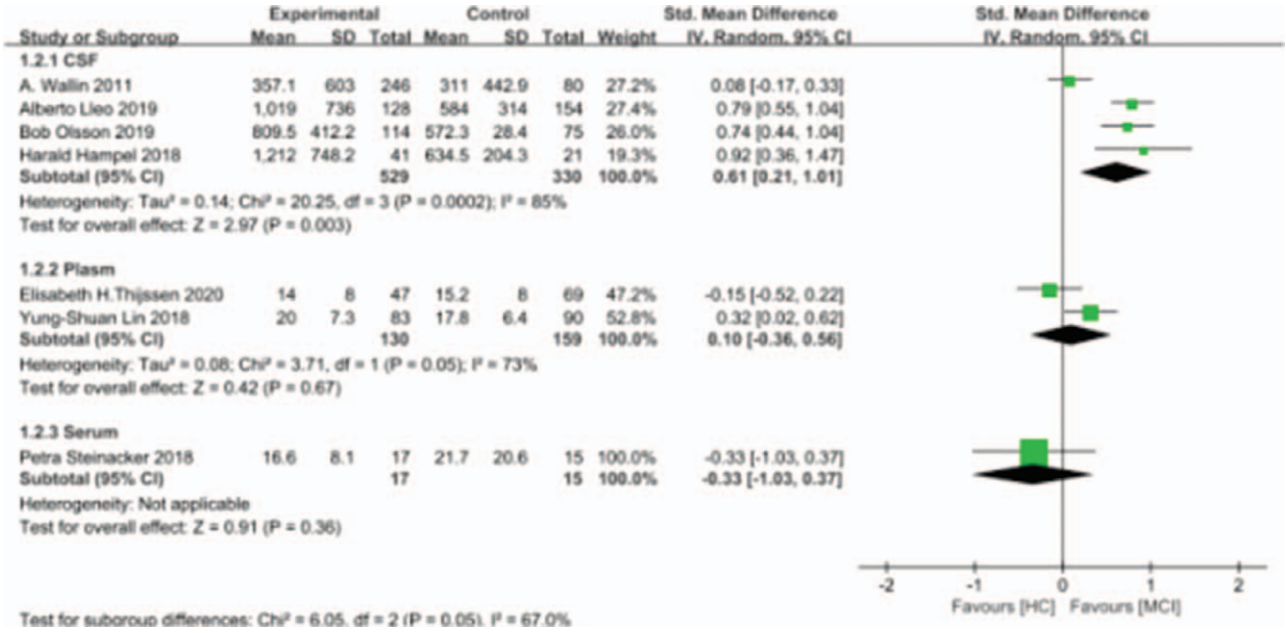


**Figure 3.** Forest plots of the association between NfL expression level and MCI individuals, subgroup analysis according to sample types. $Chi^2$ = chi-squared statistic, df = degrees of freedom, $I^2$ = I-squared heterogeneity statistic, IV = inverse variance, MCI = mild cognitive impairment, NfL = neurofilament light chain, SMD = standard mean difference, Z = Z-statistic.

association between NfL and MCI according to enzyme-linked immunosorbent assay (ELISA) and Simoa detection method. As shown in Figure 5, 4 comparisons used the ELISA method (SMD = 0.61, 95%CI: [0.21,1.01], $I^2 = 85\%$, $P < .01$) and 3 comparisons used the Simoa assay (SMD = 0.01, 95%CI: [−0.38,0.41], $I^2 = 62\%$, $P = .95$). There was a significant NfL increasing in MCI subjects in ELISA but Simoa assay method.

## 4. Discussion

The difference was evaluated in this meta-analysis in NfL concentration between individuals with MCI and controls to assess the possibility of NfL as a biomarker. Compared with HCs, MCI was associated with a significant rise of NfL expression level. Notably, subgroup analysis showed that MCI significantly increased the NfL expression level in CSF but in blood. Moreover, MCI significantly increased the NfL expression level with ELISA but with Simoa assay. There was no difference of NfL between Caucasian and Asian.

Two methods, ELISA and Simoa assay, were adopted to detect NfL expression level in CSF and blood. ELISA, although the vast majority of the studies carried out on CSF NfL have used this assay,[7] is mainly restricted to CSF because of its limited sensitivity to measure the small concentrations of NfL in blood.[13]

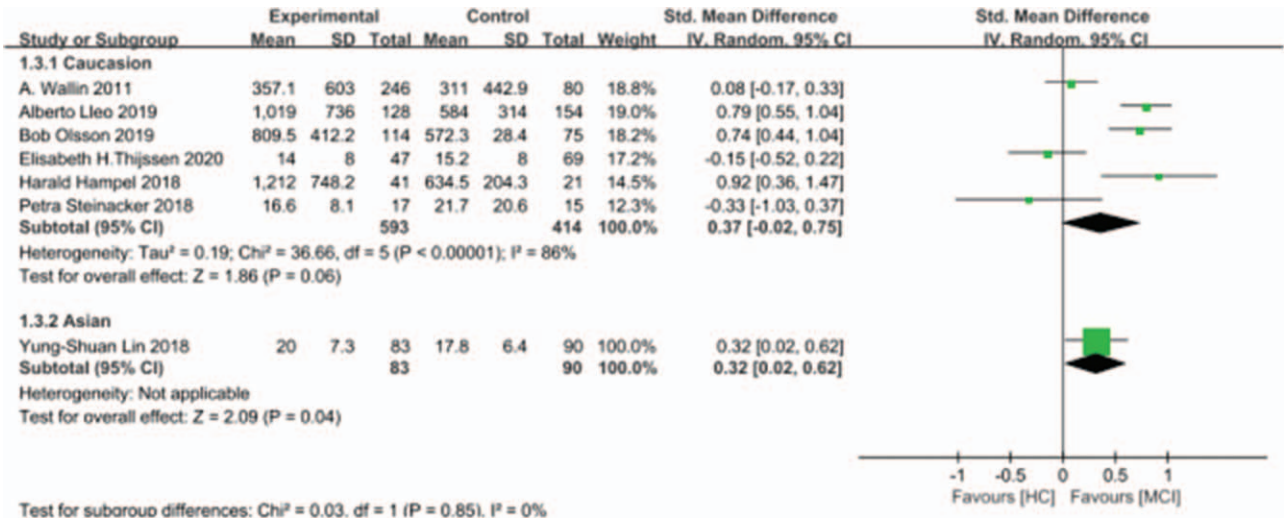


**Figure 4.** Forest plots of the association between NfL expression level and MCI individuals, subgroup analysis according to ethnicity. $Chi^2$ = chi-squared statistic, df = degrees of freedom, $I^2$ = I-squared heterogeneity statistic, IV = inverse variance, MCI = mild cognitive impairment, NfL = neurofilament light chain, SMD = standard mean difference, Z = Z-statistic.

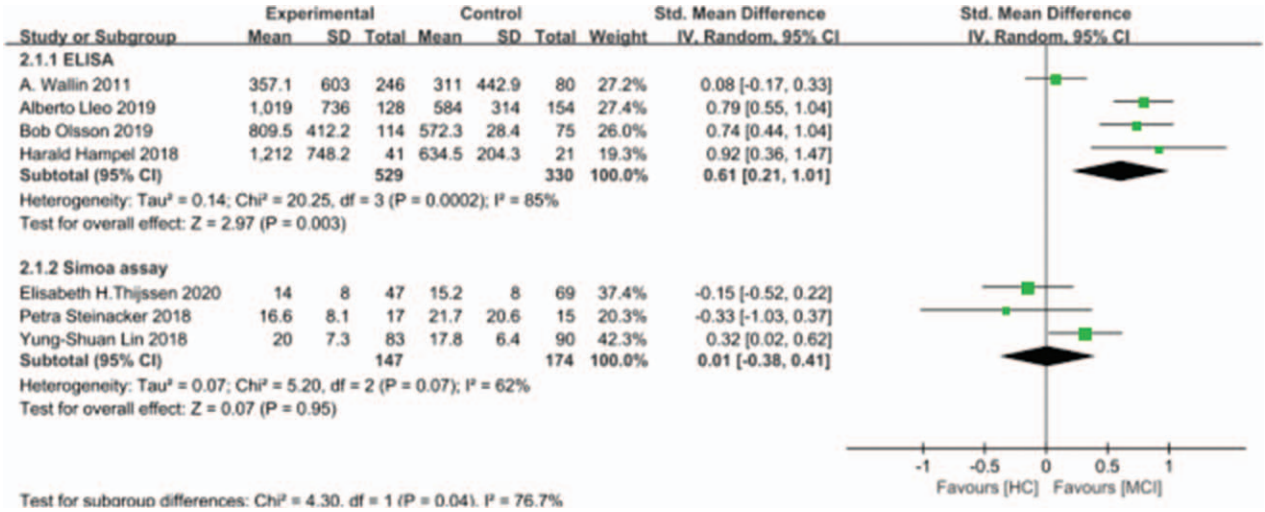


**Figure 5.** Forest plots of the association between NfL expression level and MCI individuals, subgroup analysis according to NfL analysis methods. $Chi^2$ = chi-squared statistic, df = degrees of freedom, I2 = I-squared heterogeneity statistic, IV = inverse variance, MCI = mild cognitive impairment, NfL = neurofilament light chain, SMD = standard mean difference, Z = Z-statistic.

Instead of electrochemiluminescence technique for NfL measurement in 2013,[14] Simoa technology, a new technology based on single-molecule arrays and simultaneous counting of singulated capture microbeads,[7] was introduced for NfL measurement in blood in 2016 owning to it had sharply increased the sensitivity for NfL measurement in blood and had allowed a reliable quantification in blood samples from young HCs.[15] In our meta-analysis, the NfL concentration significantly increased with ELISA but with Simoa assay.

NfL is a subunit of neurofilaments, which confer structural stability to neurons.[6] The NfL released reaches the interstitial fluid upon neuroaxonal impairment, such as inflammatory, neurodegenerative, traumatic, or vascular injury, which communicates freely with the blood and the CSF.[7] Data on NfL in MCI, however, are still limited. After a systematical review of the published literature, our study provided further evidence to support NfL in CSF as a predictive biomarker of MCI, which is accord with a recent study.[16] Previous studies have found that serum neurofilament light chain was correlated to CSF NfL levels,[17] yet NfL in blood may be not a biomarker of MCI in our study and more studies on blood NfL are needed in future.

The underlying pathophysiology linking NfL to cognitive dysfunction is unclear. Physiologically, cognitive processing speed is dependent on the integrity of long caliber brain fibers, in which NfL is abundantly expressed.[18] Age-related atrophy or reduction in brain metabolism may lead to axonal degeneration and an increase in NfL levels.[19] Recently,[20] 2 studies have discovered that NfL is a sensitive marker for micro-structural brain alterations (e.g., of white matter fiber tracts) that are related for cognitive functioning.[21] Further studies of pathogenesis of MCI are needed.

This is the first systematical review and meta-analysis about the association between NfL and MCI in our knowledge. Consistent with other meta-analysis, comprehensive research was performed for studies investigating the correlation between MCI and NfL. And a meta-analysis was performed to evaluate the role of NfL as a predictor of MCI, after the systematical review of the included studies. Ultimately, a significant result was still achieved despite heterogeneity, where a subgroup analysis was performed to elucidate the heterogeneity and a further sensitivity analysis indicating the conclusion was stable.

However, there are several obvious limitations in the present study. First, the sample sizes meeting standards were small, which would lead to bias for the consequences. Second, categorical grouping of average age, sex, and Mini-Mental State Examination of patients in included studies were not required for further subgroup analysis. Although NfL was proven to be a significant biomarker for MCI, our study failed to identify an optimal cut-off value of NfL for predicting MCI, which still needs to be further confirmed through a series of large-scale case-control research.

In summary, our study further validated NfL is a significant biomarker that distinguishes patients with MCI from controls. Moreover, compared with controls, NfL was especially increasing in CSF but in blood. And MCI significantly increased the NfL expression level with ELISA but with Simoa assay. Given the potential limitations in the study, more large-scale case-control research are needed to identify our findings.

## Author contributions

**Conceptualization:** Jing Zhang, Hongjiang Cheng.
**Data curation:** Yi Song.
**Methodology:** Wei Liu, Huimin Li.
**Validation:** Longbin Jia.
**Writing – original draft:** Jing Zhang, Hongjiang Cheng.
**Writing – review & editing:** Jing Zhang, Hongjiang Cheng.

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Cardiovascular and Interventional Radiology Society of Europe (CIRSE), Canadian Interventional Radiology Association (CIRA), Congress of Neurological Surgeons (CNS), European Society of Minimally Invasive Neurological Therapy (ESMINT), European Society of Neuroradiology (ESNR), European Stroke Organization (ESO), Society for Cardiovascular Angiography and Interventions (SCAI), Society of Interventional Radiology (SIR), Society of NeuroInterventional Surgery (SNIS), and World Stroke Organization (WSO). J Vasc Interv Radiol 2018;29: 441–53.

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