Cerebrospinal fluid kappa free light chains for the diagnosis of multiple sclerosis: A systematic review and meta-analysis

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Abstract

Background: Intrathecal immunoglobulin-G synthesis is a hallmark of multiple sclerosis (MS) which can be detected by oligoclonal IgG bands(OCB) or by κ-free light chains(κ-FLC) in cerebrospinal fluid.

Objective: To perform a systematic review and meta-analysis to evaluate whether κ-FLC index has similar diagnostic value to identify patients with clinically isolated syndrome(CIS) or MS compared to OCB, and to determine κ-FLC index cut-off.

Methods: PubMed was searched for studies that assessed diagnostic sensitivity and specificity of κ-FLC index and OCB to discriminate CIS/MS patients from control subjects. Two reviewers following PRISMA guidelines performed study eligibility assessment and data extraction. Findings from studies were analyzed with bivariate mixed models.

Results: A total of 32 studies were included in the meta-analysis to evaluate diagnostic value of κ-FLC index. Sensitivity and specificity ranged from 52-100%(weighted average:88%) and 69-100%(89%) for κ-FLC index and from 37-100%(85%) and 74-100%(92%) for OCB. Mean difference of sensitivity and specificity between κ-FLC index and OCB was 2 and -4 percentage points. Diagnostic accuracy determined by mixed models revealed no significant difference between κ-FLC index and OCB. A discriminatory cut-off for κ-FLC index was determined at 6.1.

Conclusion: The findings indicate that κ-FLC index has similar diagnostic accuracy in MS as OCB.

Introduction

Cerebrospinal fluid (CSF) analysis is of high importance in the diagnostic work-up of patients with suspected multiple sclerosis (MS) (1). Evidence of intrathecal immunoglobulin G (IgG) synthesis in the CSF, although not specific for MS, substitutes for dissemination in time according to current diagnostic criteria (2) and increases diagnostic certainty in the appropriate clinical setting (3). Currently, the gold standard to prove intrathecal IgG synthesis is the detection of CSF-restricted oligoclonal IgG bands (OCB) (4).

In the last decade, κ-free light chains (κ-FLC) in the CSF have emerged as new biomarker in MS. κ-FLC are secreted by B cells along with intact immunoglobulins and accumulate in the CSF in case of chronic intrathecal inflammation (5). In contrast to OCB, determination of κ-FLC has considerable advantages. First, κ-FLC are measured by nephelometry or turbidimetry, which are easy, reliable, labour-saving, and cost-effective methods. Second, the determination of κ-FLC returns a metric and rater-independent result (6,7).

Most studies used the κ-FLC index to prove an intrathecal synthesis and showed its high diagnostic accuracy to discriminate patients with MS from other neurological diseases (8-12). However, a strong consensus on the role of κ-FLC as biomarker in MS is still lacking. This might be due to heterogeneity between published studies ranging from different patient populations included, different assays used, to the different κ-FLC measures (e.g., κ-FLC index versus absolute CSF κ-FLC concentration) and cut-off values applied.

Therefore, we aimed to compare the diagnostic value of κ-FLC index to OCB in patients with clinically isolated syndrome (CIS) and MS and to identify an appropriate cut-off for κ-FLC index. Furthermore, we aimed to elucidate differences to other κ-FLC measures.

Methods

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline (13).

Search Strategy

A comprehensive search of the electronic database PUBMED was performed on February 1, 2022. The search included the following terms: “free light chain” and “multiple sclerosis”. “Multiple sclerosis” was searched as a MeSH Term and keyword, “free light chain” was searched as keyword. The publication date was restricted from January 1, 2000 (prior to that date there were no studies on κ-FLC in CSF as determined by nephelometry or turbidimetry) to February 1, 2022. Only original articles in English were included. Two authors (HH and FD) independently conducted the literature search, i.e., screened titles and abstracts of identified articles after removing duplicates, then independently assessed the full text of potentially relevant articles for inclusion and exclusion criteria. Discrepancies between the two authors were discussed and resolved.

Selection Criteria

Studies were included if they were original articles investigating the diagnostic value of κ-FLC index, the percentage intrathecal κ-FLC fraction ($IF_{\kappa\text{-FLC}}$), CSF κ-FLC concentration or κ-FLC quotient ($Q_{\kappa\text{-FLC}}$) in patients with CIS or MS compared to any healthy or disease control. Definition and calculation of κ-FLC index, $IF_{\kappa\text{-FLC}}$ or $Q_{\kappa\text{-FLC}}$ are provided in the supplemental material.

Patients of any age were included, with no restrictions on MS disease course, disease duration, disability, comorbidities, or treatment. Diagnosis of CIS or MS should be stated with referring to the established diagnostic criteria (2,14-16). Only studies using immunonephelometry or immunoturbidimetry to determine κ-FLC concentrations in paired CSF and serum/ plasma samples, or in the CSF only were included. When patient populations overlapped in several articles, only the one with the most complete information was included. Studies could be retrospective or prospective.

Data extraction

Data extraction forms were created. Data were extracted from selected articles independently in duplicate (HH and FD). Disagreements were resolved by consensus and if needed with another author (JW).

The following data were extracted: the first author, publication year, number of patients per disease group (i.e. CIS or MS, control group), type of samples collected (CSF, serum or plasma), method used for κ-FLC detection (principal method [nephelometry, turbidimetry], assay kit [Freelite, N Latex], and the platform, as appropriate), diagnosis and used diagnostic criteria of CIS/ MS patients, allocation of controls to one of predefined control groups (non-inflammatory neurological disease control [NINDC], inflammatory neurological disease control [INDC], peripheral inflammatory neurological disease control [PINDC], symptomatic control [SC], healthy control [HC] (17) and non-neurological disease control [NNDC]), corticosteroid treatment prior to sample collection in CIS/ MS patients, disease-modifying treatment at the time of sample collection in CIS/ MS patients, number of positive OCB test results (pattern II or pattern III) (4) in the CIS/ MS patients, number of negative OCB test results in the control subjects, number of positive test results for κ-FLC index, $IF_{κ\text{-}FLC}$, CSF κ-FLC concentration or $Q_{κ\text{-}FLC}$ in the CIS/ MS patients, number of negative test results for κ-FLC index, $IF_{κ\text{-}FLC}$, CSF κ-FLC concentration or $Q_{κ\text{-}FLC}$ in the control subjects, the applied cut-off values to define test positivity. If the number of positively or negatively tested patients and controls, respectively, was not available, the reported diagnostic sensitivity and specificity were used to back-calculate this number.

Statistical analysis

Studies with data available of diagnostic sensitivity and specificity of κ-FLC index, $IF_{κ\text{-}FLC}$, CSF κ-FLC concentration or $Q_{κ\text{-}FLC}$ to discriminate CIS or MS patients from controls were included in the quantitative meta-analysis.

Both sensitivity and specificity of each κ-FLC measure were compared to sensitivity and specificity of OCB used within the same study thereby holding the within study conditions for both parameters constant (e.g., characteristics of CIS/ MS patients and control subjects, administration of prior immune treatment). Findings are presented in forest plots separately for sensitivity and specificity. The magnitude of heterogeneity was assessed by Higgins/Thompson's I^2, which is an estimate of the variability across studies based on

heterogeneity rather than chance. I^2 ranges from 0 to 100% and low, moderate and high heterogeneity are indicated by I^2 values below 25%, 50% and 75%, respectively (18).

To consider simultaneously within study variation, between study variation and the degree of correlation between sensitivity and specificity because of the chosen cut-off point, a bivariate mixed model was employed (19). Using REML (restricted maximum likelihood) for estimation, the estimates of sensitivity and specificity and their 95% elliptical confidence interval (CI) were used to compare the accuracy of each κ-FLC measure with OCB. To ensure the validity of our meta-analysis, we did an outlier diagnostic. The estimated bivariate distribution was used to show summary receiver operating curves (sROC). The findings were checked for robustness by splitting the studies according to their different patients and control groups and performing the corresponding sub-analyses.

A power analysis was conducted (20) to investigate whether sample size was sufficient to interpret statistically non-significant findings. A significance level of 5% and the number of studies included in the meta-analysis were used. A large between study heterogeneity was assumed. A difference in sensitivity and specificity of 5% was regarded as substantial.

Cut-off values for the discrimination between CIS/ MS patients and control subjects were determined for the κ-FLC index and the CSF κ-FLC concentration. Bivariate confidence intervals of sensitivity and specificity for each of these two κ-FLC measure were computed at the 99% confidence levels. The weighted average over all cut-offs from the studies in this confidence interval was calculated. The weighting was based on the sample size of the studies. A two-sided significance level of 5% was considered statistically significant. R software (21) and the package mada (22) were used for all analyses.

Results

The search strategy identified 234 references (Figure 1). After removing duplicate records, 101 references were screened for potential relevance through titles and abstracts. This process yielded 66 potentially eligible studies that underwent full-text eligibility review. Of these, 38 studies were included in the systematic review (8-12,23-56). Thirty-two studies addressed the diagnostic value of κ-FLC index, 13 studies of $IF_{\kappa\text{-FLC}}$, 9 studies of CSF κ-FLC

concentration and 3 studies of $Q_{\kappa\text{-FLC}}$; 15 studies addressed more than one of these parameters.

κ-FLC index vs. OCB

A total of 32 studies addressed the diagnostic accuracy of κ-FLC index including 3322 patients with CIS/ MS and 5849 controls. All studies reported significantly elevated κ-FLC index in CIS/ MS patients compared to controls. Diagnostic sensitivity and specificity ranged from 52-100% (weighted average: 88%) and 69-100% (89%) for κ-FLC index and from 37-100% (85%) and 74-100% (92%) for OCB.

Studies differed with regard to demographics, clinical characteristics and laboratory methods. While 22 studies included distinct cohorts of MS patients and 8 studies patients with CIS, 8 studies analyzed mixed cohorts comprising both patients with CIS and MS. Twenty-four (75%) of 32 studies applied either the 2010 or 2017 revised McDonald criteria in CIS/ MS patients, 3 studies used earlier diagnostic criteria and 5 studies did not specify the applied criteria. Nephelometry was applied in 22 (69%) studies and turbidimetry in 9 (28%) studies; 16 (50%) studies used the Freelite assay and 15 studies (47%) the N Latex assay. One study (3%) applied different type of platform and assay in the patient and control group. Cut-off values of the κ-FLC index denoting test positivity ranged from 2.4 to 20.0. For further details on each study characteristics, we refer to Table S1.

First, we performed power analysis for a bivariate mixed model to ensure a valid interpretation for not statistically significant differences. For that, we used a significance level of 5%, a sample size of 32 studies, the studies within variance, assumed a large between heterogeneity and chose a 5% difference in sensitivity or specificity between κ-FLC index and OCB as important to detect (e.g., OCB 90% and κ-FLC index 85%). Therewith we obtained a power of 98.7% for sensitivity and of 99.9% for specificity.

Forest plots were used to visualize sensitivities and specificities and to get an overview of between study heterogeneity. They showed mostly overlapping confidence intervals and revealed low to moderate between-study heterogeneity (I^2=29.5%; [95% CI]: 0, 55.0%; Figure 2).

Mean difference of diagnostic sensitivity between κ-FLC index and OCB was 2 percentage point (pp) and -4 pp of specificity. The estimated bivariate mixed model assessed no statistically significant difference between κ-FLC index and OCB for the accuracy to discriminate CIS and MS patients from controls (Figure 3, Table S5). In addition, we evaluated a possible impact of the type of assay on the diagnostic sensitivity and specificity of κ-FLC index and observed a statistically significant lower sensitivity with the Freelite assay ($p<0.001$, Table S6). Further analysis comparing the accuracy of κ-FLC index and OCB controlling for the type of assay and excluding mixed cohorts of CIS/ MS patients (Table S7) showed that in the group of Freelite assay not only sensitivity of κ-FLC index was lowered, but also of OCB. This implies that not the type of assay, but another confounding factor is responsible for this observation. Indeed, studies using the Freelite assay included more frequently patients with CIS (5 of 13 studies), while studies using the N Latex assay were done with MS patients mainly (8 of 10 studies). The bivariate model analyzing the diagnostic accuracy of κ-FLC index and OCB controlling for the type of disease (CIS vs. MS) confirmed that patients with CIS showed a lower sensitivity than patients with MS for the κ-FLC index, but also for OCB (Table S8).

In analogy, we investigated the possible impact of the platform (nephelometry or turbidimetry) on the diagnostic sensitivity and specificity of κ-FLC index and observed at first a statistically significantly lower sensitivity for turbidimetry (Table S9). In the subgroup turbidimetry as well as in the subgroup nephelometry sensitivity of κ-FLC index and OCB did not differ, thus, a potential impact of the platform could be excluded (Table S10).

To further investigate the impact of different patient (MS, CIS, mixed CIS/MS) and control groups (non-inflammatory diseases, inflammatory and/ or non-inflammatory diseases), subgroup analyses were performed. This robustness check revealed consistent findings for all subgroups (Figure S1 and S2).

A cut-off for κ-FLC index at 6.1 was determined to discriminate CIS/MS patients from non-inflammatory disease controls (Figure S3).

Intrathecal κ-FLC fraction vs. OCB

The diagnostic accuracy of $IF_{\kappa\text{-FLC}}$ was addressed by 13 studies including 1428 CIS/ MS patients and 3299 controls. All studies reported significantly elevated $IF_{\kappa\text{-FLC}}$ in patients with CIS/ MS compared to controls. $IF_{\kappa\text{-FLC}}$ showed a diagnostic sensitivity ranging from 66-100% (weighted average: 93%) and a specificity from 53-100% (84%). In comparison, OCB had a diagnostic sensitivity of 57-97% (89%) and a specificity of 74-100% (91%).

Study characteristics concerning demographics, clinical variables and laboratory methods are detailed in Table S2. A total of 9 studies included MS patients, 3 studies CIS patients and 4 studies analyzed mixed cohorts comprising both CIS and MS patients. Eleven (85%) of 13 studies applied either the 2010 or 2017 revised McDonald criteria in CIS/ MS patients. Nephelometry was used in 11 (85%) studies, turbidimetry in one (8%) study. Four (31%) studies used the Freelite assay, while 8 studies (62%) the N Latex assay. One study applied different type of platform and assay in the patient and control group. Studies applied different formulae for the definition of the cut-off (i.e. the $Q_{lim\ \kappa\text{-FLC}}$): 6 (46%) studies applied the formula by Reiber et al (45), 5 by Presslauer et al (57), and 1 study by Senel et al (11).

Forest plots of sensitivity and specificity are shown in Figure S4 and revealed a high between-study homogeneity (I^2=5.9%; [95% CI]: 0, 57.8%).

Diagnostic sensitivity between $IF_{\kappa\text{-FLC}}$ and OCB differed on average by 4 pp and specificity by -8 pp. The diagnostic accuracy as determined by the mixed model revealed no difference between $IF_{\kappa\text{-FLC}}$ and OCB to discriminate CIS and MS patients from controls (Figure S5). We also considered different formulae (Presslauer versus Reiber formula) in the model, but did not find evidence for an impact on diagnostic sensitivity and specificity. However, the calculated power for the model was smaller than 80% due to the small number of studies.

CSF κ-FLC concentration vs. OCB

A total of 10 studies addressed the value of CSF κ-FLC including 901 patients with CIS/ MS and 2251 controls. All studies reported significantly elevated CSF κ-FLC concentration in patients with MS compared to controls. CSF κ-FLC concentration showed a diagnostic sensitivity ranging from 66-96% (weighted average: 84%) and a specificity from 70-100%

(87%). In comparison, OCB had a diagnostic sensitivity of 57-100% (86%) and a specificity of 72-100% (88%).

Seven studies included distinct groups of MS patients, 3 studies patients with CIS, while 3 studies analyzed mixed cohorts. In all studies, either the 2010 or 2017 revised McDonald criteria were applied for CIS/ MS patients. Nephelometry was used in 8 (80%) studies and turbidimetry in the remaining 2 (20%) studies; half of the studies used the Freelite assay, whereas the other half the N Latex assay. Cut-off values for the CSF κ-FLC concentration test positivity ranged from 0.3 to 7.1 mg/l. Detailed study characteristics are shown in Table S3.

Forest plots of sensitivity and specificity are provided in Figure S6. They show a low to moderate between-study heterogeneity (I^2=28.7%; [95% CI]: 0, 63.2%).

Mean difference of diagnostic sensitivity between CSF κ-FLC index and OCB was 0 pp and of specificity -3 pp. Diagnostic accuracy between CSF κ-FLC concentration and OCB to discriminate CIS/MS patients from controls was similar (Figure S7). A cut-off for CSF κ-FLC concentration of 0.96 mg/l to discriminate CIS/MS patients from controls was observed (Figure S8). However, the calculated power was smaller than 80% due to the small number of studies.

κ-FLC quotient vs. OCB

Two studies including MS patients and one study with a cohort of CIS and MS patients investigated the diagnostic accuracy of $Q_{\kappa\text{-FLC}}$. These studies included a total of 256 CIS/ MS patients and 1249 controls. Study characteristics are given in Table S4. Overall, sensitivity of $Q_{\kappa\text{-FLC}}$ ranged from 92-94% (weighted average: 93%) and specificity from 74-96% (95%), while OCB showed a sensitivity of 91-100% (96%) and a specificity of 93-100% (94%) in these studies. Mean difference of diagnostic sensitivity between $Q_{\kappa\text{-FLC}}$ index and OCB was 3 pp and of specificity -8 pp. Forest plots of sensitivity and specificity are shown in Figure S9.

Comparisons between different κ-FLC measures

Studies that applied different κ-FLC measures on the same patient cohort were eligible: 7 studies compared κ-FLC index with CSF κ-FLC concentration, 4 studies $IF_{κ\text{-}FLC}$ with CSF κ-FLC concentration and 11 studies compared κ-FLC index with $IF_{κ\text{-}FLC}$. Diagnostic accuracy between all three κ-FLC measures was similar, however, the statistical power for the comparison with the most employed studies was already less than 80% (Figure S10).

Discussion

This systematic review and meta-analysis provides evidence that the determination of intrathecal κ-FLC shows a high diagnostic accuracy to discriminate patients with CIS and MS from other neurological diseases. All approaches to capture intrathecal κ-FLC – including the κ-FLC index, the $IF_{κ\text{-}FLC}$, the $Q_{κ\text{-}FLC}$ and the absolute CSF κ-FLC concentration – showed comparable performance, which was equal to OCB testing. With high statistical power of 99%, significant evidence exists just for κ-FLC index with 32 studies performed on approximately 3300 CIS/ MS patients and 5800 control subjects.

κ-FLC in the CSF– similar to immunoglobulins or other proteins – originate either from blood by diffusion across the blood-CSF-barrier, or are produced within the intrathecal compartment under pathological conditions (58). Conceptually, it seems necessary to determine the locally synthesized κ-FLC fraction separate from the blood-derived fraction (as it is also done for IgG) to prove intrathecal B cell activity. Therefore, the majority of studies used the κ-FLC index (8-12,23-44,46-48,50,51) or the $IF_{κ\text{-}FLC}$ (9-11,33-36,38,41,49,51-53). Both approaches consider the albumin quotient (Q_{alb}) which is an established marker of the blood-CSF-barrier function (59) and correct for the absolute serum κ-FLC concentration. Few studies used the $Q_{κ\text{-}FLC}$ (11,32,34,44). Other authors determined the absolute CSF κ-FLC concentrations only (10,29,34,36,38,44,50,54-56). As the intrathecal κ-FLC fraction is greater than 80% in most CIS/ MS patients (9,45), one might argue that the contribution of blood-derived κ-FLC to the total CSF κ-FLC concentration is negligible in cases with intrathecal synthesis. In the present meta-analysis, we did not find a statistically significant difference in the diagnostic performance between both κ-FLC index and $IF_{κ\text{-}FLC}$ compared to CSF κ-FLC

concentration. However, the statistical power was below 80% and, thus, insufficient to interpret not statistically significant results with a small enough Type II error. This means that superiority of, e.g., κ-FLC index over CSF κ-FLC concentration (or even vice versa) cannot be excluded. Two studies further elaborated this research question. One study separated patients into low and high CSF κ-FLC categories (based on median values) and observed that CSF κ-FLC concentration, $Q_{\kappa\text{-FLC}}$ and κ-FLC index showed similar diagnostic performance in the high category, but not in the low category with inferiority of CSF κ-FLC and to some extent also of $Q_{\kappa\text{-FLC}}$ (60). Thus, the impact of serum κ-FLC and Q_{alb} is indeed negligible in patients with high intrathecal κ-FLC synthesis, but probably not in patients with only low or modest intrathecal κ-FLC production. This might be of importance in CIS patients who showed lower diagnostic sensitivity and lower amount of intrathecal κ-FLC (9). Another very recent large multicenter study including more than 1600 patients confirmed that κ-FLC index and $IF_{\kappa\text{-FLC}}$ performed better than absolute CSF κ-FLC concentration (ref). Anyway, further studies are required to compare the different κ-FLC measures in patients with varying degree of intrathecal B cell activity and varying blood-CSF-barrier function.

Different cut-off values for κ-FLC index, for CSF κ-FLC concentration, as well as different formulae defining the $Q_{lim\ \kappa\text{-FLC}}$ (Presslauer (57), Reiber (45), Senel (11)) for calculating the $IF_{\kappa\text{-FLC}}$ have been published. In general, different cut-off values might apply depending on the clinical question, e.g. to provide an upper reference limit determined in a (non-inflammatory) control population (17) or to differentiate MS from other INDC. Furthermore, cut-off values might vary whether the main aim is to increase diagnostic sensitivity or specificity (61). Here, we observed a discriminatory cut-off for κ-FLC index at 6.1 to differentiate CIS/ MS patients from controls, as well as at 0.96 mg/l for CSF κ-FLC concentration. Even though the cut-off for κ-FLC index (8,9,12,29) as well as for the CSF κ-FLC concentration (29,54) is in line with those identified by several large – partly multicenter – studies, we have to clearly state that this analysis was exploratory. Comparison of studies applying different non-linear formulae (11,45,57) did not reveal a difference, but the power for this analysis was low due to the

small number of studies. So far, there is one study that compared the performance of all three formulae within an independent cohort reporting a diagnostic sensitivity ranging from 96-98% in MS patients and 40-44% in CIS patients (35).

At this point, it has to be stated that studies dealt differently with samples in case of non-detectable CSF κ-FLC concentrations. Some studies used the lower detection limit, while others set these samples to “zero” or even omitted these samples from the statistical analysis. For the absolute CSF κ-FLC concentration, samples treated as “zero” or set to the lower detection limit still means that these samples are in the lower concentration range. Hence, determination of cut-off values is probably not affected, and also the clinical interpretation is clear (i.e. no intrathecal synthesis), as the lower detection limit (e.g., 0.3 mg/l) is by far lower than the cut-off (in this meta-analysis 0.96 mg/l). However, κ-FLC index values depend also on serum κ-FLC concentration and Q_{alb}, so that different handling of non-detectable CSF κ-FLC concentration might indeed lead to considerably varying index values which might then impact on cut-off values. Studies that validate the herein observed cut-offs in a multicenter setting are needed. These studies should consider different handling in case of non-detectable CSF κ-FLC values, and the potential impact of different assays and platforms as well. We did not find a statistically significant impact of assay and platform on κ-FLC index and a recent large multicentre study did also not observe any impact of the platform on κ-FLC index (ref). This might because using the ratio of the CSF and serum κ-FLC concentration (for calculating the index) is probably less prone to laboratory variations. The potentially different susceptibility of κ-FLC index and absolute CSF κ-FLC concentrations to laboratory variation should be further addressed.

Diagnostic sensitivities and specificities as reported by different studies showed a certain variability not only for κ-FLC measures, but also for OCB. This arises from a certain heterogeneity of included patients between studies. It is evident that sensitivity differs whether CIS patients or MS patients are included (62). Specificity is lowered when patients with inflammatory neurological disease (IND) were included into the control group. κ-FLC in the CSF are – similar to CSF-restricted OCB – a sign of intrathecal inflammation and thus can

support the diagnosis of MS, but they are not specific for MS. The spectrum of diseases which show an intrathecal κ-FLC synthesis is probably similar to that with CSF-restricted OCB. κ-FLC synthesis reflects IgG synthesis, but might be present also in case of intrathecal IgA or IgM synthesis. Studies on the frequency of intrathecal κ-FLC synthesis in neurological diseases other than MS are still rare. Apart from a mixture of different IND as part of control populations, dedicated disease-specific studies exists only for a few entities, e.g., neuroborreliosis (63,64). For the present meta-analysis, we applied a model considering not only between study variation, but also within study variation and used only studies using both κ-FLC measures and OCB. Therefore, potential sources of bias were reduced and allowed a reliable comparison of the above-mentioned parameters. Furthermore, robustness of findings was checked by subgroup analyses (different patient groups [CIS, MS, mixed cohorts], different control groups [non-inflammatory and inflammatory/ non-inflammatory], and different assays [Freelite, N Latex] and platforms [nephelometry, turbidimetry]).

There are some limitations of the meta-analysis. The statistical power for κ-FLC measures apart from the κ-FLC index was low, so that firm conclusions on the similar diagnostic performance of $IF_{\kappa\text{-FLC}}$, $Q_{\kappa\text{-FLC}}$, CSF κ-FLC concentration and OCB cannot yet be drawn. Most of the studies did not report how their cut-off values were obtained. This might have an impact on our estimated cut-off values for κ-FLC index and CSF κ-FLC concentration (as discussed above). Another limitation is that the analytic performance of OCB detection probably differed between studies, as different methods were used, e.g. commercial versus in-house assays; and interpretation of results is rater-dependent (ref). It cannot be excluded that OCB would have shown better performance if tested only in few, specialized laboratories. However, it has to be clearly stated that one of the clear advantages of κ-FLC is the reliable and rater-independent determination which should overcome technical difficulties and finally allow a widespread use.

In conclusion, it seems reasonable to consider intrathecal κ-FLC synthesis equally to CSF-restricted OCB, both reaching a diagnostic sensitivity and specificity of approximately 90% without significant differences when meta-analysed. Statistically sufficient power for the

comparisons exists only for κ-FLC index. The potential of κ-FLC in the CSF as new biomarker in MS was clearly demonstrated. Due to considerable methodological advantages as a fast, time- and labour-saving, rater-independent and reliable method, intrathecal κ-FLC synthesis might serve as alternative tool to measure intrathecal immunoglobulin synthesis. A detailed review of the advantages and limitations of κ-FLC and OCB, respectively, and consensus recommendations for implementation of κ-FLC in clinical routine are given in (*co-submitted manuscript*). In future, κ-FLC might be used as a screening test and in certain constellations OCB as a confirmation test, e.g., in case of borderline κ-FLC results, as already implemented by some clinical laboratories (54). Since the best algorithm to determine intrathecal κ-FLC synthesis has to be established and universal cut-off values for different platforms remain to be confirmed, the combination of both tests - intrathecal κ-FLC and OCB - might be the best option at this moment.

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Disclosure of conflicts of interest

HH has participated in meetings sponsored by, received speaker honoraria or travel funding from Bayer, Biogen, Celgene, Merck, Novartis, Sanofi-Genzyme, Siemens, Teva, and received honoraria for acting as consultant for Biogen, Celgene, Novartis and Teva.

JW has nothing to disclose.

GA has received speaking honoraria and compensation for consulting services or participation in advisory boards from Sanofi, Merck, Roche and Horizon Therapeutics; travel funding from Novartis, Roche and ECTRIMS; is the editor for Europe of Multiple Sclerosis Journal – Experimental, Translational and Clinical; and is a member of the International Women in Multiple Sclerosis (iWiMS) network executive committee.

KB has participated in meetings sponsored by, received speaking honoraria or travel funding from Roche, Biogen, Sanofi and Teva.

SG has received speaker honoraria and has been scientific boards from Biogen Idec, Genzyme, Novartis, and Merck and grant funding from Genzyme, Merck and Takeda. BK has nothing to disclose.

MK has received funding for travel and speaker honoraria from Bayer, Novartis, Merck, Biogen Idec and Teva Pharmaceutical Industries Ltd. and serves on scientific advisory boards for Biogen Idec, Merck Serono, Roche, Novartis and Gilead.

RS has nothing to disclose.

CT has a collaboration contract with ADx Neurosciences, Quanterix and Eli Lilly, performed contract research or received grants from AC-Immune, Axon Neurosciences, Bioconnect, Biogen, Bioorchstra, Brainstorm Therapeutics, Celgene, EIP Pharma, Eisai, Novo Nordisk, PeopleBio, Roche, Toyama, Vivoryon. She serves on editorial boards of Medidact Neurologie/Springer, Alzheimer Research and Therapy, Neurology: Neuroimmunology & Neuroinflammation, and is editor of a Neuromethods book Springer. Research of CET is supported by the European Commission (Marie Curie International Training Network, grant agreement No 860197 (MIRIADE), Innovative Medicines Initiatives 3TR (Horizon 2020, grant no 831434)and JPND (bPRIDE), National MS Society (Progressive MS alliance) and Health

Holland, the Dutch Research Council (ZonMW), Alzheimer Drug Discovery Foundation, The Selfridges Group Foundation, Alzheimer Netherlands, Alzheimer Association. CT is recipient of ABOARD, which is a public-private partnership receiving funding from ZonMW (#73305095007) and Health~Holland, Topsector Life Sciences & Health (PPP-allowance; #LSHM20106). ABOARD also receives funding from Edwin Bouw Fonds and Gieskes-Strijbisfonds.

HT has participated in meetings sponsored by or received honoraria for acting as an advisor/speaker for Alexion, Bayer, Biogen, Celgene, Fresenius, Genzyme-Sanofi, Jannssen, Merck, Novartis, Roche, Siemens and Teva.

LMV has served at scientific advisory boards, participated in meetings sponsored by, received speaking honoraria or travel funding or research grants from Roche, Sanofi, Merck, Biogen, Bristol Myers, and Novartis.

MAVW has received research grants from The Binding Site, Siemens Healthineers and Sebia Inc, has participated in an advisory board for Myeloma360.

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

FD has participated in meetings sponsored by or received honoraria for acting as an advisor/speaker for Alexion, Almirall, Biogen, Celgene, Genzyme-Sanofi, Merck, Novartis Pharma, Roche, and Teva. His institution has received research grants from Biogen and Genzyme Sanofi. He is section editor of the MSARD Journal (Multiple Sclerosis and Related Disorders).

Authors' contributions

HH has contributed in conception and design of the study, acquisition of data, analysis and interpretation of data, and drafting the manuscript.

JW has contributed in analysis and interpretation of data and revision the manuscript for intellectual content.

GA has contributed in revision the manuscript for intellectual content.

KB has contributed in acquisition of data and revision the manuscript for intellectual content.

SG has contributed in revision the manuscript for intellectual content.

BK has contributed in revision the manuscript for intellectual content.

MK has contributed in revision the manuscript for intellectual content.

RS has contributed in revision the manuscript for intellectual content.

CT has contributed in revision the manuscript for intellectual content.

HT has contributed in revision the manuscript for intellectual content.

LMV has contributed in revision the manuscript for intellectual content.

MAVW has contributed in revision the manuscript for intellectual content.

HZ has contributed in revision the manuscript for intellectual content.

FD has contributed in conception and design of the study, acquisition of data, analysis and interpretation of data, and revision the manuscript for intellectual content.