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Free Kappa light chains in neuroinflammatory disorders: Complement rather than substitute?

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Objectives: The detection of cerebrospinal fluid (CSF)-specific IgG oligoclonal bands (OCB) by isoelectric focusing (IEF) is widely used to help diagnose inflammatory neurological disorders (IND), including multiple sclerosis. However, the quantification of free light chains (FLC) is increasingly evaluated as a surrogate method to determine the presence of an intrathecal inflammatory process. The objective of this study was to evaluate the diagnostic performance of kappa (κ) FLC measurement in comparison with OCB detection by IEF.

Material and methods: We measured serum and CSF κFLCs by turbidimetry using the SPA_{PLUS} automated analyser and calculated the κ index in 142 samples from OCB-positive and negative MS, as well as from patients with inflammatory and non-inflammatory neurological disorders (IND and NIND).

Results: The κFLC index was significantly increased in OCB-positive MS and IND patients versus OCB-negative patients. Its performance was relatively comparable to that of IEF for MS diagnosis. When using a κFLC index cutoff value of 6.29, sensitivity increased from 61.2% to 75.7% in comparison with IEF for diagnosing IND ($P = .0051$), with a slightly lower non-statistically significant specificity (82.1% vs 100%). When considering both OCB status positivity or a κFLC index superior to 6.29 to diagnose IND status, sensitivity raised to 80.6% ($P < .05$) with an equal specificity.

Conclusion: Our results demonstrate that the κFLC index does not discriminate MS from other IND patients, but is a reliable technique to detect intrathecal inflammation. However, κFLC quantification should probably be considered as a complementary method, rather than a substitute, to OCB detection.

KEYWORDS
cerebrospinal fluid, free kappa light chains, multiple sclerosis, neuroinflammatory disorders, oligoclonal bands

1 | INTRODUCTION

The diagnosis of multiple sclerosis (MS), a chronic inflammatory demyelinating disease of the central nervous system (CNS), relies on the demonstration of spatial and temporal dissemination of disease, based on medical history, clinical examination, and results of magnetic resonance imaging, following exclusion of other diseases.[1]

Cerebrospinal fluid (CSF) analysis helps in the differential diagnosis and to indicate the inflammatory nature of the disease. Isoelectric focusing (IEF) reveals the presence of CSF-specific IgG oligoclonal bands (OCB) in more than 80% of patients, with a prevalence varying between countries due to the effect of latitude.[2] Clonally expanded B cells accumulate in the CSF of MS patients and are the likely source of OCBs.[3] The presence of OCBs has been incorporated in the most recent revision of the McDonald criteria as a substitute criteria for temporal dissemination, allowing the diagnosis of MS following a first clinical event.[4] OCBs are, however, also found in other inflammatory disorders of the central nervous system.[5]

K and λ antibody light chains are produced in excess in comparison with heavy chains, allowing their detection both in serum and in CSF. The quantification of κFLC is increasingly evaluated as an alternative method to IgG OCB detection by IEF.[6-13] This is partly due to the introduction of automated turbidimetric or nephelometric assays allowing the quantification of free chains independently of the bound chains. However, IgG OCB testing still constitutes the gold standard method used in clinical routine of neurochemistry laboratories worldwide. On the contrary, the value of λFLC quantification remains controversial, as many studies have shown that it is less performant than IgG OCB testing.[6,13-15]

In this study, we first aimed to compare the measurement of the κFLC index in patients with MS and other inflammatory or non-inflammatory neurological disorders (IND or NIND). We next evaluated the diagnostic performance of κFLC measurement in comparison with OCB status determination for diagnosing MS or IND. Finally, we compared κFLC index determination versus κFLC IEF in OCB-negative patients with clinical and/or radiological suspicion of IND.

2 | MATERIAL AND METHODS

2.1 | Study subjects and sample preparation

Cerebrospinal fluid and serum pairs from 142 patients were retrospectively included in the study. The patients had undergone a lumbar puncture in the Neurology department of the Cliniques Universitaires Saint-Luc between 2013 and 2018, as part of the routine diagnostic procedure. Hemorrhagic samples were excluded. CSF samples were collected and directly sent to the Neurochemistry laboratory of the University. Preanalytical aspects and storage were carried out in accordance with the consensus protocol proposed by Teunissen et al.[16]

Patients were classified in distinct subgroups according to the BioMS-eu consortium definitions.[17] Among the 142 subjects, 59 were diagnosed with MS. Eight subjects with primary progressive MS and 3 with secondary progressive MS were included in the study. Based on their clinical diagnosis, 44 patients were classified as inflammatory neurological disease controls (INDC) and 39 patients as non-inflammatory disease controls (NINDC). Diagnoses of the control subjects are listed in Table S1. Patient characteristics and demographic data are displayed in Table 1. Patients admitted to the Cliniques Universitaires Saint-Luc sign an internal regulatory document, stating that leftovers from biological samples used for routine diagnostic procedures can be used for retrospective academic studies, without an additional informed consent (ethics committee approval 2007/10SEP/233).

2.2 | Protein quantification

Quantitative measurement of κFLC was performed by turbidimetry on a SPA_{PLUS} analyser, using the FreeLite Human Kappa Free kit (The Binding Site). Serum samples were diluted 1/10 while CSF samples were not diluted, as recommended by the manufacturer. The lower limit of quantification (LOQ) in the CSF was 0.1 mg/L. A significant part of the cohort had no detectable CSF λFLC, preventing the use of this marker in our study. The κFLC index and κFLC ratio were calculated as follows: $(CSF_{FLC}/Serum_{FLC})/(CSF_{Albumin}/Serum_{Albumin})$ and $(CSF_{FLC}/Serum_{FLC})$, respectively. CSF as well as serum IgGs and albumin were routinely quantified by the Neurochemistry laboratory of the Cliniques Universitaires Saint-Luc using the IMMAGE® 800 (Beckman Coulter).

2.3 | Isoelectric focusing for detection of IgG oligoclonal bands

Isoelectric focusing of IgG was routinely performed by affinity-mediated capillary blotting on agarose gel.[18] According to guidelines, a positive OCB test is defined by the presence of at least two CSF-specific bands.[19] Therefore, mixed patterns were considered to be positive when meeting this criterion. On the other hand, mirror patterns, sometimes encountered in systemic inflammatory diseases, were considered to be negative.

TABLE 1 Demographic and paraclinical characteristics of patient groups

	MS	INDC	NINDC
Number	59	44	39
Mean age (years) ± SD	45.8 ± 14.3	46.7 ± 16.0	48.7 ± 14.2
% of women	57.6	65.9	58.9
Positive OCB n = (%)	46 (78.0)	17 (38.6)	0 (0)
Median Albumin quotient*10^3 (Interquartile range)	5.3 (3.5-7.5)	5.6 (3.6-9.8)	4.5 (3.6-7.2)
Median serum κFLC (mg/L) (Interquartile range)	9.36 (7.17-12.63)	8.22 (6.16-13.87)	8.05 (5.55-10.75)
Median CSF κFLC (mg/L) (Interquartile range)	2.68 (0.74-10.92)	0.48 (0.18-1.65)	0.17 (0.11-0.25))
Median κFLC Index (Interquartile range)	57.36 (14.2-184.4)	8.25 (4.46-20.63)	4.19 (2.72-5.92)
Median κFLC ratio (Interquartile range)	0.335 (0.080-0.977)	0.062 (0.029-0.136)	0.022 (0.015-0.037)

FLC, free light chains; INDC, inflammatory neurological disease control; MS, multiple sclerosis; NINDC, non-inflammatory neurological disease controls; SD, standard deviation.

2.4 | Imprecision and linearity evaluation of the assay

Precision was assessed by performing ten repeated measurements of two CSF samples (low and high values). The mean, standard deviation (SD) and coefficient of variation (CV) were calculated. A linearity study was performed using a CSF sample with a high concentration of κFLC that was serially diluted with a pool of CSF samples with undetectable Kappa levels (<0.1 mg/L). Six serial dilutions were prepared and measured in duplicate. Linearity was assessed by weighted linear regression and mean recovery as well as standard deviation were calculated.

2.5 | Isoelectric focusing of Kappa free chains

The presence of CSF oligoclonal κFLC bands was determined by affinity-mediated capillary blotting on agarose gel. CSF samples from 29 OCB-negative patients (14 MS and 15 INDC) were analyzed using the previously established method.[18]

2.6 | Statistical analysis

Statistical analysis was performed using the GraphPad Prism 7 software. To test for differences between patient populations, non-parametrical Kruskal-Wallis tests followed by Dunn's multiple comparison tests were performed. ROC curves were used to assess the performance of κFLC index quantification for MS or inflammatory neurological disease (IND) diagnosis. *P*-values ≤ .05 (two-tailed) were considered as statistically significant. The McNemar test was used to compare the sensitivity and specificity between κFLC measure and OCB status determination, as described previously.[20] Cohen's Kappa test was also used to evaluate the agreement between κFLC index and OCB detection.

3 | RESULTS

3.1 | Precision and linearity

A simple precision measurement study, representing repeatability, gave satisfactory results. The CVs for measures of a low (mean: 0.49 mg/L) and high Kappa concentration sample (mean: 13.70 mg/L) were 1.96% and 2.08% respectively. Sustained linearity over a 0.17-9.22 mg/L tested range was also observed. The linear equation of linearity was $y = 1.0012x - 0.1268$ with $r^2 = 0.998$ and a mean recovery (SD) of 93% (13.3%) was obtained: therefore, no significant matrix effect was observed.

3.2 | OCB status and κFLC quantification

Sixty-three patients (46 MS and 17 INDC) had CSF-specific IgG OCBs. Conversely, 13 MS and 27 INDC were OCB-negative. All 39 NINDC cases were negative for OCB testing. We quantified κFLC in paired CSF and serum samples of patients presenting with neurological diseases for which OCB testing had been prescribed. κFLC were above the LOQ in 94.3% and 100% of the CSF and serum samples respectively. CSF samples presenting a κFLC value below the LOQ were rounded to this value (0.1 mg/L) to allow calculation of the κFLC Index (in 1 MS OCB−, 1 INDC OCB− and 6 NINDC samples).

3.3 | FLC index in MS and other neurological diseases

Our next aim was to investigate whether the κFLC index could discriminate MS from other neurological diseases. To do so, patients were classified according to their clinical diagnosis and to the presence or not of CSF-specific IgG OCBs. Kruskal-Wallis test followed by Dunn's multiple comparisons revealed statistically significant differences between the OCB+ and OCB− subgroups ($P < .001$, Figure 1). MS patients with OCBs had a higher κFLC index in comparison with OCB− MS patients, as well as OCB− INDC patients ($P < .001$ and

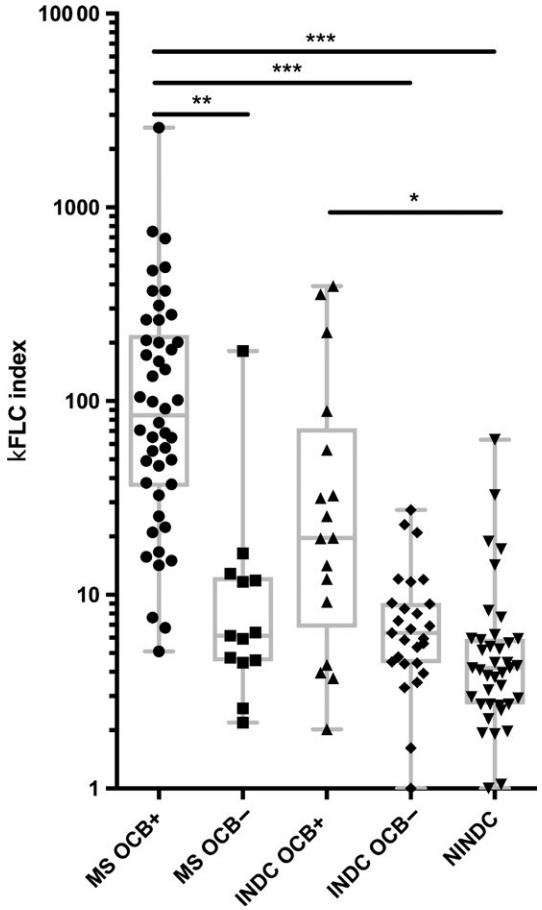

FIGURE 1 κFLC indexes in OCB+ and OCB−, MS, IND and NINDC patients. IND, inflammatory neurological disease; MS, multiple sclerosis; NINDC, non-inflammatory neurological disease controls. OCB: CSF-specific IgG oligoclonal bands. Kruskal-Wallis test followed by Dunn's multiple comparison tests were performed. Whisker intervals correspond to the 2.5 and 97.5 percentiles. Individual values are also represented. *, ** and *** indicate *P*-values of ≤.01, ≤.001 and ≤.0001, respectively

$P < .0001$ respectively). The κFLC indices of OCB+ MS and INDC patients were significantly higher than that of NINDC patients ($P < .0001$ and $P = .0012$ respectively). In the MS, INDC and NINDC subgroups, non-Gaussian distribution was observed with median (interquartile ranges) of 57.36 (14.20-184.4), 8.25 (4.46-20.63) and 4.19 (2.72-5.92) (Table 1). One MS patient had a κFLC index of 2487 due to a high κFLC concentration in the CSF (84.32 mg/L; Figure 1). At the time of sampling, this patient presented a spinal cord relapse with numerous gadolinium-enhancing lesions. One NIND case, with a clinical diagnosis of amyotrophic lateral sclerosis (ALS), had a κFLC index of 63.12 with a CSF κFLC concentration of 4.13 mg/L. Intrathecal inflammation has rarely been reported in ALS, as well as blood-CSF barrier breakdown as seen in our case (Albumin quotient: 9.9×10^3).[21] There was, however, no statistically significant difference between the κFLC index levels of OCB+ MS patients and that of OCB+ INDC patients. However, when comparing the MS and INDC groups, independently from OCB status, a statistically significant difference was observed ($P < .0001$, Figure S1). The κFLC index of OCB− MS patients was comparable to that of OCB− INDC or NINDC subjects.

3.4 | Performance of κFLC index quantification for MS or inflammatory neurological disease diagnosis

The performance of κFLC quantification for all IND including MS or exclusively for MS diagnosis was evaluated using ROC analysis and compared to that of CSF-specific OCB detection by IEF. The sensitivities of intrathecal OCBs for MS or all IND in our cohort were 78% (95% confidence interval (IC_{95}): 65.3%-87.7%) and 61.2% (IC_{95} :51.6%-70.1%) respectively (Table 2). Specificity of IgG OCBs for IND was 100%.

The optimal threshold for MS diagnosis was a κFLC index above 12.45, yielding a sensitivity of 78.0% and specificity of 77.1%, globally comparable to that of CSF-specific IgG OCB detection (Table 2, Figure 2). Following the McNemar test, the sensitivity and specificity of the FLC index were comparable to that of CSF-specific OCB determination for MS diagnosis ($P > .05$; Table S2). According to Cohen's Kappa test, the strength of agreement is considered to be "good" with a Kappa value of 0.77 (IC_{95}: 0.66-0.89).

For IND diagnosis, the optimal cutoff was 6.29 for the κFLC index, 0.045 for the κFLC ratio and 0.28 mg/L for κFLC CSF concentration. Areas under the curve were 0.84, 0.85 and 0.86, respectively (Table 1, Figure 3). When choosing 6.29 as cutoff for the κFLC index, a sensitivity of 75.7% (IC_{95} : 66.3%-83.6%) and a specificity of 82.1% (IC_{95} : 66.5%-92.5%) were observed (Table 2, Figure 2). The sensitivity of the κFLC index for IND diagnosis was significantly higher than that of intrathecal OCB detection ($P = .0051$), whereas its specificity was comparable ($P > .05$; Table S2). When applying the formula from Presslauer et al,[8] determining an upper threshold line for the κFLC ratio according to the albumin quotient, the sensitivity obtained was 82.5% (IC_{95}: 73.8%-89.3%). However, the specificity of this approach was significantly lower at 71.8% (IC_{95}: 55.1%-85.0%; Figure S2).

TABLE 2 Assessment of CSF-specific OCBs and κFLC index for MS or IND diagnosis

	Optimal Cutoff	All patients (n = 142)	IND[a] (n = 103)	MS (n = 59)	Sensitivity	Specificity	Positive predictive value	Negative predictive value	AUC	95% CI	P-value
OCBs for MS	NA	NA	NA	46	78.0	79.5	73	83.5	NA	NA	NA
κFLC index for MS	>12.45	65	NA	46	78.0	77.1	70.8	83.1	0.84	0.77-0.90	<.0001
OCBs for IND[a]	NA	63	63	NA	61.2	100	100	71.8	NA	NA	NA
κFLC index for IND[a]	>6.29	85	78	51	75.7	82.1	91.8	56.1	0.84	0.77-0.90	<.0001

AUC, area under the curve; CI, confidence interval; FLC, free light chains; IND, inflammatory neurological disorders; MS, multiple sclerosis; NA, not applicable; OCB, CSF-specific IgG oligoclonal bands.
[a]IND including MS.

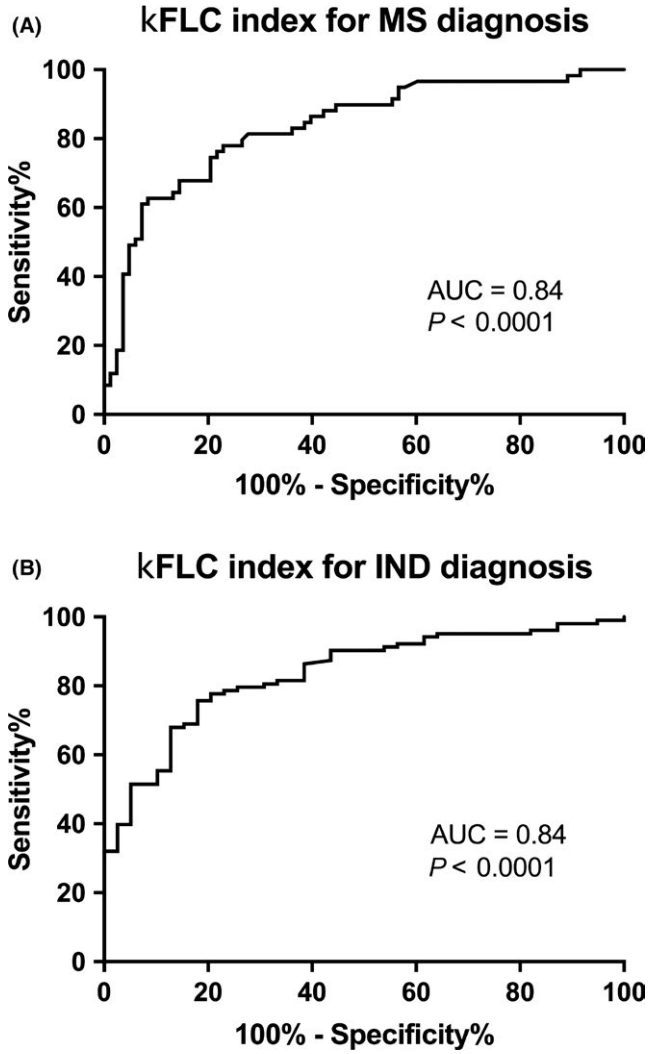

FIGURE 2 Receiver operating characteristic (ROC) curves for determining the optimal cut-off of the κFLC index for MS (A) or IND diagnosis (B). AUC, area under the curve; FLC, free light chains; IND, inflammatory neurological disease; MS, multiple sclerosis

The combination of OCB positivity or a κFLC index above 6.29 as diagnostic criteria allowed the detection of 6 additional cases in our cohort in comparison with κFLC index alone. Sensitivity raised, modestly but significantly (P = .041), from 75.7% to 80.6% (IC_{95}: 71.6%-87.7%) with a specificity maintained at 82.1%.

3.5 | Comparison of κFLC quantification with κFLC isoelectric focusing

Isoelectric focusing of κFLC was performed for 29 patients (14 MS and 15 INDC) with negative IgG OCBs as part of the routine diagnostic procedure. The κFLC index was also calculated. Oligoclonal κFLC bands were detected in the CSF of 13 (45%) samples (10 MS and 3 INDC). 8 of these (62%) had a κFLC index above the threshold of 6.29, defined in the previous section for detecting inflammatory neurological disorders. On the other hand, 7 of 16 patients (44%) with no detectable κFLC by IEF had an κFLC index superior to 6.29.

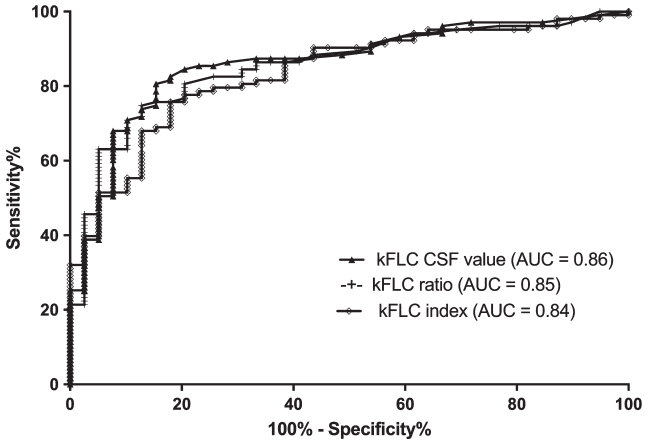

FIGURE 3 Receiver operating characteristic (ROC) curves for IND diagnosis using κFLC CSF value (mg/L), κFLC ratio ([κFLC]CSF/[κFLC]Serum) or κFLC index (([κFLC]CSF/[κFLC]Serum)/(AlbuminCSF/AlbuminSerum)). AUC, area under the curve; FLC, free light chains; IND, inflammatory neurological disease

When combining both approaches, 20 of 29 patients (69%) were positive for at least one of the two criteria suggesting an intrathecal inflammatory process.

4 | DISCUSSION

Several studies have investigated the diagnostic value of κFLC measurement in replacement of testing for IgG OCBs by IEF.[6-9,11-14] Automated measurement of CSF κFLC in the setting of neurological diseases has repeatedly demonstrated a relatively comparable sensitivity and specificity of κFLC assessment in comparison with the determination of OCBs. Some studies suggest that κFLC quantification could be a surrogate marker for CSF-specific IgG OCBs in patients presenting with a diagnosis of MS or a clinically isolated syndrome (CIS), corresponding to a first clinical episode compatible with a demyelinating event.[13]

On the other hand, some analytical concerns have emerged regarding κFLC measurements. Firstly, lot-to-lot variability in precision has been reported.[22] Indeed, different batches of sheep-derived polyclonal antibodies do not necessarily have the same cross-reactivity with a heterogeneous mixture of human FLC. Secondly, FLC can be monomeric or polymeric; these latter may form oligomeric complexes leading to an overestimation of measured concentration, possibly by binding at several antigenic sites on the FLC protein.[23] Non-linearity of the assay has also been reported, but this last problem seems to affect mostly monoclonal FLC.[22] Nevertheless, pending further standardization, these concerns have not yet been addressed for CSF. Of note, in our experiment, sustained linearity was observed without a significant matrix effect.

Since the introduction of κFLC and λFLC quantification kits, different calculation methods have been introduced to determine diagnostic thresholds. The lack of consensus to evaluate intrathecal FLC production makes comparison between the different studies

difficult. Some studies simply use the absolute intrathecal FLC concentration.[13,24] However, in the setting of MS or other IND, a possible blood-CSF barrier breakdown has to be taken into account. Consequently, other means of normalization have been developed such as the κFLC/λFLC ratio and FLC indices. Interestingly, we obtained close diagnostic performance for IND diagnosis using these three approaches. Furthermore, some groups have applied Reiber's formula to determine κFLC intrathecal synthesis.[10-12] The use of this method, developed for IgG, raised the question of whether such an equation is applicable for smaller proteins.[25] Indeed, IgG have a molecular weight of 150 kDa while κFLC have a molecular weight of approximatively 22 kDa. Therefore, those proteins have different diffusion coefficients through the blood-CSF barrier. Using a large cohort of control patients, Presslauer et al[8] have determined an upper threshold line for the κFLC ratio according to the albumin ratio, covering 98% of the control subjects. The determination of this threshold allowed the calculation of the relative fraction of κFLC in an individual CSF sample.[7] Due to the smaller number of samples included in our study, it was not possible for us to establish such a formula. According to the threshold defined by Presslauer's formula, the sensitivity of the κFLC ratio for IND diagnosis in our cohort was comparable (85.2%), whereas its specificity was lower (71.8%). This discrepancy with previously published results can be possibly explained by the use of a different automated analyzer in our study, as different platforms can give distinct results, as reported previously.[26] While there was a significant correlation between the CSF κFLC concentration and the albumin quotient in NIND patients (probably reflecting higher κFLC diffusion in case of blood-CSF breakdown), this was not observed for the κFLC index (Data not shown). Therefore, we chose rather to use the κFLC index in this study, taking into account the blood-CSF barrier status. The threshold maximizing both sensitivity and specificity, based on ROC curve analysis, was calculated. The κFLC index is significantly increased in OCB-positive cohorts (MS and INDC) in comparison with OCB-negative and NINDC patients. No significant difference between the κFLC index of OCB-positive MS and INDC patients was found. However, when omitting OCB status, a statistically significant difference was observed between the κFLC index of MS and INDC patients. As a significant number of OCB-negative MS patients were selected for this study, the sensitivity of intrathecal OCB detection in our cohort was lower than previously reported.[2] Specificity of intrathecal OCBs was also lower due to the inclusion of many patients with other inflammatory neurological disorders in our cohort, as reported by Petzold.[27]

It has been suggested that FLC quantification could help in discriminating MS from inflammatory disorders other than MS.[12] We wanted to further investigate this question by comparing the diagnostic performance of the κFLC index to that of intrathecal OCB determination in MS patients or patients with IND (including MS). ROC analysis showed that a cutoff of 12.45 for the κFLC index discriminated MS patients with comparable sensitivity (78.0% for both tests) and specificity (77.1% vs 79.5%) in our patient cohort. The sensitivity of the κFLC index for diagnosing IND with a cutoff of 6.29 was however superior to OCB testing (75.7% vs 61.2%), with lower specificity, although non-statistically significant (78.7% vs 100%, $P > .05$).

More than 80% of MS patients have CSF-specific IgG OCBs that will persist lifelong.[2] Oligoclonal κFLC can, however, be detected in a subset of these OCB-negative patients.[28] Therefore, IEF specific for κFLC can be performed in patients with a clinical and/or radiological suspicion of CIS/MS. A recently published report compared the performance of several κFLC quantification methods to κFLC IEF. They did not observe major differences regarding the diagnostic performances of the different techniques.[29] No firm conclusion can be drawn from our results, due to the small number of samples analyzed by both techniques. Of note, 5 of 13 samples positive for oligoclonal κFLC by IEF had a κFLC index below the threshold of 6.29 suggestive for IND, again probably highlighting the complementarity of both techniques.

In conclusion, despite the retrospective design of this study, we have challenged the diagnostic performance of the κFLC index by including a significant number of OCB-negative MS and IND cases. We have shown that κFLC index measurement had comparable sensitivity for MS diagnosis to that of intrathecal IgG OCB determination. κFLC was however more sensitive than OCB detection for the diagnosis of IND. However, the κFLC index did not allow the distinction between OCB-positive MS and IND cases. Finally, in most cases, the κFLC index could be an adequate surrogate measure for intrathecal IgG OCBs. However, in some cases, when the clinical suspicion of inflammatory neurological disorder of the central nervous system is high, the combination of both techniques might still prove useful, as reported by our results. Alternatively, OCB detection, thanks to its high specificity, could be used to confirm positive κFLC cases, as suggested by Crespi et al.[30]

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare related to this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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