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Original Research Paper

Kappa free light chains is a valid tool in the diagnostics of MS: A large multicenter study

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Abstract

Objective: To validate kappa free light chain (KFLC) and lambda free light chain (LFLC) indices as a diagnostic biomarker in multiple sclerosis (MS).

Methods: We performed a multicenter study including 745 patients from 18 centers (219 controls and 526 clinically isolated syndrome (CIS)/MS patients) with a known oligoclonal IgG band (OCB) status. KFLC and LFLC were measured in paired cerebrospinal fluid (CSF) and serum samples. Gaussian mixture modeling was used to define a cut-off for KFLC and LFLC indexes.

Results: The cut-off for the KFLC index was 6.6 (95% confidence interval (CI) = 5.2–138.1). The cut-off for the LFLC index was 6.9 (95% CI = 4.5–22.2). For CIS/MS patients, sensitivity of the KFLC index (0.88; 95% CI = 0.85–0.90) was higher than OCB (0.82; 95%CI = 0.79–0.85; $p < 0.001$), but specificity (0.83; 95% CI = 0.78–0.88) was lower (OCB = 0.92; 95% CI = 0.89–0.96; $p < 0.001$). Both sensitivity and specificity for the LFLC index were lower than OCB.

Conclusion: Compared with OCB, the KFLC index is more sensitive but less specific for diagnosing CIS/MS. Lacking an elevated KFLC index is more powerful for excluding MS compared with OCB but the latter is more important for ruling in a diagnosis of CIS/MS.

Keywords: Multiple sclerosis, KFLC, OCB, CSF, biomarkers

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Introduction

Cerebrospinal fluid (CSF) assessment is often part of the diagnostic workup for multiple sclerosis (MS), and its value is supported by the latest 2017 revisions of the McDonalds criteria.[1] CSF examination is frequently performed in diagnosing MS for excluding alternative diagnoses, although, in the current MS criteria,[2] the assessment of oligoclonal IgG bands (OCB) plays a limited role. However a recent study showed the added value of OCB in the MS diagnostic criteria.[3] In the 2017 revisions, the OCB have a prominent role in patients with a clinically isolated syndrome (CIS). The presence of both magnetic resonance imaging (MRI) criteria for dissemination in space (DIS) and CSF-specific OCB will enable to establish the MS diagnosis in patients with a single clinical episode suggestive of central nervous system (CNS) inflammatory demyelinating disease. The OCB assessment also has important prognostic value in CIS and MS.[4,5] However, the assessment of OCB is labor-intensive, requires trained personnel, and is in some cases examiner- and method-dependent, which may affect its reliability.

Alongside intact immunoglobulins, which are composed of two heavy and two light chains, plasma cells produce and secrete immunoglobulin free light chains (FLC) of either kappa (KFLC) or lambda (LFLC) chains. KFLC and LFLC can be detected in both CSF and serum.[6–10] Since the late 1970s,

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multiple studies have reported increased CSF levels of KFLC in MS.[6–14] The analytical specificity of the earlier methods (e.g. radioimmunoassay,[15,16] quantitative enzyme-linked immunosorbent assay)[8,17] was insufficient, but with the recent emergence of the more sensitive nephelometric and turbidimetric FLC assays, research in this field has been revived. Nephelometric (and turbidimetric) FLC level determination has the additional advantage compared to OCB of being assessed by an automated procedure and being quantifiable.[18]

Using the FLC assay,[19] recent studies showed that both CSF KFLC levels and the KFLC index are increased in patients with CIS or relapsing-remitting MS (RRMS) compared with controls.[8,14,18,20–22] The use of an index measure is necessary, for example [CSF KFLC/serum KFLC]/[CSF albumin/serum albumin], to include blood-CSF barrier permeability.[10,14] The KFLC index has comparable sensitivity and specificity to OCB for diagnosis of MS and CIS.[14,21,23] However, large-scale studies comparing diagnostic performance of the two methods and to define the cut-off of FLC are lacking. The main aim of this study was to validate KFLC and LFLC indices as a diagnostic biomarker in MS compared with OCB in a large multicenter study including samples from 18 MS centers across Europe.

Methods

Patients and controls

Eighteen MS centers participated, located in the Netherlands, Spain, France, Belgium, Hungary, Italy, Poland, Turkey, Denmark, Serbia, Austria, and Switzerland. We selected 745 paired CSF/serum samples from patients with known OCB status, diagnosed as CIS ($n=242$), RRMS ($n=235$), primary-progressive MS (PPMS) ($n=41$), and secondary-progressive MS (SPMS) ($n=8$). We also included inflammatory neurological disease controls (INDC) ($n=67$), non-inflammatory neurological disease controls (NINDC) ($n=76$), symptomatic controls (SC) ($n=49$), and healthy controls (HC) ($n=27$) as defined previously.[24] The different control groups were pooled into one control group ($n=219$). The CIS and MS patients were also pooled (CIS/MS) ($n=526$).

The large majority (84%) of the CIS/MS patients fulfilled the 2010 McDonald criteria[2] but, in some cases, the patients were diagnosed according to the 2005 McDonald criteria[25] (16%). Table 1 presents the demographic and clinical characteristics of the patients and controls.

CSF and serum samples

Only CSF samples that were immediately centrifuged and stored in polypropylene tubes within 2 hours at −80°C, at the local center, were included. The assessment of OCB had been performed by isoelectric focusing (on agarose or polyacrylamide gel), followed by immunofixation by the centers as part of the diagnostic workup.

Samples were taken between 2005 and 2016, with a median age of 2.9 years (IQR = 1.7–5.7).

We used fresh aliquots, and in our lab, we did not freeze and thaw the samples during the analyses. As far as we know, no effects of freezing and thawing have been reported.

KFLC, LFLC, and albumin analysis

KFLC, LFLC, and albumin concentrations in CSF and serum samples were analyzed using the turbidimetric analyzer SPAplus® (The Binding Site, Birmingham, UK) with the serum free light chain immunoassay (Freelite®, The Binding Site, Birmingham, UK) according to the manufacturer's instructions. All samples were measured centrally in the Neurochemistry Laboratory of the Department of Clinical Chemistry of the VU University Medical Center (VUmc), Amsterdam, the Netherlands. All samples were run blinded for the clinical data.

To verify the QC data supplied by the manufacturer, we calculated intra-assay coefficient of variation (CV) by taking the mean CVs of four replicates of five samples (CSF/serum) within one run. We calculated inter-assay CV based on $n=5$ samples (CSF/serum) measured in 5 different days. CV values for KFLC and LFLC were all found to be lower than those supplied by the manufacturer (Supplemental Table 1). CV values for albumin were comparable to those supplied by the manufacturer. Assay linearity was experimentally confirmed for albumin (serum and CSF) and the FLC assays in serum and showed that recalculated values varied by 25.7% (KFLC) and 14.2% (LFLC) from the original value.

For 29 samples, CSF albumin results were below detection. Here, we assigned a random uniform value between 35 mg/mL (lowest detected value in undiluted rerun) and 175 mg/mL (formal detection limit).

FLC indices

We determined the CSF/serum quotients (Q FLC) of KFLC and LFLC and calculated indices in order to

Table 1. Demographic and clinical characteristics of the included patients and controls at the time of lumbar puncture.

Disease group	*N* = 745	Sex (female) *N* (%)	Age (years) Mean ± SD	Stage/type of disease RR/SP/PP	Disease duration (months) Median (IQR)	No. of patients using treatment	No. of patients on corticosteriods Median (IQR)	EDSS Median (IQR)	OCB positive *N* (%)
CIS	242	177 (73.1)	35 ± 10	n.a.	0.8 (0.3–3.0)	1	4	2.0 (1.0–2.5)[a]	186 (76.9)
MS	284	170 (59.9)	38 ± 11	235/8/41	13.1 (2.4–48.1)	3[b]	10	2.0 (1.5–3.5)[c]	245 (86.3)
Controls	219	130 (59.4)[d]	42 ± 12	n.a.	n.d.	1	1	n.a.	17 (7.8)
- NINDC	76	49 (64.5)	45 ± 13	n.a.	n.d.	0	0	n.a.	4 (5.3)
- INDC	67	33 (49.3)	42 ± 13	n.a.	n.d.	1[e]	1[e]	n.a.	13 (19.4)
- SC	49	32 (65.3)	39 ± 10	n.a.	n.d.	0	0	n.a.	0 (0)
- HC	27	16 (59.3)	41 ± 10	n.a.	n.d.	0	0	n.a.	0 (0)

RR: relapsing-remitting; SP: secondary progressive; PP: primary progressive; disease duration: time between CSF lumbar puncture and date of onset first neurological complaints; EDSS: Expanded Disability Status Scale, OCB: oligoclonal IgG bands; CIS: clinically isolated syndrome; MS: multiple sclerosis; n.a.: not applicable; n.d.: not determined; NINDC: non-inflammatory neurological disease control; INDC: inflammatory neurological disease controls; SC: symptomatic control; HC: healthy control; CSF: cerebrospinal fluid; CLIPPERS: chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids.
All participants gave written informed consent at the center where the CSF/serum was conducted.
[a]In 178 CIS patients, an EDSS was available.
[b]Disease-modifying treatment in combination with corticosteroids was used in two MS patients. In one patient, only disease-modifying treatment.
[c]In 107 MS patients, an EDSS was available.
[d]In six control patients, the sex was unknown.
[e]One INDC (CLIPPERS syndrome) patient had steroids and methotrexate at the time of lumbar puncture.

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Table 2. FLC in CIS/MS and controls.

A	CIS/MS ($n=526$)	Controls ($n=219$)	p-value
CSF KFLC (mg/L)	3.7 (1.0–10.1)	0.2 (0.1–0.4)	<0.001
Serum KFLC (mg/L)	12.4 (9.8–15.7)	13.7 (10.6–16.9)	0.001
KFLC index	75.6 (21.8–197.0)	2.8 (2.2–4.9)	<0.001
KFLC index ⩾ 6.6	460 (87.5)	38 (17.4)	–
B	**CIS/MS ($n=543$)**	**Controls ($n=202$)**	**p-value**
CSF LFLC (mg/L)	0.5 (0.2–1.3)	0.2 (0.2–0.3)	< 0.001
Serum LFLC (mg/L)	11.3 (9.2–13.7)	12.1 (9.8–15.4)	0.001
LFLC index	11.6 (5.3–33.3)	3.3 (2.5–4.7)	< 0.001
LFLC index ⩾ 6.9	359 (68.3)	30 (13.7)	–

FLC: free light chains; CIS: clinically isolated syndrome; MS: multiple sclerosis; A: KFLC in CIS/MS and controls; CSF: cerebrospinal fluid; KFLC: kappa free light chain; index: FLC quotient/albumin quotient; B: LFLC in CIS/MS and controls; LFLC: lambda free light chains; IQR: interquartile range.
Values are given as *n* (%) or as median (IQR).

take possible blood-CSF diffusion into account. The FLC indices were defined as [Q FLC] × [serum albumin/CSF albumin].

Statistics

Differences in demographics, clinical characteristics, FLC concentrations, and FLC indices were tested via the Mann–Whitney *U* test for comparison of two groups of non-normally distributed data. For comparison of more than two groups, Kruskal–Wallis test with post hoc Dunn's multiple comparison test was applied. For normally distributed continuous variables, an independent-samples *t* test or one-way analysis of variance (ANOVA) with post hoc Bonferroni correction was applied. For binary variables, a chi-square test was performed. These statistical analyzes were performed in SPSS 22.0 (IBM Crop., Armonk, NY, USA).

Gaussian mixture modeling was used to define cut-offs for abnormal FLC indices using the R statistical software program version 3.2.1 mixtools package. First, the number of distributions that best described the data was determined with the R boot.comp function. Next, we defined a data-driven cut-off as the point where the lines of two fitted normal distributions crossed each other. The main analyses included all subjects. Data were log-transformed because FLC indices were not normally distributed. Based on the defined cut-off, subjects were classified as positive or negative for kappa or lambda FLC as binary result, similar as the available results for OCB status. As extra comparisons, we combined the different tools to compare with the single measurement OCB. The three combinations were as follows: KFLC with OCB, LFLC with OCB, and KFLC with LFLC. We defined the outcome of the combination as followed: when one of the measurements is positive, the combination is positive, when both are negative, the combination is negative.

To compare the sensitivity, specificity, and accuracy between the two different diagnostic tools (OCB and FLC), the McNemar test was used (SPSS 22.0 (IBM Crop., Armonk, NY, USA)). The positive and negative predictive values (PPVs and NPVs) were compared using the R package DTcompair. The p values < 0.05 were considered statistically significant.

Results

Paired CSF and serum samples from a total of 745 patients were included in this multicenter study (see Supplemental Figure 1 for the flowchart of patient selection). The patient groups ($n=526$) consisted of 242 CIS and 284 MS patients. The control group ($n=219$) consisted of 76 NINDC, 67 INDC, 49 SC, and 27 HC. The control group was older (mean = 42 ± 12 years) than the CIS/MS patient group (mean = 35 ± 10 years, $p < 0.001$). There was no significant difference in sex distribution between the two groups ($p=0.20$). Only 7.7% of the control group had a positive OCB status. The diagnoses of these patients are as follows: Tolosa hunt ($n=2$), meningitis ($n=1$), neurosarcoidosis ($n=2$), lupus ($n=1$), transient global amnesia ($n=1$), stroke ($n=1$), post-infectious myelitis ($n=1$), myelitis ($n=1$), encephalitis ($n=1$), epilepsy ($n=1$), ADEM ($n=1$), neurodegeneration ($n=1$), INDC, exact diagnosis unknown ($n=2$), and neuritis optica ($n=1$).

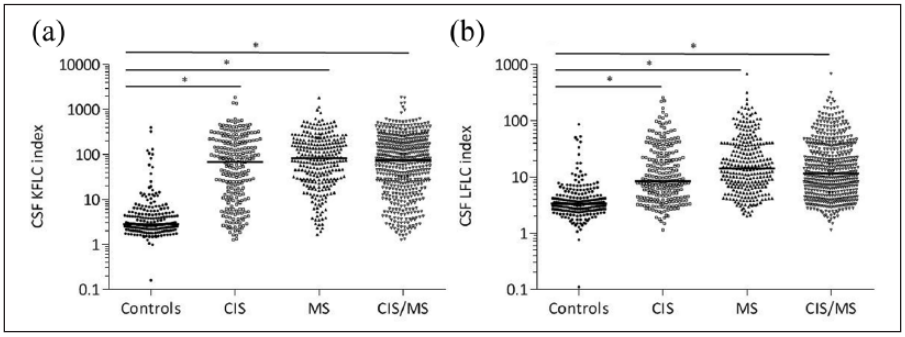


Figure 1. CSF KFLC and LFLC indices of CIS, MS, CIS/MS and controls. (a) Levels of KFLC indices, (b) levels of LFLC indices. Horizontal bars in the scatter dot plot represent the median.
CSF: cerebrospinal fluid; KFLC: kappa free light chains; LFLC: lambda free light chains; CIS: clinically isolated syndrome; MS: multiple sclerosis.
*$p \leq 0.001$.

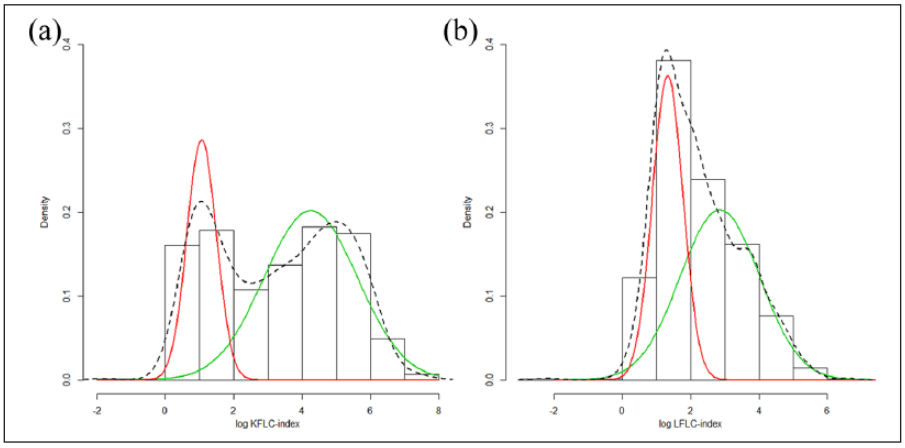


Figure 2. Cerebrospinal fluid (a) KFLC and (b) LFLC cut-off values based on mixture modeling. The red (low values) and green (high values) lines are the individual components of the estimated mixture distributions, the dotted line is the combined estimated mixture distribution. The cut-off is defined as the point where the red and green lines cross.

For a detailed list including the diagnoses of non-inflammatory neurological diseases and inflammatory neurological diseases included in the groups, see Supplemental Tables 2A and 2B.

FLC concentrations and FLC indices

Table 2 shows the levels and indices of the KFLC and LFLC in CIS/MS patients and controls. CSF KFLC and CSF LFLC concentrations were significantly increased in CSF of CIS/MS patients compared to controls (both $p < 0.001$). In addition, KFLC and LFLC concentrations in CSF were higher in MS patients than CIS patients (both $p < 0.001$).

KFLC and LFLC serum concentrations were significantly higher in the control group than CIS/MS (both, $p = 0.001$) but did not differ significantly between CIS and MS (KFLC $p = 0.33$, LFLC $p = 1.00$).

See Figure 1(a) for the KFLC indices per subgroup and Figure 1(b) for the LFLC indices per subgroup. KFLC and LFLC indices were significantly increased in CIS/MS compared to controls (both $p < 0.001$). In addition, FLC indices were higher in MS than in CIS (KFLC $p = 0.07$, LFLC $p < 0.001$).

FLC index cut-off

In the total cohort ($n = 745$) (all diagnostic subgroups), a bimodal distribution of the log-transformed KFLC index values fitted the data best. This yielded a cut-off for the log-KFLC index of 1.89 (95% confidence interval (CI) = 1.65–4.92) (Figure 2(a)), which corresponds to an KFLC index of 6.6 (95% CI = 5.2–138.1) on the original scale.

For the LFLC index, a bimodal distribution yielded an optimal cut-off for the log-LFLC index of 1.9 (95%

Table 3. Diagnostic sensitivity and specificity of the FLC indices compared to OCB.

	OCB	KFLC	*p*-value	LFLC	*p*-value
CIS/MS vs controls					
Sensitivity	0.82 (95% CI = 0.79–0.85)	0.88 (95% CI = 0.85–0.90)	< 0.001	0.66 (95% CI = 0.62–0.70)	< 0.001
Specificity	0.92 (95% CI = 0.89–0.96)	0.83 (95% CI = 0.78–0.88)	< 0.001	0.86 (95% CI = 0.81–0.91)	0.019
Accuracy	0.85 (95% CI = 0.82–0.88)	0.86 (95% CI = 0.84–0.89)	1.000	0.74 (95% CI = 0.70–0.77)	< 0.001
PPV	0.96 (95% CI = 0.94–0.98)	0.92 (95% CI = 0.90–0.95)	< 0.001	0.92 (95% CI = 0.90–0.95)	0.001
NPV	0.68 (95% CI = 0.63–0.73)	0.73 (95% CI = 0.68–0.79)	0.010	0.53 (95% CI = 0.48–0.58)	< 0.001
CIS vs controls					
Sensitivity	0.77 (95% CI = 0.72–0.82)	0.81 (95% CI = 0.76–0.86)	0.150	0.60 (95% CI = 0.54–0.66)	< 0.001
Specificity	0.92 (95% CI = 0.89–0.96)	0.83 (95% CI = 0.78–0.88)	< 0.001	0.86 (95% CI = 0.82–0.91)	0.019
Accuracy	0.84 (95% CI = 0.80–0.88)	0.82 (95% CI = 0.78–0.85)	0.149	0.73 (95% CI = 0.68–0.77)	< 0.001
PPV	0.92 (95% CI = 0.88–0.95)	0.84 (95% CI = 0.79–0.88)	< 0.001	0.83 (95% CI = 0.77–0.89)	0.001
NPV	0.78 (95% CI = 0.73–0.83)	0.79 (95% CI = 0.74–0.84)	0.56	0.66 (95% CI = 0.61–0.72)	< 0.001
MS vs controls					
Sensitivity	0.86 (95% CI = 0.82–0.90)	0.93 (95% CI = 0.90–0.97)	< 0.001	0.75 (95% CI = 0.70–0.80)	< 0.001
Specificity	0.92 (95% CI = 0.89–0.96)	0.83 (95% CI = 0.78–0.88)	< 0.001	0.86 (95% CI = 0.81–0.91)	0.019
Accuracy	0.89 (95% CI = 0.86–0.92)	0.89 (95% CI = 0.86–0.92)	1.000	0.80 (95% CI = 0.77–0.84)	< 0.001
PPV	0.94 (95% CI = 0.91–0.97)	0.88 (95% CI = 0.84–0.91)	< 0.001	0.88 (95% CI = 0.84–0.92)	0.002
NPV	0.84 (95% CI = 0.79–0.88)	0.91 (95% CI = 0.86–0.95)	0.001	0.73 (95% CI = 0.68–0.78)	< 0.001

FLC: free light chains; OCB: oligoclonal IgG bands; KFLC: kappa free light chains; LFLC: lambda free light chains; CIS: clinically isolated syndrome; MS: multiple sclerosis; PPV: positive predictive value; NPV: negative predictive value.
To compare the sensitivity and specificity between the two different diagnostic tools (OCB and FLC), the McNemar test was used.

CI = 1.5–3.1) (Figure 2(b)), which corresponds to an LFLC index of 6.9 (95% CI = 4.5–22.2) on the original scale.

Diagnostic sensitivity and specificity of the indices compared to OCB

KFLC index. When pooling CIS and MS as one group (CIS/MS), the sensitivity to identify CIS/MS from controls of the KFLC index (0.88) was significantly higher than of OCB (0.82; $p < 0.001$) at the cost of a significantly lower specificity (KFLC index = 0.83, OCB = 0.92; $p < 0.001$) (see Table 3).

When identifying CIS patients from controls, the sensitivities did not differ significantly between KFLC and OCB (KFLC index = 0.81, OCB = 0.77; $p = 0.15$), but the specificity of OCB was significantly higher (KFLC index = 0.83, OCB = 0.92, $p < 0.001$). Identifying MS patients from controls, the sensitivity of the KFLC index (0.93) was significantly higher than of OCB (0.86; $p < 0.001$) but the specificity (0.83) was significantly lower (0.92; $p < 0.001$).

The accuracies were similarly high for both biomarkers for all three comparisons. The PPVs were higher for OCB (in all three comparisons $p < 0.001$). The NPV was the same for both markers ($p = 0.56$) in CIS

versus controls, but when pooling CIS/MS and in MS alone, the NPVs were slightly higher for KFLC ($p = 0.010$, $p < 0.001$) (see Table 3).

LFLC index. When pooling CIS and MS in one group (CIS/MS), the sensitivity to identify CIS/MS from controls of the LFLC index (0.66) was significantly lower than that of OCB (0.82; $p < 0.001$). The specificity to discriminate CIS/MS from controls was also significantly lower (0.86) than for OCD (0.92; $p = 0.019$).

When identifying CIS patients from controls, the sensitivities did differ; the LFLC index was significantly lower than OCB (LFLC index = 0.60; OCB = 0.77; $p < 0.001$). The specificity of LFLC in CIS was lower than OCB (LFLC index = 0.86; OCB = 0.92; $p = 0.019$). Identifying MS patients from controls, the sensitivity of the LFLC index (0.75) was significantly lower than OCB (0.86; $p < 0.001$). The specificity (0.86) was also significantly lower than OCB (0.92; $p = 0.019$). The accuracies and NPV were significantly lower for LFLC than OCB for all three comparisons. The PPV was significantly lower for LFLC than OCB when comparing MS with controls, and in the other comparisons, the PPV of LFLC was similar to OCB (see Table 3).

Combination of the different tools compared to single measurement

Three combinations were made, KFLC index–OCB, LFLC index–OCB, and KFLC index–LFLC index, and all were compared with the analysis of OCB alone. Sensitivity and specificity were calculated for all the subgroups compared to the control group. The sensitivity of the combination OCB with the KFLC index increased to 0.88, and the specificity of this combination decreased to 0.83. The same results were obtained for the combinations of LFLC index–OCB and KFLC index–LFLC index compared to single OCB, showing a slightly higher sensitivity (LFLC index–OCB = 0.87, KFLC–LFLC index = 0.87) and a lower specificity (0.83 and 0.80, respectively).

Sensitivities and specificities in alternative subgroups

Including patients with the diagnosis CIS (according to McDonald 2005 criteria) as RRMS patients resulted in lower sensitivities of OCB, KFLC, and LFLC that were seen when comparing CIS patients with controls. However, the *p* value did not change relevantly when comparing the new sensitivities for OCB, KFLC, and LFLC. No relevant differences were seen when comparing MS to control group.

No relevant differences were seen when we exclude patients with the CIS diagnosis according to McDonald 2005 criteria.

When excluding INDC from the control group, higher specificities were seen for OCB, KFLC, and LFLC when comparing CIS/MS with the controls. However, the *p* value did not change relevantly when comparing the new specificities of OCB, KFLC, and LFLC (data not shown).

Discussion

Our study indicates that the KFLC index is a valid test for diagnosing CIS/MS. Compared to OCB, the KFLC index is more sensitive at the cost of a lower specificity. This trade-off resulted in a higher NPV for the KFLC index compared to OCB, but a lower PPV. In addition, our results indicate that the LFLC index is not a valid test for diagnosing CIS/MS.

Our sensitivity and specificity for the KFLC index in CIS/MS were lower than a few much smaller previous studies,[18,23,26] which reported sensitivity in the range of 0.93–0.95 and specificity in the range of 0.91–1.00. The study of Desplat-Jégo et al.[9] showed a lower sensitivity of 0.70 and a lower specificity of 0.82 for the KFLC index in MS patients. The more recent study of Vasilj et al.[27] showed a lower sensitivity of 0.71, however a higher specificity of 0.98. The results of the comparison of KFLC and OCB are in line with results of a recent multicenter study,[14] where a cut-off of 5.9 was employed to validate KFLC in CSF as a diagnostic biomarker in 60 CIS patients, 60 MS patients compared to 60 OND, reporting a higher sensitivity of the KFLC index (0.78) compared to OCB (0.72) for diagnosis of CIS. In MS, the sensitivity of the KFLC and OCB was comparable (0.93 vs 0.93). However, the specificity (0.95) in CIS and MS was higher compared to our study. Another paper used the same 5.9 cut-off; this resulted in a sensitivity and specificity of 0.96 and 0.98, respectively, in MS patients.[21]

This discrepancy in the crude sensitivity and specificity of the KFLCs may be due to the more heterogeneous control group in our study compared to the previous studies, due to pooling of the CIS/MS group or the inclusion of not only clinical definite MS patients. For example, similar as for the OCB, KFLCs can be elevated in inflammatory controls,[28] and thus specificity will be lower when included. Nevertheless, this is a very relevant control group in differential diagnosis of MS. The unprecedented large number of patients and the large heterogeneous control group in this study gave us a reflection of the real-life clinical

situation, thus avoiding spectrum bias, and allowed us to give a more representative sensitivity and specificity for OCB, KFLC, and LFLC indices.

Noticeable is that the sensitivity and specificity of OCB to discriminate MS patients from controls were lower in our study than previously reported.[10,29,30] However, a meta-analysis published in 2013[31] (13,467 patients) showed that the diagnostic specificity of OCB diminished if other inflammatory etiologies were considered. Therefore, the lower specificities in our study may also be due to inclusion of various control groups.

The cut-off in this study was calculated using a data-driven Gaussian mixture modeling approach. We chose a different approach compared to other studies that applied, for example, receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) values,[9,10,18,23,27,32] because we reasoned that the cut-off should be defined by biological levels (data driven) and not based on clinical diagnosis, which is an imperfect golden standard. We determined a cut-off of 6.6 for abnormal KFLC indices and 6.9 for abnormal LFLC indices. Our cut-off for KFLC is in line with a previous multicenter study showing a KFLC index cut-off of 5.9.[10] This almost comparable cut-off for the KFLC index in two multicenter studies supports its robustness and implies that it can be used as an universal cut-off.

There are some limitations in this study. One limitation was that not all patients were diagnosed based on the same MS criteria; most patients by McDonald 2010 (84%) but a few with McDonald 2005, which may have influenced the diagnosis of CIS patients particularly. CIS patients diagnosed before 2010 may very well be MS patients according to McDonald 2010, because in the 2005 criteria, MS diagnosis was more stringent. None of the patients were diagnosed by the new 2017 criteria, because of the retrospective setup, and thus imaging information was not collected. We address this problem by pooling all CIS and MS patients. Another reason for pooling CIS and MS is that we did not have the data to test CIS converting to MS versus non-converting CIS, because we did not have follow-up data. We performed several sensitivity analyses in CIS or MS patients separately, and by reclassifying and excluding specific clinical groups (Table 3). In these analyses, similar results were observed, suggesting that our results are robust for the total population. Another limitation is that we did not repeat the OCB analysis per patient centrally, but relied on the original local outcomes. However, we received the samples and OCB status from expertise MS centers (participating in the BioMSeu consortium) using standardized protocols.[33] Moreover, inter-laboratory agreement is reported to be good for OCB, for example an inter-laboratory agreement of kappa > 0.8 between 19 participating laboratories in Spain was observed.[34]

One more important note is that the best set up for the study would have been if the test population should be suspected MS cases and not already diagnosed with MS. Still, as provided in Supplemental Table 2B, we included various INDC and quite some patients initially suspected for demyelinating disease.

Alongside the sensitivity and specificity results of the indices, we found significantly increased FLC concentrations and quotients in CSF of CIS/MS patients compared to the control groups. However, our main focus in this study was in the FLC indices and not in the concentrations of the FLC. To control for blood-CSF barrier function, we used indices instead of concentrations.

Combination of different markers (KFLC index–OCB, LFLC index–OCB, and KFLC index–LFLC index) compared to the single measurement OCB, showed that the combination KFLC index–OCB compared to single OCB gave a slightly higher sensitivity (0.88). However, the specificity became lower (0.83). The same results were seen in the combination LFLC index–OCB and KFLC index–LFLC index compared to single OCB. By definition, the sensitivity become higher and the specificity lower when you decide beforehand that the combination test will be positive when the test is positive in one of the two.

For clinical practice, the KFLC index is more accurate in excluding CIS/MS compared to OCB but for ruling in a diagnosis of CIS/MS, analysis of OCB appears to be more accurate. If we replace OCB by KFLC in diagnostic practice, there is a slightly higher chance that a patient with a diagnosis different from MS will get the diagnosis of MS and maybe unnecessarily exposed to potential negative side effects of early treatment. Since the KFLC index is more sensitive at the cost of a lower specificity, we should stress that replacement of OCB by the KFLC index is not optimal to arrive at high diagnostic certainty. However, with the higher sensitivity of KFLC, an earlier treatment start may be considered. Whether it is an option to start treatment based on the KFLC result and clinical/MRI findings according to the novel McDonald criteria or whether the treatment may be adapted after a first-year evaluation is subject of further studies and discussions.

In conclusion, this study indicates that the KFLC index is a valid tool in the diagnostic process of MS. Since this marker is measured by a faster and rater-independent analytical procedure, it should be considered as a potential cost-effective replacement of the OCB, especially when CSF analysis will regain a more prominent role in the 2017 revisions of the McDonald criteria.

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Author Contributions

C.E.L. contributed to study design, collecting all data, statistical analysis, interpretation of the data, and drafting the manuscript. H.A.M.T. contributed to KFLC and LFLC data analyses, interpretation of the data, and revising the manuscript for intellectual content. B.I.L.-W. contributed to statistical analysis of the data (Biostatistician). B.M.J.U., J.K., C.B., and C.T. contributed to study design, interpretation of the data, and revising the manuscript for intellectual content. All other authors helped by selecting and collecting samples and clinical information. All other authors helped by revising the manuscript for intellectual content.

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