



Kappa index for multiple sclerosis diagnosis: an accurate biomarker of intrathecal synthesis

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Abstract

Background Cerebrospinal fluid (CSF) kappa-free light chains (KFLC) are becoming a diagnostic biomarker for multiple sclerosis (MS).

Objectives We aimed to compare the diagnostic performance of intrathecal synthesis biomarkers to that of oligoclonal bands (OB) in diagnosing MS, radiological and clinical isolated syndromes (RIS-CIS) on a large cohort of patients collected over 10 years.

Methods We collected 1124 patients (58% females) in 10 years who underwent CSF analysis for intrathecal synthesis in the diagnostic work-up, and they were classified according to their diagnosis as 417 MS, 287 with other neurological inflammatory disorders (including 76 RIS-CIS), and 420 non-inflammatory diseases (excluding lymphoproliferative and infective diagnosis).

Results MS patients significantly differ from all other groups (including if considering the RIS-CIS cohort) for CSF KFLC, KFLC intrathecal fraction (IF), Kappa index, and OB. Evaluating the diagnostic performance, the Kappa index cut-off was 6.4 for diagnosing MS and 5.7 for predicting OB. A diagnostic algorithm could avoid IEF if the Kappa index is higher than 20.

Conclusions The KFLC index confirmed its accuracy for MS diagnosis in this large Italian cohort, adding information also in the RIS-CIS population.

Keywords Kappa index · Kappa free light chains · Intrathecal synthesis

Introduction

Multiple sclerosis (MS) diagnostic criteria include the detection of immunoglobulin (Ig) type G oligoclonal bands (OB) in the cerebrospinal fluid (CSF) to fulfil the dissemination in time (DIT) criterion in case of single clinical attacks with dissemination in space (DIS) [1]. The presence of OB is routinely assessed qualitatively with isoelectric focusing (IEF) on an agarose gel followed by immunoblotting

or immunofixation [2] and remains a supportive biomarker with a high sensitivity (ranging from 82% to almost 100%) and specificity (86–96%) [3]. However, IEF is a time-consuming and technically demanding technique with a strongly rater-dependent interpretation [4].

Alternative quantitative measures have been employed, and the most known is the IgG or Link index, calculated as the ratio between IgG ($Q_{IgG} = \text{IgG}_{\text{CSF}} / \text{IgG}_{\text{serum}}$) and albumin quotients ($Q_{\text{Alb}} = \text{Albumin}_{\text{CSF}} / \text{Albumin}_{\text{serum}}$) to normalize for blood-CSF barrier dysfunction [5]. The Link index correlates with OB detection [6] but is less sensitive (about 75%) [7], and test performances do not improve if combined with OB (versus OB alone) [8]. Several studies have assessed kappa-free light chains (KFLC) with ultrasensitive immunoassays on turbidimetric or nephelometric platforms [9] and compared CSF values and related indices to OB with variable cut-offs [8, 10], showing a diagnostic relevance. Subsequently, a recent meta-analysis determined a Kappa index cut-off of 6.1 to discriminate clinically isolated syndrome (CIS) and MS patients from controls with sensitivity

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and specificity of almost 90%, similar to OB detection [11]. Moreover, the Kappa index showed a higher diagnostic performance than the Link index [12]. Nevertheless, indices require a linear relationship between albumin and the protein of interest, regardless of the difference in the size of the molecules [13]. Other mathematical models have been proposed to express the intrathecal synthesis of Ig or FLC: an extended version of the IgG index [14], its logarithmic correction (LogIndex) [15], Tourtellotte's formula [16], Schuller's equation [17], or the hyperbolic Reiber's function, or Reibergram [18]. This latter formula corrects for the molecular size, predicting faster diffusion of smaller proteins [19]. Some authors reviewed the most frequently available metrics to express intrathecal synthesis, showing a higher sensitivity of hyperbolic functions despite a greater risk of false positive results [13].

For a decade, our group focused on the diagnostic, predictive, and prognostic roles of KFLC in MS patients. In our first two studies, the Kappa index showed high sensitivity and specificity compared to OB and the IgG index, with an optimal cut-off of 5.0 [20]. Consequently, we proposed a sequential diagnostic work-up using the Kappa index as a screening, followed by IEF only if above 5.0 [21]. Nevertheless, some authors support the use of solely OB accounting for higher sensitivity compared with KFLC-related parameters or propose to use OB as a screening method [22]. At present, our aim is a comprehensive re-evaluation of the diagnostic performance of intrathecal synthesis biomarkers and, more specifically, of KFLC, in a large cohort to re-define an optimal diagnostic cut-off and to evaluate the best metrics for CSF biomarkers. Firstly, we focused on discriminating MS from non-MS subjects. Secondly, we distinguished MS from radiologically and clinically isolated syndrome (RIS-CIS) cases.

Methods

Patient recruitment and clustering

We collected a total of 1392 paired CSF and serum samples upon a request from clinicians for assessing intrathecal synthesis, from April 2014 to May 2024. The exclusion criteria were: repetitive samples ($N=50$ cases), no definite diagnosis at the end of the diagnostic work-up (154), IEF or nephelometric results not available due to technical or sample-related issues (9), final diagnoses of central nervous system (CNS) infections (49) and/or lymphomas (6). Consequently, we finally included 1124 patients in the final analysis, classified as: (0) symptomatic controls ($N=53$), (1) clinically definite MS according to the McDonald criteria [1] (417), (2) RIS-CIS (79), (3) other CNS inflammatory diseases (107: acute disseminated encephalomyelitis, ADEM, autoimmune

encephalitis, Hashimoto's encephalopathy, neuromyelitis optica or NMO, rheumatologic involvement of the CNS, including neuroBehçet, lupus and vasculitis), (4) inflammatory diseases of the peripheral nervous system (PNS) (101: autoimmune polyneuropathies, cryoglobulinemic polyneuropathy, myasthenia gravis, vasculitic PNS disorders), (5) non-inflammatory PNS diseases (74: compressive, diabetic, hereditary, nutritional or paraneoplastic polyneuropathies), (6) other non-inflammatory neurological diseases (293: CNS primary tumor, cerebrovascular disorders, dementia, epilepsy, iatrogenic cerebral lesions, headache, metabolic encephalopathies, amyotrophic lateral sclerosis, parkinsonism, pseudotumor cerebri, radiculopathies).

These categories were pooled into three groups: a definite MS (417 patients), other inflammatory neurological disorders including RIS-CIS group (OIND: 287 patients), and other diagnoses (OD: 420 patients) (Fig. 1). For comparative analyses, we pooled the OIND and OD groups in the non-MS group (707 subjects). All patients signed an informed consent form for both diagnostic and research purposes. The study was approved by the Ethics Committee of Maggiore della Carità University Hospital of Novara (references: CE1804 and CE262/2022). Of note, part of this cohort was already included in previous studies published by our research group [20, 21, 23].

Sample collection and analytical techniques

All paired CSF and serum samples were centrifuged for 6 min at 3600 rpm, and, unless analyzed immediately, they were stored at 2–8 °C for up to one week or frozen at – 80 °C for long-term preservation.

OB detection was performed by IEF on an agarose gel followed by immunofixation with a mammalian peroxidase-labeled anti-IgG antiserum using the HYDRAGEL 3 CSF ISOFOCUSING or HYDRAGEL 9 CSF ISOFOCUSING kits by Sebia on the semiautomatic HYDRASYS 2 system (IgG band detection limit: 0.31 mg/L). Serum and CSF samples were always analyzed in parallel and pre-diluted, if needed, to adjust the IgG concentration to the same level recommended by the manufacturer (10–20 mg/L). CSF and serum quantitative measurements were performed by immunonephelometric assays on the BN II System by Siemens (Siemens Healthcare Diagnostics, Marburg, Germany). Albumin was determined using the N Antiserum to Human Albumin kit (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) containing rabbit polyclonal anti-human albumin antibodies (lower detection limits, or LOD: 17.7 mg/L in CSF and 0.355 g/L in serum). KFLC and LFLC were measured with the N Latex FLC kappa and N Latex FLC lambda kits by Siemens using polystyrene particles coated with anti-KFLC or anti-LFLC murine monoclonal antibodies (LOQ: 0.034 mg/L in CSF and 0.195 mg/L

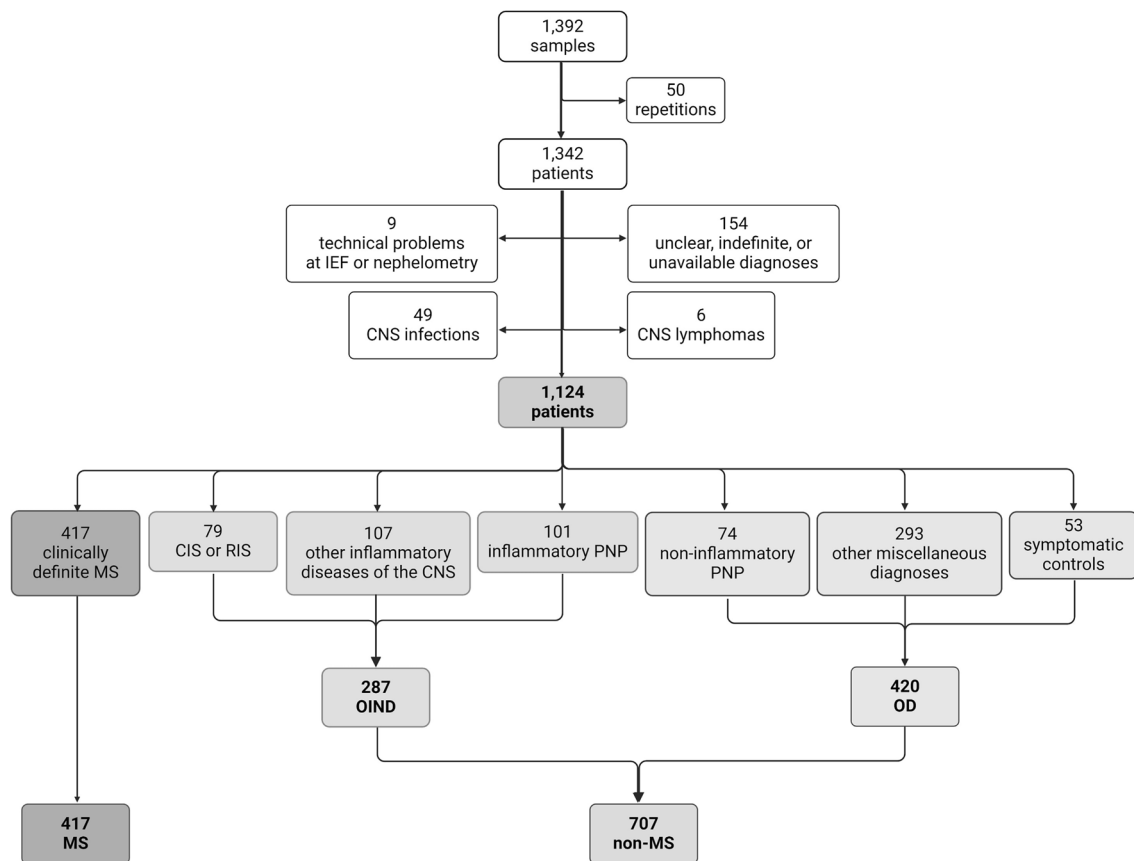


Fig. 1 Flowchart of sample collection, exclusion criteria, and patient clustering into separate diagnostic categories. CIS, clinically isolated syndrome; CNS, central nervous system; IEF, isoelectric focusing; MS, multiple sclerosis, OD, other diagnoses (non-inflammatory);

OIND, other inflammatory neurological disorders; PNP, peripheral neuropathy; RIS, radiologically isolated syndrome. Created with BioRender.com

in serum for KFLC and 0.480 mg/L in CSF and 0.532 mg/L in serum for LFLC). Total IgG concentration was measured using the N Antiserum to Human IgG by Siemens, containing rabbit polyclonal anti-human IgG antibodies (LOQ: 3.6 mg/L in CSF and 0.07 g/L in serum). Formulas to obtain linear and non-linear values of intrathecal synthesis are presented in Supplementary Table 1. Concerning non-linear functions, we specifically referred to the ones proposed by Reiber [24] and Tourtellotte [25].

Statistical analysis

Statistical analysis was performed using MedCalc Software version 22.021. Most data were not normally distributed according to the Shapiro–Wilk test and, consequently, they were all expressed as the median and interquartile range (IQR), and non-parametric tests were then employed. Quantitative variables were compared using the non-parametric Wilcoxon–Mann–Whitney *U* test. For multiple comparisons, the Chi-squared test was adopted for categorical variables while for quantitative data the non-parametric Kruskal–Wallis test

was chosen, followed by a post-hoc analysis using the Dunn test. *p* Values less than 0.05 were considered statistically significant. To assess the diagnostic performance, the online Bayesian calculator (version 1.2) [26] offered by SIPMEL (Italian Society of Clinical Pathology and Laboratory Medicine) was employed to calculate sensitivity, specificity, likelihood ratio for a positive test (LR+), likelihood ratio for a negative test (LR–), positive predictive value (PPV), negative predictive value (NPV), overall diagnostic accuracy, and number needed to diagnose (NND) with 95% confidence intervals (CI). The best cut-offs for each parameter were defined through ROC curve analysis, which reported an area under the curve (AUC) with a 95% CI. All ROC curves were obtained after setting a type I error at 0.05 and a type II error at 0.20.

Results

Demographic and clinical characteristics of the study population

Demographic and CSF features of each diagnostic category (from 0 to 6) are presented in Supplementary Table 2.

Our cohort included 655 females (58%) and 468 males (42%). 417 (37%) patients were diagnosed with MS, and 79 (7%) with RIS-CIS (included in the OIND group). The median age at the first spinal tap was 48 years (min–max range: 1–91), 38 (12–79) in the MS group, 50 (1–91) in the OIND category, and 56 (1–91) in the OD group. Age was significantly different among the groups, and the median age of MS patients was significantly lower than that of all categories except RIS-CIS.

The prevalence of OB positivity was 42% (473/1124) in the global cohort, 90% (374/417) in the MS group, and 25% (71/287) in the OIND category. However, considering the RIS-CIS patients as a separate group, the prevalence increased to 42% (33/79) but this subgroup did not fulfil the DIS criterion according to the McDonald criteria [1].

Comparison of diagnostic categories and of MS vs non-MS patients

We evidenced a significant difference for MS patients in comparison with each diagnostic category for CSF FLC-related parameters: absolute CSF KFLC concentration, Q_{KFLC} , Q_{LFLC} , KFLC_{Loc}, LFLC_{Loc}, KFLC IF, LFLC IF, Kappa index, Lambda index, and KFLC/LFLC ratio. In the post-hoc analysis, all KFLC-related parameters and some LFLC-related parameters persisted in differentiating MS from other groups (including group 2). On the other hand, the IgG index was not effective in discriminating between MS and RIS-CIS.

As a next step, we clustered patients into an MS group versus a non-MS group, including OIND and OD. However, given the peculiarity of RIS-CIS diagnoses, we also extracted the pure RIS-CIS patients from the non-MS group to compare them with MS subjects in a separate analysis (Supplementary Table 3 and Supplementary Fig. 1). Apart from CSF albumin, all parameters were significantly different between MS and non-MS patients. Interestingly, in discriminating between MS and RIS-CIS, only the FLC-related parameters showed the most statistically significant difference (p value < 0.0001).

Diagnostic performance of quantitative and qualitative biomarkers of intrathecal synthesis

To assess the diagnostic performance of the main biomarkers of intrathecal synthesis, including the qualitative detection of OB at IEF, ROC curve analysis and contingency

tables were applied to the whole cohort to define the optimal cut-offs and to calculate sensitivity, specificity, PPV, NPV, LR+, LR–, NNT, and overall diagnostic accuracy at each threshold. It was decided not to include the absolute concentrations of IgG, KFLC, and LFLC since they are prone to be influenced by plasma proteins in case of blood-CSF barrier dysfunction. Table 1 displays the diagnostic performance of all these parameters in their ability to discriminate clinically definite MS from all other diagnoses. We then assessed the performance of quantitative parameters in predicting OB detection at IEF (Table 2). Figure 2 shows the ROC curves of the three most widely used biomarkers of intrathecal synthesis, namely the IgG index, the Kappa index, and the Lambda index. In discriminating MS from non-MS subjects, the highest AUC (0.900) was associated with the Kappa index, with a discriminative cut-off of 6.4, immediately followed by the KFLC IF (0.899) and other KFLC-related parameters. Also LFLC-associated biomarkers showed a high AUC (0.803), while the performance of IgG-related biomarkers was markedly lower (AUC 0.683 for the IgG index, 0.691 for IgG IF, 0.679 for IgG_{Loc}, and 0.644 for IgG_{Tourtellotte}). OB detection at IEF also showed good diagnostic performance (AUC 0.878) and slightly higher sensitivity, specificity, PPV, and NPV compared with the Kappa index (for OB: Sn 89.7%, Sp 86.0%, PPV 79.1%, NPV 93.4%, for the Kappa index: Sn 87.3%, Sp 84.7%, PPV 77.1%, NPV 91.9%). Overall, OB detection had the highest diagnostic accuracy (87.4%).

The same trend was observed when assessing the diagnostic power of intrathecal synthesis biomarkers in predicting OB detection at IEF. The Kappa index and the Kappa IF confirmed themselves as the best diagnostic parameters with AUC of 0.975 and 0.976, respectively. This time, the best cut-off for the Kappa index was 5.7. In the second place, there were LFLC-related biomarkers, whereas the performance of IgG-associated parameters was much lower, with sensitivities as low as 55.6% and 45.7% for IgG_{Tourtellotte} and IgG_{Loc}, respectively.

Discussion

Our study performed a multiple comparison analysis of qualitative and quantitative biomarkers of the intrathecal immune response among all diagnostic categories, and the parameters for which MS patients differed with the highest level of significance were related to CNS-restricted FLC (CSF KFLC, Q_{KFLC} , Q_{LFLC} , KFLC_{Loc}, LFLC_{Loc}, KFLC IF, LFLC IF, Kappa index, Lambda index, and KFLC/LFLC ratio). Conversely, markers of intrathecal IgG synthesis did not differ between MS and RIS-CIS patients, implying that FLC synthesis could represent a better discriminator in this context, as we confirmed in the bivariate comparison of MS

Table 1 ROC curve analyses and Bayesian calculations to assess the performance of qualitative and quantitative biomarkers of intrathecal synthesis in discriminating between MS and non-MS patients

| Biomarker of intrathecal synthesis | Cut-off | AUC (95% CI) | p value | Sensitivity, % (95% CI) | Specificity, % (95% CI) | LR+ (95% CI) | LR- (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) | Diagnostic accuracy, % (95% CI) | NND (95% CI) |
|--|----------|----------------------|----------|-------------------------|-------------------------|-------------------|--------------------|-------------------|-------------------|---------------------------------|-------------------|
| OB, N | ≥ 2 | 0.878 (0.858; 0.897) | < 0.0001 | 89.7 (86.4; 92.3) | 86.0 (83.2; 88.4) | 6.41 (5.32; 7.71) | 0.12 (0.09; 0.16) | 79.1 (75.2; 82.5) | 93.4 (91.2; 95.1) | 87.4 (85.3; 89.2) | 1.32 (1.24; 1.44) |
| $Q_{IgG} \times 10^3$ | > 3.1 | 0.572 (0.542; 0.601) | < 0.0001 | 63.1 (58.2; 67.7) | 53.0 (49.3; 56.8) | 1.34 (1.21; 1.50) | 0.70 (0.60; 0.80) | 44.2 (41.6; 46.9) | 70.9 (67.8; 73.8) | 56.8 (53.8; 59.6) | 6.21 (4.12; 13.0) |
| $Q_{KFLC} \times 10^3$ | > 40.4 | 0.881 (0.860; 0.899) | < 0.0001 | 86.6 (82.9; 89.7) | 83.0 (80.1; 85.7) | 5.10 (4.31; 6.03) | 0.16 (0.13; 0.21) | 75.1 (71.8; 78.1) | 91.3 (89.1; 93.1) | 84.3 (82.1; 86.3) | 1.44 (1.33; 1.59) |
| $Q_{LFLC} \times 10^{3a}$ | > 31.3 | 0.750 (0.722; 0.777) | < 0.0001 | 58.6 (53.2; 63.8) | 82.6 (79.4; 85.5) | 3.37 (2.78; 4.10) | 0.50 (0.44; 0.57) | 65.6 (61.1; 69.8) | 77.9 (75.6; 80.1) | 74.0 (71.1; 76.6) | 2.42 (2.04; 3.05) |
| IgG _{Loc} mg/dL | > - 0.47 | 0.679 (0.651; 0.706) | < 0.0001 | 47.5 (42.6; 52.4) | 82.0 (79.0; 84.8) | 2.64 (2.19; 3.19) | 0.64 (0.58; 0.71) | 60.9 (56.4; 65.3) | 72.6 (70.6 - 74) | 69.2 (66.5; 71.8) | 3.39 (2.71; 4.59) |
| KFLC _{Loc} mg/dL | > 0.02 | 0.895 (0.876; 0.913) | < 0.0001 | 86.6 (82.9; 89.7) | 84.9 (82.0; 87.4) | 5.72 (4.78; 6.84) | 0.16 (0.12; 0.20) | 77.1 (73.8; 80.1) | 91.5 (89.3; 93.2) | 85.5 (83.3; 87.4) | 1.40 (1.30; 1.54) |
| LFLC _{Loc} ^a mg/dL | > - 0.01 | 0.805 (0.779; 0.830) | < 0.0001 | 71.8 (66.8; 76.5) | 76.3 (72.7; 79.6) | 3.03 (2.59; 3.54) | 0.37 (0.31; 0.44) | 63.1 (59.4; 66.7) | 82.7 (80.1 - 85) | 74.7 (71.9; 77.3) | 2.08 (1.79; 2.52) |
| IgG IF, % | > - 22.0 | 0.691 (0.663; 0.718) | < 0.0001 | 54.0 (49.0; 58.8) | 80.6 (77.5; 83.5) | 2.78 (2.34; 3.32) | 0.57 (0.51; 0.64) | 62.2 (58.0; 66.2) | 74.8 (72.7; 76.8) | 70.7 (68.0; 73.3) | 2.89 (2.38; 3.75) |
| KFLC IF, % | > 35.3 | 0.899 (0.880; 0.916) | < 0.0001 | 90.9 (87.7; 93.5) | 82.3 (79.2; 85.0) | 5.13 (4.36; 6.03) | 0.11 (0.082; 0.15) | 75.2 (72.1; 78.1) | 93.9 (91.8; 95.4) | 85.5 (83.3; 87.4) | 1.37 (1.28; 1.49) |
| LFLC IF ^a , % | > - 22.7 | 0.802 (0.776; 0.827) | < 0.0001 | 73.9 (68.9; 78.4) | 77.1 (73.6; 80.4) | 3.23 (2.76; 3.78) | 0.34 (0.28; 0.41) | 64.6 (60.9; 68.1) | 83.9 (81.3; 86.2) | 75.9 (73.1; 78.5) | 1.96 (1.71; 2.35) |
| IgG index | > 0.5 | 0.683 (0.655; 0.710) | < 0.0001 | 59.5 (54.6; 64.2) | 71.0 (67.5; 74.3) | 2.05 (1.78; 2.36) | 0.57 (0.50; 0.65) | 54.7 (51.3; 58.2) | 74.8 (72.4; 77.1) | 66.7 (63.9; 69.4) | 3.28 (2.61; 4.50) |
| Kappa index | > 6.4 | 0.900 (0.881; 0.917) | < 0.0001 | 87.3 (83.7; 90.3) | 84.7 (81.9; 87.3) | 5.71 (4.79; 6.82) | 0.15 (0.12; 0.19) | 77.1 (73.8; 80.1) | 91.9 (89.8; 93.6) | 85.7 (83.5; 87.6) | 1.39 (1.29; 1.52) |
| Lambda index ^a | > 3.2 | 0.803 (0.776; 0.827) | < 0.0001 | 77.0 (72.2; 81.3) | 73.9 (70.2; 77.3) | 2.95 (2.55; 3.41) | 0.31 (0.26; 0.38) | 62.5 (59.0; 65.8) | 85.0 (82.4; 87.4) | 75.0 (72.2; 77.6) | 1.97 (2.35; 1.72) |
| KFLC/LFLC ratio | > 1.0 | 0.803 (0.777; 0.828) | < 0.0001 | 80.5 (75.9; 84.5) | 73.1 (69.4; 76.5) | 2.99 (2.60; 3.43) | 0.27 (0.21; 0.33) | 62.8 (59.5; 66.0) | 86.9 (84.2; 89.2) | 75.7 (72.9; 78.3) | 1.87 (1.65; 2.20) |
| IgG _{Tourtelotte} ^a mg/day | > 0.32 | 0.644 (0.615; 0.672) | < 0.0001 | 51.6 (46.6; 56.4) | 74.0 (70.6; 77.2) | 1.98 (1.70; 2.31) | 0.65 (0.59; 0.73) | 53.9 (50.0; 57.7) | 72.1 (69.9; 74.3) | 65.7 (62.8; 68.4) | 3.92 (3.00; 5.75) |

AUC, area under the curve; CI, confidence interval; IF, Intrathecal fraction; IgG, immunoglobulin G; KFLC, kappa free light chain; LFLC, lambda free light chain; Loc, concentration of intrathecally synthesized protein (Reiber's formula); LR+, likelihood ratio for a positive test; LR-, likelihood ratio for a negative test; N, number; NND, Number needed to diagnose; NPV, negative predictive value; OB, Oligoclonal bands; PPV, positive predictive value

^aSerum and CSF LFLC were measured only in 964/1124 patients due to technical issues

Table 2 ROC curve analyses and Bayesian calculations to assess the performance of quantitative biomarkers of intrathecal synthesis in predicting OB detection at IEF

| Biomarker of intrathecal synthesis | Cut-off | AUC (95% CI) | p value | Sensitivity, % (95% CI) | Specificity, % (95% CI) | LR+ (95% CI) | LR- (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) | Diagnostic accuracy, % (95% CI) | NND (95% CI) |
|--|------------|----------------------|----------|-------------------------|-------------------------|--------------------|-------------------|-------------------|-------------------|---------------------------------|-------------------|
| $Q_{IgG} \times 10^3$ | > 2.9 | 0.599 (0.569; 0.627) | < 0.0001 | 68.9 (64.5; 73.1) | 50.8 (46.9; 54.8) | 1.40 (1.27; 1.55) | 0.61 (0.52; 0.71) | 50.5 (48.0; 52.9) | 69.2 (65.9; 72.4) | 58.5 (55.6; 61.3) | 5.06 (3.62; 8.60) |
| $Q_{KFLC} \times 10^3$ | > 33.6 | 0.958 (0.944; 0.969) | < 0.0001 | 92.6 (89.9; 94.8) | 89.1 (86.4; 91.4) | 8.49 (6.81; 10.6) | 0.08 (0.06; 0.11) | 86.1 (83.2; 88.5) | 94.3 (92.3; 95.8) | 90.6 (88.7; 92.1) | 1.22 (1.16; 1.31) |
| $Q_{LFLC} \times 10^{3a}$ | > 31.9 | 0.811 (0.785; 0.835) | < 0.0001 | 62.7 (57.7; 67.5) | 88.8 (86.0; 91.3) | 5.61 (4.40; 7.16) | 0.42 (0.37; 0.48) | 79.3 (75.0; 83.0) | 77.7 (75.3; 79.9) | 78.2 (75.5; 80.7) | 1.94 (1.71; 2.29) |
| IgG _{Loc} mg/dL | > - 0.34 | 0.712 (0.684; 0.738) | < 0.0001 | 45.7 (41.1; 50.3) | 89.9 (87.3; 92.1) | 4.50 (3.51; 5.78) | 0.60 (0.55; 0.66) | 76.6 (71.8; 80.8) | 69.5 (67.6; 71.3) | 71.3 (68.6; 73.8) | 2.82 (2.37; 3.51) |
| KFLC _{Loc} mg/dL | > 0.01 | 0.968 (0.956; 0.977) | < 0.0001 | 94.9 (92.5; 96.7) | 91.9 (89.5; 93.8) | 11.7 (9.00; 15.10) | 0.06 (0.04; 0.08) | 89.4 (86.7; 91.6) | 96.1 (94.4; 97.4) | 93.2 (91.5; 94.5) | 1.15 (1.11; 1.22) |
| LFLC _{Loc} ^a mg/dL | > - - 0.01 | 0.863 (0.839; 0.884) | < 0.0001 | 76.7 (72.2; 80.8) | 83.3 (79.9; 86.2) | 4.58 (3.79; 5.54) | 0.28 (0.23; 0.34) | 75.8 (72.1; 79.1) | 84.0 (81.4; 86.3) | 80.6 (78.0; 83.0) | 1.67 (1.50; 1.91) |
| IgG IF, % | > - 22.0 | 0.741 (0.714; 0.766) | < 0.0001 | 56.2 (51.6; 60.8) | 85.3 (82.3; 87.9) | 3.81 (3.12; 4.66) | 0.51 (0.46; 0.57) | 73.5 (69.4; 77.2) | 72.8 (70.7; 74.9) | 73.0 (70.4; 75.6) | 2.41 (2.07; 2.94) |
| KFLC IF, % | > 41.3 | 0.976 (0.965; 0.984) | < 0.0001 | 94.7 (92.3; 96.6) | 93.5 (91.4; 95.3) | 14.6 (10.9; 19.6) | 0.06 (0.04; 0.08) | 91.4 (88.8; 93.5) | 96.0 (94.3; 97.3) | 94.0 (92.5; 95.3) | 1.13 (1.09; 1.20) |
| LFLC IF ^a , % | > - 22.7 | 0.862 (0.838; 0.883) | < 0.0001 | 78.0 (73.6; 82.0) | 83.8 (80.5; 86.7) | 4.81 (3.96; 5.83) | 0.26 (0.22; 0.32) | 76.6 (73.0; 79.9) | 84.8 (82.2; 87.1) | 81.4 (78.9; 83.8) | 1.62 (1.46; 1.85) |
| IgG index | > 0.5 | 0.736 (0.709; 0.762) | < 0.0001 | 62.0 (57.4; 66.3) | 75.4 (71.9; 78.7) | 2.52 (2.17; 2.93) | 0.50 (0.45; 0.57) | 64.7 (61.1; 68.1) | 73.2 (70.7; 75.5) | 69.8 (67.0; 72.4) | 2.68 (2.23; 3.39) |
| Kappa index | > 5.7 | 0.975 (0.965; 0.984) | < 0.0001 | 94.3 (91.8; 96.2) | 94.0 (91.9; 95.7) | 15.7 (11.6; 21.4) | 0.06 (0.04; 0.09) | 92.0 (89.4; 93.9) | 95.8 (94.0; 97.0) | 94.1 (92.6; 95.4) | 1.13 (1.09; 1.19) |
| Lambda index ^a | > 3.2 | 0.860 (0.837; 0.882) | < 0.0001 | 81.3 (77.1; 85.1) | 80.6 (77.2; 83.8) | 4.20 (3.53; 4.99) | 0.23 (0.19; 0.29) | 74.1 (70.7; 77.3) | 86.4 (83.7; 88.7) | 80.9 (78.3; 83.3) | 1.61 (1.46; 1.84) |
| KFLC/LFLC ratio | > 1.0 | 0.855 (0.832; 0.877) | < 0.0001 | 84.4 (80.4; 87.9) | 79.8 (76.2; 83.0) | 4.17 (3.52; 4.93) | 0.20 (0.15; 0.25) | 74.0 (70.6; 77.1) | 88.2 (85.6; 90.4) | 81.6 (79.1; 84.0) | 1.56 (1.42; 1.76) |
| IgG _{Tourtellone} ^a mg/day | > 0.05 | 0.690 (0.663; 0.717) | < 0.0001 | 55.6 (51.0; 60.1) | 76.2 (72.7; 79.4) | 2.34 (1.99; 2.74) | 0.58 (0.52; 0.65) | 62.9 (59.1; 66.6) | 70.3 (67.9; 72.5) | 67.5 (64.7; 70.2) | 3.15 (2.54; 4.19) |

AUC, area under the curve; IF, intrathecal fraction; IgG, immunoglobulin G; KFLC, kappa free light chain; LFLC, lambda free light chain; Loc, concentration of intrathecally synthesized protein (Reiber's formula); LR+, likelihood ratio for a positive test; LR-, likelihood ratio for a negative test; NPV, negative predictive value; OB, oligoclonal bands; PPV, positive predictive value

^aSerum and CSF LFLC were measured only in 964/1124 patients due to technical issues

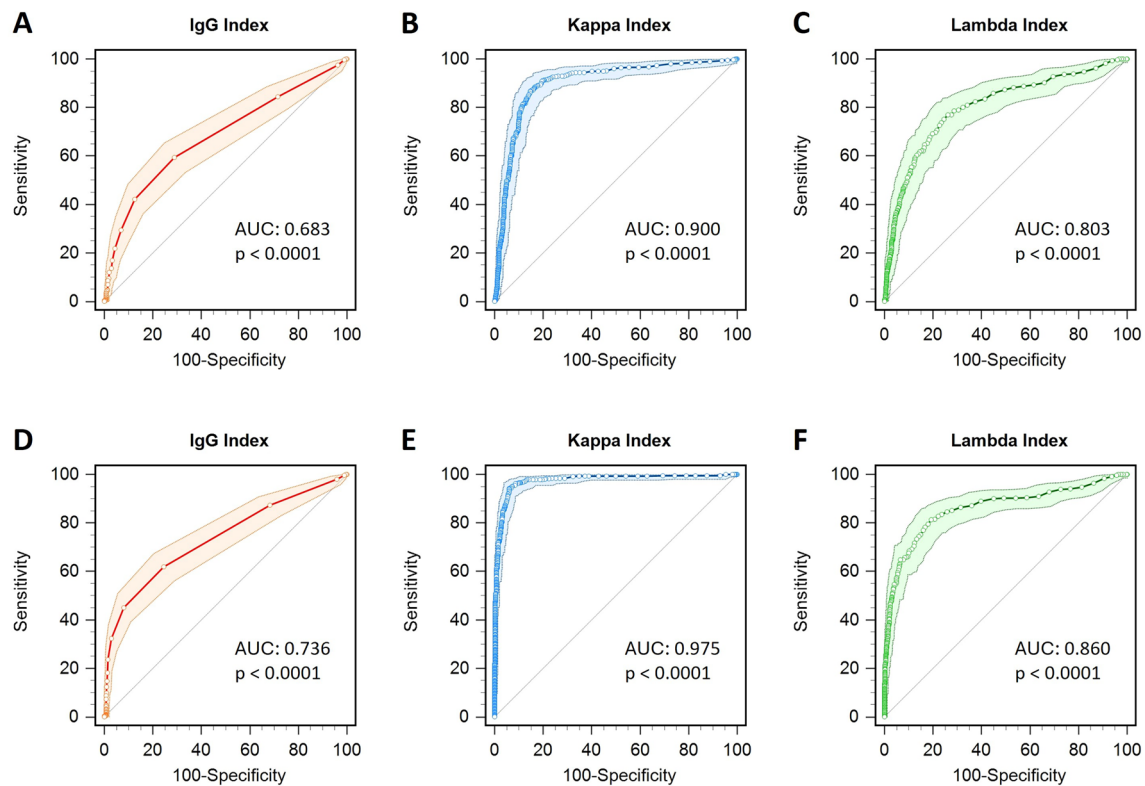


Fig. 2 ROC curve analysis for IgG index, Kappa index, and Lambda index to diagnose MS (A-C) and to predict OB (D-F). AUC, area under the curve; IgG, immunoglobulin G; MS, multiple sclerosis; OB, oligoclonal bands; ROC, receiver operating characteristic

versus non-MS patients and MS versus RIS-CIS groups. Indeed, all parameters except CSF albumin discriminate between MS and non-MS subjects. However, FLC-related biomarkers emerged in discriminating RIS-CIS patients from MS. Globally, our data suggest that the intrathecal Ig synthesis proposed as a supportive criterion in MS diagnosis [1] should be re-discussed considering biomarkers of intrathecal FLC synthesis.

Secondly, we assessed the diagnostic performances of intrathecal synthesis parameters to find the most informative, to define optimal cut-offs, and to evaluate the use of linear or non-linear functions. In this large cohort, the Kappa index cut-off in predicting OB detection at IEF did not differ significantly compared to the one originally proposed by our group (5.0 [20] versus new cut-off: 5.7), but it resulted higher for predicting MS diagnosis (new cut-off: 6.4). Anyway, the Kappa index confirmed an only slightly lower sensitivity in diagnosing MS if compared to IEF (87.3 versus 89.7), preserving reasonable specificity (84.7), and showing an accurate performance also at predicting OB. Other KFLC-related biomarkers, namely Q_{KFLC} , $KFLC_{Loc}$, and KFLC IF, also showed very good diagnostic accuracy and high AUC, especially in predicting OB detection at IEF. However, the Kappa index is easier to interpret than non-linear calculations. Consideration is needed for the graphical

use of Reiber's formulas with the aid of informatic tools able to generate a graph after simply importing the nephelometric measurements of the absolute concentrations of intrathecal synthesis biomarkers. Indeed, the visual impact of a graph might become at least an interpretative aid for clinicians, especially when the IEF interpretation is quite challenging.

Concerning the other biomarkers, those with the worst performance were all related to IgG intrathecal synthesis, i.e. Q_{IgG} , IgG_{Loc} , IgG IF, IgG index, and $IgG_{Tourtelotte}$. These data corroborate previous findings [7, 8, 27, 28], confirming that adding IgG-related biomarkers to qualitative CSF analysis does not improve the overall diagnostic accuracy.

Regarding the controversial role of LFLC, apart from Q_{LFLC} , all biomarkers of intrathecal LFLC synthesis ($LFLC_{Loc}$, LFLC IF, and Lambda index) showed satisfactory AUC at the ROC curve analysis, both for MS diagnosis (but below 80%) and for prediction of OB. However, LFLC were not measured in all patients (only 964/1124 had LFLC results available due to technical issues), so comparisons of their diagnostic performance were limited by the different sample size. Overall, these results seem to confirm what was already known from the literature [10, 29–31], i.e. the fact that LFLC does not provide any added value to MS diagnosis and are never superior to KFLC-related biomarkers. In the future, it would be interesting to investigate

whether LFLC could have a prognostic significance during MS patient follow-up [31].

Notably, the choice of the best cut-off is also strongly influenced by the study population and especially by the exclusion methodology used to stratify patients into different diagnostic categories. It is thus not surprising that the thresholds proposed in the literature are so variable [3]. For instance, some authors consider CIS together with MS [8], while others exclude all peripheral neurological disorders to better focus only on the CNS [10], resulting in considerable variability when it comes to the evaluation of test performance. As it relates to this study, we preferred to be not so strict during sample selection, excluding only infections and CNS lymphomas while keeping all other diagnoses to better reflect the real population referring to our institution. Perhaps a better standardization in the recruitment protocols would determine an improvement in the inter-study concordance and add value to the choice of shared decisional limits.

The adoption of a 5.7 cut-off for the Kappa index to predict OB is associated with a 95.8% NPV and appears a reasonable decisional limit should a sequential diagnostic algorithm be applied to the patient work-up, as previously proposed by our group and called a rule-out algorithm [20, 23] (Fig. 3). This cut-off would likely exclude OB with sufficient confidence to avoid performing IEF in all cases, reserving it only to those with a Kappa index > 5.7 as a confirmatory test. Using this algorithm on the actual cohort, 27 patients (2.4% of the total cohort) with a Kappa index ≤ 5.7 were OB+, of whom 11 with MS (2.6% of all MS patients; according to the McDonald criteria [1], the DIT criterion was fulfilled even without the need of CSF analysis) and 2 with RIS-CIS (despite intrathecal synthesis, according to the McDonald criteria [1]). On the other hand, 39 patients (3.4% of the entire cohort) had a Kappa index > 5.7 but were OB-, of whom 5 had MS (1.2% of all MS patients) and 6 had RIS-CIS. Of note, using this algorithm, 639 patients would not be tested with IEF (57%), resulting in savings between 7600 and 14,400 euros (pending on the kit types)

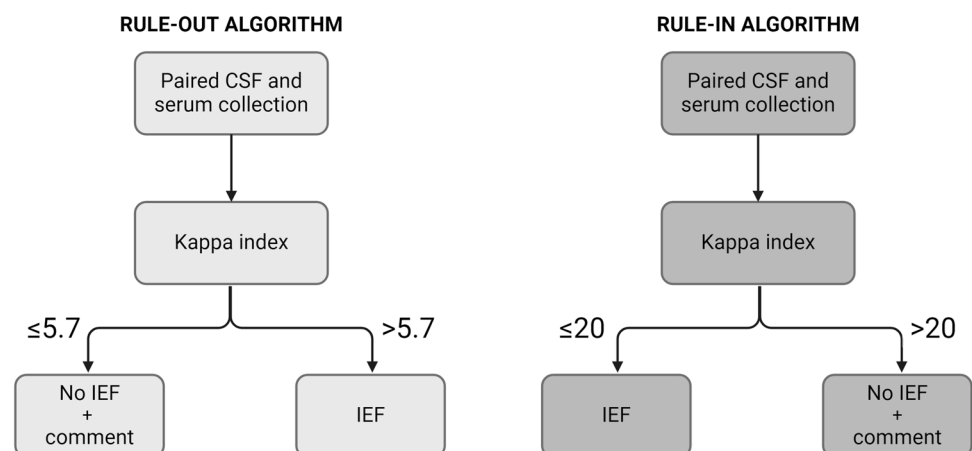
when considering only the costs related to the kit purchase and not those deriving from staff time, since it would have been too difficult to provide an accurate estimate.

Another approach to discuss would be to avoid IEF in patients with a Kappa index above a certain threshold, relying on a high PPV, called a rule-in algorithm. To reach a 100% PPV, the cut-off would be inflated to 121.4, dropping the sensitivity to 13.5% (95% CI 10.6–16.9). Consequently, we considered the highest cut-off published for the Kappa index, i.e., the value of 20 by Desplat-Jégo et al. [11, 30], which showed a sensitivity of 69.7% and a specificity of 81.8% in the original paper and a specificity of 98.8% (95% CI 97.6–99.5) and a PPV of 97.4% (95% CI 94.9–98.7) applied to our cohort in a rule-in diagnostic algorithm. In this scenario, 8 patients with a Kappa index > 20 were OB- and, of these, 2 had MS. In this case, we would have avoided 303 IEF analyses (27% of the total) and a cost saving ranging from 3800 to 7200 euros (Fig. 3). According to these speculations, we might update our diagnostic algorithm to a rule-in method, but an interdisciplinary consensus is hence needed according to the resource availability.

Conclusions

Our study firstly confirmed the role of quantitative measures of intrathecal synthesis for MS diagnosis on a large Italian cohort evaluating the diagnostic performance of CSF biomarkers and re-defining cut-offs to optimize the diagnostic work-up. Globally, OB detection remains the gold standard for MS diagnosis when clinical and radiological diagnostic criteria are not fully satisfied. Notwithstanding this, quantitative biomarkers of intrathecal synthesis represent a valid adjunct to CSF qualitative analysis, and, among these, the Kappa index is confirmed to have a diagnostic accuracy similar to that of OB. Regarding the best metrics to express biomarkers of intrathecal synthesis, using linear indices appear the most appropriate both to correct for any

Fig. 3 Diagnostic algorithms proposed for the clinical laboratory. CSF, cerebrospinal fluid; IEF, isoelectric focusing. Created with BioRender.com



potential extra-CNS protein contribution and to facilitate calculations and result interpretation. A rule-in algorithm, that would avoid IEF in patients with a Kappa index above 20, could be considered for clinical practice. Further discussion is needed to determine the most accurate approach to avoid IEF in some cases, and a role for LFLC has not been defined in the MS work-up yet.

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Availability of data and material Data available on request.

Declarations

Conflicts of interest None for all authors for this work.

Ethics approval Local Ethical Committee approvals (Comitato Etico Interaziendale AOU “Maggiore della Carità” di Novara, ASL BI, ASL NO, ASL VCO): CE 060/2022 and 260/2022). Consent to participate/consent for publication: written consent obtained from all participants.

References

- Thompson AJ, Banwell BL, Barkhof F et al (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol* 17(2):162–173. [https://doi.org/10.1016/S1474-4422\(17\)30470-2](https://doi.org/10.1016/S1474-4422(17)30470-2)
- Freedman MS, Thompson EJ, Deisenhammer F et al (2005) Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. *Arch Neurol* 62(6):865–870. <https://doi.org/10.1001/ARCHNEUR.62.6.865>
- Christiansen M, Gjelstrup MC, Stilund M, Christensen T, Petersen T, Møller HJ (2019) Cerebrospinal fluid free kappa light chains and kappa index perform equal to oligoclonal bands in the diagnosis of multiple sclerosis. *Clin Chem Lab Med* 57(2):210–220. https://doi.org/10.1515/CCLM-2018-0400/DOWNLOADASSET/SUPPL/CCLM-2018-0400_SUPPL.ZIP
- Franciotta D, Lolli F (2007) Interlaboratory reproducibility of isoelectric focusing in oligoclonal band detection. *Clin Chem* 53(8):1557–1558. <https://doi.org/10.1373/CLINCHEM.2007.089052>
- Tibbling G, Link H, Öhman S (1977) Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. *Scand J Clin Lab Invest* 37(5):385–390. <https://doi.org/10.1080/00365517709091496>
- Mayringer I, Timeltaler B, Deisenhammer F (2005) Correlation between the IgG index, oligoclonal bands in CSF, and the diagnosis of demyelinating diseases. *Eur J Neurol* 12(7):527–530. <https://doi.org/10.1111/J.1468-1331.2005.00997.X>
- Fortini AS, Sanders EL, Weinschenker BG, Katzmann JA (2003) Cerebrospinal fluid oligoclonal bands in the diagnosis of multiple sclerosis. Isoelectric focusing with IgG immunoblotting compared with high-resolution agarose gel electrophoresis and cerebrospinal fluid IgG index. *Am J Clin Pathol* 120(5):672–675. <https://doi.org/10.1309/Y5VFF2UAWORK5W63>
- Gurtner KM, Shosha E, Bryant SC et al (2018) CSF free light chain identification of demyelinating disease: comparison with oligoclonal banding and other CSF indexes. *Clin Chem Lab Med* 56(7):1071–1080. https://doi.org/10.1515/CCLM-2017-0901/DOWNLOADASSET/SUPPL/CCLM-2017-0901_SUPPL.ZIP
- Bradwell AR, Carr-Smith HD, Mead GP et al (2001) Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem* 47(4):673–680. <https://doi.org/10.1093/clinchem/47.4.673>
- Levrant M, Laurent-Chabalier S, Ayrignac X et al (2023) Kappa free light chain biomarkers are efficient for the diagnosis of multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* 10(1):e200049. <https://doi.org/10.1212/NXI.000000000200049>
- Hegen H, Walde J, Berek K et al (2022) Cerebrospinal fluid kappa free light chains for the diagnosis of multiple sclerosis: a systematic review and meta-analysis. *Mult Scler J*. <https://doi.org/10.1177/13524585221134213>. (published online February 1, 2022)
- Matute-Blanch C, Villar LM, Álvarez-Cermeño JC et al (2018) Neurofilament light chain and oligoclonal bands are prognostic biomarkers in radiologically isolated syndrome. *Brain* 141(4):1085–1093. <https://doi.org/10.1093/BRAIN/AWY021>
- Schwenkenbecher P, Konen FF, Wurster U et al (2019) Reiber's diagram for kappa free light chains: the new standard for assessing intrathecal synthesis? *Diagnosics* 9(4):194–200. <https://doi.org/10.3390/DIAGNOSTICS9040194>
- Öhman S, Forsberg P, Nelson N, Vrethem M (1989) An improved formula for the judgement of intrathecally produced IgG in the presence of blood brain barrier damage. *Clin Chim Acta* 181(3):265–272. [https://doi.org/10.1016/0009-8981\(89\)90232-5](https://doi.org/10.1016/0009-8981(89)90232-5)
- Luxton RW, McLean BN, Edward JT (1990) Isoelectric focusing versus quantitative measurements in the detection of intrathecal local synthesis of IgG. *Clin Chim Acta* 187(3):297–308. [https://doi.org/10.1016/0009-8981\(90\)90115-9](https://doi.org/10.1016/0009-8981(90)90115-9)
- Tourtellotte W (1970) On cerebrospinal fluid immunoglobulin-G (IgG) quotients in multiple sclerosis and other diseases: a review and a new formula to estimate the amount of IgG synthesized per day by the central nervous system. *J Neurol Sci* 10(3):279–304. [https://doi.org/10.1016/0022-510X\(70\)90156-5](https://doi.org/10.1016/0022-510X(70)90156-5)
- Schuller E, Sagar HJ (1981) Local synthesis of CSF immunoglobulins. *J Neurol Sci* 51(3):361–370. [https://doi.org/10.1016/0022-510X\(81\)90114-3](https://doi.org/10.1016/0022-510X(81)90114-3)
- Reiber H (1994) Flow rate of cerebrospinal fluid (CSF)—a concept common to normal blood–CSF barrier function and to dysfunction in neurological diseases. *J Neurol Sci* 122(2):189–203. [https://doi.org/10.1016/0022-510X\(94\)90298-4](https://doi.org/10.1016/0022-510X(94)90298-4)
- Senel M, Mojib-Yezdani F, Braisch U et al (2019) CSF free light chains as a marker of intrathecal immunoglobulin synthesis in multiple sclerosis: a blood–CSF barrier related evaluation in a large cohort. *Front Immunol* 10:641–649. <https://doi.org/10.3389/fimmu.2019.00641>
- Crespi I, Sulas MG, Mora R et al (2017) Combined use of kappa free light chain index and isoelectrofocusing of cerebrospinal fluid in diagnosing multiple sclerosis: performances and costs. *Clin Lab* 63(3):551–559. <https://doi.org/10.7754/Clin.Lab.2016.160930>
- Crespi I, Vecchio D, Serino R et al (2019) K index is a reliable marker of intrathecal synthesis, and an alternative to igG index in multiple sclerosis diagnostic work-up. *J Clin Med* 8(4):2–8. <https://doi.org/10.3390/jcm8040446>

22. Schwenkenbecher P, Konen FF, Wurster U et al (2018) The persisting significance of oligoclonal bands in the dawning era of kappa free light chains for the diagnosis of multiple sclerosis. *Int J Mol Sci* 19(12):3803. <https://doi.org/10.3390/IJMS19123796>
 23. Vecchio D, Crespi I, Virgilio E et al (2019) Kappa free light chains could predict early disease course in multiple sclerosis. *Mult Scler Relat Disord* 30:81–84. <https://doi.org/10.1016/J.MSARD.2019.02.001>
 24. Reiber H, Peter JB (2001) Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. *J Neurol Sci* 184(2):101–122. [https://doi.org/10.1016/S0022-510X\(00\)00501-3](https://doi.org/10.1016/S0022-510X(00)00501-3)
 25. Tourtellotte W, Baumhefner R, Syndulko K et al (1988) The long march of the cerebrospinal fluid profile indicative of clinical definite multiple sclerosis; and still marching. *J Neuroimmunol* 20(2–3):217–227. [https://doi.org/10.1016/0165-5728\(88\)90163-4](https://doi.org/10.1016/0165-5728(88)90163-4)
 26. Società Italiana di Patologia Clinica e Medicina di Laboratorio. <https://www.sipmel.it/it/risorse/strumenti/calcolatore>. Accessed 3 Sept 2024
 27. Sindic CJM, Van Antwerpen MP, Goffette S (2001) The intrathecal humoral immune response: laboratory analysis and clinical relevance. *Clin Chem Lab Med* 39(4):333–340. <https://doi.org/10.1515/CCLM.2001.052/MACHINEREADABLECITATION/RIS>
 28. Presslauer S, Milosavljevic D, Brücke T, Bayer P, Hübl W (2008) Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis. *J Neurol* 255(10):1508–1514. <https://doi.org/10.1007/S00415-008-0954-Z/METRICS>
 29. Desplat-Jégo S, Feuillet L, Pelletier J, Bernard D, Chérif AA, Boucraut J (2005) Quantification of immunoglobulin free light chains in cerebrospinal fluid by nephelometry. *J Clin Immunol* 25(4):338–345. <https://doi.org/10.1007/S10875-005-5371-9>
 30. Leurs CE, Twaalfhoven HAM, Lissenberg-Witte BI et al (2020) Kappa free light chains is a valid tool in the diagnostics of MS: a large multicenter study. *Mult Scler* 26(8):923. <https://doi.org/10.1177/1352458519845844>
 31. Voortman MM, Stojakovic T, Pirpamer L et al (2017) Prognostic value of free light chains lambda and kappa in early multiple sclerosis. *Mult Scler* 23(11):1496–1505. <https://doi.org/10.1177/1352458516681503>
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