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Kappa Free Light Chain Biomarkers Are Efficient for the Diagnosis of Multiple Sclerosis

A Large Multicenter Cohort Study

Michael Levraut, MD, Sabine Laurent-Chabalier, PhD, Xavier Ayrignac, MD, PhD, Kévin Bigaut, MD, Manon Rival, MD, Sanae Squalli, MD, Hélène Zéphir, MD, PhD, Tifanie Alberto, MD, Jean-David Pekar, MD, Jonathan Ciron, MD, Damien Biotti, MD, Bénédicte Puissant-Lubrano, PharmD, PhD, Jean-Philippe Camdessanché, MD, PhD, Yannick Tholance, PharmD, PhD, Olivier Casez, MD, Bertrand Toussaint, MD, PhD, Jeanne Marion, MD, Thibault Moreau, MD, PhD, Daniela Lakomy, MD, PhD, Audrey Thomasset, MD, Elisabeth Maillart, MD, Delphine Sterlin, PharmD, PhD, Aude Maurousset, MD, Auriane Rocher, MD, David Axel Laplaud, MD, PhD, Edith Bigot-Corbel, PharmD, PhD, Pierre-Olivier Bertho, MD, Jean Pelletier, MD, PhD, Joseph Boucraut, MD, PhD, Pierre Labauge, MD, PhD, Thierry Vincent, MD, PhD, Jérôme De Sèze, MD, PhD, Isabelle Jahn, MD, Barbara Seitz-Polski, MD, PhD, Eric Thouvenot, MD, PhD, and Christine Lebrun-Frenay, MD, PhD, on behalf of Société Francophone de la Sclérose En Plaques (SFSEP)

Correspondence
Dr. Levraut
michael.levraut@gmail.com

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Abstract

Background and Objectives

Kappa free light chains (KFLC) seem to efficiently diagnose MS. However, extensive cohort studies are lacking to establish consensus cut-offs, notably to rule out non-MS autoimmune CNS disorders. Our objectives were to (1) determine diagnostic performances of CSF KFLC, KFLC index, and KFLC intrathecal fraction (IF) threshold values that allow us to separate MS from different CNS disorder control populations and compare them with oligoclonal bands' (OCB) performances and (2) to identify independent factors associated with KFLC quantification in MS.

Methods

We conducted a retrospective multicenter study involving 13 French MS centers. Patients were included if they had a noninfectious and nontumoral CNS disorder, eligible data concerning CSF and serum KFLC, albumin, and OCB. Patients were classified into 4 groups according to their diagnosis: MS, clinically isolated syndrome (CIS), other inflammatory CNS disorders (OIND), and noninflammatory CNS disorder controls (NINDC).

Results

One thousand six hundred twenty-one patients were analyzed (675 MS, 90 CIS, 297 OIND, and 559 NINDC). KFLC index and KFLC IF had similar performances in diagnosing MS from nonselected controls and OIND ($p = 0.123$ and $p = 0.991$ for area under the curve [AUC] comparisons) and performed better than CSF KFLC ($p < 0.001$ for all AUC comparisons). A KFLC index of 8.92 best

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From the Unité de Recherche Clinique Cote d'Azur-UR2CA URRIS (M.L., C.L.-F.), Centre Hospitalier Universitaire de Nice; Département de Biostatistiques (S.L.-C.), Epidémiologie clinique et Santé Publique, Centre Hospitalier Universitaire de Nîmes; Département de Neurologie (X.A., P.L.), Centre Hospitalier Universitaire de Montpellier, Montpellier; Département de Neurologie (K.B., J.D.S.), Centre Hospitalier Universitaire de Strasbourg; Service de Neurologie (M.R., E.T.), Centre Hospitalier Universitaire de Nîmes; Laboratoire de Biochimie (S.S.), Centre Hospitalier Universitaire de Nîmes; Département de Neurologie (H.Z., T.A.), Centre Hospitalier Universitaire de Lille; Centre Hospitalier Universitaire de Lille (J.-D.P.), Service de Biochimie Automatisée, Protéines (UF 8833), Lille; Département de Neurologie (J.C., D.B.), Centre Hospitalier Universitaire de Toulouse, Hôpital Pierre-Paul Riquet, CRC-SEP, F-31059, Toulouse Cedex 9; Laboratoire d'Immunologie (B.P.-L.), Centre Hospitalier Universitaire de Toulouse; Département de Neurologie (J.-P.C.), Centre Hospitalier Universitaire de Saint-Etienne; Laboratoire de Biochimie (Y.T.), Centre Hospitalier Universitaire de Saint-Etienne; Département de Neurologie (O.C.), Centre Hospitalier Universitaire de Grenoble Alpes; Univ. Grenoble Alpes (B.T.), CNRS, UMR 5525, VetAgro Sup, Grenoble INP, CHU Grenoble Alpes, TIMC; Laboratoire de Biochimie (B.T., J.M.), Centre Hospitalier Universitaire de Grenoble Alpes; Département de Neurologie (T.M.), Centre Hospitalier de Dijon; Laboratoire de Biochimie (D.L., A.T.), Centre Hospitalier Universitaire de Dijon; Département de Neurologie (E.M.), Assistance-Publique des Hôpitaux de Paris, Centre Hospitalier Universitaire de la Pitié-Salpêtrière; Laboratoire d'Immunologie (D.S.), Assistance Publique des Hôpitaux de Paris, Centre Hospitalier Universitaire de la Pitié-Salpêtrière, Paris; Département de Neurologie (A.M., A.R.), Centre Hospitalier Universitaire de Tours; Université de Nantes (D.A.L.), C2RTI Inserm UMR1064, service de Neurologie, CHU Nantes; Département de Biochimie (E.B.-C., P.-O.B.), Centre Hospitalier Universitaire de Nantes; Aix Marseille Univ (J.P.), APHM, Hôpital de la Timone, Pôle de Neurosciences Cliniques, Service de Neurologie; Laboratoire de Biochimie (J.B.), Assistance Publique des Hôpitaux de Marseille, Centre Hospitalier Universitaire de La Conception; Laboratoire d'Immunologie (T.V.), Centre Hospitalier Universitaire de Montpellier; Laboratoire d'Immunologie (I.J.), Centre Hospitalier Universitaire Strasbourg; Laboratoire d'Immunologie (B.S.-P.), Centre Hospitalier Universitaire de Nice; Institut de Génomique Fonctionnelle (E.T.), Université de Montpellier, CNRS, INSERM, Montpellier; and Département de Neurologie (C.L.-F.), Centre Hospitalier Universitaire de Nice, France.

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Glossary

AUC = area under the curve; **CIS** = clinically isolated syndrome; **FLC** = free light chain; **IF** = intrathecal fraction; **KFLC** = Kappa free light chain; **LDL** = lower detectable limit; **LFLC** = lambda FLC; **NINDC** = noninflammatory CNS disorder control; **OCB** = oligoclonal band; **OIND** = other inflammatory CNS disorder; **SC** = symptomatic control.

separated MS/CIS from the entire nonselected control population, with better performances than OCB ($p < 0.001$ for AUC comparison). A KFLC index of 11.56 best separated MS from OIND, with similar performances than OCB ($p = 0.065$). In the multivariate analysis model, female gender ($p = 0.003$), young age ($p = 0.013$), and evidence of disease activity ($p < 0.001$) were independent factors associated with high KFLC index values in patients with MS, whereas MS phenotype, immune-modifying treatment use at sampling, and the FLC analyzer type did not influence KFLC index.

Discussion

KFLC biomarkers are efficient tools to separate patients with MS from controls, even when compared with other patients with CNS autoimmune disorder. Given these results, we suggest using KFLC index or KFLC IF as a criterion to diagnose MS.

Classification of Evidence

This study provides Class III evidence that KFLC index or IF can be used to differentiate patients with MS from nonselected controls and from patients with other autoimmune CNS disorders.

MS is the most common chronic inflammatory and demyelinating CNS disease worldwide. It mainly presents as relapsing subacute clinical demyelinating events that progressively lead to neurologic disabilities. For the past 2 decades, clinical research has focused on how to diagnose MS as early as possible and prevent relapse and handicap by initiating disease-modifying treatments as soon as possible.

In the last MS diagnostic criteria update,[1] biomarkers have an essential role in the diagnostic workup of patients presenting with a typical first demyelinating event. Clinical and MRI abnormalities can ensure MS diagnosis to fulfill dissemination in space and time criteria. CSF oligoclonal IgG bands (OCB) detection can avoid fulfilling the dissemination in time to treat patients with early MS. OCB detection reflects intrathecal B-cell activity, which are critical but nonspecific, effector cells in MS.[2,3] Nevertheless, the level of intrathecal B-cell activity could be an exciting field of research to separate MS from other CNS inflammatory diseases[4] and target treatment. As OCB are a qualitative biomarker, their detection does not permit quantification of the intrathecal B-cell activity.[5] Moreover, isoelectric focusing is a time-consuming procedure that requires an experienced biologist to provide reliable results.[6] Therefore, novel diagnostic biomarkers are being investigated, including blood and CSF free light chains (FLCs), small immunoglobulin compounds that may reflect and quantify B-cell activity. Kappa FLC (KFLC) has shown good overall performance in diagnosing MS, whereas lambda FLC (LFLC) performance seems to be lower than OCB.[7-15] FLC measurement has the advantage of being an automatized, quantitative, easy to perform, and labor- and time-saving procedure.[16]

In practice, evaluation of the CSF and serum KFLC and albumin provides the level of intrathecal KFLC synthesis by calculating the KFLC index or KFLC intrathecal fraction (KFLC IF), including blood-CSF barrier permeability data.[7,17] Both biomarkers could help diagnose OCB negative,[18] and IgA or IgM OCB positive patients with MS[19] and identify intrathecal immunoglobulin synthesis in patients presenting with one isolated band on isoelectric focusing.[20] KFLC could also predict the risk of developing a second clinical attack in patients presenting with a first clinical demyelinating event.[21-25] However, the use of either the KFLC index, which refers to linear modeling of a locally synthesized fraction of KFLC, or the KFLC IF, which refers to hyperbolic modeling of KFLC intrathecal synthesis, is not clear.[26,27] Even if the KFLC IF seems closer to the pathophysiology, some studies show that both biomarkers perform equally in diagnosing MS.[27-29] There is no consensus concerning the KFLC index or KFLC IF cut-off to use in daily practice to confirm MS-related immunoglobulin intrathecal synthesis. Moreover, only a few studies have investigated the performance of KFLC in ruling out non-MS CNS inflammatory disorders.[30] Finally, studies comparing the performance of the KFLC index and KFLC IF in a large cohort of patients are lacking.

The primary research question of this study was: Does KFLC biomarkers (CSF KFLC, KFLC index, and KFLC IF) allow to separate patients with MS from different controls (a nonselected CNS disorder population, and a non-MS autoimmune CNS disorder population) accurately? And compare their performances to OCB.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

According to French laws, the patients received transparent, fair, and appropriate research information, and nonopposition

participation was available. The study received approval from the institutional review board of the University Hospital of Nice, and the protocol was registered on ClinicalTrial.gov (NCT05088473).

Patients and Controls

Thirteen French MS centers participated in this retrospective cohort study. Patients were eligible if they had (1) a diagnostic workup for a suspected CNS disorder and (2) eligible data for CSF and serum KFLC, albumin, and a known OCB status, up to March 31, 2021. Patients were excluded if the final diagnosis was (1) peripheral neurologic disorder, (2) infectious CNS disease, (3) CNS tumor, or if they had a monoclonal gammopathy or severe chronic kidney injury (glomerular filtration rate < 30 mL/min/1.73 m^2) to avoid FLC misinterpretation.[31]

Peripheral neurologic diseases, CNS infections, and tumors were excluded so that the other inflammatory CNS disorder (OIND) group was as close as possible to clinical and/or radiologic MS mimickers. According to the final diagnoses, patients were separated into groups according to previously published classification criteria[32]: MS, clinically isolated syndrome (CIS), other inflammatory neurologic disorder (OIND), noninflammatory neurologic disorder (NIND), and symptomatic control (SC) groups. Patients classified as SC and NIND were grouped into a noninflammatory neurologic disorder control (NINDC) group. All patients with MS fulfilled the 2017 McDonald criteria.

Collected Data

The following data were collected based on patients' medical records: age, sex, immune modifying drug ongoing at sampling, final diagnosis, CSF and serum albumin, white blood cells, IgG, OCB status, and KFLC. When available, LFLC data were collected. For patients with MS/CIS, the type of clinical demyelinating event and evidence of disease activity (combined variable of the presence of an ongoing clinical demyelinating symptom at CSF sampling and/or gadolinium-enhanced T1 lesions on a full 30-day time lag MRI) were collected.

Laboratory Features

FLC were measured in each center's laboratory using a turbidimetric or nephelometric analyzer; 9 centers used the turbidimeter Optilite (The Binding Site, Birmingham, UK), 2 used the turbidimeter SPAPLUS (The Binding Site, Birmingham, UK), one the nephelometer BN ProSpec (Siemens Healthcare Diagnostics Products GmbH), and one the nephelometer BNII (Siemens Healthcare Diagnostics Products GmbH) with the serum-free light chain immunoassay Freelite (The Binding Site, Birmingham, UK). FLC measurements were performed according to the manufacturer's instructions. Serum and CSF albumin levels were determined with the same turbidimetric or nephelometric analyzer. Four of the 13 centers used FLC as part of the diagnostic workup, and data were measured on fresh samples. For the other centers, paired CSF and serum were immediately centrifuged after sampling and stored in polypropylene tubes within 2 hours at −80°C until assay. eTable 1 (links.lww.com/NXI/A753) summarizes the laboratory data according to each center. The assessment of OCB was performed by isoelectric focusing and subsequent immunoglobulin using IgG-specific antibody staining, according to each center's laboratory routine care, fulfilling the recommended standards for CSF analysis.[5] In each center, OCB patterns were evaluated by experienced biologists and classified as negative or positive. A cut-off ≥ 2 CSF-restricted bands were used to define OCB positivity.

Free Light Chain Biomarkers Determination

The FLC index and the FLC IF were determined for each patient.

a. The FLC index was calculated with the formula: FLC index=Q_{FLC} /Q_{alb} with Q_{FLC} = CSF FLC/serum FLC and Q_{alb}= CSF albumin/serum albumin
b. The FLC IF was calculated with the formulas[17,27]: FLC IF=(FLC $_{loc}$ /CSF FLC)×100 with FLC $_{loc}$ = (Q_{FLC} /Q_{FLC}(lim)) × serum FLC and Q_{KFLC}(lim)=3.27 (Q_{alb}2 + 33)0.5 − 8.2 (×10-3) Q_{LFLC}(lim) = 3.1276 × Q_{alb}0.8650

For patients whose CSF FLC concentration was below the analyzer's lower detectable limit (LDL), we assigned an empirical value equal to the LDL divided by 2.[17] The attributed CSF KFLC concentrations were 0.17 mg/L for the Optilite and the BNII, 0.19 mg/L for the SPAPLUS, and 0.03 mg/L for the BN ProSpec.

Statistical Analysis

Categorical variables were expressed as counts with percentages, and continuous variables as means and SD or medians and interquartile ranges because of their distribution. Normality and heteroskedasticity of the baseline demographic, clinical, and biology characteristics were assessed using the Shapiro-Wilk and Levene tests, respectively.

Differences between groups were tested using a Wilcoxon-Mann-Whitney test to compare 2 groups of non-normally distributed data. The Kruskal-Wallis test with post hoc Conover was applied to reach more than 2 groups. For normally distributed continuous variables, a Student *t*-test was used. A Fischer exact test or a χ^2 test was performed for categorical variables. All analyses were 2-tailed.

To evaluate FLC biomarkers' diagnostic performance, we first pooled all patients presenting with a demyelinating disease (MS and CIS groups) and compared them with control groups (OIND and NINDC). Then, the MS group was compared with OIND. Logistic regressions were implemented for each population to obtain the ROC curves used to measure the diagnostic capacity (area under the curve [AUC], 95% CI) of the different FLC biomarkers. The DeLong method assessed the comparison of each FLC biomarker by comparing their areas under the ROC curves with their 95% CI. The Youden index allowed the definition of each biomarker's optimal threshold values.

The patients were classified as positive or negative for each FLC biomarker to define categorical variables from the obtained thresholds. The diagnostic capacity of each FLC biomarker of interest was compared with OCB via the categorical variables. Comparisons of sensitivities and specificities were made using the McNemar test, and comparisons of positive and negative predicted values were made using the Moskowitz and Pepe[33] method.

The identification of independent clinical factors associated with KFLC index values in patients with MS was first performed using a univariate linear regressions implementation for each element to be tested (age, sex, type of analyzer, type of sample, type of MS, disease activity, immune treatment ongoing at sampling, and type of clinical event). The clinical factors whose p value was less than 0.2 were retained for the multivariate analysis in the univariate analysis.

The selection of variables in the multiple linear regression was made step by step, removing the least nonsignificant variable (p value > 0.05). The model retained all the variables whose p value was below the significance level of 5%. For all comparisons, p values <0.05 were considered statistically significant. Comparisons of sensitivity, specificity, and positive and negative predictive values were made using the R statistical software version 3.2.1 mix tools package (sesp.mcnemar function and pv.rpv function of the R DTComPair packages). All other analyses were made with SAS version 9.4 (SAS Institute, Inc). Figures were done using the online application EasyMedStat (version 3.14, easymedstat.com).

Data Availability

Data not provided in the article because of space limitations may be shared (anonymized) at the request of the corresponding author to replicate procedures and results.

Results

Paired CSF and serum samples were available for 1,917 eligible patients, and 1,621 patients (MS group [n = 675], CIS group [n = 90], OIND group [n = 297] and NINDC group [n = 559]) were included in the analysis (eFigure 1, links.lww.com/NXI/A753). Serum and CSF LFLC were available for 825 patients.

Definite diagnoses are available in supplementary data (eTable 2, links.lww.com/NXI/A753). Both MS and CIS groups had comparable baseline characteristics except for the OCB status (84 vs 63% positivity, respectively, $p < 0.001$) and IgG index values (median of 0.82 vs 0.64, respectively, $p = 0.001$). Both control groups had different baseline characteristics compared with patients with MS. In particular, immune-modifying drugs at sampling were more frequent in the OIND group (17%) than in the MS group (9%), $p = 0.003$. All baseline characteristics are available in Table 1, and details concerning disease-modifying

Table 1 Demographic and Clinical Data of Patients at Serum/CSF Sampling

	MS (n = 675)	CIS (n = 90)	*p* Value MS vs CIS	OIND (n = 297)	*p* Value MS vs OIND	NINDC (n = 559)	*p* Value MS vs NINDC
Age (y), median [IQR]	37 [29; 48]	37 [28; 50]	0.873[a]	46 [35; 60]	**<0.001**[a]	54 [39; 69]	**<0.001**[a]
Sex (women), n (%)	481 (71)	68 (76)	0.455[b]	169 (56.9)	**<0.001**[b]	349 (62)	**0.001**[b]
Immune treatment at sampling, n (%)	57 (9.3)	4 (4.9)	0.217[b]	45 (16.6)	**0.003**[b]	23 (4.5)	**0.002**[b]
CSF protein (g/L), median [IQR]	0.35 [0.28; 0.46]	0.36 [0.28; 0.41]	0.363[a]	0.41 [0.29; 0.54]	**<0.001**[a]	0.36 [0.28; 0.48]	**0.012**[a]
CSF WBC (/mL), median [IQR]	2 [0; 6]	1 [0; 8]	0.667[a]	3 [0; 8]	**<0.001**[a]	0 [0; 1]	**<0.001**[a]
Albumin quotient (%), median [IQR]	0.51 [0.37; 0.69]	0.51 [0.36; 0.62]	0.177[a]	0.61 [0.44; 0.88]	**<0.001**[a]	0.54 [0.39; 0.75]	**0.001**[a]
IgG index, median [IQR]	0.82 [0.62; 1.25]	0.64 [0.60; 0.87]	**0.001**[a]	0.56 [0.48; 0.66]	**<0.001**[a]	0.52 [0.47; 0.58]	**<0.001**[a]
Positive OCB, n (%)	570 (84.4)	57 (63.3)	**<0.001**[b]	64 (21.6)	**<0.001**[b]	19 (3.4)	**<0.001**[b]
Serum KFLC (mg/L), median [IQR]	13.5 [10.8; 16.4]	12.6 [10.6; 15.4]	0.073[a]	13.8 [10.9; 17.8]	**<0.001**[a]	14.9 [11.9; 19.4]	**<0.001**[a]
Serum LFLC (mg/L), median [IQR]	11.3 [9.1; 14.2]	11.1 [9.1; 13.1]	0.303[a]	12.4 [9.9; 15.0]	**0.013**[a]	12.5 [10.1; 15.7]	**0.001**[a]

Bold text indicates p values that reach statistical significance (p <0.05).
Abbreviations: CIS = clinically isolated syndrome; IQR = interquartile range; MS = multiple sclerosis; NINDC = noninflammatory neurologic disease control; OCB = oligoclonal band; OIND = other inflammatory neurologic disease; sKFLC = serum kappa free light chain; sLFLC = serum lambda free light chain; WBC = white blood cell.
[a] Wilcoxon-Mann-Whitney test.
[b] Fischer exact test.

treatment are available in supplementary data (eTable 3, links.lww.com/NXI/A753).

Patients With MS and CIS Presented Higher FLC Biomarkers Than Both Control Groups

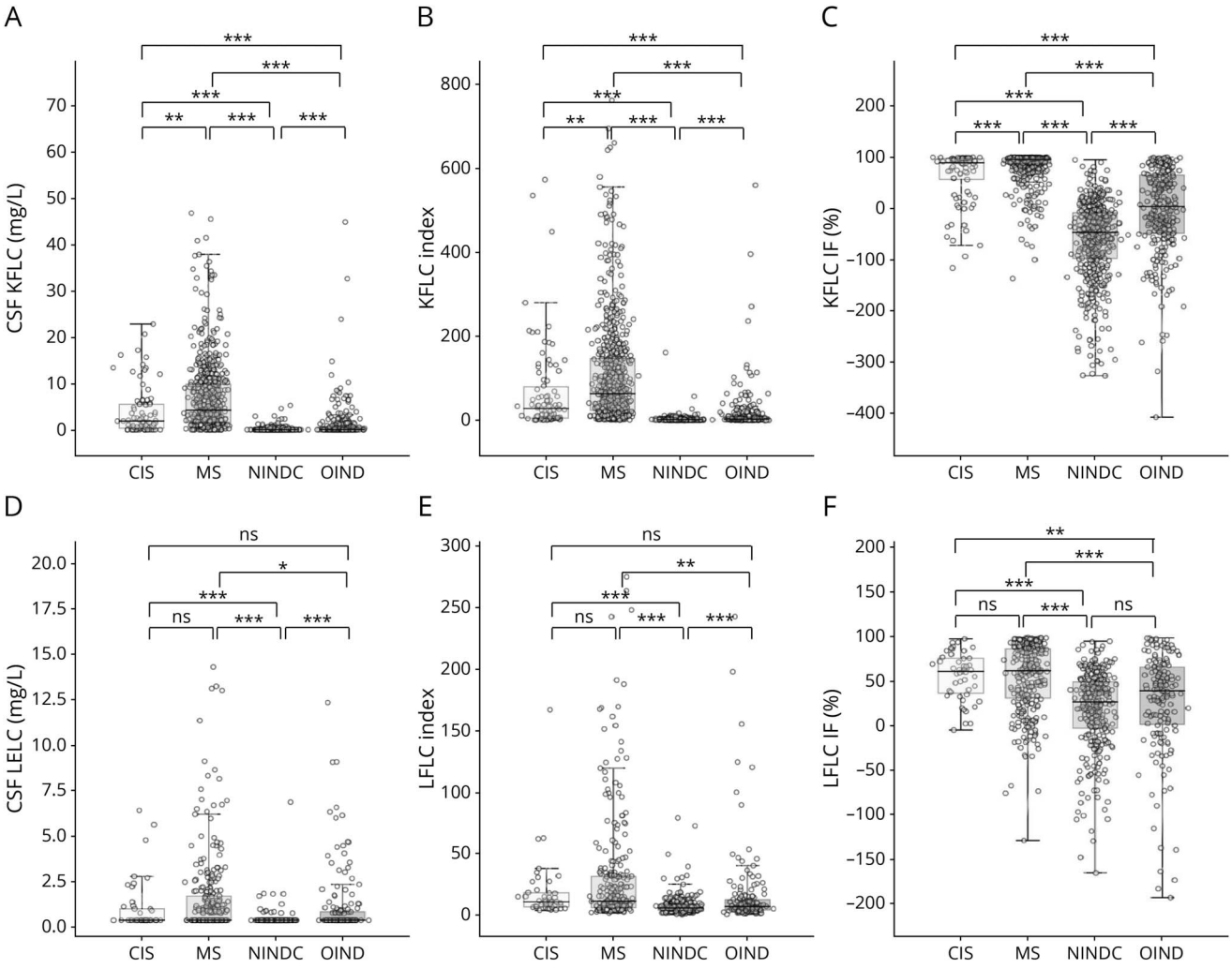
The CSF KFLC (Figure 1A), KFLC index (Figure 1B), and KFLC IF (Figure 1C) values were higher in the MS group (median = 4.36 mg/L [1.75–9.96], 64.2 [24.4–147.5], and 94.6% [86.7–97.6], respectively) than in the CIS group (median = 2.04 mg/L [0.46–5.66], 29.0 [6.3–80.5], and 88.3% [55.8–96.2], respectively), the OIND group (median = 0.17 mg/L [0.17–0.93], 3.8 [2.2–9.5], and 3.2% [−48.9 to 64.5], respectively), and the NINDC group (median = 0.17 mg/L [0.17–0.17], 2.4 [1.6–3.5], and −47.2% [−98.3 to 8.8], respectively). p Value was <0.001 for all MS or CIS vs OIND and NINDC comparisons. Although focusing on MS phenotype, progressive patients with MS (n = 83) presented with similar median CSF KFLC concentration than relapsing patients with MS (n = 592), $p = 0.292$, and lower KFLC index ($p = 0.023$) and KFLC IF ($p = 0.043$) values. All data are presented in eFigure 2 (links.lww.com/NXI/A753).

The CSF LFLC (Figure 1D), LFLC index (Figure 1E), and LFLC IF (Figure 1F) values were higher in the MS group than in the NINDC group, p Value <0.001 for all comparisons. Median values of all 3 LFLC biomarkers were similar between MS and CIS groups.

Both KFLC Index and KFLC IF Performed Better Than Other FLC Biomarkers in Diagnosing MS

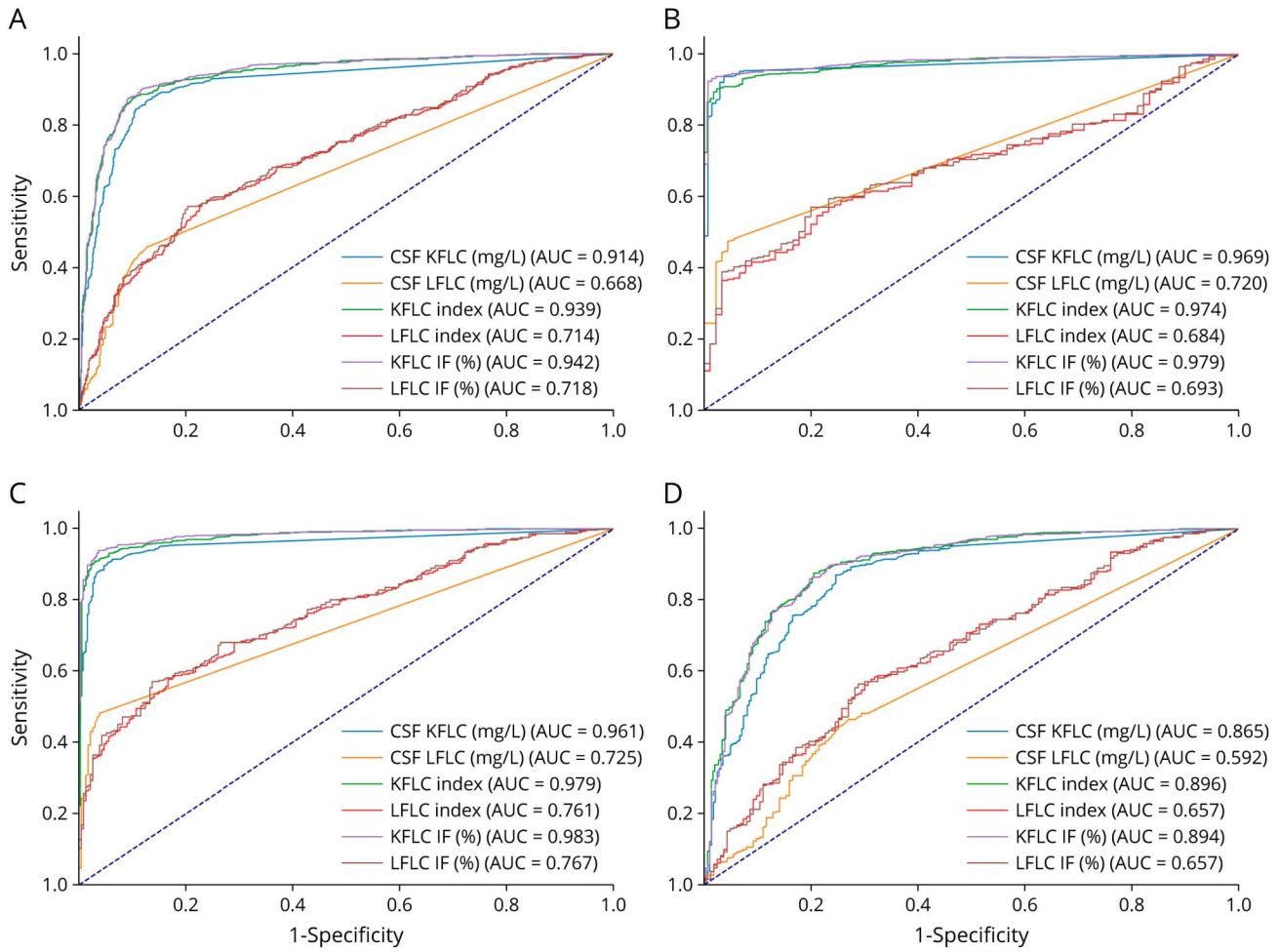
MS/CIS groups were compared with OIND/NINDC groups to evaluate the diagnostic performance of each biomarker in the entire cohort. KFLC index (AUC 0.939) and KFLC IF (AUC 0.942) had similar diagnostic performances, $p = 0.123$, and performed better than CSF KFLC (AUC 0.914, $p < 0.001$

Figure 1 Comparison of the Different FLC Biomarkers Values Between Groups



CIS = clinically isolated syndrome; CSF KFLC = CSF kappa free light chains; CSF LFLC = CSF lambda free light chains; IF = intrathecal fraction; KFLC IF = kappa free light chains IF; KFLC index = kappa free light chains index; LFLC IF = lambda free light chains intrathecal fraction; LFLC index = lambda free light chains index; MS = multiple sclerosis; NINDC = noninflammatory neurologic disorder controls; ns = nonsignificant; OIND = other inflammatory neurologic disorder. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure 2 FLC Biomarkers ROC Curve Analysis and Ability to Separate MS (±CIS) From Other Control Populations



Panel A shows diagnostic performances to separate MS and CIS from patients with NINDC and OIND (n = 1,621 for KFLC biomarkers and n = 811 for LFLC biomarkers). Panel B shows diagnostic performances to separate MS from patients with SC (n = 842 patients for KFLC biomarkers and n = 415 for LFLC biomarkers). Panel C shows diagnostic performances to separate MS from patients with NIND (n = 1,067 for KFLC biomarkers and n = 541 for LFLC biomarkers). Panel D shows diagnostic performances to separate MS from patients with OIND (n = 972 patients for KFLC biomarkers and n = 479 for LFLC biomarkers). CIS = clinically isolated syndrome; FLC = free light chain; IF = intrathecal fraction; KFLC = Kappa free light chain; LFLC = lambda free light chain; NINDS = noninflammatory CNS disorder control; OIND = other inflammatory CNS disorder.

for both comparisons) in diagnosing MS/CIS (Figure 2A). Optimal cut-off values were 8.92 and 67.3% for KFLC index and KFLC IF, respectively (eTable 4, links.lww.com/NXI/A753).

Patients with MS were then compared with those with SC (Figure 2B). In this situation, KFLC IF performed better than the KFLC index (p = 0.008), and optimal cut-off values were 9.11 for KFLC index and 46.3% for KFLC IF (eTable 5, links.lww.com/NXI/A753). When patients with MS were compared with patients with NIND (Figure 2C), the KFLC IF performed better than the KFLC index (p = 0.002), and optimal cut-off values were 8.39 and 38.1% for KFLC index and KFLC IF, respectively (eTable 6, links.lww.com/NXI/A753).

Patients with MS were then compared with patients with OIND (Figure 2D). In this situation, the KFLC index (AUC 0.896) and KFLC IF (AUC 0.894) had similar diagnostic performance (p = 0.991) and performed better than CSF KFLC (AUC 0.865, p < 0.001 for both comparisons). Optimal cut-off values were 11.56 and 67.9% for KFLC index and KFLC IF, respectively (eTable 7, links.lww.com/NXI/A753). In all situations, CSF LFLC, LFLC index, and LFLC IF had lower performances than KFLC biomarkers.

Because different threshold values were obtained depending on the chosen control population, we compared the performances of the different thresholds in other situations to ensure their relevance. When applying KFLC biomarker thresholds obtained in separating MS from NIND to separate MS from OIND, the AUC of all 3 biomarkers decreased (p = 0.025 for CSF KFLC, p = 0.045 for KFLC index, and p < 0.001 for KFLC IF comparisons). The same results were obtained when applying KFLC biomarker thresholds obtained in separating MS from SC to separate MS from OIND. All data are shown in Table 2.

Table 2 Comparison of the Different CSF KFLC, KFLC Index, and KFLC IF Obtained Thresholds Across Groups

	MS vs OIND								
	CSF KFLC			**KFLC index**			**KFLC IF**		
Threshold	0.64	0.94	0.47	8.38	11.56	9.11	38.1	67.9	46.3
AUC	0.791 [0.761; 0.820]	0.8112 [0.784; 0.840]	0.767 [0.737; 0.797]	0.819 [0.791; 0.848]	0.836 [0.809; 0.863]	0.823 [0.795; 0.851]	0.777 [0.748; 0.807]	0.832 [0.805; 0.859]	0.791 [0.762; 0.821]
***p* Value**	**0.025**[a]			**0.045**[a]			**<0.001**[a]		
		<0.001[a]			**0.049**[a]			**<0.001**[a]	
	MS vs NIND								
	CSF KFLC			KFLC index			KFLC IF		
Threshold	0.94	0.64	0.47	11.56	8.38	9.11	67.9	38.1	46.3
AUC	0.918 [0.902; 0.934]	0.926 [0.909; 0.942]	0.908 [0.890; 0.927]	0.929 [0.915; 0.943]	0.939 [0.926; 0.953]	0.937 [0.923; 0.951]	0.939 [0.926; 0.952]	0.952 [0.939; 0.965]	0.949 [0.936; 0.962]
***p* Value**	0.212[a]			**0.022**[a]			**0.021**[a]		
		0.007[a]			0.235[a]			0.235[a]	

Abbreviations: AUC = area under the curve; MS = multiple sclerosis; NIND = noninflammatory neurologic disorder; OIND = other neurologic disease.
Bold text indicates *p* values that reach statistical significance (p <0.05).
[a] AUC comparison by DeLong method.

KFLC Index and KFLC IF Performed Better Than OCB in Diagnosing MS/CIS From All Controls

For this analysis, we evaluated the performances of binary KFLC biomarkers (according to the obtained threshold values) in separating MS/CIS from OIND/NINDC and compared them to OCB. A KFLC index >8.92 (AUC 0.887) and a KFLC IF > 67.3% (AUC 0.892) performed better than OCB (AUC 0.861) in diagnosing MS/CIS (p < 0.001 for KFLC index vs OCB comparison, and for KFLC IF vs OCB comparison). These results are translated by a higher sensitivity and the same specificity, favoring KFLC biomarkers instead of OCB for MS/CIS diagnosis. Data are shown in Table 3.

KFLC Index and KFLC IF Tend to Perform Better Than OCB in Separating MS From OIND

To evaluate the ability of KFLC biomarkers to separate MS from OIND, KFLC binary variables, according to obtained cut-off, were compared with OCB status. A KFLC index >11.56 (AUC 0.835) and KFLC IF > 67.9% (AUC 0.831) tend to perform better than OCB (AUC 0.814) in diagnosing MS (p = 0.065 for KFLC index vs OCB comparison, and p = 0.138

Table 3 CSF KFLC, KFLC Index, KFLC IF, and OCB Diagnostic Performances in Separating MS/CIS From Controls (n = 1,621)

Variable	OCB	CSF KFLC >0.74 mg/L	*p* Value OCB vs CSF KFLC	KFLC index >8.92	*p* Value OCB vs KFLC index	KFLC IF >67.3%	*p* Value OCB vs KFLC-IF
AUC	0.861 [0.844; 0.878]	0.870 [0.853; 0.887]	0.265[a]	0.887 [0.872; 0.903]	**<0.001**[a]	0.892 [0.876; 0.908]	**<0.001**[a]
Sensitivity (%)	81.99 [79.21; 84.77]	87.72 [85.35; 90.1]	**<0.001**[b]	88.24 [85.95; 90.52]	**<0.001**[b]	87.59 [85.2; 89.97]	**<0.001**[b]
Specificity (%)	90.16 [88.1; 92.22]	86.43 [84.06; 88.79]	**<0.001**[b]	89.36 [87.29; 91.42]	0.339[b]	90.91 [88.92; 92.9]	0.446[b]
Positive predictive value (%)	88.38 [85.97; 90.79]	85.51 [82.99; 88.02]	**0.008**[c]	88.12 [85.83; 90.41]	0.852[c]	89.79 [87.57; 92.01]	0.169[c]
Negative predictive value (%)	84.58 [82.16; 87.00]	88.52 [86.29; 90.75]	**<0.001**[c]	89.46 [87.4; 91.52]	**<0.001**[c]	88.92 [86.77; 91.06]	**<0.001**[c]
Well classified patients (%)	86.4	87.0		88.8		89.3	

Abbreviations: AUC = area under the curve; IF = intrathecal fraction; K = kappa; KFLC = kappa free light chain; OCB = oligoclonal band.
Bold text indicates *p* values that reach statistical significance (p <0.05).
[a] AUC comparison by DeLong method.
[b] McNemar test.
[c] Package DTComPair.

Table 4 CSF KFLC, KFLC Index, KFLC IF, and OCB Performances in Separating MS (n = 675) From Other Inflammatory CNS Disorders (n = 297)

Variable	OCB	CSF KFLC >0.94 mg/L	p Value OCB vs CSF KFLC	KFLC index >11.56	p Value OCB vs KFLC index	KFLC IF >67.9%	p Value OCB vs KFLC IF
AUC	0.814 [0.787; 0.841]	0.810 [0.782; 0.839]	0.999[a]	0.835 [0.809; 0.861]	0.065[a]	0.831 [0.803; 0.858]	0.138[a]
Sensitivity (%)	84.26 [81.46; 87.06]	86.73 [84.12; 89.34]	0.095[b]	87.56 [85.07; 90.05]	**0.026**[b]	89.35 [86.98; 91.73]	**<0.001**[b]
Specificity (%)	78.09 [73.27; 82.91]	75.62 [70.62; 80.62]	0.307[b]	79.73 [75.15; 84.31]	0.527[b]	77.03 [72.13; 81.93]	0.647[b]
Positive predictive value (%)	89.80 [87.4; 92.21]	89.06 [86.63; 91.5]	0.462[c]	90.78 [88.56; 93.01]	0.331[c]	89.91 [87.58; 92.23]	0.913[c]
Negative predictive value (%)	68.42 [63.35; 73.49]	71.33 [66.22; 76.45]	0.195[c]	73.75 [68.93; 78.57]	**0.021**[c]	75.96 [71.01; 80.9]	**<0.001**[c]
Well classified patients (%)	82.6	83.3		85.1		85.5	

Abbreviations: AUC = area under the curve; IF = intrathecal fraction; K = kappa; KFLC = kappa free light chain; MS = multiple sclerosis; OCB = oligoclonal band; OIND = other inflammatory neurologicneurological disorder.
Bold text indicates p values that reach statistical significance (p <0.05).
[a] AUC comparison by DeLong method.
[b] McNemar test.
[c] Package DTComPair.

for KFLC IF vs OCB comparison). Again, these results are translated by a higher sensitivity and the same specificity, favoring KFLC biomarkers instead of OCB for MS diagnosis. All data are shown in Table 4.

Adding OCB to KFLC Biomarkers as a Combinate Variable Does Not Increase Diagnostic Performances but Increases Specificity for MS Diagnosis

Because KFLC biomarkers and OCB's diagnostic performances were similar in separating MS from OIND, we analyzed whether a combined variable adding OCB to KFLC biomarkers performed better than KFLC biomarkers alone. The combined KFLC index variable performed similarly to KFLC index alone (AUC = 0.835 vs 0.826, p = 0.231), as for the KFLC IF (AUC = 0.831 vs 0.828, p = 0.666). Nonetheless, the double positive OCB/KFLC combined variable had higher specificity (p < 0.001) and lower sensitivity (p < 0.001) for MS diagnosis. The results are shown in eTable 8 (links.lww.com/NXI/A753).

Young Age, Female Gender, and Evidence of Disease Activity Were Independent Factors Associated With a High KFLC Index in MS

We then evaluated whether some clinical factors of interest independently influenced KFLC index values in patients with MS. In a univariate analysis, age, female gender, evidence of disease activity, MS phenotype, and the type of analyzer influenced KFLC index values, whereas the type of clinical event and immune-modifying drug consumption at sampling did not. In the multivariate analysis model, only young age (p = 0.013), female gender (p = 0.003), and evidence of disease activity (p < 0.001) were associated with high KFLC index values in patients with MS (Table 5).

To better appreciate if age and gender influenced our results, we compared the performances of the KFLC thresholds between men and women and between 3 ranges of age (<30, between 30 and 55, and >55 years old). We found that gender did not influence our threshold diagnostic performances, whereas the AUC of all KFLC biomarkers were not statistically different between men and women (eTable 9, links.lww.com/NXI/A753). Similar results were found when comparing KFLC biomarker performances according to age. As shown in eTable 10, the sensitivity of KFLC biomarkers decreased with advanced age, whereas specificity increased.

KFLC Biomarkers Seem to Be Reproducible Across Centers

To evaluate the reproducibility of KFLC biomarkers, we compared, in patients with MS, median CSF KFLC, KFLC index, and KFLC IF values between each center that included at least 40 patients. The other centers were pooled together as another center. There was no statistical difference in median KFLC biomarker values between Marseille, Montpellier, Nantes, Nice, and other centers (p = 0.067, p = 0.268, and p = 0.091, for CSF KFLC, KFLC index, and KFLC IF comparisons, respectively) (eTable 11, links.lww.com/NXI/A753).

Second, the KFLC index and KFLC IF threshold values were applied separately in each center that included at least 100 patients with available data for MS, NINDC, and OIND groups. All KFLC thresholds seemed to separate MS from the dedicated control populations similarly (eTable 12, links.lww.com/NXI/A753).

Table 5 Clinical Factors Influencing KFLC Index Values in MS

	Univariate analysis		Multivariate analysis			
	Spearman correlation coefficient		Removing variable order	β coefficient	IC95	*p* Value
Age (n = 675)	ρ = −0.14			−1.0154	-1.82;-0.21]	**0.013**
	Median [IQR]	*p* Value				
Sex		**<0.001**[a]			-53.26; −10.73]	**0.003**
Women (n = 481),	73.7 [27.1; 163.8]			*Ref Women*		
Men (n = 194)	48.3 [18.2; 99.9]			−31.99		
Evidence of disease activity		**<0.001**[a]			-62.25; −17.31]	**<0.001**
Yes (n = 425)	80.8 [33.1; 172.3]			*Ref Yes*		
No (n = 177)	45.7 [15.0; 115.6]			−39.78		
Type of analyzer		**0.042**[b]	1		-26.31; 49.46]	0.549
Optilite (n = 581)	65.3 [25.5; 147.5]			*Ref Optilite*		
SPAPLUS (n = 43)	92.8 [24.2; 164.6]			11.57		
BN ProSpec/BNII (n = 51)	39.1 [11.5; 62.8]					
MS type		**<0.001**[b]	2			
RR-MS (n = 591)	68.8 [27.7; 158.3]			*Ref RR-MS*		
SP-MS (n = 14)	76.1 [18.8; 127.9]			−23.39	-91.34; 44.56]	0.234
PP-MS (n = 69)	36.1 [12.1; 76.3]			−22.106	-58.56; 14.35]	0.499
Type of sample						
Fresh (n = 370)	68.0 [24.6; 153.8]	0.765[a]		—	—	—
Thawed (n = 305)	62.7 [24.4; 143.8]					
Immune treatment ongoing at sampling		0.251[a]		—	—	—
Yes (n = 57)	53.2 [16.0; 130.3]					
No (n = 556)	65.8 [25.4; 147.7]					
Type of clinical event		0.867[b]	—	—	—	—
Myelitis (n = 273)	71.0 [30.4; 144.1]					
Optic neuritis (n = 128)	80.4 [27.5; 147.4]					
Infratentorial (n = 100)	66.3 [24.9; 185.1]					
Supratentorial (n = 69)	59.9 [17.7; 135.7]					
>1 location (n = 37)	67.5 [25.5; 158.0]					

Abbreviation: IQR = interquartile range.
Bold text indicates *p* values that reach statistical significance ($p < 0.05$).
[a] Wilcoxon-Mann-Whitney test.
[b] Kruskal-Wallis test.

Primary Research Question

Does KFLC biomarkers correctly separate patients having MS from patients having other CNS disorders, particularly other autoimmune CNS disorders? This study provides Class III evidence that KFLC index or IF can be used to differentiate patients with MS from nonselected controls and from patients with other autoimmune CNS disorders.

Discussion

Our study shows that the KFLC index and KFLC IF are valuable tools to detect MS, even compared with other noninfectious and nontumoral inflammatory neurologic disorders. As reported previously, LFLC biomarkers could not separate MS from controls with a reasonable accuracy.[7,13,15] KFLC index and KFLC IF performed better than CSF KFLC,

reinforcing the need to weight FLC values with a blood-brain barrier permeability factor such as albumin quotient to increase diagnostic performances.

In this cohort, a KFLC index >8.92 or a KFLC IF > 67.3% permit to separate MS from controls, with better sensitivity and the same specificity as OCB. However, these KFLC thresholds seemed similar to OCB in separating MS from OIND, with a trend that favors KFLC biomarkers when increasing threshold values. Moreover, predictive studies[23,34] found that KFLC index predict second clinical attack or MRI dissemination in space and time, in patients with CIS, with a good accuracy, slightly better than OCB.[23,34] Because KFLC measurement is an easier way to prove intrathecal B-cell activity, with at least as good diagnostic performances than OCB, KFLC index or KFLC IF could be used in clinical practice instead of OCB as first-line MS biomarker. Nonetheless, using a combination of KFLC and OCB significantly increases specificity for MS diagnosis. Therefore, adding OCB to KFLC biomarkers may be helpful in atypical cases. Based on our results, the results of other diagnostic studies,[7-14] the ones of predictive studies,[21,23,34] and the knowledge into MS red flags,[1,35,36] we propose an algorithm that integrate KFLC biomarkers to diagnose MS (eFigure 3, links.lww.com/NXI/A753).

Even if our threshold values seem close to each other, we found that using lower KFLC thresholds to separate MS from OIND statistically decreases diagnostic performance. As already reported, it highlights that some controls may present pathologic intrathecal B-cell activity.[28,30,37,38] There is growing evidence that CNS B-cell activity is higher in MS than in other autoimmune disorders, as pointed out by pathologic analyses, showing a substantial perivascular B-cell infiltrate in MS compared with other inflammatory diseases.[4] Süße et al.[30] reported similar results using KFLC IF, showing that using a 78.6% KFLC IF cut-off could separate MS-related myelitis (n = 26) from NMOSD-related myelitis (n = 9) with a good accuracy. These constants reinforce the interest in using quantitative biomarkers to measure CSF B-cell activity in suspected patients with MS. Of note, threshold values will always depend on the chosen control population. Therefore, the preciseness of our results should be used with caution in clinical practice, and the use of a 11.6 or 12 KFLC index threshold could be valuable to separate MS from OIND.

Our KFLC index thresholds seem consistent with currently published ones ranging from 3.045 to 10.62.[7-13] The different chosen control populations can explain such discrepancy. For example, Leurs et al.[7] identified a 6.6 KFLC index threshold to separate MS/CIS from controls. Only 30% of their 219 control patients had an inflammatory neurologic disorder explaining the lower cut-off obtained in this study. Another explanation could be the poor performance of the analyzers in detecting a low amount of FLC. For example, Sanz Diaz et al.[12] attributed a practical value of 0.0001 mg/L to all patients with undetectable CSF KFLC concentrations (157/197 controls), leading to the report of a low KFLC index cut-off of 3.045. In our cohort, for undetectable CSF KFLC data, we assessed an empirical value based on the LDL of the analyzer divided by 2. In doing so, we may not have underestimated threshold values. Recently, Saadeh et al.[14] reported, on a series of 1,359 patients (1,204 patients without MS), a median CSF KFLC value of 0.16 mg/L for controls, which seems similar to our empirical attributed value (0.17 mg/L).

Only a few studies focused on establishing a KFLC IF cut-off value to separate MS from controls.[30,37] Most of the studies focused on the "presence or absence" of intrathecal KFLC synthesis based on a QKFLC>QKFLC(lim), leading to a KFLC IF>0% as a binary result. However, many patients without MS may present with low intrathecal B-cell activity resulting in a lack of specificity in such an approach. Our results support that using a KFLC IF > 67.9% to assess an MS-specific intrathecal B-cell activity separates patients more accurately.

The use of either the KFLC index or KLFC IF to prove intrathecal immunoglobulin synthesis is debated. Nonetheless, both biomarkers use CSF and serum KFLC concentration and include the albumin quotient as the blood-brain barrier permeability correction variable. In our study, both performed equally to separate MS from controls and OIND. These results are in line with others.[27] Based on these results, both the KFLC index or KFLC IF can be used in daily practice.

In MS, OCB positivity is slightly modified over disease duration or disease-modifying treatment use.[39,40] This is a crucial point to consider while a diagnostic biomarker needs to be efficient at the diagnostic workup. In our study, nearly 10% of the patients took an immune-modifying treatment at sampling, which did not influence KFLC index values. Moreover, we found that the KLFC index was not modified by the type of clinical demyelinating event nor by the MS phenotype (progressive vs relapsing), which allows its use in any clinical situation raising suspicion of MS. However, patients with evidence of MS activity at sampling presented with higher KFLC index values. This result is in line with predictive studies that showed that KFLC could predict MS disability[20] and second clinical attack in patients with CIS.[24,34,41,42]

Of note, added to evidence of disease activity, we found that age and gender were independent KFLC index influencing factors in MS. KFLC sensitivity for MS diagnosis decreased in men, older patients, and inactive disease at sampling. Therefore, clinicians should be careful when interpreting a KFLC biomarker in an older man with suspected inactive MS, as a result, it could be negative. These findings may explain the observed difference in KFLC values between progressive and relapsing MS in the univariate analysis (and not in the multivariate analysis), whereas patients with progressive MS were older and more often men. Finally, we pointed out that median KFLC biomarkers values in patients with MS were similar between centers. The obtained KFLC thresholds permitted to correctly separate patients in each available center. These findings reinforce the reproducibility of such a biologic tool and its use in MS diagnostic workup. Nonetheless, dedicated prospective studies must confirm these results.

Our study is one of the first that evaluate the diagnostic performance of FLC biomarkers in a large multicenter cohort of MS and non-MS inflammatory patients. However, it has a few drawbacks. First, MS diagnosis was made according to the 2017 criteria, and dissemination in time data was not collected. Therefore, some patients with CIS could have been diagnosed with MS because of an OCB positive status, leading to a possible bias in comparing OCB and KFLC biomarker performance. However, this limitation tended to overestimate the OCB positivity in the MS group, which could not lead to an overestimation of KFLC biomarkers' diagnostic performance. We pooled patients with MS and CIS in the first analysis to avoid this limitation. Second, KFLC was measured using different analyzers and samples, in different laboratories.

We included the analyzer and sample types in the multivariate analysis model to evaluate their impact on our results to avoid this limitation. We found that both did not influence KFLC index values. However, the distribution of samples between the different analyzers was heterogeneous. Therefore, dedicated analyzers' comparative studies must be performed in MS to evaluate the consistency of FLC measurement, as it has been shown, in other disorders, that there were differences between the FLC measurement platforms.[43-45] Third, we assigned an empirical FLC value equal to the LDL of the analyzer divided by 2 in patients with nondetectable FLC concentrations. We may not have overestimated KLFC biomarkers performances, whereas most patients with undetectable KFLC were not in the MS group. Finally, we excluded patients with CNS infections and/or tumors. Some of these patients may present elevated CSF B-cell activity, and their exclusion may have influenced KFLC biomarkers thresholds in this cohort. However, infections and tumors are not typical MS-mimicking diseases, and many other examinations allow to distinguish MS from such disorders.

In conclusion, KFLC is an automated and reliable variable that permits quantifying the CSF B-cell activity by calculating the KFLC index or KFLC IF. As supported by our results and the findings of other studies, the higher the KFLC biomarker value is, the higher the specificity for MS diagnosis will be. Based on these findings, we propose to use either a KFLC index >11.56 or a KFLC IF > 67.9% in patients presenting with atypical clinical demyelinating event suggestive of an inflammatory CNS disorder to favor MS from OIND. Otherwise, a KFLC index >8.92 or a KFLC IF > 67.3% can be used to favor MS diagnosis.

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Appendix 1 Authors

Name	Location	Contribution
Michael Levraut, MD	Unité de Recherche Clinique Cote d'Azur-UR2CA URRIS, Centre Hospitalier Universitaire de Nice, Nice, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Sabine Laurent-Chabalier, PhD	Département de Biostatistiques, Epidémiologie clinique et Santé Publique, Centre Hospitalier Universitaire de Nîmes, Nîmes, France	Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data
Xavier Ayrignac, MD, PhD	Département de Neurologie, Centre Hospitalier Universitaire de Montpellier, Montpellier, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Kévin Bigaut, MD	Département de Neurologie, Centre Hospitalier Universitaire de Strasbourg, Strasbourg, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Manon Rival, MD	Service de Neurologie, Centre Hospitalier Universitaire de Nîmes, Nîmes, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Sanae Squalli, MD	Laboratoire de Biochimie, Centre Hospitalier Universitaire de Nîmes, Nîmes, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Continued

Appendix 1 *(continued)*

Name	Location	Contribution
Hélène Zéphir, MD, PhD	Département de Neurologie, Centre Hospitalier Universitaire de Lille, Lille, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Tifanie Alberto, MD	Département de Neurologie, Centre Hospitalier Universitaire de Lille, Lille, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Jean-David Pekar, MD	Centre Hospitalier Universitaire de Lille, Service de Biochimie Automatisée, Protéines (UF 8833), Lille, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Jonathan Ciron, MD	Département de Neurologie, Centre Hospitalier Universitaire de Toulouse, Hôpital Pierre-Paul Riquet, CRC-SEP, Toulouse Cedex 9, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Damien Biotti, MD	Département de Neurologie, Centre Hospitalier Universitaire de Toulouse, Hôpital Pierre-Paul Riquet, CRC-SEP, Toulouse Cedex 9, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Bénédicte Puissant-Lubrano, PharmD, PhD	Laboratoire d'Immunologie, Centre Hospitalier Universitaire de Toulouse, Toulouse, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Jean-Philippe Camdessanché, MD, PhD	Département de Neurologie, Centre Hospitalier Universitaire de Saint-Etienne, Saint-Etienne, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Yannick Tholance, PharmD, PhD	Laboratoire de Biochimie, Centre Hospitalier Universitaire de Saint-Etienne, Saint-Etienne, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Olivier Casez, MD	Département de Neurologie, Centre Hospitalier Universitaire de Grenoble Alpes, Grenoble, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Bertrand Toussaint, MD, PhD	Univ. Grenoble Alpes, CNRS, UMR 5525, VetAgro Sup, Grenoble INP, CHU Grenoble Alpes, TIMC, Grenoble, France; Laboratoire de Biochimie, Centre Hospitalier Universitaire de Grenoble Alpes, Grenoble, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Jeanne Marion, MD	Laboratoire de Biochimie, Centre Hospitalier Universitaire de Grenoble Alpes, Grenoble, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Appendix 1 *(continued)*

Name	Location	Contribution
Thibault Moreau, MD, PhD	Département de Neurologie, Centre Hospitalier de Dijon, Dijon, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Daniela Lakomy, MD, PhD	Laboratoire de Biochimie, Centre Hospitalier Universitaire de Dijon, Dijon, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Audrey Thomasset, MD	Laboratoire de Biochimie, Centre Hospitalier Universitaire de Dijon, Dijon, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Elisabeth Maillart, MD	Département de Neurologie, Assistance-Publique des Hôpitaux de Paris, Centre Hospitalier Universitaire de la Pitié-Salpêtrière, Paris, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Delphine Sterlin, PharmD, PhD	Laboratoire d'Immunologie, Assistance Publique des Hôpitaux de Paris, Centre Hospitalier Universitaire de la Pitié-Salpêtrière, Paris, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Aude Maurousset, MD	Département de Neurologie, Centre Hospitalier Universitaire de Tours, Tours, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Auriane Rocher, MD	Département de Neurologie, Centre Hospitalier Universitaire de Tours, Tours, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
David Axel Laplaud, MD, PhD	Université de Nantes, C2RTI Inserm UMR1064, service de Neurologie, CHU Nantes, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Edith Bigot-Corbel, PharmD, PhD	Département de Biochimie, Centre Hospitalier Universitaire de Nantes, Nantes, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Pierre-Olivier Bertho, MD	Département de Biochimie, Centre Hospitalier Universitaire de Nantes, Nantes, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Jean Pelletier, MD, PhD	Aix Marseille Univ, APHM, Hôpital de la Timone, Pôle de Neurosciences Cliniques, Service de Neurologie, Marseille, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Joseph Boucraut, MD, PhD	Laboratoire de Biochimie, Assistance Publique des Hôpitaux de Marseille, Centre Hospitalier Universitaire de La Conception, Marseille, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Appendix 1 *(continued)*

Name	Location	Contribution
Pierre Labauge, MD, PhD	Département de Neurologie, Centre Hospitalier Universitaire de Montpellier, Montpellier, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Thierry Vincent, MD, PhD	Laboratoire d'Immunologie, Centre Hospitalier Universitaire de Montpellier, Montpellier, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Jérôme De Sèze, MD, PhD	Département de Neurologie, Centre Hospitalier Universitaire de Strasbourg, Strasbourg, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Isabelle Jahn, MD	Laboratoire d'Immunologie, Centre Hospitalier Universitaire Strasbourg, Strasbourg, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Barbara Seitz-Polski, MD, PhD	Laboratoire d'Immunologie, Centre Hospitalier Universitaire de Nice, Nice, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design
Eric Thouvenot, PhD	Service de Neurologie, Centre Hospitalier Universitaire de Nîmes, Nîmes, France; Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS, INSERM, Montpellier, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Christine Lebrun-Frenay, MD, PhD	Unité de Recherche Clinique Cote d'Azur-UR2CA URRIS, Centre Hospitalier Universitaire de Nice, Nice, France; Département de Neurologie, Centre Hospitalier Universitaire de Nice, Nice, France	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Appendix 2 Coinvestigators

Name	Location	Role	Contribution
Audoin Bertrand, MD, PhD	Marseille, France	SFSEP investigator	Acquisition of data
Bodisteanu Ruxanda, PhD	Nice, France	SFSEP investigator	Acquisition of data
Bost Chloé, PhD	Toulouse, France	SFSEP investigator	Acquisition of data
Boutière Clémence, MD	Marseille, France	SFSEP investigator	Acquisition of data
Bresch Saskia, MD	Nice, France	SFSEP investigator	Acquisition of data
Carra Dallière Clarisse, MD	Montpellier, France	SFSEP investigator	Acquisition of data

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Charif Mahmoud, MD	Montpellier, France	SFSEP investigator	Acquisition of data
Cognot Chantal, PhD	Montpellier, France	SFSEP investigator	Acquisition of data
Cohen Mikaël, MD	Nice, France	SFSEP investigator	Acquisition of data
Delalande Loreen, MD	Nantes, France	SFSEP investigator	Acquisition of data
Delourme Adrien, MD	Toulouse, France	SFSEP investigator	Acquisition of data
Dos Santos Amélie, MD	Nantes, France	SFSEP investigator	Acquisition of data
Dufat Laurant, PharmD	Paris, France	SFSEP investigator	Acquisition of data
Ghillani-Dalbin Pascale, PharmD, PhD	Paris, France	SFSEP investigator	Acquisition of data
Jentzer Alexandre, MD, PhD	Montpellier, France	SFSEP investigator	Acquisition of data
LeGouellec Audrey, MD	Grenoble, France	SFSEP investigator	Acquisition of data
Louapre Céline, MD, PhD	Paris, France	SFSEP investigator	Acquisition of data
Maarouf Adil, MD, PhD	Marseille, France	SFSEP investigator	Acquisition of data
Onraed Brigitte, PhD	Lille, France	SFSEP investigator	Acquisition of data
Papeix Caroline, MD	Paris, France	SFSEP investigator	Acquisition of data
Pinna Frédéric, M.	Montpellier, France	SFSEP investigator	Acquisition of data
Pontal Daniel, M.	Montpellier, France	SFSEP investigator	Acquisition of data
Rico Audrey, MD, PhD	Marseille, France	SFSEP investigator	Acquisition of data
Russello Mélanie, Ms.	Montpellier, France	SFSEP investigator	Acquisition of data
Theis Cédric, M.	Montpellier, France	SFSEP investigator	Acquisition of data
Wiertlewski Sandrine, MD	Nantes, France	SFSEP investigator	Acquisition of data

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