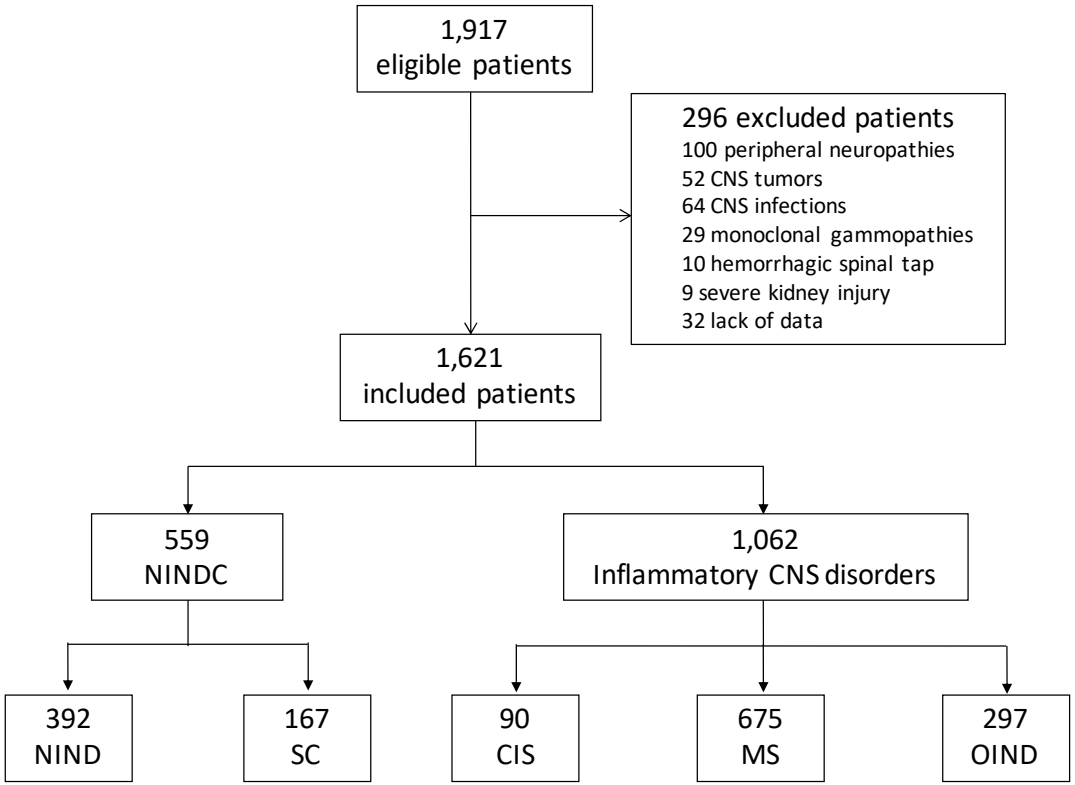
eTable 1: Data concerning patients and FLC measurement in each center

Center	All patients (*n*=1,621)	MS/CIS patients (*n*=765)	OIND patients (*n*=297)	NINDC patients (*n*=559)	Type of analyzed sample	Type of analyzer
Dijon	36	36	0	0	Thawed	Optilite®
Grenoble	66	43	12	11	Thawed	Optilite®
Lille	84	32	13	39	Thawed	SPAplus®
Marseille	151	151	0	0	Fresh	Optilite®
Montpellier	116	58	25	33	Thawed	Optilite®
Nantes*	182	80	32	70	Thawed	Optilite®
Nice*	556	205	122	229	Fresh	Optilite®
Nîmes	168	23	27	118	Fresh	Optilite®
Paris	27	15	11	1	Thawed	Optilite®
Saint-Etienne*	73	27	13	33	Thawed	BN Prospec®
Strasbourg	60	35	8	17	Fresh	BNII®
Toulouse	81	39	34	8	Thawed	Optilite®
Tours	21	21	0	0	Thawed	SPAplus®

CIS: Clinically isolated syndrome; MS: Multiple sclerosis; NINDC: Non-inflammatory neurological disorder controls; OIND: Other inflammatory neurological diseases.

* centers that performed lambda free light chains analysis.

eFigure 1. Flow Chart



MS: Multiple sclerosis; CIS: Clinically isolated syndrome; CNS: Central nervous system; OIND: Other inflammatory neurological disorder; NIND: Non-Inflammatory neurological disorder; NINDC: Non-inflammatory neurological disorder control; SC: Symptomatic control.

eTable 2. Definite diagnoses according to the different groups

Group	Number of patients		Diagnoses (*n*)
MS	675		RR-MS (*n*=591); SP-MS (*n*=14); PP-MS (*n*=69)
CIS	90		Myelitis (*n*=39); Optic neuritis (*n*=30); Infratentorial attack (*n*=13); Supra-tentorial attack (*n*=8)
OIND	297		MOGAD (*n*=26); NMOSD (*n*=18); ADEM (*n*=10); idiopathic myelitis (*n*=29); Idiopathic optic neuritis (*n*=29); Neurosarcoidosis (*n*=17); CNS vasculitis (*n*=21); Sjögren syndrome (*n*=5); Neurolupus (*n*=5); Behçet's disease (*n*=5); Idiopathic LETM (*n*=10); Undefined demyelinating disease (*n*=17); Autoimmune encephalitis (*n*=72); Pachy/Leptomeningitis (*n*=7); Aseptic meningitis/meningoradiculitis (*n*=10); Tolosa-Hunt (*n*=4); Vogt-Koyanagi-Harada syndrome (*n*=3); CAAri (*n*=6); Relapsing meningoencephalomyelitis (*n*=2); Hypophysitis (*n*=1)
NINDC	559	SC (n=167)	Non organic symptoms (n=62); Neuropathic pain (*n*=41); Migraine (*n*=32); Headaches (*n*=20); Vertigo (*n*=11); Transient memory loss (*n*=1)
		NIND (n=392)	Degenerative disorder (*n*=98); Epilepsy (*n*=44); Stroke/Small vessel disease (*n*=69); Non-inflammatory optic neuritis (*n*=26); Non-inflammatory myelopathy (*n*=18); Non-inflammatory encephalopathy (*n*=9); ALS (*n*=20); Idiopathic/Genetic cerebellar syndrome (*n*=19); Idiopathic intracranial hypertension (*n*=21); Normal pressure hydrocephalus (*n*=18); Other neurological disorders (*n*=50)

ADEM: acute disseminated encephalomyelitis; ALS: Amyotrophic lateral sclerosis; CAAri: Cerebral amyloid angiopathy related inflammation; CIS: Clinically isolated syndrome; LETM: Longitudinally and extensive transverse myelitis; MOGAD: Myelin oligodendrocyte antibody

associated disease; MS: Multiple sclerosis; NIND: Non-inflammatory neurological disease; NINDC: Non-inflammatory neurological disease controls; NMOSD: Neuromyelitis optica spectrum disorder; OIND: Other inflammatory neurological diseases; PP: Primary progressive; RR: Relapsing-remitting; SC: Symptomatic controls; SP: Secondary progressive.

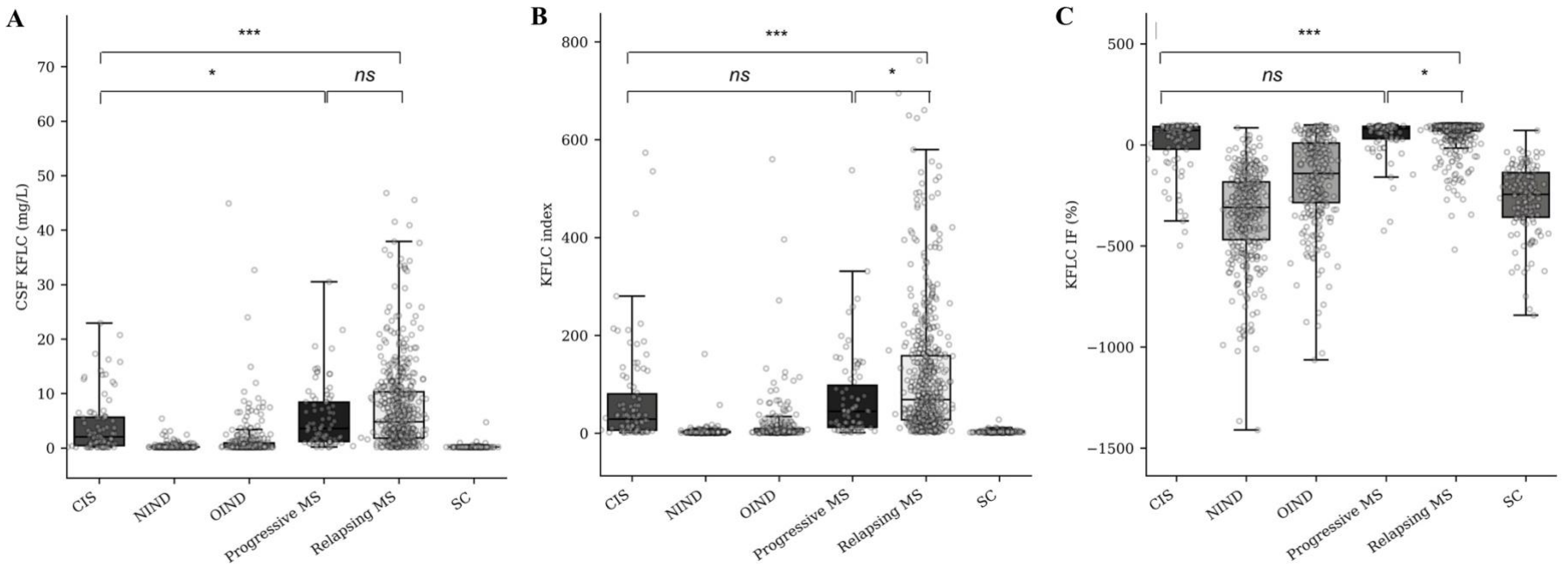
eTable 3. Type of immune modifying treatment use at sampling in each group

	MS group (*n*=57)	CIS group (*n*=4)	OIND group (*n*=45)	NINDC group (*n*=23)	Total *n* (%)
Steroids	26	2	25	9	62 (48.1)
Immunosuppressors					59 (45.7)*
Anti-CD20	2	0	4	1	
Natalizumab	3	0	0	0	
Teriflunomide	8	0	0	0	
Fingolimod	6	0	0	0	
TNFα blocker	3	0	1	2	
Cyclophosphamide	0	0	3	0	
Methotrexate	1	1	3	2	
Azathioprine	1	0	2	1	
Mycophenolate mofetil	0	0	2	1	
Tocilizumab	0	0	2	1	
Other	4	0	6	4	
Immunoregulators					11 (8.5)
Dimethyl Fumarate	0	1	0	0	
Glatiramer acetate	2	0	0	0	
β interferon	2	0	0	0	
Hydroxychloroquine	1	0	3	2	
Plasma exchange	0	1	1	0	2 (1.6)
Chemotherapy	0	0	1	2	3 (2.3)
IV immune globulin	0	0	2	1	3 (2.3)

CIS: clinically isolated syndrome; MS: multiple sclerosis; NINDC: non-inflammatory neurological disease controls; OIND: other inflammatory neurological disorder.

Other immunosuppressors: Ciclosporin (*n*=2), Mitoxantrone (*n*=1), IL-17A monoclonal antibody (*n*=1), JAK inhibitor (*n*=2), immune check point inhibitor (*n*=4), Leflunomide (*n*=1), Tyrosin kinase inhibitor (*n*=3); * 5 of the 59 patients had 2 immunosuppressive treatments.

eFigure 2. Comparison of the KFLC biomarkers values in each subgroup of patients



In MS subgroups, patients were distributed as follow: relapsing MS (*n*=592, median age of 35 [28;44] years-old, male to female ratio of 0.27, with 78% of patients presenting with an active disease at sampling), progressive MS (*n*=83, median age 53 [51;56] years-old, male to female ratio of 0.42, with 14% of patients presenting with an active disease at sampling). The panel A shows that CSF KFLC were not statistically different between progressive MS (median= 3.6 [1.2;8.4] mg/L) and relapsing MS (median= 4.8 [1.8;10.3] mg/L), p=0.292; but both MS subgroups presented with higher CSF KFLC concentrations than CIS patients (median= 2.0 [0.5;5.7] mg/L), p=0.012 for CIS vs. progressive MS comparison, and p<0.001 for relapsing MS vs. CIS comparison. The panel B shows that relapsing MS patients had higher KFLC index (median= 68.8 [27.7;158.3]) than progressive MS (median= 44.6 [12.1;98.0]), p=0.023, and CIS (median=29.0 [6.3;80.5]), p<0.001. Both progressive MS and CIS presented with similar median KFLC index values, p=0.241. The panel C shows that relapsing MS patients had higher KFLC IF (median= 86.9 [69.1;94.1]

%) than progressive MS (median= 80.3 [29.7;91.8] %), p=0.043, and CIS (median= 71.6 [-21.8;90.1] %), p<0.001. Both progressive MS and CIS presented with similar median KFLC index values, p=0.259.

Progressive MS, relapsing MS, and CIS patients presented higher CSF KFLC, KFLC index, and KFLC IF values than NIND, or SC, and all differences were statistically significant (p<0.001 for all comparisons).

CIS: Clinically isolated syndrome; MS: Multiple sclerosis; NIND: Non-inflammatory neurological disorder; OIND: Other inflammatory neurological disorder; SC: Symptomatic control.

ns: non-significant ; *: p-value < 0.05 ; ***: p-value < 0.001

eTable 4. KFLC biomarkers diagnosis performance to separate MS and CIS patients (*n*=765) from OIND and NINDC patients (*n*=856)

Variable	CSF KFLC	KFLC index	KFLC IF
AUC	0.9142[a]	0.9391[a]	0.9416[a]
95% CI	[0.8995; 0.9289]	[0.9274; 0.9508]	[0.9298; 0.9534]
Model (y = ax+b)	-1.3162+0.609*CSF KFLC	-1.4808+0.0561*KFLC index	-1.7281+0.0398*KFLC IF
Younden index	0.7437	8.9248	67.3251
Sensitivity	87.9 [85.3; 90.1]	88.2 [85.7; 90.4]	87.6 [85.0; 89.9]
Specificity	86.3 [83.8; 88.6]	89.3 [87.0; 91.3]	90.8 [88.6; 92.7]
PPV	85.4 [82.7; 87.9]	88.0 [85.5; 90.2]	89.7 [87.2; 91.8]
NPV	88.6 [86.2; 90.8]	89.5 [87.2; 91.4]	88.9 [86.6; 91.0]

AUC: area under the curve; NPV: negative predictive value; PPV: positive predictive value.

[a]: AUC comparison by Delong method: the KFLC index AUC and the KFLC IF AUC were higher than the CSF KFLC AUC, $p<0.001$ for both comparisons. KFLC index AUC and KFLC IF AUC values did not differ statistically, $p=0.123$.

eTable 5. KFLC biomarkers diagnosis performance to separate MS patients (*n*=675) from SC patients (*n*=167)

Variable	CSF KFLC	KFLC index	KFLC IF
AUC	0.967[a]	0.9737[a]	0.9790[a]
95% CI	[0.9554; 0.9786]	[0.9645; 0.9830]	[0.9701; 0.9879]
Model (y = ax+b)	-1.8012+4.4598*CSF KFLC	-2.5344+0.4200*KFLC index	-0.3801+0.0504*KFLC IF
Younden index	0.4704	9.1072	46.2631
Sensitivity	93.8 [91.7; 95.6]	90.4 [87.9; 92.5]	93.2 [91.0; 95.0]
Specificity	96.3 [91.6; 98.8]	97.6 [94.0; 99.3]	98.5 [94.8; 99.8]
PPV	99.2 [98.1; 99.7]	99.4 [98.3; 99.8]	99.7 [98.8; 99.9]
NPV	76.5 [69.4; 82.6]	71.5 [65.2; 77.3]	75.1 [68.1; 81.3]

AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value

[a]: AUC comparison by Delong method: the KFLC IF AUC was higher than the KFLC index AUC, p=0.008. The KFLC IF AUC and the KFLC index AUC were higher than the CSF KFLC AUC, p<0.001 for both comparisons.

eTable 6. KFLC biomarkers diagnosis performance to separate MS patients (*n*=675) from NIND patients (*n*=392)

Variable	CSF KFLC	KFLC index	KFLC IF
AUC	0.9613[a]	0.9787[a]	0.9831[a]
95% CI	[0.9504; 0.9722]	[0.9709; 0.9866]	[0.9765; 0.9897]
Model (y = ax+b)	-2.3461+3.0548*CSF KFLC	-2.3934+0.2637*KFLC index	-1.1775+0.0532*KFLC IF
Younden index	0.6400	8.3850	38.1203
Sensitivity	91.5 [89.1; 93.5]	.91.0 [88.5; 93.0]	94.0 [91.9; 95.7]
Specificity	93.8 [90.9; 96.0]	96.9 [94.7; 98.4]	96.4 [94.0; 98.0]
PPV	96.1 [94.3; 97.5]	98.1 [96.7; 99.0]	97.8 [96.3; 98.8]
NPV	86.8 [83.1; 89.9]	86.2 [82.6; 89.3]	90.5 [87.2; 93.2]

AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value

[a]: AUC comparison by Delong method: the KFLC IF AUC was higher than the KFLC index AUC, p=0.002. The KFLC index AUC and the KFLC IF AUC were higher than the CSF KFLC AUC, p<0.001.

eTable 7. KFLC biomarkers diagnosis performance to separate MS patients (*n*=675) from OIND patients (*n*=297)

Variable	CSF KFLC	KFLC index	KFLC IF
AUC	0.8645[a]	0.8962[a]	0.8943[a]
95% CI	[0.8380; 0.8910]	[0.8737; 0.9187]	[0.8708; 0.9178]
Model (y = ax+b)	-0.2421+0.3466*CSF KFLC	-0.4208+0.0331*KFLC index	-1.146+0.0341*KFLC IF
Younden index	0.94	11.5593	67.8591
Sensitivity	87.0 [84.2; 89.5]	87.6 [84.8; 90.0]	89.5 [86.9; 91.8]
Specificity	75.4 [69.9; 80.3]	79.5 [74.4; 83.9]	0.76.8 [71.4; 81.6]
PPV	88.9 [86.3; 91.3]	90.6 [88.1; 92.8]	89.8 [87.2; 92.0]
NPV	71.8 [66.3; 76.9]	73.8 [68.6; 78.5]	76.2 [70.9; 81.0]

AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value

[a]: AUC comparison by Delong method: the KFLC IF AUC and the KFLC index AUC were not statistically different, p=0.991. The KFLC index AUC and the KFLC IF AUC were higher than the CSF KFLC AUC, p<0.001.

eTable 8. Diagnosis performances of adding OCB to KFLC biomarkers in separating MS from OIND

MS vs. OIND *n*=972			
	AUC	Sensitivity	Specificity
KFLC index > 11.56	0.835 [0.809; 0.861]	87.6 [85.1; 90.1]	79.7 [75.2; 84.3]
KFLC index > 11.56 + positive OCB	0.826 [0.801; 0.849]	79.4 [76.2; 82.4]	85.8 [81.3; 89.6]
P-value	0.231[a]	**<0.001**	**<0.001**
KFLC IF > 67.9%	0.831 [0.803; 0.858]	89.4 [86.0; 91.7]	77.0 [72.1; 81.9]
KFLC IF > 67.9% + positive OCB	0.828 [0.802; 0.851]	79.9 [76.6; 82.9]	85.8 [81.3; 89.6]
P-value	0.666[a]	**<0.001**	**<0.001**

[a] : AUC comparison by Delong method

[b] : McNemar test

MS: Multiple sclerosis; OIND: Other inflammatory neurological disorder.

eTable 9. Application of the KFLC index and KFLC IF obtained cut-off values in female and male gender patients

	MS/CIS vs. OIND/NINDC						MS vs. OIND					
	KFLC index > 8.92			KFLC IF > 67.3%			KFLC index > 11.56			KFLC IF > 67.9%		
	AUC	Sensitivity (%)	Specificity (%)	AUC	Sensitivity (%)	Specificity (%)	AUC	Sensitivity (%)	Specificity (%)	AUC	Sensitivity (%)	Specificity (%)
Female, n=1,067	0.897 [0.876; 0.915]	89.3 [86.4; 91.7]	89.8 [86.8; 92.3]	0.897 [0.877; 0.915]	79.1 [75.4; 82.5]	94.5 [92.1; 96.3]	0.847 [0.817; 0.875]	88.8 [85.6; 91.5]	79.9 [73.0; 85.6]	0.838 [0.806; 0.866]	82.0 [78.1; 85.4]	85.3 [78.9; 90.3]
Male, n=554	0.875 [0.844; 0.902]	73.9 [67.4; 79.8]	94.0 [90.7; 96.3]	0.882 [0.852; 0.909]	85.6 [80.3; 90.0]	88.5 [84.6; 91.7]	0.811 [0.763; 0.853]	84.5 [78.7; 89.3]	78.9 [70.8; 85.6]	0.816 [0.769; 0.858]	87.2 [81.6; 91.6]	77.9 [69.5; 84.9]
P-value	0.180[f]	**<0.001**[b]	**0.032**[b]	0.361[f]	**0.041**[b]	**0.002**[b]	0.155[f]	0.126[b]	0.836[b]	0.399[f]	0.165[b]	0.107[b]

[f] : independent AUC comparison by Delong method

[b] : McNemar test

CIS: Clinically isolated syndrome; MS: Multiple sclerosis; NINDC: Non-inflammatory neurological disorder control; OIND: Other inflammatory neurological disorder.

eTable 10. Application of the KFLC index and KFLC IF obtained cut-off values to different range of age

	MS/CIS vs. OIND/NINDC						MS vs. OIND					
	KFLC index > 8.92			KFLC IF > 67.3%			KFLC index > 11.56			KFLC IF > 67.9%		
	AUC	Sensitivity (%)	Specificity (%)	AUC	Sensitivity (%)	Specificity (%)	AUC	Sensitivity (%)	Specificity (%)	AUC	Sensitivity (%)	Specificity (%)
age < 30 group, *n*=355 (32/192/51/80)*	0.867 [0.827; 0.900]	90.2 [85.5; 93.7]	83.2 [75.7; 89.2]	0.866 [0.825; 0.900]	88.3 [83.2; 92.3]	84.8 [77.3; 90.6]	0.764 [0.705; 0.816]	88.0 [82.6; 92.3]	74.7 [50.1; 77.6]	0.767 [0.707; 0.820]	90.2 [84.9; 94.1]	63.3 [48.3; 76.7]
30 < age < 55 group, *n*=799 (43/403/142/211)*	0.888 [0.864 ;0.909]	88.6 [85.2; 91.4]	89.0 [85.2; 92.0]	0.891 [0.866; 0.912]	87.9 [84.4; 90.8]	90.3 [86.6; 93.3]	0.862 [0.830; 0.890]	89.3 [85.9; 92.2]	83.1 [75.9; 88.9]	0.847 [0.813; 0.876]	89.9 [86.4; 92.7]	79.4 [71.6; 85.9]
55 < age group, *n*=467 (15/80/104/268)*	0.870 [0.836 ;0.899]	82.3 [73.2; 89.3]	91.7 [88.4; 94.3]	0.891 [0.858; 0.918]	84.8 [75.8; 91.4]	93.4 [90.3; 95.8]	0.798 [0.732; 0.853]	77.8 [67.2; 86.3]	81.7 [73.0; 88.6]	0.824 [0.760; 0.877]	85.0 [75.3; 92.0]	79.8 [70.5; 87.2]
P-value	0.346 [f]	0.116 [b]	**0.026** [b]	0.268 [f]	0.647 [b]	**0.016** [b]	**0.014** [f]	**0.013** [b]	**0.016** [b]	0.082 [f]	0.375 [b]	**0.049** [b]

* in order, the values represents the number of patients in CIS group, MS group, OIND group, and NINDC group, respectively.

[f] : independent AUC comparison by Delong method: in eTable 11, the shown p-value for AUC comparison is the lowest p-value obtained by the different age groups comparisons (*n*=3 comparison for each AUC p-value).

[b] : McNemar test

CIS: Clinically isolated syndrome; MS: Multiple sclerosis; NINDC: Non-inflammatory neurological disorder control; OIND: Other inflammatory neurological disorder.

eTable 11. CSF KFLC, KFLC index, and KFLC IF median values across centers in MS patients

	Marseille (*n*=146)	Montpellier (*n*=45)	Nantes (*n*=68)	Nice (*n*=174)	Other centers (*n*=242)	P-value
CSF KFLC (mg/L), median [IQR range]	4.88 [1.83; 10.83]	4.85 [2.64; 7.27]	7.15 [2.50; 12.88]	4.67 [1.77; 10.16]	3.52 [1.72; 8.99]	0.067[e]
KFLC index, median [IQR range]	71.53 [23.83; 155.62]	63.88 [41.24; 124.16]	96.35 [30.46; 191.43]	67.25 [30.46; 147.43]	56.95 [23.43; 143.79]	0.268[e]
KFLC IF (%), median [IQR range]	95.67 [86.36; 97.74]	94.64 [91.91; 97.07]	96.56 [88.86; 98.32]	94.83 [86.83; 99.24]	93.77 [86.66; 97.33]	0.091[e]

[e] : Kruskal Wallis test

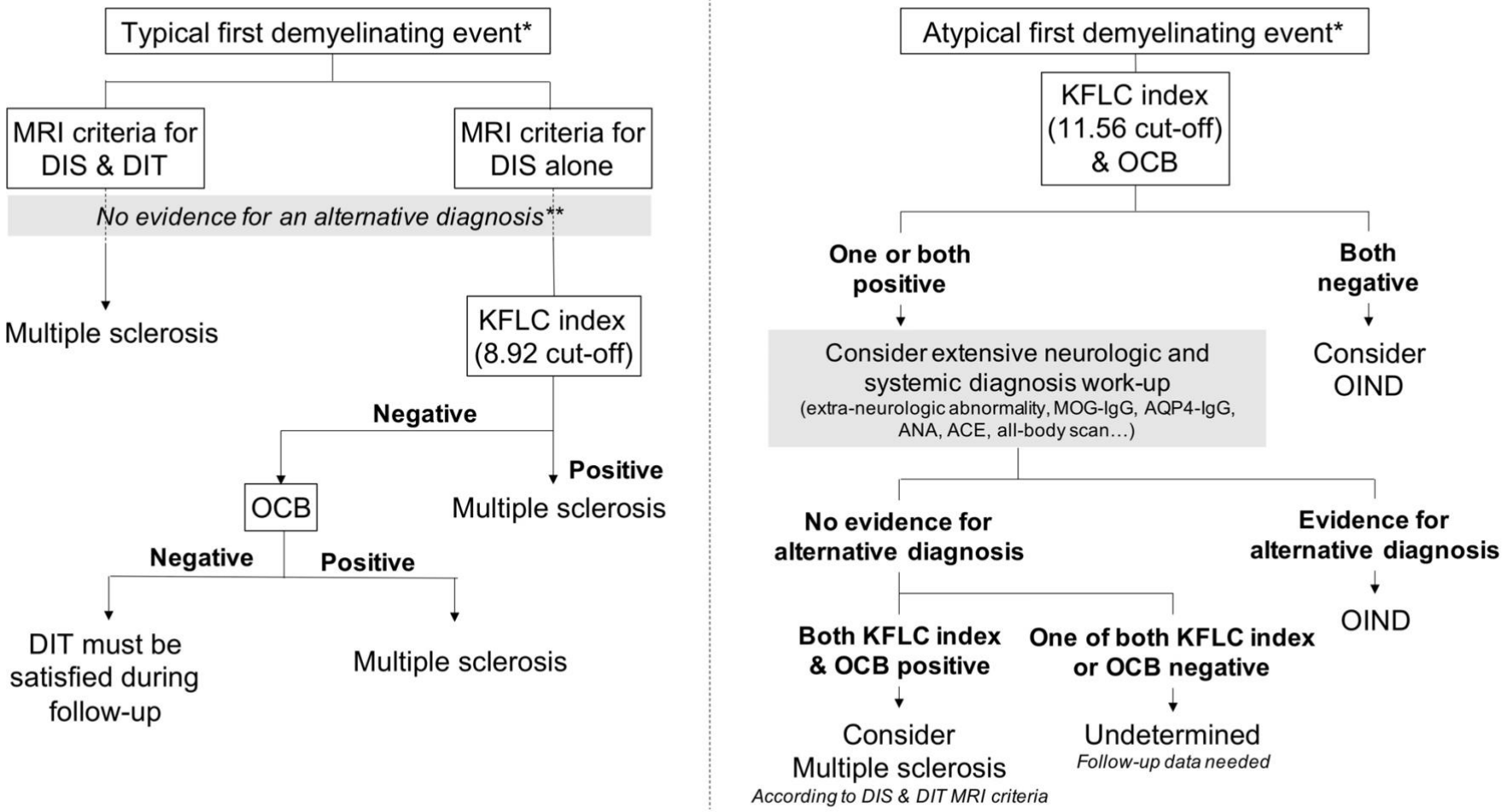
eTable 12. Application of the KFLC index and KFLC IF cut-off values to each available center.

			Nantes	Nice	Nîmes	Montpellier	P-value
MS + CIS vs. NINDC + OIND	KFLC index > 8.92	AUC	0.901 [0.848; 0.940]	0.908 [0.881; 0.931]	0.832 [0.766; 0.885]	0.897 [0.826; 0.945]	0.1272[f]
		Sensitivity (%)	90.0 [81.2; 95.6]	89.3 [84.2; 93.2]	73.9 [51.6; 89.8]	82.8 [70.6; 91.4]	0.107[b]
		Specificity (%)	89.5 [81.5; 94.8]	92.0 [88.7; 94.6]	92.4 [86.8; 96.2]	96.5 [87.9; 99.6]	0.546[b]
	KFLC IF > 67.3%	AUC	0.888 [0.834; 0.930]	0.912 [0.885; 0.934]	0.835 [0.770; 0.888]	0.897 [0.826; 0.945]	0.1211[f]
		Sensitivity (%)	88.8 [79.5; 94.7]	89.3 [84.2; 93.2]	73.9 [51.6; 89.8]	82.8 [70.6; 91.4]	0.157[b]
		Specificity (%)	89.5 [81.5; 94.8]	92.9 [89.7; 95.3]	92.4 [86.8; 96.2]	96.5 [87.9; 99.6]	0.502[b]
MS vs. OIND	KFLC index > 11.56	AUC	0.832 [0.744; 0.899]	0.874 [0.830; 0.909]	0.775 [0.633; 0.882]	0.904 [0.810; 0.962]	0.0704[f]
		Sensitivity (%)	88.2 [78.1; 94.8]	92.0 [86.7; 95.5]	77.3 [54.6; 92.2]	90.0 [78.2; 96.7]	0.187[b]
		Specificity (%)	72.0 [50.6; 87.9]	82.0 [74.0; 88.3]	77.8 [57.7; 91.4]	92.0 [74.0; 99.0]	0.491[b]
	KFLC IF > 67.9%	AUC	0.847 [0.761; 0.911]	0.871 [0.828; 0.907]	0.738 [0.593; 0.853]	0.904 [0.810; 0.962]	**0.0242**[f]
		Sensitivity (%)	91.2 [81.8; 96.7]	93.1 [88.3; 96.4]	77.3 [54.6; 92.2]	90.0 [78.2; 96.7]	0.106[b]

		Specificity (%)	72.0 [50.6; 87.9]	80.3 [72.2; 87.0]	66.7 [46.0; 83.5]	92.0 [74.0; 99.0]	0.258 [b]

[f] : independent AUC comparison by Delong method: in eTable 9, the shown p-value for AUC comparison is the lowest p-value obtained by the different centers comparisons (*n*=6 comparison for each AUC p-value).
[b] : McNemar test
CIS: Clinically isolated syndrome; MS: Multiple sclerosis; NINDC: Non-inflammatory neurological disorder control; OIND: Other inflammatory neurological disorder.

eFigure 3. Proposed diagnostic algorithm including KFLC index, depending on clinical and radiological presentation of suspected multiple sclerosis patients



ACE: Angiotensin converting enzyme; ANA: Anti-nuclear antibody; AQP4-IgG: Aquaporin 4 antibody; DIS: Dissemination in space; DIT: Dissemination in time; KFLC: Kappa free light chains; MOG-IgG: Myelin oligodendrocyte antibody; MRI: Magnetic resonance imaging; OCB: Oligoclonal bands; OIND: Other inflammatory neurological disorders.

***** Patients are classified as typical or atypical demyelinating event depending on the presence of clinical or magnetic resonance imaging "red flags" for multiple sclerosis diagnosis, according to references [1, 36-37].

** Diagnosis work-up that allow to exclude alternative diagnosis should refer to the 2017 McDonald criteria. [1]