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Free light chains in cerebrospinal fluid of multiple sclerosis patients negative for IgG oligoclonal bands

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ABSTRACT

The detection of IgG oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) is, as yet, the recommended biochemical marker for the diagnosis of multiple sclerosis (MS). Aim of this study was to investigate the behaviour of free light chains (FLC) in OCBs negative (OCBs-) MS patients compared with that in OCBs positive (OCBs+) MS patients and in a control group (CG) of subjects without cerebrospinal inflammatory disease. At multiple comparisons between the three groups, statistically significant differences ($p < .001$ for all) were found for κFLC. Conversely, λFLC values evidenced a greater overlapping in the three groups. Receiver operating characteristics (ROC) curves made with κFLC values, evidenced the greater differences of areas under curves (AUCs) between OCBs- and OCBs+ (AUCs: κFLC 0.98, QκFLC 0.98, κFLC index 0.96) with respect to the differences between OCBs- and CG (AUCs: κFLC 0.77, QκFLC 0.86, κFLC index 0.77): indeed > 50% of MS OCBs- subjects studied evidenced the same values of κFLC, QκFLC and κFLC index found in CG. Conversely, if the aim is to select MS subjects while avoiding undertaking the more complex isoelectrofocusing test, values with absolute specificity for MS (QκFLC = 15, sensitivity = 0.76 and κFLCindex = 3.09, sensitivity = 0.72) could be used. The values found in this study call for confirmation with data from more subjects, including those with other CSF inflammatory diseases. Anyway, the most important finding was that, for some OCBs- subjects, κFLC are more effective than OCBs in diagnosing MS.

1. Introduction

The presence of free light chains (FLC) in cerebrospinal fluid (CSF) has been well known since the late seventies, when studies [1–3] identified these proteins in association with CSF-immunoglobulin (Ig) synthesis in patients with cerebrospinal inflammatory diseases, in particular, those with multiple sclerosis (MS). From a pathophysiological viewpoint, when an antigenic stimulation occurs in CSF, plasma cells secrete oligoclonal intact Ig, together with an excess of FLC, which can be detected with specific antibodies. Since quantitative methods were not available qualitative FLC-oligoclonal patterns were compared with IgG-oligoclonal bands patterns (OCBs).

Although the sensitivity of OCBs as a MS marker [4] varies depending on different methodological factors, it is very high, albeit not absolute. It is usually higher than the sensitivity of quantitative data, such as IgG index [5]. Therefore OCBs continue to be considered the gold standard. On the other hand, there are drawbacks to the detection of OCBs: the technique is time consuming and expensive, and calls for personnel who have expertise in its performance and the interpretation of results. Moreover, its specificity in diagnosing MS can vary depending on whether the comparison group includes patients with different inflammatory conditions [6], oligoclonal IgG synthesis being common to many other inflammatory and/or autoimmune diseases.

Recently, the use of CSF-FLC has been proposed as a quantitative marker for MS.[7–9]. Since OCBs are not specific for MS and not all MS patients evidence positive OCBs patterns, the present study was conducted to evaluate FLC in MS patients in order to verify whether this marker could provide additional information, in particular in patients with negative oligoclonal IgG patterns (OCBs-), that are not of diagnostic utility.

Abbreviations: OCBs, oligoclonal bands; CSF, cerebrospinal fluid; MS, multiple sclerosis; FLC, free light chains; OCBs-, OCBs oligoclonal negative bands; OCBs+, OCBs oligoclonal positive bands; CG, control group; QκFLC, CSF-κFLC/serum-FLC; κFLC index, QκFLC/Qalb; Qalb, CSF-albumin/serum-albumin.; ROC, receiver operating characteristics; AUCs, areas under curves

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2. Materials and methods

One hundred and six serum and CSF paired samples collected within January 2014 and March 2017 and stored at −80 °C, were enrolled retrospectively in this study. Patients performed the lumbar puncture for diagnosis and while no disease-modifying treatment (including steroids) was on-going. No comorbidity was reported.

Samples were classified and then selected on the basis of the most recent diagnostic criteria [10] retrospectively applied.

The diagnostic workup included brain and spinal cord magnetic resonance imaging (MRI) (T1, T2, fluid-attenuated inversion recovery (FLAIR), double inversion recovery (DIR), phase-sensitive inversion recovery (PSIR), and gadolinium-enhancing sequences), complete cerebrospinal fluid (CSF) examination (including the search for OCBs and evaluation of quantitative IgG indexes), visual evoked potential, serum biochemical analysis (including B12, folates), and immunological screening (including anti-nuclear antibody, anti-neutrophil cytoplasmic antibody, anti-extractable nuclear antigens, anti-double stranded DNA, IgM and IgG anti-beta2 glycoprotein I, IgM and IgG anti-cardiolipin, lupus anticoagulant, anti-acquaporin-4 and anti-MOG detections, and angiotensin-converting enzyme).

Sixty-eight out of 106-paired samples were from relapsing-remitting MS (RRMS) patients: 38 were OCBs positive (OCBs+) and 30 negative (OCBs-). Positivity criterium for OCBs was defined as ≥2 CSF restricted IgG oligoclonal bands.

OCBs- MS subjects represent a particular small group of MS subjects characterized by the pathology who, conversely, are negative for oligoclonal IgG synthesis: These subjects were selected and studied in comparison both with subjects suffering from the same pathology but positive for the OCBs, and with subjects negative for OCBs suffering from different diseases, which represent the comparison group (CG). The CG consisted of 38 subjects complaining of tension headache ($n = 8$), subjective transient sensory symptoms ($n = 14$), and psychosomatic disorders ($n = 16$), who underwent the previous described diagnostic workup in order to rule out neurological and/or systemic disease; all subjects were OCBs-.The basic characteristics of the three studied groups are listed in Table 1.

All paired samples were tested for both κFLC and λFLC with Freelite assay (The Binding Site Group Birmingham, UK) applied on BNII analyzer (Siemens Healthcare Diagnostics). Albumin, IgG, and IgM concentrations were measured on a Dimension Vista analyzer (Siemens Diagnostics), using an immunonephelometric method.

The evaluation of OCBs was performed on agarose isoelectric focusing followed by transfer to nitrocellulose membrane, IgG specific immunofixation and amplification with avidin-biotin and peroxidase staining [11].

The statistical analysis was performed using the Analyse-it program.

The study was approved by the local Ethics Committee.

3. Results

Results are expressed as FLC absolute concentrations (mg/L), FLC concentrations quotients (QFLC = CSF-FLC/serum-FLC) and FLC index (QFLC/Qalb; Qalb = CSF-albumin/serum-albumin); in all three studied groups the values obtained both of κFLC as λFLC did not evidence a normal distribution (Shaphiro-Wilk $p < .001$).

Table 2 reports the values (medians and interquartile ranges) obtained in the three studied groups; at Kruskal-Wallis analysis (Bonferroni's adjusted p values), a statistically significant difference was found in the two to two comparison between the three groups for κFLCs, expressed as absolute values, as well as QκFLC and κFLC index.

Conversely, λFLC evidenced no significant difference between CG and OCBs- when expressed as λFLC index.

In order to evaluate whether an FLC value could evidence MS OCBs- patients with respect to MS OCBs+ patients and CG, data obtained from κFLC values were plotted as receiver operating characteristics (ROC) curves (Fig. 1): the highest areas under curves (AUC) were obtained on comparing OCBs- *vs* OCBs+ values (Fig. 1C) and all MS (OCBs+ and OCBs-) *vs* CG values (Fig. 1A). Significant values for the AUC were also obtained on comparing MS OCBs- *vs* CG values (Fig. 1B): QκFLC evidenced the greatest AUC, but the difference between it and κFLC and κFLC index did not attain statistical significance. Fig. 2 shows the ranges of values obtained and the corresponding number of subjects of each group, with the aim to highlight the overlapping of values in the three studied groups.

Table 1
Main characteristics (median values and interquartile ranges into brakets) of the studied groups.

	CG	OCBs-	OCBs+
Age median (range)	43.30 (22.62–53.84)	41.75 (27.70–55.48)	38.71 (25.61–48.62)
Gender (Female/Male)	24/6	15/15	20/18
Disease Duration (months)	not applicable	58 (0–95)	17*^ (0 − 30)
Serum albumin (g/L)	42.10 (40.90–44.51)	42.85 (38.90–45.92)	42.10 (39.89–45.72)
CSF albumin (mg/L)	193 (155–239)	205 (159–303)	220 (159–296)
Q_{Alb}	4.56 (3.52–5.64)	4.90 (3.77–7.64)	5.10 (3.70–6.64)
Blood –brain barrier damage	0 (0%)	9 (30.0%)	8 (22.9%)*
Serum IgG (g/L)	11.65 (10.37–13.10)	9.65 (8.81–11.42)***	9.96 (8.88–11.73)****
CSF IgG (mg/L)	24.50 (18.00–33.20)	24.50 (18.90–33.20)	37.50 (32.80—58.20)*^
Q_{IgG}	2.16 (1.61–2.71)	2.57 (1.92–3.67)	3.89 (3.08–5.73)*^
IgG index	0.47 (0.45–0.50)	0.49 (0.46–0.54)	0.75 (0.60–1.09)*^
IgG index > 0.7	0	1 (3.3%)	19 (47.5%)^^^***
IgG_{LOC} (mg/dL)	0.0 (0.0–0.0)	0.01 (0.0–0.04)	1.1 (0.0–4.0)*^
IgG_{IF}(%)	0 (0–0)	0.1 (0–1.5)	8 (0–35) *^
$IgG_{IF} > 0$	0	1 (3.3%)	20 (50.0%)^^
Serum IgM (g/L)	1.24 (0.87–1.77)	1.25 (0.79–1.68)	1.07 (0.85–1.44)
CSF IgM (mg/L)	0.19 (< 0.15–0.30)	0.20 (< 0.15–0.44)	0.25 (< 0.15–0.71)

Quantitative variable: Kruskal-Wallis analysis (Bonferroni's adjusted p values), qualitative variable chi-squared test.Symbols * evidence p values *vs* CG; symbols ^ evidence p values *vs* OCBs-; for Blood-Brain Barrier Damage, p-value is expressed for the comparison between the 3 groups by means of Chi-Square (the value in CG was setted to 1).
(*p < .0001; **p < .001***; p = .01; **** $p = .0185$; ^ $p < .0001$; ^^ $p < .001$: ^^^ $p < .005$)

4. Discussion

In most previous studies demonstrating the utility of CSF-FLC in the diagnosis of MS, a comparison was made between MS and other CSF inflammatory diseases [9.12]. In the present study, a subgroup of MS patients (MS OCBs-), for whom OCBs were not informative, was examined in order to evaluate the behaviour of FLC, in particular with respect to MS OCBs+ subjects.

Our results are in agreement with those in previous studies [13,14], which underline the greater diagnostic performance of κFLC with respect to λFLC in MS, irrespective of whether OCBs test results are positive or negative (Table 1).

Despite the fact that the statistical approach could provide significant differences (Kruskal-Wallis analysis and Fig. 1), there is a wide overlapping between OCBs- and CG in lower values and between OCBs- and OCBs+ in higher values; indeed only CG *vs* OCBs+ evidenced a

Table 2
Median values and interquartile ranges (into brakets) of FLC in Oligoclonal Positive (OCBs+), Oligoclonal negative (OCBs-) patients and comparison group (CG).

	CSFκFLC, mg/L	QκFLCx10^3	κFLC Index
CG	0.07 (< 0.06–0.09)	5.63 (4.84–7.26)	0.99 (0.87–1.44)
OCBs-	0.14* (0.09–0.33)	10.90* (7.56–29.75)	1.82** (1.07–5.87)
OCBs+	4.04*^ (2.59–9.98)	298.74*^ (175.47–1171.20)	63.26*^ (25.90–206.20)
	CSFλFLC, mg/L	**QλkFLC**	**λFLC index**
CG	0.07 (0.06–0.10)	6.58 (5.68–8.59)	1.45 (1.18–1.68)
OCBs-	0.09*** (0.07–0.13)	9.24**** (4.54–13.59)	1.58 (1.19–2.17)
OCBs+	0.23*^^ (0.10–0.78)	20.52*^ (10.79–108.09)	4.90*^ (2.43–19.73)

Samples with FLC values lower than assay sensitivity were not included in the QFLC and FLC index calculation: 18 samples from CG and 4 samples from OCBs- group provided CSF κFLC < 0.06 mg/L; 8 samples from CG (in 7 out of 8 samples both kappa and lambda were undetectable) and 3 samples from OCBs- group (in 1 both kappa and lambda were undetectable) provided CSFλFLC < 0.05 mg/L.
Symbols * evidence p values *vs* CG; symbols ^ evidence p values *vs* OCBs- (*p < .0001;** $p = .0017$;*** $p = .039$ **** $p = .0061$;^p < 00001;^^ $p = .0003$).

completely different distribution of values for κFLC, QκFLC and κFLC index (Table 2, Fig. 2).

AUC at ROC analysis highlighted a greater difference between OCBs- and OCBs+ (Fig. 1C) than between OCBs- and CG (Fig. 1B), although in the last case, QκFLC seems to distinguish better between the two groups (AUC = 0.86). It has to be considered that few aspects could explain the discrepancy between QκFLC and κFLC index: i) κFLC index corrects CSF concentrations for Blood Brain Barrier dysfunction; ii) QκFLC and QAlb are not linearly associated, which means that exponential function is the best approach to estimate intrathecal κFLC origin (8).

Different approaches could be used if the aim is to have diagnostic information about MS disease (known to include OCBs+ and OCBs- subjects) or to select OCBs+, in order to avoid undertaking the more complex isoelectrofocusing test.

Our results demonstrate that, in order to "detect" MS patients, even the lowest κFLC value (< 0.06 mg/L) does not attain the maximum value for sensitivity: in our study, 4/30 OCBs- subjects presented values < 0.06 mg/L (Fig. 2A). Conversely, QκFLC = 4.3 and κFLC index = 0.84 (Fig. 2B and C) enable the maximum value of sensitivity to be attained, but show an extremely low specificity (15 and 20%, respectively), and, consequently, low accuracy. Previous studies [12,15] proposed different values for diagnosing of MS: such differences may be due in part to the choice of the comparison group, and to the prevalence of OCBs- subjects enrolled.

Applying κFLC index = 2.91, proposed by Valencia-Vera et al. in our studied subjects, we can reach the maximum value of specificity in diagnosing MS, but would "miss" 17/26 = 65% of OCBs- MS subjects. On the other hand, κFLC index = 5.9 proposed by Presslauer et al. as cut off value in MS diagnosis, would fail in diagnosing 20/26 = 77% of OCBs- MS subjects of our study, but would identify 37/38 OCBs+ MS subjects.

Conversely, if the aim is to avoid undertaking the more complex isoelectrofocusing test, without "losing" MS patients, values allowing absolute specificity for MS could be used (QκFLC = 15, sensitivity = 0.76 and κFLC index = 3.09, sensitivity = 0.72). Such values

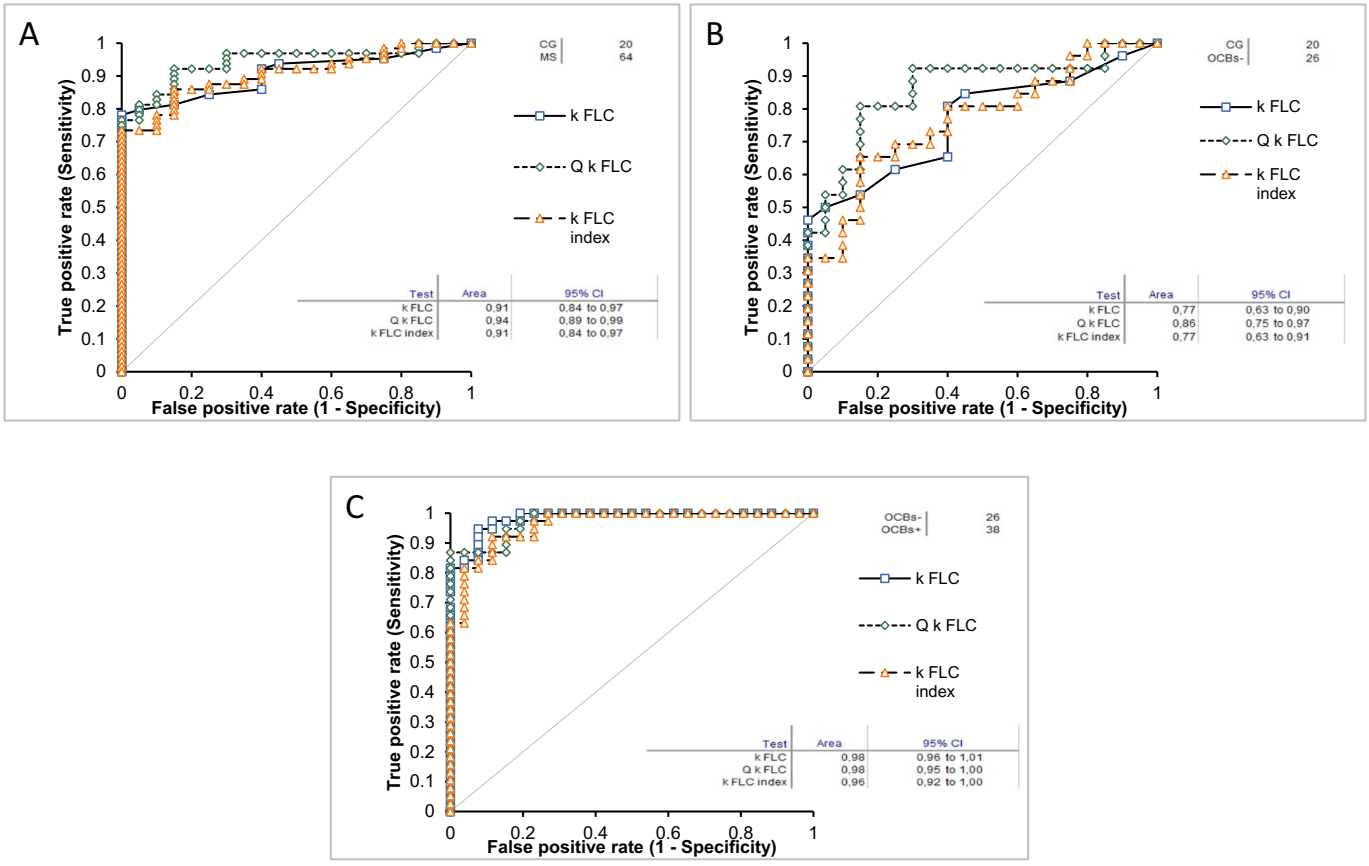

Fig. 1. ROC curves obtained comparing the values of three studied groups. A) MS (OCBs+ and OCBs-) *vs* CG; B) MS OCBs- *vs* CG; C) MS OCBs- *vs* MS OCBs+.

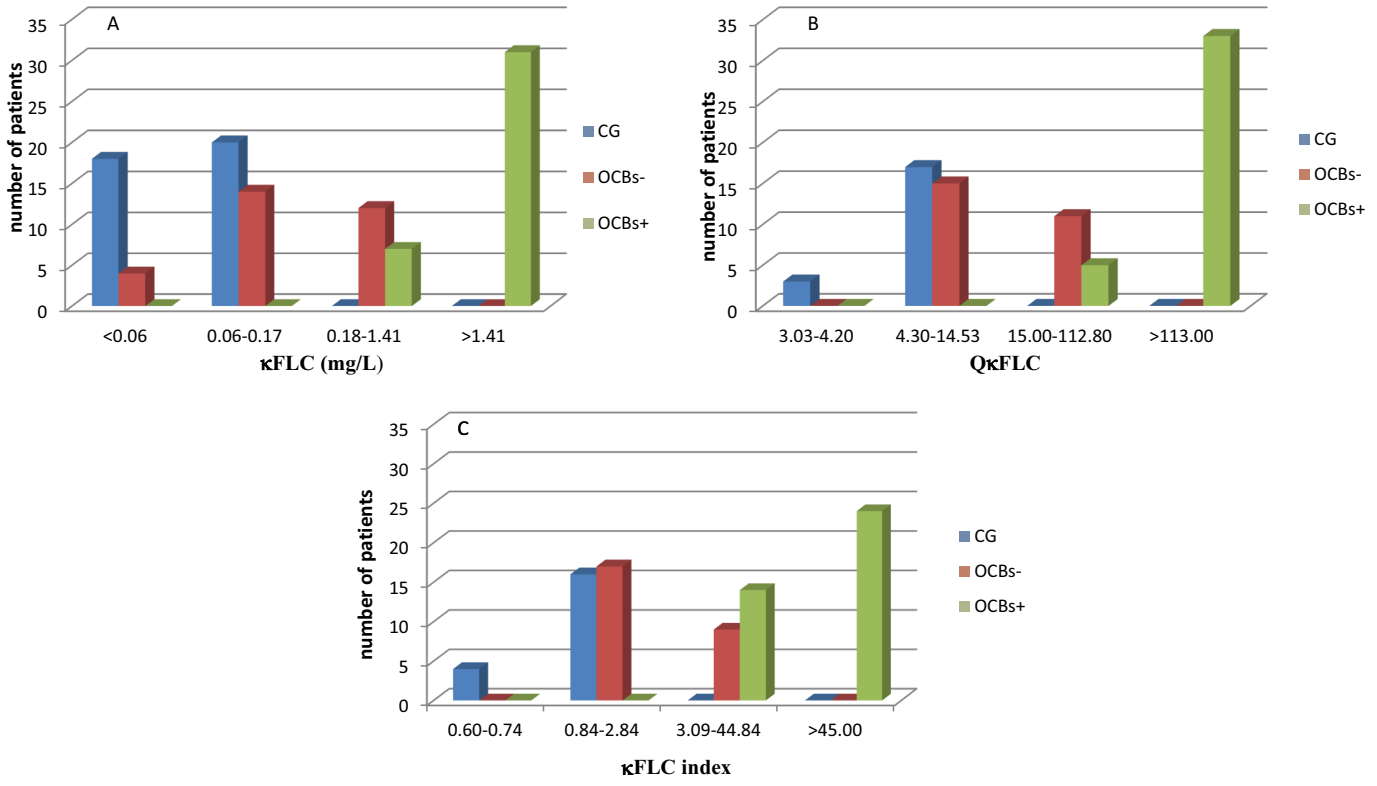

Fig. 2. Number of subjects of each studied group and ranges of values obtained for κFLC A), QκFLC B), κFLC index C).

will include all MS OCBs+, and more than one/third of OCBs- MS patients (10/26 = 38% using QκFLC and 9/26 = 35% using κFLC index).

On the one hand, OCBs can be found in diseases other than MS, and, on the other, not all MS patients are OCBs+. This study was focused on OCBs- MS subjects characterized by the pathology but negative for oligoclonal IgG synthesis, in order to evaluate if a different marker of cerebrospinal inflammatory disease could be more informative than oligoclonal IgG synthesis. Our results evidenced that in > 35% of OCBs- subjects, κFLC are of greater utility than OCBs in making a diagnosis of MS. It should be of interest to further investigate whether κFLC might provide prognostic information, and to verify whether OCBs-subjects with higher κFLC values (similar to those found in OCBs+ patients) might have a worse prognosis than those with lower values, similar to those found in CG subjects .

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