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ORIGINAL COMMUNICATION

Validation of CSF free light chain in diagnosis and prognosis of multiple sclerosis and clinically isolated syndrome: prospective cohort study in Buenos Aires

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Abstract

Background The objective was to evaluate the precision of kappa and lambda free light chains (KFLC and LFLC) in CSF for the diagnosis of multiple sclerosis (MS) and prognosis of clinically isolated syndrome (CIS).

Methods CSF and serum samples from CIS, MS and other neurological non-MS disease were collected between 2015 and 2017. FLC concentrations were measured using immunoassay Freelite™. Results were correlated with the patients' diagnoses and ROC curve analysis was used to determine accuracy. In CIS patients, analysis of FLC were compared in CIS converters vs. non-converter during follow-up.

Results In the MS group ($n=41$), the optimal cut-off for KFLC determined was 7 mg/L, with a diagnostic sensitivity and specificity of 95% and 97%, respectively. The optimal cut-off for LFLC was 0.7 mg/L, with a diagnostic sensitivity and specificity of 71% and 81%, respectively. 36 CIS patients were included; mean follow-up time was 28 ± 9 months, and 22 (61.1%) patients converted to MS. The median concentration of CSF K and LFLCs at CIS diagnosis was slightly higher in CIS-converters compared to non-converters, but this did not reach statistical significance (KFLC: median 7 ± 5.3 mg/L vs. 5 ± 2.3 mg/L, $p=0.11$; LFLC 0.7 ± 0.33 mg/L vs. 0.5 ± 0.23 mg/L $p=0.16$). A strong correlation was observed between the concentration of K and L FLCs at diagnosis and the change in PBVC during follow-up ($r=0.72$ and $r=0.65$, respectively).

Conclusion KFLCs have a high sensitivity and specificity for the diagnosis of MS. FLC concentrations at CIS diagnosis were not significantly higher in CIS-converters.

Keywords Multiple sclerosis · Clinically isolated syndrome · Free light chains · Progression · Diagnosis

Introduction

Multiple sclerosis (MS) is a chronic degenerative disease that affects young adults between 18 and 40 years of age, being the first cause of physical disability of non-traumatic origin in several countries [1, 2].

The presence of immunoglobulin (Ig) G oligoclonal bands (OB) in cerebrospinal fluid (CSF) is known to support a diagnosis and prognosis of multiple sclerosis (MS) [3, 4]. However, its evaluation is difficult in some regions, including Latin America, due to the qualitative, subjective assessment that it implies [5, 6].

Measuring the levels of CSF Ig free light chains (FLC) kappa (KFLC) and lambda (LFLC) has been proposed as a potential alternative to the qualitative assessment of OB and showed comparable diagnostic sensitivity and specificity, as well as a potential role in prognosis of conversion from

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clinically isolated syndrome (CIS) to MS [7, 8]. The extent to which quantification of FLC may support the diagnosis and progression prediction of MS has been investigated in only a few studies and not at all in the Latin American region.

Due to the relevance of a quantitative result to aid in the diagnosis of MS, as well as a prognosis marker in CIS in our region, the objective of this study was to determine the sensitivity and specificity of kappa and lambda free light chains (KFLC and LFLC) in CSF.

Methods

Patients, materials and methods

This study was approved by the ethics committee of the Hospital Italiano de Buenos Aires, and all participants gave written informed consent.

Paired CSF and serum samples from patients, including CIS, MS and other neurological non-disease MS, were collected between 2015 and 2017 at the MS center of Buenos Aires (CEMBA) and fulfilled the following criteria: (1) a diagnosis of CIS suggestive of MS or relapsing-remitting multiple sclerosis (RRMS) according to available criteria [9, 10], including non-CIS/MS patients as a control group; (2) availability of CSF and serum samples; (3) no MS-specific treatment prior to sampling or corticosteroids; (4) MRI examination close to sampling; and (5) clinical follow-up of at least 3 years.

Controls consisted of individuals evaluated at the outpatient clinic, matching the following profile: (1) diagnosis of a neurological disease of non-inflammatory etiology (cranial/peripheral palsy, non-inflammatory neurological disease controls, headache or sensory disturbances and symptomatic controls); (2) availability of CSF and serum samples ; (3) all routine-diagnostic variables measured in CSF and serum within normal range; and (4) no immunomodulatory or immunosuppressive treatment prior to sampling.

Clinical assessment and follow-up

Recorded demographic and clinical data included age, gender, age at disease onset, time between the diagnosis of CIS and conversion to CDMS (upon second relapse or radiological conversion) [9, 10], and degree of disability as determined by the Expanded Disability Status Scale (EDSS). Subsequent to sampling and diagnosis, patients were followed by experienced neurologists during scheduled follow-up visits. Relapses were recorded over time according to the aforementioned definition, that is, at least one neurological symptom (re)appears or a previous symptom attributed to MS worsens for at least 24 h following a stable or improving neurological state during at least 30 days.

CSF and serum sampling and analyses at lumbar puncture

A total volume of 10 mL of CSF and 8 mL of peripheral blood were obtained from each patient. After routine diagnostic work-up, excess volumes of CSF/serum pairs were stored immediately at − 80 °C until further analyses. All samples were handled and stored according to international consensus guidelines, and sample analyses were performed by trained analysts blinded to clinical information.

Routine diagnostic work-up of CSF and serum were performed. KFLC and LFLC were measured in serum and CSF by immunoturbidimetry on a SPA PLUS analyzer (Freelite®, The Binding Site Group Ltd., Birmingham, UK).

MR assessment

For the MR analysis, patients had to have at least one brain MR within the first 30 days as of study entry. The MR was made with Siemens 1.5 T MR device with standardized image-taking techniques for patients with demyelinating diseases (proton density, conventional T2, FLAIR, T1 with and without IV contrast), and volumetric 3D sequence [11]. An analysis was made of each CIS patient's MR performed at study entry and annually during 3 years of follow-up. Brain volumes were measured on T1-weighted images using the cross-sectional version of the SIENA software, SIENAX, which forms a part of FSL [12]. SIENAX allows global measurements of normalized total brain volume (NTBV) as well as selective measurements of normalized cortical volume (NCV) and normalized white matter volume (NWMV) [13]. To avoid misclassification due to WM lesions, this was masked out and refilled with intensities matching the surrounding normal-appearing WM (NAWM) before each tissue-class segmentation analysis [14, 15]. For atrophy measurement, 3D T1-weighted images sequence (MPRAGE, 176 partitions; flip angle 15°, 1.2 mm slices, matrix size, 256 × 256, voxel size, 1 × 1 × 1 mm^3, repetition time (TR) 1900 ms; echo time (TE) 4.0 ms; inversion time (TI) 300 ms) was used. The images obtained and stored in standard storage format (DICOM) were subsequently included and processed in a completely automated way by SIENA and SIENAX software [12, 13]. For the analysis with this software, we incorporated the sagittal sequences of T1 without contrast and compared brain volume between the MR performed at the beginning of the disease with that performed after a period of time, detecting the percentage of brain volume loss in that period. We manually delineated and subsequently inpainted white matter (WM) lesions in the

native T1-weighted scans to prevent a possible confound of WM lesions on tissue segmentation [16].

All imaging analyses were performed by trained and experienced MRI technicians blinded to clinical data.

Statistical analyses

Statistical analyses were performed using Stata 10.1. Group differences were determined by either Chi-square test for categorical data or Mann–Whitney *U* test for continuous variables. Differences between more than two groups were defined by applying the Kruskal–Wallis test. We performed Receiver operating characteristic (ROC) curve analysis to determine the optimal sensitivity and specificity of the parameters investigated. FLC concentrations were compared in CIS-MS converters vs. CIS non-converters and expressed as median ± SD, and the correlation between FLC and PBVC was assessed by binary logistic and Cox regression analyses. Significance level was set at 5% ($p < 0.05$).

Results

The patient group ($n = 77$) consisted of 36 patients with CIS and 41 patients with RRMS. The control group ($n = 42$) consisted of 7 patients with cranial/peripheral palsy (non-inflammatory neurological disease controls) and 35 with headache complaints. The demographic and clinical data of both groups are shown in Table 1. The mean age of MS patients at the time of disease onset was 35 ± 6 years, and 65.5% of the patients were women. In CIS patients, the mean age at disease onset was 31 ± 4 years. Mean follow-up time of the CIS sample included in the analysis was 40 ± 4.3 months. No patient was lost during follow-up. Patients and controls were comparable regarding age and gender distribution (Table 1).

CSF samples were obtained at the time of diagnosis in all included patients. Regarding the FLC in CSF and serum in patients and controls, data are fully provided in Table 2.

FLC accuracy in the diagnosis of MS

Regarding the accuracy of FLC in the diagnosis, in the MS group ($n = 41$) CSF KFLCs were highly elevated (7.5 ± 3.2 mg/L) while CSF LFLCs were moderately elevated (0.4 ± 0.22 mg/L) when compared to K and LFLCs in controls with other neurological diseases (1.7 ± 2.3 mg/L and 0.2 ± 0.03 mg/L, respectively) (Table 2). A total of 93% of MS patients and 9.5% of the non-MS were OB positive, resulting in a diagnostic sensitivity and specificity of 93% and 90.4%. The optimal cut-off for KFLCs determined by ROC analysis was 7 mg/L, with a diagnostic sensitivity and specificity of 95% and 97%, respectively. The optimal cut-off for LFLCs was 0.7 mg/L, with a diagnostic sensitivity and specificity of 71% and 81%, respectively (Table 2; Fig. 1).

FLC and CIS prognosis

In the CIS group ($n = 36$) a median age of 31 ± 4 years was observed. The median follow-up time was 40 ± 4.3 months, during which 22 (61.1%) patients converted to MS during the follow-up (Table 3). The median concentration of FLCs at CIS diagnosis was slightly higher in CIS-converters compared to non-converters, but this did not reach statistical significance (KFLC 7 ± 5.3 mg/L vs. 5 ± 2.3 mg/L, $p = 0.11$; LFLC 0.7 ± 0.33 mg/L vs. 0.5 ± 0.23 mg/L, $p = 0.16$) (Tables 3, 4). However, a strong correlation was observed between the concentration of K and LFLCs at diagnosis and the change in PBVC during follow-up ($r = 0.72$ and $r = 0.65$, respectively). An inverse correlation between KFLC/LFLC ratio and the increase in PBVC was also found ($r = -0.54$) (Table 4).

Table 1 Baseline characteristics of included patients

	CIS, $N = 36$	RRMS, $N = 41$	Controls, $N = 42$
Female, *n* (%)	21 (60)	27 (65.5)	23 (55)
Mean age at LP ± SD (years)	32 ± 3	36 ± 2	36 ± 3
Mean age at disease onset ± SD (years)	31 ± 4	35 ± 6	–
Disease duration ± SD (years)	1.3 ± 1	3 ± 1.3	–
EDSS at LP ± SD	1 ± 0.5	1.5 ± 1	–
OB in CSF, *n* (%)	32(90)	38(93)	4 (9.5)
Mean follow-up time ± SD (months)	40 ± 4.3	32 ± 3.1	–
Total brain volume × 10^6 mm^3	1.60 ± 0.08	1.58 ± 0.09	–
Neocortical gray matter volume × 10^6 mm^3	0.67 ± 0.09	0.65 ± 0.06	–
Total gray matter volume × 10^6 mm^3	0.81 ± 0.1	0.83 ± 0.05	–
White matter volume × 10^6 mm^3	1.01 ± 0.04	0.97 ± 0.07	–

CIS clinically isolated syndrome, *RRMS* relapsing remitting multiple sclerosis, *LP* lumbar puncture, *EDSS* expanded disability status scale, *OB* oligoclonal bands

Table 2 FLC accuracy in the diagnosis of MS

	Controls, $N=42$	RRMS, $N=41$			p	
OB in CSF (%)	4 (9.5)	38 (93)			< 0.01	
CSF KFLC (mg/L)	1.7 ± 2.3	7.5 ± 3.2			< 0.01	
CSF LFLC (mg/L)	0.2 ± 0.03	0.4 ± 0.22			< 0.01	
Serum KFLC (mg/L)	10 ± 8	12 ± 6			0.17	
Serum LFLC (mg/L)	11 ± 6	12 ± 4			0.35	
	Sensitivity	Specificity	LR+	LR−	ROC	Cut-off
OB	93	90.4	10	1	0.91	–
KFLC CSF	95	97	12	0	0.95	7.1
LFLC CSF	71	81	5	3	0.82	0.7
KFLC + LFLC CSF	95	91	10	1	0.95	-

KFLC kappa free light chain (mg/L), *LFLC* lambda free light chain (mg/L), *OB* oligoclonal bands

Discussion

In our study, we found that KFLCs have a higher sensitivity and specificity for the diagnosis of MS than traditional OCB testing, and that FLC analysis has the additional advantage of being an automated immunoassay that is simple to perform and interpret. We also found that FLC concentrations at CIS diagnosis were not significantly higher in CIS-converters compared to CIS non-converters, but patients with higher CSF FLC concentrations tended to have increased brain atrophy during follow-up (Fig. 2).

Our findings are in line with previous studies performed in other regions. Presslauer et al. reported that OB detection has a sensitivity of 94% in the diagnosis of MS [8]. Regarding the KFLC index threshold line, the study showed a sensitivity of 97%. Based on the results, the authors concluded that FLC quantification determine intrathecal plasma cell activity with high sensitivity and specificity, outperforming IgG-index calculation, and is a fast, automated laboratory method that can be easily integrated into routine diagnostics [8]. Regarding prognosis, the Presslauer et al. study showed no association between elevated KFLC index and disease progression in 58 MS and CIS patients during the evaluation period [8]. Using an arbitrary cut-off, authors concluded that in the study the absolute value of an elevated KFLC index has no prognostic significance on MS or CIS disease course [8].

In another study, Vortman et al. assessed the prognostic value of FLC in OCB-positive patients with clinically isolated syndrome suggestive of MS and early MS [4]. Authors determined FLC kappa (KFLC) and lambda (LFLC) in CSF and serum and underwent 3T magnetic resonance imaging (MRI) at the baseline and follow-up (median time interval 2.2 years; IQR 1.0–3.7 years) to determine T2 lesion load (T2LL) and the percentage of brain volume change (PBVC) during follow-up [4]. They found that CSF FLC were significantly increased in CIS/MS compared to controls (all $p < 0.001$). They also found that a lower KFLC/LFLC CSF ratio was associated with CIS clinically definite multiple sclerosis (CDMS) conversion [hazard ratio (HR) 2.89; 95% confidence interval (CI) 1.17–7.14; $p < 0.05$] [4]. Authors did not identify correlations between FLC variables and T2 lesion load on MR or PBVC [4]. They concluded that the study confirms increased intrathecal synthesis of FLC in CIS/MS, supporting their diagnostic contribution, and that KFLC/LFLC CSF ratio appears to have a prognostic value in CIS beyond OB [4]. Recently, Puthenparampil et al. described that in RRMS patients, intrathecal KFLC or LFLC synthesis was present in respectively 66% and 43% of the cases and the K/L ratio was significantly higher compared to HC (17.0 ± 31.3 vs. 0.79 ± 0.20, $p < 0.001$), improving the

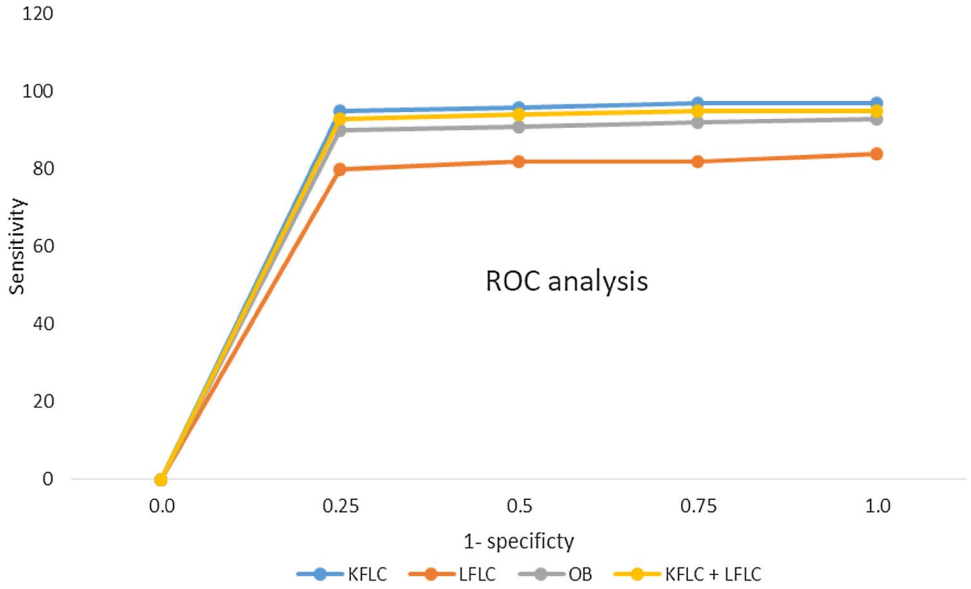

Fig. 1 ROC tab analysis of FLC in the diagnosis of MS. *KFLC* kappa free light chain, *LFLC* lambda free light chain/, *OB* oligoclonal bands

Table 3 FLC and CIS prognosis

	CIS without progression	CIS with progression	*p*
N	14	22	
Age	35 ± 5	36.1 ± 4	0.34
Female (%)	10 (71.4)	19 (86.3)	0.02
DMD (%)	12 (85)	20 (90)	0.25
OB (%)	12 (85)	22 (100)	0.01
CSF KFLC	5.3 ± 2.3	7 ± 5.3	0.11
CSF LFLC	0.55 ± 0.23	0.73 ± 0.33	0.16
Ratio K/L CSF	7 ± 1.2	5 ± 1.4	0.07
T2 lesion volume, mm3	353 ± 67	401 ± 165	0.12
Follow-up time (months)	38 ± 8	35 ± 7	0.19
PBVC at final analysis	− 1.2 ± 0.2%	− 1.7 ± 0.3%	0.03

Table 4 Logistic regression analysis of FLC and PBVC

	p	HR	IC 95%
Age	0.25	0.9	0.65–1.34
Female	0.11	1.1	0.76–1.45
DMD	0.18	0.85	0.68–1.13
OB	0.07	1.23	0.96–1.67
KFLC	0.13	1.12	0.89–1.23
Ratio K/L CSF	0.08	0.75	0.65–1.02
LFLC	0.22	1.01	0.67–1.28
T2 lesion volume, mm^3	0.02	1.56	1.23–1.98

KFLC kappa free light chain, *LFLC* lambda free light chain/, *OB* oligoclonal bands, *PBVC* percentage brain volume change

diagnostic usefulness of CSF examination in RRMS [17]. Authors found no association of KFLC and LFLC with any MRI parameter (cortical lesion number and volume, white matter lesion number and volume, gad + lesions, and cortical thickness) [17]. The diagnostic role of FLC was also evaluated in a pediatric population [18]. In a study that included 21 pediatric MS patients and 35 non-MS demyelinating or inflammatory neurological disorders, abnormally elevated levels of KFLC monomers and dimers in the CSF of MS patients showed a higher sensitivity (90.5%) and specificity (91.4%) for discrimination between MS and non-MS patients [18]. Finally, in a multicenter study, Presslauer et al. validated the diagnostic accuracy for MS of intrathecal KFLC synthesis. Diagnostic sensitivity of intrathecal KFLC synthesis, was 95% in patients with MS and 82% in patients with CIS. Specificity of intrathecal KFLC synthesis was 95% and 98% for all other measures providing strong support for the diagnostic value of intrathecal KFLC synthesis in CIS and MS patients and demonstrated a valid, easier and rater-independent alternative to OCB detection [19].

Our study has some limitations. First, the fact that all patients in our cohort only underwent a single lumbar

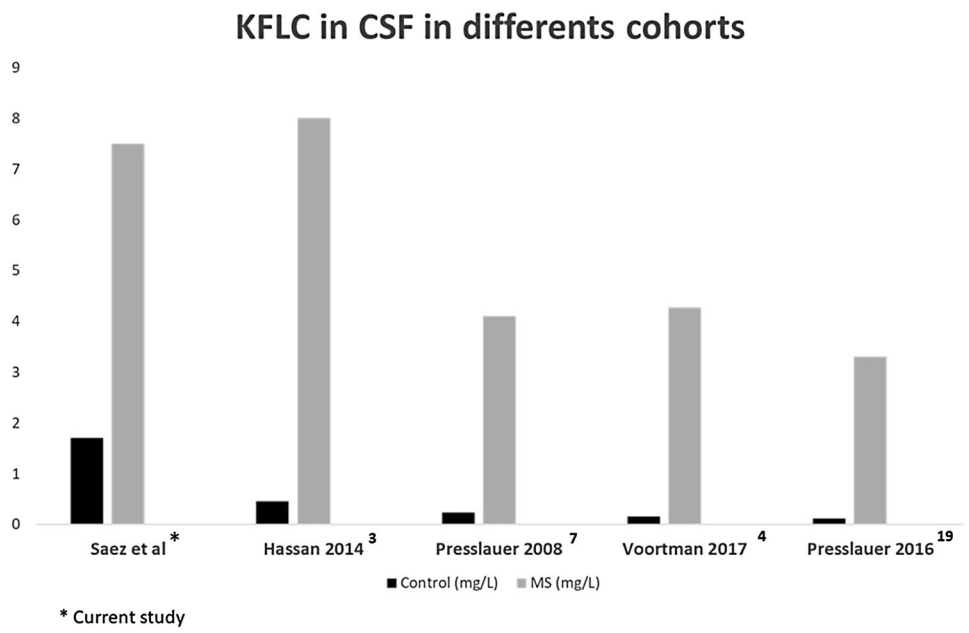

Fig. 2 KFLC in CSF in different cohorts reported. *KFLC* kappa free light chain

puncture; we, therefore, have no data on fluctuations of CSF FLC levels over time. Second, there is a modest number of patients included. And finally, it is worth mentioning that follow-up time could be increased to improve the prognosis role of FLC in CIS patients.

In conclusion, we identified that KFLCs have a higher sensitivity and specificity for the diagnosis of MS than traditional OCB testing, and that FLC analysis has the additional advantage of being an automated immunoassay that is simple to perform and interpret. We also found that higher CSF FLC concentrations in CIS patients tended to increased brain atrophy during follow-up. This is the first study performed in Latin American patients to evaluate the role of FLC in diagnosis and prognosis. Considering that the identification of OB in Latin America is difficult due to technical issues, a validation of the method in our region could provide a more specific and automated test to support the diagnosis and provide information regarding the prognosis. More research should be done to validate our findings and promote the use of FLC in our local medical practice.

Compliance with ethical standards

Conflicts of interest Authors declare no conflict of interest with the research performed.

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