

# Vloga indeksov prostih lahkih verig pri diagnozi multiple skleroze in njihova povezava s kliničnimi znaki

## Diagnostic relevance of free light chain indices and their relation to the clinical presentation of multiple sclerosis

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### Izvleček

**Namen:** Proste lahke verige (PLV) v cerebrospinalni tekočini (CST) predstavljajo obetajoč bioznačevalec za diagnozo multiple skleroze (MS), vendar je njihovo vključitev v diagnostična merila potrebno podpreti z dodatnimi študijami. Prav tako je slabo proučena njihova povezava s kliničnimi znaki ob prvem zagonu MS. Namen raziskave je bil opredeliti diagnostični pomen indeksov PLV in raziskati njihovo povezavo s funkcionalnimi sistemi (FS) pri novonastali MS.

**Metode:** V raziskavi je sodelovalo 71 bolnikov, ki so jim bili odvzeti parni vzorci CST in seruma, iz katerih so bili izračunani kapa in lambda indeksi PLV. Vključeni so bili bolniki z nevnetnimi nevrološkimi boleznimi (ne-MS, n

### Abstract

**Introduction:** Cerebrospinal fluid (CSF) free light chains (FLC) are of promising diagnostic importance in patients with multiple sclerosis (MS). However, research is required to confirm their utility as part of the diagnostic criteria for MS. In addition, very few publications can be found addressing the relationship of FLCs to the clinical presentation at the onset of MS. We aimed to evaluate the diagnostic performance of the FLC kappa and lambda indices, and explored their relationship to the number and type of functional systems (FS) involved. **Methods:** FLC indices were determined in paired CSF and serum samples from 71 patients with noninflammatory neurological diseases (non-MS, n = 29) and new-onset MS (MS, n = 42). The

= 29) in novonastalo MS (MS, n = 42). Slednji so bili glede na število začetnih simptomov razdeljeni na mono- in polisimptomatske. Prizadetost FS je bila opredeljena v skladu z razširjeno lestvico stopnje prizadetosti (angl. Expanded Disability Status Scale, EDSS).

**Rezultati:** Pri sočasnem upoštevanju obeh indeksov PLV, spola in starosti bolnikov je bila diagnostična natančnost večja (AUC 0,991, senzitivnost 95,5 %, specifičnost 95,8 %) kot pri posamezni uporabi lambda (AUC 0,677, senzitivnost 68,0 %, specifičnost 60 %) ali kapa (AUC 0,957, senzitivnost 88,5 %, specifičnost 88,0 %) indeksa. Med mono- in polisimptomatskimi bolniki ni bilo razlik v lambda ( $U = 78,0$ ,  $p = 0,749$ ) ali kapa ( $U = 820$ ,  $p = 0,885$ ) indeksih. Indeksa prav tako nista povezana z EDSS.

**Zaključek:** Indeksa PLV sta potencialno uporabna diagnostična bioznačevalca. Ne razlikujeta med mono- in polisimptomatskim začetkom bolezni. Prav tako nista povezana z oceno po EDSS lestvici.

latter were further divided into two groups with mono- and polysymptomatic presentation. FS involvement was evaluated in accordance with the Expanded Disability Status Scale (EDSS).

**Results:** Using both FLC indices along with the age and sex of patients, the diagnostic accuracy of MS was higher (AUC, 0.991; sensitivity, 95.5%; specificity, 95.8%) than that observed when using either the lambda index (AUC, 0.677; sensitivity, 68.0%; specificity, 60.0%) or kappa index (AUC, 0.957; sensitivity, 88.5%; specificity, 88.0%) alone. No differences were observed in the lambda ( $U = 78.0$ ;  $p = 0.749$ ) or kappa indices ( $U = 82.0$ ;  $p = 0.885$ ) between mono- and polysymptomatic patients. Nor was a correlation found between FLC indices and EDSS scores.

**Conclusion:** FLC indices could be useful diagnostic markers for MS. However, such indices do not differentiate between clinical presentations at disease onset and are not correlated with baseline EDSS scores.

## INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS), characterized by inflammation, demyelination, and neurodegeneration (1). Its diagnosis is based on the temporal and spatial dissemination of the disease by clinical, imaging, and laboratory evidence (2).

Ninety percent of patients initially present as a clinically isolated syndrome (CIS), lacking the requisite feature of dissemination in time (DIT), and thus, precluding the immediate diagnosis and treatment of MS (3). Not all patients convert to clinically definite MS (CDMS), and the increasingly strong focus on timely treatment has led to misdiagnoses and consequent treatment-related morbidity (4,5).

Magnetic resonance imaging (MRI) is not sufficiently sensitive to detect the earliest stages of the disease (6,7). Therefore, cerebrospinal fluid (CSF) oligoclonal bands (OCB) have been reintroduced into the newest (2017) iteration of the McDonald criteria as independent predictors of the conversion from CIS to

CDMS. In the appropriate clinical setting of a typical CIS and fulfilment of clinical or MRI criteria for DIStwo or more OCBs that are unique to CSF may be substituted in place of the requirement of DIT (3,8–10). However, the accepted method of OCB detection (agarose gel electrophoresis with isoelectric focusing and immunoblotting or immunofixation for IgG) is technically demanding, time-consuming, qualitative, and difficult to standardize. Even experienced analysts can have trouble identifying OCBs because of their low resolution and weak visualization. Thus, the results are ambiguous and subject to investigator bias.

Because the misinterpretation of a single OCB can make a difference between a positive and negative result, the need for alternative biomarkers has arisen (6,10–14).

Intrathecal free light chains (FLC) of the kappa (KFLC) and lambda (LFLC) isotypes produced alongside intact immunoglobulins have always been of great scientific interest in the field of neurology,

but their clinical utility, especially for MS diagnosis, has not been determined due to technical limitations.

Recent technological advances have led to the development of a sensitive, rapid, automated, and easy-to-standardize immunonephelometric method to quantify CSF FLCs; thus, paving the way for new diagnostic capabilities (10,15–17). Studies have revealed the comparable diagnostic and predictive performances of FLCs, and in particular KFLCs, compared to the established method of OCB detection. The potential of FLCs as MS biomarkers is further supported by the fact that they can be detected in OCB-negative MS patients (6,14–16,18–23). Nevertheless, their relationship to clinical signs at MS onset has yet to be determined.

The aim of this study was to assess the utility of FLCs on MS diagnosis, and explore whether FLCs, as markers of intrathecal inflammation, are indicative of baseline Expanded Disability Status Scale (EDSS) scores and functional system (FS) involvement.

## MATERIALS AND METHODS

### Patients

The research protocol was approved by the Medical Ethics Committee of the Maribor University Medical Centre in Slovenia (UKC-MB-KME-18-04/16).

The study was a retrospective analysis of demographic, clinical, and paraclinical data collected from the electronic medical records of 104 patients who underwent a diagnostic lumbar puncture from 2015 to 2018 in the Department of Neurology at the Maribor University Medical Centre. The collected pairs of CSF and serum were analysed in the hospital's Department of Laboratory diagnostics. Of the 104 patients, 66 were female (63.5%) and 38 were male (36.5%).

Table 1 shows the descriptive statistics of measurements and patient demographics.

**Table 1.** Descriptive statistics of measurements and patient demographics.

	Lambda index	Kapa index	EDSS	Age in years
N	79	80	42	104
Missing	25	24	62	0
Mean	47.4	77.5	1.86	42.2
Median	30.2	22.4	2.00	42.5
Standard deviation	48.6	138	1.41	15.7
Minimum	0.00	0.00	0.00	12
Maximum	220	918	6.00	84

Upon reviewing patients' electronic medical records, three diagnostic groups were established, including new-onset multiple sclerosis (MS, n = 28), clinically isolated syndrome (CIS, n = 14), and other neurological diseases (non-MS, n = 65). The MS group encompassed the following phenotypes: relapsing-remitting (n = 40) and secondary progressive (n = 2) MS. All patients were OCB positive. The data from patients diagnosed with CIS were reassessed in accordance with the updated McDonald criteria, and resulted in the allocation of 12 patients to the relapsing-remitting MS group.

Due to possible intrathecal plasma cell activity and inflammatory changes in the blood-CSF barrier, the remaining two patients with CIS were excluded with 36 patients from the non-MS group with the following diagnoses: neuromyelitis optica, neuroborreliosis, neurosarcoidosis, myelitis, meningitis, encephalitis, inflammatory demyelinating polyneuropathy, Guillain-Barre syndrome, Miller-Fisher syndrome, stiff-person syndrome, GAD antibody-associated cerebellar ataxia, and facial nerve palsy.

The remaining 29 patients with noninflammatory neurological conditions were selected as the control group. The diagnoses included primary headache disorders (n = 6), post-dural-puncture headache (n = 1), microvascular leukoencephalopathy (n = 5), motor

neuron disease (n = 3), somatization disorder (n = 1), dissociative disorder (n = 1), acute vertigo (n = 2), brain tumor (n = 1), epilepsy (n = 1), fibromyalgia (n = 1), ischaemic stroke (n = 2), essential tremor (n = 1), myelopathy (n = 3), and atypical neck pain (n = 1).

The aforementioned changes resulted in the formation of two clinically homogenous patient groups that were the easiest to compare: non-MS (n = 29) and MS (n = 42). Based on the number of symptoms presenting at disease onset, the latter group was further divided into mono- (n = 12) and polysymptomatic (n = 27) subgroups.

### Sample collection and storage

Paired CSF and peripheral blood samples were obtained within a 2-hour period. CSF was collected through a lumbar puncture at the L3/L4 or L4/L5 interspinous level into clear conical screw cap tubes. Haemorrhagic, haemolytic, and turbid samples were excluded from the analysis. Peripheral blood was collected through venepuncture of the cubital vein into red cap tubes containing clot activator. Haemolytic samples were excluded from the analysis.

The inclusion criterion for sample analysis was a minimum volume of 1 mL each of CSF and serum. After centrifugation, samples were either analysed or stored at -25°C for a maximum of 6 months. Upon thawing, samples were used only once.

### Albumin determination

Albumin in CSF and serum was measured by nephelometry (Siemens BN ProSpec®) within 7 days of sample collection. The results were interpreted according to the manufacturer's instructions.

### KFLC and LFLC determination

KFLC and LFLC in CSF and serum were measured by nephelometry (Siemens N Latex FLC kappa and lambda assays on the Siemens BN ProSpec®) using monoclonal antibodies. The analysis was performed within 6 months of sample collection and the results were interpreted according to the manufacturer's instructions.

### Clinical disability and functional system involvement

The degree of disability was calculated in accordance with the Expanded Disability Status Scale (EDSS),

which is based on a rating of seven functional systems (FS), including visual, brainstem, pyramidal, cerebellar, cerebral, sensory, and bowel and bladder functions. The number and type of FSs involved at disease onset were gathered from patient records. If the presenting symptoms were characteristic of a single FS, patients were considered monosymptomatic, whereas patients with symptoms indicative of multiple FS were deemed polysymptomatic.

## Theory and calculations

### KFLC and LFLC index calculations

To accurately assess the intrathecal concentration of FLCs, two factors were considered:

a. The physiological passive diffusion of small amounts of FLCs from the serum into the CSF. Passive transfer of FLCs was evaluated by applying CSF/serum FLC quotients.

b. The influence of potential blood-CSF barrier disruption. Blood-CSF barrier permeability was evaluated by applying albumin CSF/serum quotients. Albumin is exclusively synthesised in the liver and passively diffuses into the CSF when the barrier is compromised.

As a result, kappa and lambda indices were defined as the quotients of KFLC and LFLC concentrations in CSF and serum, divided by the respective albumin concentrations in CSF and serum (24,25).

$$\text{FLC index} = \frac{\frac{\text{FLC (CSF)}}{\text{FLC (serum)}}}{\frac{\text{Albumin (CSF)}}{\text{Albumin (serum)}}} = \frac{\text{Q(FLC)}}{\text{Q(albumin)}}$$

### Statistical analysis

Our research objective was to establish whether (1) there was a relationship between kappa and lambda FLC indices; (2) there were differences in FLC indices between the MS and non-MS groups; (3) controlling for age and sex was required for determining such differences; (4) there were differences in FLC indices between mono- and polysymptomatic patients; (5) there were differences in FLC indices between FSs; and (6) there was a correlation between FLC indices

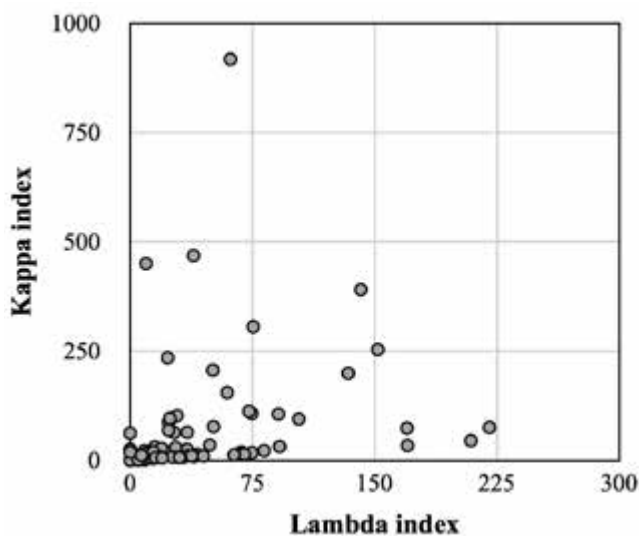
and the baseline EDSS score.

The statistical methods included the Shapiro-Wilk test to determine if the data followed a normal distribution. The Mann-Whitney U test was used to compare two independent groups of numerical measurements. Binomial multiple logistic regression was used to determine the differences between groups, while controlling for specific variables. The Spearman's correlation coefficient was used to test the relationship between measurements. The significance level  $\alpha$  (p-value cut-off) used in all statistical tests was set to 0.05.

## RESULTS

### Relationship between FLC indices

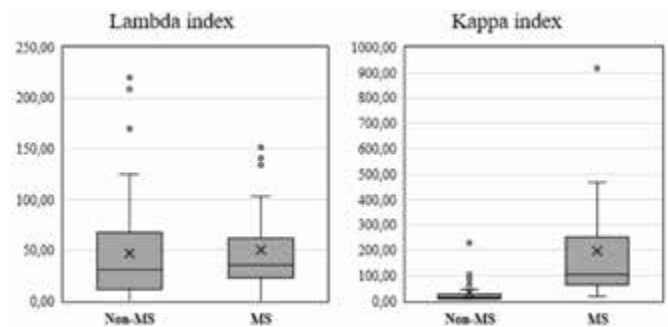
First, an analysis of the relationship between kappa and lambda indices was conducted. The Shapiro-Wilk test of normality determined that the assumption of a normal distribution of either measurement was not met ( $p < 0.001$  for both). Therefore, the Spearman's correlation coefficient was used to assess the relationship between the two variables, revealing a positive and moderate relationship ( $\rho = 0.472$ ;  $p < 0.001$ ; Figure 1). As this could have been detrimental to further analyses, the variables were centered to reduce the chances of multicollinearity.



**Figure 1.** Relationship between kappa and lambda indices.

### Differences in FLC indices between MS and non-MS groups

Next, the possible difference in kappa and lambda indices between MS and non-MS patients was evaluated. Again, the Shapiro-Wilk test of normality showed that the measurements of FLC indices for both diagnostic groups did not adhere to the normal distribution ( $p < 0.05$  in all cases). As such a finding violated one of the assumptions of parametric testing, the non-parametric Mann-Whitney U test was used to determine the difference between the two independent groups. The Mann-Whitney U test showed that there was no statistically significant difference in the lambda index between MS and non-MS patients ( $U = 302.0$ ;  $p = 0.848$ ). Conversely, MS and non-MS patients differed significantly in their kappa indexes ( $U = 30.0$ ;  $p < 0.001$ ). Figure 2 compares boxplots of the lambda and kappa indices between the two patient groups.



**Figure 2.** Comparison of lambda (left) and kappa (right) indices between MS and non-MS patients.

### Controlling for the age and sex of patients

Further experiments were conducted while controlling for the age and sex of patients.

### Combination of kappa index, age, and sex

Binomial multiple logistic regression was used to predict the presence or absence of MS, which were dependent variables. The kappa index, age, and sex of patients were independent variables. The backwards step-wise approach was used to gradually eliminate variables from the model until the Akaike information criterion (AIC), used to evaluate out-of-sample prediction error of our statistical model, was reduced. In addition, the predictors were centered to reduce the collinearity between them. The logistic

regression model was built with the three predictors mentioned previously and was statistically significant in determining the presence or absence of MS ( $\chi^2 = 37.0$ ;  $p < 0.001$ ). The model explained 52.4% of the variance in the presence of MS (McFadden's  $R^2 = 0.524$ ). The predictive ability of the model in regards to determining the presence or absence of MS was also measured. The overall classification accuracy was 88.2%, specificity was 88.5%, sensitivity was 88.0%, and the AUC was 95.7% when considering the optimal 0.67 dividing point of logistic regression between the two groups of dependent variables (non-MS and MS).

**Combination of lambda index, age, and sex**

The model considering only the lambda index, age, and sex of patients performed comparatively worse than the previously-described model. Using the backwards step-wise approach, sex was removed from the model. The resulting combination of the lambda index and age was statistically significant ( $\chi^2 = 6.51$ ;  $p = 0.039$ ). This model explained only 9.39% of the variance of the dependent variables (McFadden's  $R^2$ ). The classification metrics were as follows: classification accuracy of 64.0%, specificity of 68.0%, sensitivity of 60.0%, and AUC of 67.7% with a cut-off of 0.5 as the dividing point of logistic

regression between the two groups of dependent variables (non-MS and MS).

**Combination of both indices, age, and sex**

The inclusion of both FLC indices, age, and sex of the patients resulted in a statistically significant logistic regression model ( $\chi^2 = 53.4$ ;  $p < 0.001$ ). The model explained 83.9% of the variance in the presence of MS (McFadden's  $R^2 = 0.839$ ) and correctly classified 93.5% of patients. The sensitivity of the model was 95.5%, specificity was 95.8%, and the AUC was 99.1% using a cut-off of 0.7 as the dividing point of logistic regression between the two groups of dependent variables (non-MS and MS).

In summary, the best performing model included both FLC indices, age, and sex of the patients. It displayed the highest quality in terms of the predicted variance, and all four classification metrics. Thus, we used this model as the final model. Out of the four predictors of MS, only lambda index was statistically significant ( $p = 0.038$ ). Thus, the higher the lambda index, the higher the probability of the patient having MS. Table 2 shows the estimates of the predictors. Age, sex, and kappa index were not of statistical significance. As the starting model (combining both indices with age and sex) resulted in the lowest AIC, none of the predictors were removed in the final model.

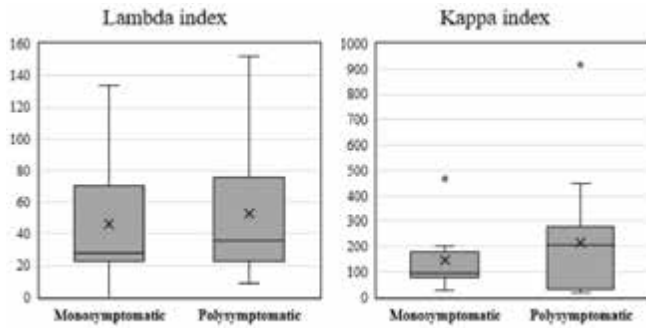
**Table 2.** Coefficients of binomial multivariate logistic regression.

						95% Confidence Interval	
Predictor	Estimate	SE	Z	p	Odds ratio	Lower	Upper
Intercept	-167.582	97.478	-1.719	0.086	5.27e-8	2.66e-16	10.46
Age (centered)	-0.0563	0.0823	-0.684	0.494	0.945	0.80442	1.11
Sex (Female-Male)	-13.057	20.259	-0.645	0.519	0.271	0.00511	14.37
Kappa index (centered)	0.3555	0.1947	-1.826	0.068	0.701	0.47847	1.03
Lambda index (centered)	0.1312	0.0634	2.071	0.038	1.140	100.706	1.29

**Differences in FLC indices between mono- and polysymptomatic patients**

Within the MS group, differences between the FLC indices between mono- and polysymptomatic patients were assessed. The Shapiro-Wilk test of normality revealed that none of the groups conformed to a

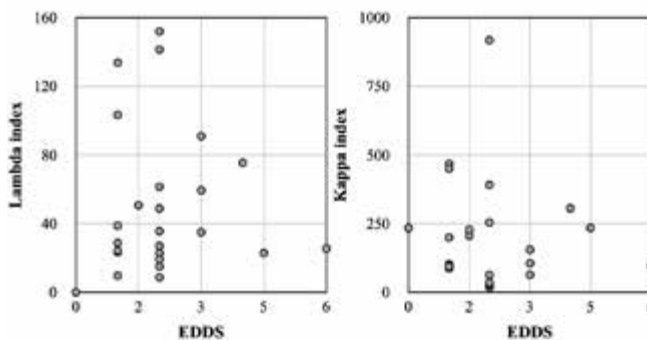
normal distribution ( $p < 0.05$  in all cases). The Mann-Whitney U test showed that there was no statistically significant difference in the lambda ( $U = 78.0$ ;  $p = 0.749$ ) or kappa ( $U = 82.0$ ;  $p = 0.885$ ) indices between mono- and polysymptomatic patients (Figure 3).



**Figure 3.** Comparison of lambda (left) and kappa (right) indices between mono- and polysymptomatic patients.

**Relationship between FLC indices and EDSS**

The relationship between the two FLC indices and EDSS score was evaluated within the MS group. The Shapiro-Wilk test found a statistically significant difference between all measurements and the normal distribution. Therefore, the Spearman’s correlation coefficient was used to explore the relationship between EDSS and both indices (Figure 4), and revealed no statistically significant correlations between EDSS and the lambda ( $\rho = 0.138$ ;  $p = 0.530$ ) or kappa ( $\rho = -0.121$ ;  $p = 0.582$ ) indices.



**Figure 4.** Relationship between EDSS and lambda index (left) and EDSS and kappa index (right).

**Relationship between FLC indices and FS**

The existence of a possible relationship between the lambda or kappa indices and the type of FSs involved at the onset of MS was also evaluated. MS patients were split into six groups, based on the type of FS affected. None of the patient subgroups conformed to the normal distribution. Consequently, the Mann-Whitney U test was used to test the differences between the types of FSs involved at the onset of MS and kappa and lambda indices (Table 3).

**Table 3.** Results of the Mann-Whitney U test assessing the relationship between FLC indices and FS.

Independent measurements	Dependent measurements	U	p
Pyramidal FS	Lambda index	73.0	0.705
	Kappa index	75.0	0.823
Cerebellar FS	Lambda index	63.0	0.515
	Kappa index	83.0	0.923
Brainstem FS	Lambda index	84.0	0.865
	Kappa index	83.0	0.568
Sensory FS	Lambda index	59.0	-0.275
	Kappa index	81.0	0.689
Bowel and bladder FS	Lambda index	11.0	0.239
	Kappa index	22.0	0.762
Visual FS	Lambda index	40.0	0.718
	Kappa index	38.0	0.547

Thus, the results from Table 3 show that there were no statistically significant differences between any of the six FSs in either of the indices ( $p > 0.05$  in all comparisons).

**DISCUSSION**

In recent years, research on FLC indices has predominantly focused on validating the diagnostic role of the kappa index. Our results on its sensitivity (88.5%) and specificity (88.0%) are consistent with several other studies that reported sensitivities from 80 to 96% and specificities from 77 to 100% (9,11,16,25–27).

The lambda index sensitivity (60.0%) and specificity (68.0%) were inferior to those reported by Passerini et al. (82.1% and 75.0%, respectively). However, in the current study, as well as that of Passerini et al., the kappa index outperformed the lambda index (9).

The binomial multivariate logistic regression model designed in this study unexpectedly revealed the relevance of the lambda index in predicting MS. When considering both FLC indices, age, and sex of patients, the diagnostic accuracy was higher than that observed when incorporating only the kappa or lambda indices. The model exhibited statistical significance in predicting both the presence and absence of MS ( $\chi^2 =$

53.4;  $p < 0.001$ ). As was evident from the estimates, only the lambda index had a statistically significant ability to predict MS ( $p = 0.038$ ). Thus, the higher the lambda index, the higher the probability of a patient having MS. Surprisingly, the kappa index displayed no such characteristic ( $p=0.068$ ), which may be attributable to the moderate correlation between the two indices, as shown by the Spearman's correlation coefficient ( $\rho = 0.472$ ;  $p < 0.001$ ).

Despite the statistically insignificant effect of the kappa index, age, and sex, removing any of the predictors from the model resulted in a decrease of AIC values, the percentage of the explained variance of the dependent variables, as well as all the classification metrics (sensitivity, specificity, accuracy and AUC). Models using individual indices performed inferiorly compared to the model combining both indices, which may hint to an existence of latent but statistically significant variables, the effects of which were not measured by our experiment. Nevertheless, the resulting logistic model should be used as it is until other possible measurements are evaluated and can be included. Thus, further research on variables not yet identified, and the inclusion of more demographic data is necessary.

The second half of our analysis focused on evaluating the relationship between FLC indices and clinical parameters. Multiple studies have suggested that FLCs, especially KFLCs, play a role in predicting a more severe disease evolution in terms of disability progression as determined by the EDSS. Makshakov et al. found a significant correlation between the concentration of KFLC and the level of EDSS progression after two years in patients with CIS who converted to MS within two years after their first relapse (19).

Rudick et al. also demonstrated a relationship between KFLC values and increased risk of progression, as defined by the EDSS (28).

The study of Rinker et. al. indicated that KFLC predicted a high likelihood of requiring ambulatory assistance (unilateral, bilateral, and wheelchair) within ten years and over the disease course. Similarly, KFLC predicted greater disability in terms of EDSS relative to disease duration, as estimated by the Multiple Sclerosis Severity Score (MSSS) (29).

Since EDSS data were limited and the prognostic value of FLCs could not be assessed, we explored whether FLC indices correlated with the baseline disability level of patients. No correlation between FLC indices and baseline EDSS scores was found. This may have been due to an insufficient number of patients analysed, as well as the inclusion of new-onset MS presenting with minimal disability (MedianEDSS = 2). A longitudinal follow-up including more advanced stages of the disease could uncover significant correlations between FLC indices and EDSS scores. Comparable findings were demonstrated by another study that explored the relationship between FLCs and EDSS score (30). However, the analysis had the same study limitations as ours, which could explain the similarity between our results.

We also entertained the idea of a possible connection between FLC indices and the baseline disease presentation in terms of FS involvement. To our knowledge, this has not been addressed before. We did not find a correlation between FLC indices and mono-/polysymptomatic presentation, or the involvement of any type of FS (visual, brainstem, pyramidal, cerebellar, sensory, bowel and bladder system).

The limitations of our study included the following. The study was conducted on a small sample of Caucasian patients from a single medical centre. Other neurological inflammatory diseases were excluded as a relevant control group for the diagnosis of MS. Thus, the inclusion of a larger number of patients and a more heterogenous control group is necessary to achieve more representative results.

## CONCLUSIONS

The main advantage of our study was its holistic approach that used a model combining multiple variables, and thus, resembled a more realistic and natural state, as opposed to analysing variables in isolation.

When considered on its own, the lambda index was of limited diagnostic importance. However, evaluation of the kappa index, age, and sex of the patient presented the most significant prediction model for MS.



Our results suggested that the proposed model, if validated in a larger sample of patients, could be a beneficial addition to the diagnostic work-up of patients with suspected MS displaying an elevated lambda index. Future studies should focus on the

integration of more variables and demographic data. To establish the prognostic value of FLC indices, extending the follow-up time and including the later stages of MS is necessary.

## REFERENCES

- Sospedra M, Martin R. Immunology of multiple sclerosis. *Semin Neurol*. 2016;36:115–27.
- Kaplan B, Ganelin-Cohen E, Golderman S, Livneh A. Diagnostic utility of kappa free light chains in multiple sclerosis. *Expert Rev Mol Diagn*. 2019;19.
- Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. 2018;17:162–73.
- Kaisey M, Solomon AJ, Luu M, Giesser BS, Sicotte NL. Incidence of multiple sclerosis misdiagnosis in referrals to two academic centers. *Mult Scler Relat Disord*. 2019;30:51–6.
- Solomon AJ, Bourdette DN, Cross AH, Applebee A, Skidd PM, Howard DB et al. The contemporary spectrum of multiple sclerosis misdiagnosis. *Neurology*. 2016;87(13):1393–9.
- Vasilj M, Basic V, Vrkic N, Vukasovic I. Relevance of KFLC quantification to differentiate clinically isolated syndrome from multiple sclerosis at clinical onset. *Clin Neurol Neurosurg*. 2018;174:220–9.
- Villar LM, Espiño M, Costa-frossard L, Muriel A, Jiménez J, Álvarez-cermeño JC. High levels of cerebrospinal fluid free kappa chains predict conversion to multiple sclerosis. *Clin Chim Acta*. 2012;413(23–24):1813–6.
- Reiber H, Zeman D, Hradl P, Zapletalova O, Kurkova B, Masjuan J et al. Kappa free light chains in cerebrospinal fluid as markers of intrathecal immunoglobulin synthesis. *PLoS One*. 2018;25(4):576–8.
- Passerini G, Costa GD, Sangalli F, Moiola L, Colombo B, Locatelli M et al. Free light chains and intrathecal B cells activity in multiple sclerosis: A prospective study and meta-analysis. *Mult Scler Int*. 2016;1–9.
- Jeli N, Šolji V. Kappa free light chains in cerebrospinal fluid of patients with identified oligoclonal immunoglobulin G. *Psychiatr Danub*. 2017;29:124–8.
- Valencia-Vera E, Garcia-Ripoll Martinez-Escribano A, Enguix A, Abalos-Garcia C, Segovia-Cuevas MJ. Application of  $\kappa$  free light chains in cerebrospinal fluid as a biomarker in multiple sclerosis diagnosis: development of a diagnosis algorithm. *Clin Chem Lab Med*. 2017;56(4):609–13.
- Suesse M, Hannich M, Petersmann A, Zylla S, Pietzner M, Nauck M et al. Kappa free light chains in cerebrospinal fluid to identify patients with oligoclonal bands. *Eur J Neurol*. 2018;25(9):1134–9.
- Rathbone E, Durant L, Kinsella J, Parker AR, Hassan-Smith G, Douglas MR et al. Cerebrospinal fluid immunoglobulin light chain ratios predict disease progression in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2018;89(10):1044–9.
- Presslauer S, Milosavljevic D, Brücke T, Bayer P, Hübl W. Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis. *J Neurol*. 2008;255(10):1508–14.
- Presslauer S, Milosavljevic D, Huebl W, Abouelenin-Djamshidian F, Krugluger W, Deisenhammer F et al. Validation of kappa free light chains as a diagnostic biomarker in multiple sclerosis and clinically isolated syndrome: A multicenter study. *Mult Scler J*. 2015;1–9.
- Puthenparampil M, Altinier S, Stropparo E, Zywicki S, Poggiali D, Cazzola C et al. Intrathecal K free light chain synthesis in multiple sclerosis at clinical onset associates with local IgG produc-

- tion and improves the diagnostic value of cerebrospinal fluid examination. *Mult Scler Relat Disord*. 2018;25:241–5.
17. Basile U, Gulli F, Gragnani L, Napodano C, Pocino K, Rapaccini GL et al. Free light chains: Eclectic multipurpose biomarker. *J Immunol Methods*. 2017;451:11–9.
  18. Presslauer S, Milosavljevic D, Huebl W, Parigger S, Schneider-Koch G, Bruecke T. Kappa free light chains: Diagnostic and prognostic relevance in MS and CIS. *PLoS One*. 2014;9(2):e89945.
  19. Makshakov G, Nazarov V, Kochetova O, Surkova E, Lapin S, Evdoshenko E. Diagnostic and prognostic value of the cerebrospinal fluid concentration of immunoglobulin free light chains in clinically isolated syndrome with conversion to multiple sclerosis. *PLoS One*. 2015;10(11):e0143375.
  20. Krakauer M, Schaldemose Nielsen H, Jensen J, Sellebjerg F. Intrathecal synthesis of free immunoglobulin light chains in multiple sclerosis. *Acta Neurol Scand*. 1998;98(3):161–5.
  21. Senel M, Tuman H, Lauda F, Presslauer S, Mojib-Yezdani R, Otto M et al. Cerebrospinal fluid immunoglobulin kappa light chain in clinically isolated syndrome and multiple sclerosis. *PLoS One*. 2014;9(4):e88680.
  22. Menéndez-Valladares P, García-Sánchez MI, Martínez MA, García De Veas Silva JL, Bermudo Guitarte C, Izquierdo Ayuso G. Validation and meta-analysis of kappa index biomarker in multiple sclerosis diagnosis. *Autoimmun Rev*. 2019;18(1):43–9.
  23. Hassan-Smith G, Durant L, Tsentemidou A, Assi LK, Faint JM, Kalra S et al. High sensitivity and specificity of elevated cerebrospinal fluid kappa free light chains in suspected multiple sclerosis. *J Neuroimmunol*. 2014;276(1–2):175–9.
  24. Kroksveen AC, Opsahl JA, Gulbrandsen A, Oveland E, Torkildsen Ø Berven FS. Cerebrospinal fluid proteomics in multiple sclerosis. *Biochim Biophys Acta*. 2014;1854(7):746–56.
  25. Duranti F, Pieri M, Centonze D, Buttari F, Bernardini S, Dessi M. Determination of kFLC and K Index in cerebrospinal fluid: A valid alternative to assess intrathecal immunoglobulin synthesis. *J Neuroimmunol*. 2013;263(1–2):116–20.
  26. Ramsden D. Multiple sclerosis: assay of free immunoglobulin light chains. *Ann Clin Biochem*. 2017;54(1):5–13.
  27. Zeman D, Hradl P, Zapletalova O, Kurkova B. Assessment of intrathecal free light chain synthesis: Comparison of different quantitative methods with the detection of oligoclonal free light chains by isoelectric focusing and affinity-mediated immunoblotting. *PLoS One*. 2016;11(11):e0166556.
  28. Rudick R, Medendorp S, Namey M, Boyle S, Fischer J. Multiple sclerosis progression in a natural history study: Predictive value of cerebrospinal fluid free kappa light chains. *Mult Scler*. 1995;1:150–5.
  29. Rinker JR, Trinkaus K, Cross AH. Elevated CSF free kappa light chains correlate with disability prognosis in multiple sclerosis. *Neurology*. 2006;67(7):1288–90.
  30. Voortman MM, Stojakovic T, Pirpamer L, Jehna M, Langkammer C, Scharnagl H et al. Prognostic value of free light chains lambda and kappa in early multiple sclerosis. *Mult Scler*. 2017;23(11):1496–505.