Original Article

Multiple sclerosis diagnosis: free light chain testing as a possible valuable marker in cerebrospinal fluid

Diagnosi di sclerosi multipla: il contributo del test delle catene leggere libere nel liquido cefalorachidiano come valido marcatore

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Key words: multiple sclerosis, diagnostic performance, FLC indices, oligoclonal bands.

ABSTRACT

Background: the aim of the study was to assess the importance of free light-chain analysis in the Cerebrospinal Fluid (CSF) for the diagnosis of Multiple Sclerosis (MS) in patients visited at the General Hospital of Alessandria.
Materials and Methods: detecting κFLC and λFLC levels in CSF and serum using Freelite tests on Optilite analyser (The Binding Site) and Isoelectric Focusing (IEF) on Hydrasis 2 analyser (Sebia). Statistical analysis was conducted by GraphPad statistical software and Excel statistical tools. Calculation of p-value by Kruskal-Wallis test and χ^2 test. Evaluation of the Free Light Chain (FLC) indices' performance through the construction of a Receiver Operating Characteristics (ROC) curve.
Results: 132 CSF and serum samples were collected, and 25% of the patients were diagnosed with MS. Through the ROC analysis, the optimal cut-off value was found to be 6,56 for the K index, 10,12 for the λ index, and 0,65 for the IgG index. FLC indices were higher in MS patients than in others.
Conclusions: the study shows that the K index has an equivalent reliability and a higher predictive value, but with lower costs and times than Oligoclonal Bands (OCB). The λ index could have the potential to become a further test used for differential diagnosis of MS, but more in-depth studies via more sensitive techniques are needed to evaluate real performance.

Background: il presente studio è finalizzato alla valutazione dell'importanza dell'analisi delle catene leggere libere nel Liquido Cefalorachidiano (LCR) per la diagnosi di sclerosi multipla nei pazienti visitati presso l'Azienda Ospedaliera Universitaria di Alessandria SS. Antonio e Biagio e C. Arrigo.
Materiali e Metodi: rilevamento dei livelli di κFLC e λFLC nel LCR e nel siero utilizzando i test Freelite sull'analizzatore turbidimetrico Optilite (*The Binding Site*) e l'Isoelettrofocalizzazione (IEF) sull'analizzatore Hydrasis 2 (Sebia). L'analisi statistica è stata condotta tramite l'utilizzo del software statistico GraphPad e del pacchetto statistico di Microsoft Excel. Per il calcolo del p-value sono stati utilizzati il test di Kruskal-Wallis e il test del χ^2. La valutazione delle prestazioni degli indici FLC è stata effettuata tramite la costruzione di una curva Receiver Operating *Characteristic* (ROC).
Risultati: sono stati raccolti 132 campioni di LCR e siero e il 25% dei pazienti è stato diagnosticato con sclerosi multipla. Attraverso l'analisi ROC, il valore soglia ottimale è stato trovato essere 6,56 per l'indice K, 10,12 per l'indice λ e 0,65 per l'indice Immunoglobuline G (IgG). Gli indici FLC erano più alti nei pazienti con sclerosi multipla rispetto agli altri.
Conclusioni: lo studio mostra come l'indice K abbia un'affidabilità equivalente e un valore predittivo superiore, ma con costi e tempi inferiori rispetto alle Bande Oligoclonali (OCB). L'indice λ potrebbe avere il potenziale per diventare un ulteriore test utilizzato per la diagnosi differenziale di sclerosi multipla, ma sono necessari studi più approfonditi attraverso tecniche più sensibili per valutarne le reali prestazioni.

Introduction

Multiple Sclerosis (MS) is a chronic inflammatory autoimmune disease that affects the white matter of the Central Nervous System (CNS), causing the progressive loss of myelin sheaths of axons and the formation of hardened lesions (plaques) within the white matter of the spinal cord and the brain.[1]

Like all autoimmune diseases, MS also has a multifactorial etiology, but the triggering cause is, to date, still unknown.[2] Therefore, it's clear the importance of an early diagnosis that allows to act on the progression of the disease and its symptoms.

In this context, spatial and temporal dissemination are key concepts used for differential diagnosis of MS. Dissemination in Space (DIS) refers to the presence of characteristic MS lesions in at least two distinct areas of CNS. Dissemination in Time (DIT) indicates the appearance of new lesions or symptoms over time.[3]

To improve the diagnostic process, a set of guidelines, known as the "McDonald diagnostic criteria", has been published and is periodically updated.[4] The diagnosis is based on four elements: symptoms reported by the patient, neurological examination (evoked potentials), instrumental analysis (magnetic resonance), and biological analysis (blood and cerebrospinal fluid).[3]

This disease is characterized by an aberrant immune reaction due to the presence of self-reactive T lymphocytes that recognize the proteins of the myelin sheath as not-self. B cells also participate in this reaction: they are activated in plasma cells and release antibodies directed against myelin proteins.[5] The synthesis of these antibodies, especially IgG type, within the CNS can be evaluated through the research of Oligoclonal Bands (OCB) IgG by Isoelectric Focusing (IEF), the current gold standard in laboratory diagnostics, or through the recently introduced Free Light Chain (FLC) indices (K index, λ index and IgG index).[6]

The purpose of this study is to assess the importance of FLC analysis in CSF for the diagnosis of MS. Therefore, on the samples taken into consideration, it was decided to carry out: i) an assessment of how much the current cut-off relative to the liquoral FLC indices is predictive through the construction of a Receiver Operating Characteristic (ROC) curve in order to determine the change of sensitivity and specificity with varying threshold values; ii) an analysis of patients with positive K index (≥5) and negative or doubtful OCB pattern for intrathecal synthesis; iii) an analysis of patients with Neuroinflammatory Diseases (IND) with negative K index (<5) and positive λ index (≥3,5); iv) an analysis of FLC and OCB indices in patients under 18 years old with neurological disorders.

Materials and Methods

For this study, 132 samples of CSF and serum were collected at the Complex Unit (C.U.) Analysis Laboratory of the General Hospital of Alessandria SS. Antonio and Biagio e C. Arrigo from 22-03-2019 to 31-12-2022, on which the research of oligoclonal bands by IEF was carried out.

A table has been constructed (Table 1) containing the following data: concentrations of albumin, IgG, IgM, κFLC, and λFLC, measured in CSF and serum by turbidimetric quantitative analysis; Link index (also known as IgG index), K index and λ index, where it has been possible to determine them; profile resulting from the research of oligoclonal bands by IEF on agarose gel, followed by immunodetection with anti-IgG antibodies; fulfillment of the McDonald's criteria; clinical data on suspected incoming diagnostics, final diagnosis, any updates after discharge.

Clinical information has allowed patients to be divided into five subpopulations according to the type of neurological pathology diagnosed at discharge: MS, Non-Inflammatory Neurological Disorders (NIND), Inflammatory Neurological Disorders (IND), Peripheral Inflammatory Neurological Disorders (PIND), Symptomatic Controls (SC).[7] The characteristics of each of these groups were examined and, for each one, the median and the interquartile deviation were calculated.

The levels of κFLC and λFLC in liquor and serum were determined using Freelite tests on the Optilite turbidimeter analyser (The Binding Site Group, Birmingham, UK). The dosages were inserted in a special worksheet for the calculation of the serum dilution in order to make it comparable to the associated CSF. Later, IEF was

Table 1. Demographics and laboratory characteristics of patients of the studied population.

Characteristics	MS (n=33)	IND (n=33)	PIND (n=25)	NIND (n=28)	SC (n=13)	p-value
Sex (M/F)	11/22	18/15	20/5	18/10	4/9	ns
Age (years), median (IQR)	38,0 (26,0-50,22)	42,0 (21,0-59,02)	52,0 (31,0-60,0)	52,5 (47,75-69,0)	55,0 (45,0-69,4)	0,01
Positive OCB, number (%)	32 (97 %)	5 (15 %)	0 (0 %)	2 (7 %)	3 (23 %)	<0,0001
Serum κFLC (mg/dL), median (IQR)	1,35 (0,87-1,63)	1,20 (0,89-1,89)	1,82 (1,51-2,23)	1,71 (1,05-1,95)	2,09 (1,02-3,67)	0,005
CSF κFLC (mg/dL), median (IQR)	0,28 (0,14-0,78)	0,18 (0,07-0,27)	0,06 (0,04-0,10)	0,09 (0,03-0,10)	0,056 (0,042-0,12)	0,0002
Serum λFLC (mg/dL), median (IQR)	0,98 (0,81-1,29)	1,13 (0,89-1,47)	1,86 (1,39-2,59)	2,49 (2,04-3,25)	0,74 (0,68-2,54)	0,003
CSF λFLC (mg/dL), median (IQR)	0,13 (0,10-0,16)	0,11 (0,10-0,13)	0,10 (0,09-0,17)	0,17 (0,15-0,23)	0,13 (0,10-0,13)	ns
κFLC index, median (IQR)	44,96 (20,65-76,65)	5,53 (1,89-33,52)	1,63 (1,16-2,72)	2,04 (1,65-2,95)	4,07 (2,17-9,85)	<0,0001
λFLC index, median (IQR)	35,86 (12,93-49,93)	19,96 (10,40-34,46)	5,61 (1,53-11,60)	7,56 (4,39-13,94)	24,23 (14,54-28,45)	0,007
Serum albumin (mg/dL), median (IQR)	3830 (3600-4200)	3600 (3100-4040)	3500 (3200-3800)	3650 (3175-3940)	3500 (2900-3700)	ns
CSF albumin (mg/dL), median (IQR)	20,30 (17,30-28,30)	25,90 (19,48-48,55)	31,0 (21,80-62,50)	25,60 (22,22-38,63)	24,30 (18,10-27,50)	0,03
QAlb, median (IQR)	0,005 (0,004-0,008)	0,007 (0,005-0,014)	0,009 (0,007-0,017)	0,008 (0,006-0,011)	0,007 (0,006-0,008)	0,0078
IgG index, median (IQR)	0,76 (0,69-0,97)	0,62 (0,51-0,67)	0,57 (0,51-0,67)	0,59 (0,52-0,63)	0,50 (0,46-0,56)	<0,0001

MS, Multiple Sclerosis; IND, Inflammatory Neurological Disease; PIND, Peripheral Inflammatory Neurological Disease; NIND, Non-Inflammatory Neurological Disease; SC, Symptomatic Controls; NS, Not Significant; OCB, Oligoclonal Bands; FLC, Free Light Chain; CSF, Cerebrospinal Fluid.

performed on the Hydrasys 2 analyser (Sebia, Bagno a Ripoli, Italy).

The statistical analysis was conducted using the statistical software GraphPad and Excel statistical tools. The Kruskal-Wallis test and the χ^2 test were used to calculate the p-value.

A ROC curve was constructed to assess the sensitivity and specificity performance of the K index for a diagnosis of MS.

Results

As it can be seen from Table 1, the gender distribution of all patients shows a slight prevalence of males, with 71 individuals compared to 61 females. Considering only patients diagnosed with MS, however, we see a marked prevalence of women (67%).

Taking into account the age of the patients at the time of the lumbar puncture, we can notice a progressive increase in cases that finds its maximum in the population between 47 and 62 years (39 patients). During this period, 16 cases corresponding to patients under the age of 18 were collected.

Regarding the subpopulations identified by the classification of the pathology diagnosed at the time of discharge, half of the patients received a diagnosis of an inflammatory disease of the CNS (IND or MS). Among these, half, corresponding to 25% of the total number of cases collected, received a diagnosis of MS.

The presence of CSF's exclusive OCB (positive OCB pattern type 2 and 3) was found in 97% of patients with MS and 15% of IND patients.

Three patients belonging to the SC group received a positive OCB result: they were discharged patients diagnosed with Clinically Isolated Syndrome (CIS) and subjected to periodic clinical checks; one of these subjects was diagnosed with MS at later times.

The p-value results calculated for the different characteristics were almost always significant (<0,05).

The concentration of κFLC in CSF and the K index are much higher in patients with MS than in those who have received a different diagnosis (p=0,0002 and p<0,0001, respectively). Serum κFLC levels are higher in the SC subpopulation.

The values of λFLC in CSF were not significant, while the highest concentration in serum is found in patients belonging to the NIND group. The λ index is significantly higher in MS patients.

The albumin quotient (QAlb = CSF albumin/serum albumin), an indicative marker of damage to the blood-brain barrier, has significantly different values in the different subpopulations considered (p=0,0078), whereas the link index is considerably higher in patients with MS (p<0,0001).

To evaluate the diagnostic performance of FLC liquoral indices for MS diagnosis, a ROC analysis was performed, the results of which are shown in Figure 1. In terms of Area Under the Curve (AUC), the K index showed better diagnostic performance than the λ and IgG indices. The optimal cut-off value for the K index, identified on the data collected, is 6.56. For the λ index, a cut-off of 10.12 has been identified, while for the IgG index, the most suitable threshold value corresponds to 0.65.

For 5 patients, it was possible to collect updates after discharge. They are exclusively women, aged between 16 and 52 years, who have presented a positive OCB pattern (type 2) and a generally high K index in their CSF analysis. Only for one case was it possible to calculate the λ index, while the others had a concentration of λFLC in CSF below the analytical sensitivity of the method. None of the patients met the McDonald's criteria at the time of admission; as a result, upon discharge, 80% of them received a diagnosis of IND, and one received a diagnosis of CIS. The diagnosis of MS has been attributed within a year from the execution of the lumbar puncture because of the presentation of new symptoms and, therefore, of the necessity to perform a control MRI; only for a patient, the diagnosis arrived after 18 months, always at a control MRI.

High K index values and positive OCB patterns were found in 91,7% of patients. The cases in which, instead, these values were discordant, with an OCB pattern negative for intrathecal synthesis (types 1, 4, 5) or dubious (presence of a single exclusively liquoral band) and a positive K index K (≥ 5), are collected in Table 2. The case study identified 11 patients with the above characteristics: 5 women and 6 men. Three subjects had a type 1 pattern and were diagnosed with IND; 5 patients showed a pattern of type 4 and received diagnoses of different pathologies that allowed their placement in the subgroups PIND (cases 2-B and 2-C), SC (case 2-E) and IND (cases 2-H and 2-I); one patient (2-F) revealed a type 5 OCB pattern, while two cases received a dubious result. Patient 2-F turns out to be a particular case, as the presented symptomatology is compatible with the experimental therapy (protocol MOLTO) they had decided to undergo. This correlation becomes evident and excludes the occurrence of an autoimmune demyelinating disease due to the presence of a negative link index, a borderline K index value, and an incalculable λ index.

Patients 2-L and 2-M had a dubious result at the IEF and were diagnosed with neurosyphilis and relapsing-remitting MS, respectively. In both cases, K index values were high. It was possible to calculate the values of λ index for three cases.

For some patients, it was possible to detect the concentrations of λFLC in serum and CSF and, accordingly, calculate the relative λ index. In Table 3 are listed the patients diagnosed with IND with positive λ index (≥ 3,5) and negative K index (< 5). This subpopulation includes 3% of our cohort, as the dosage was above the sensitivity of the method currently used in 4 patients. Interestingly, patient 3-B, the youngest in this group, has the highest λ index.

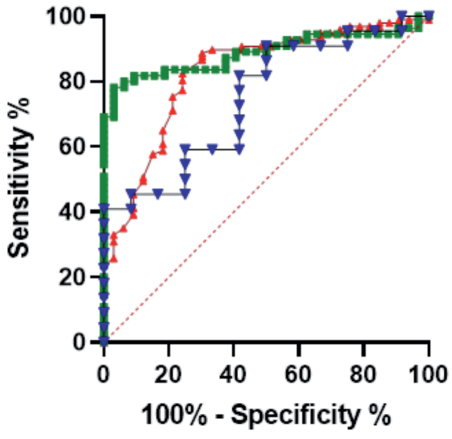

Figure 1. Receiver Operating Characteristic (ROC) curve for K index (green), λ index (blue) and IgG index (red).
Areas Under the Curve (AUCs) were calculated for K index (0,8875), λ index (0,7462) and IgG index (0,8276).
The optimal cut-off value was found to be 6,56 for the K index (sensitivity: 70,91%, specificity: 96,88%), 10,12 for the λ index (sensitivity: 45,45%, specificity: 75%), 0,65 for the IgG index (sensitivity: 75,26%, specificity: 78,79%).

Table 2. Characteristics of patients with negative or doubtful Oligoclonal Bands (OCB) pattern and positive K index (≥5).

Patient	Sex	Age	OCB Pattern	IgG index	K index	Λ index	Diagnosis	Class.
2-A	M	35	1	0,57	115,2	NC	Post infectious encephalopathy with autoimmune genesis	IND
2-B	M	60	4	11,87	183,8	NC	Neurosyphilis	PIND
2-C	F	40	4	0,54	9,67	NC	Sjogren syndrome	PIND
2-D	F	45	1	0,59	7,49	NC	Demyelinating disease	IND
2-E	F	83	4	0,61	7,28	NC	Suspected expanding left cerebral peduncle lesion	SC
2-F	F	67	5	0,56	5,97	NC	Nonspecific demyelinating inflammatory disease non sclerosis (compatible with side effects of MOLTO protocol)	IND
2-G	M	8	1	0,67	28,9	NC	Meningoencephalitis	IND
2-H	M	2	4	0,82	44,66	NC	Acute Disseminated Encephalomyelitis (ADEM)	IND
2-I	F	52	4	0,68	5,08	12,72	Possible neurosarcoidosis	IND
2-L	M	56	Dubious	0,77	7,15	6,76	Neurosyphilis	PIND
2-M	M	47	Dubious	0,63	5,76	NC	RRMS	MS

MS, Multiple Sclerosis; IND, Inflammatory Neurological Disease; PIND, Peripheral Inflammatory Neurological Disease; NIND, Non-Inflammatory Neurological Disease; SC, Symptomatic Controls; NC, Not Classifiable; OCB, Oligoclonal Bands.

Table 3. Characteristics of Inflammatory Neurological Disease (IND) patients with negative K index (<5) and positive λ index (≥3,5).

Patient	Sex	Age	OCB pattern	IgG index	K index	λ index	Diagnosis	Class.
3-A	M	13	1	0,49	NC	26,27	Autoimmune encephalitis	IND
3-B	F	2	1	0,69	NC	51,77	Acute myelitis during varicella infection	IND
3-C	F	59	1	0,63	NC	13,65	Dorsal myelitis of probable viral origin	IND
3-D	M	51	1	0,64	NC	28,69	Cervical myelitis of probable spondylogenic origin	IND

MS, Multiple Sclerosis; IND, Inflammatory Neurological Disease; PIND, Peripheral Inflammatory Neurological Disease; NIND, Non-Inflammatory Neurological Disease; SC, Symptomatic Controls; NC, Not Classifiable; OCB, Oligoclonal Bands.

Table 4. Characteristics of patients under 18 years of age.

Patient	Sex	Age	OCB pattern	IgG index	K index	λ index	McDonald's criteria	Diagnosis	Class.
4-A	M	10	1	0,68	NC	NC	NO	Meningoencephalitis of possible viral origin	IND
4-B	M	11	1	0,64	NC	NC	NO	Viral encephalitis	IND
4-C	F	16	2	0,49	10,71	NC	NO	Clinically Isolated Syndrome (CIS)	SC
4-D	F	9	1	0,51	NC	NC	NO	Medullary stroke	NIND
4-E	M	13	1	NC	NC	NC	NO	Acute Disseminated Encephalomyelitis (ADEM)	IND
4-F	F	16	2	1,11	35,7	NC	SI	MS	MS
4-G	M	8	1	0,67	28,9	NC	NO	Meningoencephalitis	IND
4-H	M	2	4	0,82	44,66	NC	NO	Acute Disseminated Encephalomyelitis (ADEM)	IND
4-I	M	13	2	0,57	12,28	53,61	SI	MS	MS
4-L	M	15	1	0,57	1,12	NC	NO	Guillain Barrè syndrome	PIND
4-M	M	10	1	0,46	4,98	24,23	NO	Migraine with aura	SC
4-N	F	2	1	0,69	NC	51,77	NO	Acute myelitis during chickenpox infection	IND
4-O	F	16	2	1,37	67,79	279,21	SI	MS	MS
4-P	M	13	1	0,49	NC	26,27	NO	Autoimmmune encephalitis	IND
4-Q	M	6	1	0,53	NC	32,80	NO	Guillain Barrè syndrome	PIND
4-Q	M	6	1	0,62	1,5	5,61	NO	Guillain Barrè syndrome	PIND

MS, Multiple Sclerosis; IND, Inflammatory Neurological Disease; PIND, Peripheral Inflammatory Neurological Disease; NIND, Non-Inflammatory Neurological Disease; SC, Symptomatic Controls; NC, Not Classifiable; OCB, Oligoclonal Bands..

In our population, a total of 15 patients under the age of 18 were also identified (Table 4). Patient 4-Q, in fact, is reported twice as it has undergone two different lumbar punctures, both with the request for research of OCB through IEF. Since the population is too small to carry out a specific statistical analysis, it was decided to treat the data obtained from the analysis of CSF and serum of these patients together with those of adult patients (Table 1). Among the most relevant features, we can note that three patients received a diagnosis

of MS as characterized by a positive OCB pattern, high K index (≥ 5), and satisfaction with McDonald's criteria. In addition, for patients 4-I and 4-O, it was possible to calculate the λ index, which was also positive (≥3,5). It is worth mentioning the patient 4-C because, according to her medical record, after an initial diagnosis of CIS, she developed MS. At the time of the CSF analysis for OCB, she had a type 2 pattern, positive for intrathecal synthesis and a high K index.

Discussion

MS is a serious neuroinflammatory and autoimmune disease of the CNS that mainly affects women in their thirties,[8] and the data collected in this report confirm the incidence of the disease in subjects with these characteristics. In addition, the presence of significant p-values for most of the parameters analysed in Table 1 ensures the validity of these data and excludes distributions attributable to the case.

Following the review of McDonald's criteria in 2017,[9] laboratory analysis of CSF for MS diagnoses has regained importance. The interest was particularly focused on the K index, which is the expression of an increased synthesis of free light chains type κ of immunoglobulins at the intrathecal level. In literature, the published studies are not unambiguous about the threshold values to be used: depending on the population considered and the characteristics, in terms of sensitivity and specificity, that are intended to provide the test, the range of cut-off values for the K index varies from 2,4 to 20.[10] the General Hospital of Alessandria uses a cut-off value of 5, which was chosen following a study carried out in 2020 involving three of the most important Piedmontese laboratories.[11] In this paper, it was decided to consider only the case studies analysed at the General Hospital SS. Antonio and Biagio and C. Arrigo of Alessandria to try to understand how much such threshold value is predictive.

To carry out this analysis, the ROC curve represented in green in Figure 1 was constructed. The most appropriate K index value as a threshold for MS prediction, according to our data, was found to be 6,56. Values above this limit were present in 32/33 (97%) of MS patients, compared to 16/99 (16%) of non-MS patients, with a sensitivity of 70,91%, a specificity of 96,88%, and an AUC of 0,8875. The threshold value set at 5, cited previously, has a sensitivity of 93,9% and a specificity of 77,4%, which allows the result of this test to be used both at the diagnostic level and as a screening test. The threshold value of 6,56 identified in this work is higher than that currently used at the Laboratory, favouring specificity to sensitivity. This result is mainly attributable to the specific and small population considered and the diagnosis attributed to each patient. In fact, our data also includes subjects who, over time, have had a different diagnosis from that received close to the lumbar puncture, a factor that can affect the sensitivity and specificity of a diagnostic test. The threshold value set at 6,56 would then allow the use of the K index as a complementary test to the OCB search by IEF. Considering the incidence of patients with suspected MS at the General Hospital of Alessandria and the availability of trained staff, the κFLC test is not used as a screening examination.

A ROC analysis was also carried out on the λ index, which made it possible to identify a more suitable threshold value at 10,12, correlating with a sensitivity of 45,45% and a specificity of 75%. This value, however, is not very representative, as it is obtained from an approximate ROC curve due to the small case studies (26% of the population) for which it was possible to dose λFLC. In addition, there are some published studies that are strongly divergent on the threshold value that can be attributed to this index, ranging from a value of 4,3 to a value of 17.[12-14] This variability is mainly due to the technique (turbidimetry) currently used and to the different antibody-based kits available (Freelite kit with polyclonal antibodies and N Latex kit with monoclonal antibodies).

The third ROC curve that has been constructed is related to the IgG index. The ideal cut-off was identified as 0,65, with a sensitivity of 75,26 %, a specificity of 78,79%, and an AUC of 0,8276. Although in the past it has been widely used to investigate the presence of intrathecal synthesis, the IgG index has lower sensitivity and specificity than the K index or the search for OCB by IEF. Consequently, as other work has already suggested,[15,16] its clinical use for the diagnosis of MS is not recommended. For the same reasons, the most recent revision of the McDonald's criteria[9] also suggests that it should be used with particular care.

Due to the diversity of samples collected, it was possible to make further considerations about FLC indices. In particular, data from the population showing a high K index (≥5) and a negative or doubtful OCB pattern (Table 2) show that the K index is more sensitive and has the same diagnostic accuracy as OCB for a diagnosis of MS or inflammatory or infectious disease of CNS.[17] The higher K index values are certainly due to the presence of an inflammatory/infectious disease which, as already mentioned, causes a marked increase in the synthesis of κFLC.[18] In addition, the presence of two cases with dubious OCB patterns and high K index values further supports the diagnostic sensitivity of this parameter and highlights its highly predictive value.

Regarding the subpopulation of patients diagnosed with a neuroinflammatory disease and having values of K index subthreshold but high λ index (Table 3), it is important to highlight that, while KFLC is almost identical to OCB in terms of clinical information, λFLCs appear to be elevated in CSF even in patients with conditions different from MS. This finding is potentially due to the fact that λFLC has a tendency to dimerize and polymerize. Lambda dimers cannot cross the blood-brain barrier and have a significantly increased biological half-life in CSF, therefore even very weak inflammatory events are able to increase λFLC levels.[18] In addition, the levels of λFLC are strongly affected by the pre-analytic treatments to which the sample is subjected, so only the development of new and more sensitive analytical techniques could allow future use of this index alongside the K index.[18,19]

With regard to patients under the age of 18, there are numerous instances that report an increase in the diagnosis of Pediatric-Onset Multiple Sclerosis (POMS). This is demonstrated by the fact that, despite the small case histories, 4/15 (27%) patients have been diagnosed with MS. The onset of the disease in children has significant long-term implications, which could affect physical and cognitive development and, more generally, the quality of life of these children.[20] When compared with adult patients, pediatric patients with MS tend to develop inflammatory disease with more frequent relapses, but a slower accumulation of disability.[21] These characteristics are attributable to high post-relapse recovery due to increased repair and neo-synthesis of myelin and increased plasticity of the developing brain.[22] The diagnosis of POMS is complicated, due to the similarity of signs and symptoms with other pathologies, not only of inflammatory nature. In order to make a differential diagnosis, the International Pediatric Study Group has established a set of criteria[23]

that have been used in most pediatric patient studies. In addition, the presence of CSF-specific OCB is another criterion to help distinguish between various demyelinating syndromes and attribute MS diagnosis when MRI results do not fully meet the DIT criteria. Another viable alternative for early diagnosis of MS in pediatric patients is dosing the inflammatory cytokines in CSF, as the high levels of inflammation typical of POMS change the permeability of the blood-brain barrier, stimulating the migration of T cells and other cells of inflammation.[24] This could explain the high levels of FLC that were detected in some pediatric patients, which were the highest of the entire population studied.

Conclusions

Tests based on FLC in the CSF used for intrathecal synthesis research are becoming increasingly important in laboratory medicine due to their high diagnostic sensitivity and specificity. In particular, the K index has equivalent reliability to OCB, but with significantly lower costs and processing times, and a very high predictive value, thus able to detect lesions associated with the onset of multiple sclerosis or a neuroinflammatory disease of the CNS. To date, the K index is not yet universally accepted, but, thanks to its characteristics, the scientific community has proposed to include it in the McDonald diagnostic criteria at the next revision, as a complementary test to OCB, the current gold standard. If, on one hand, the IgG index is less and less used, the λ index intrigues as a possible additional test to support the differential diagnosis of MS, but further studies and the development of more sensitive techniques are needed to assess its clinical potential.

Medicine is gradually evolving towards personalized clinical approaches, tailored to each patient. From this point of view, new research has emerged[25,26] and has hypothesized the diagnostic and prognostic role of serum levels of light-chain neurofilaments (sNfL), which would allow more effective monitoring of the disease, predicting its course and, accordingly, create an appropriate therapeutic plan for each patient. Although further research is needed to establish this possibility, it may hold the key to treating a significant illness like multiple sclerosis in a more focused manner.

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