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Validation and meta-analysis of kappa index biomarker in multiple sclerosis diagnosis

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ABSTRACT

The importance of studying the cerebrospinal fluid (CSF) in Multiple Sclerosis (MS) is included in the last McDonald criteria (2018). The study of oligoclonal IgG bands (OCGB) assay is strongly recommended in some situations in which MS diagnosis is uncertain. New biomarkers are developed during the last years. Kappa free light chains (FLC) can predict conversion to MS in patients with Clinically Isolated Syndrome (CIS).

The aim of this work is to validate the clinical usefulness of the kappa index, and to establish the actual state of knowledge for kappa index as a biomarker of conversion in CIS patients by a meta-analysis. Kappa index seems more relevant than the mere concentration of kappa FLC in CSF.

In the validation study, 334 patients were included; in which 100 were CIS patients. Patients were divided in two groups according kappa index cut-off of 10.62: group 1 (kappa index > 10.62); group 2 (kappa index < 10.62). In group 1 more patients had positive OCGB, IgG index > 0.56 and fulfilled magnetic resonance imaging (MRI) criteria. In contrast, in group 2, more patients showed negative OCGB, IgG index < 0.56 and did not fulfilled MRI criteria. While 67.6% of patients from group 1 converted to MS, only 12.5% of patients from group 2 converted to MS. An HR of 6.02 was obtained in the Kaplan-Meier analysis.

In the meta-analysis, 8 studies were finally included. The SROC curve revealed a high diagnostic performance for the kappa index as a MS diagnostic biomarker. Despite heterogeneity found between studies, the global OR revealed a good discriminatory capacity of kappa index.

In conclusion, kappa index has a great clinical sensitivity and specificity as a support in MS diagnosis. High kappa index increase the probability of CIS to MS conversion. A correct sample processing in the preanalytical stage is key to obtain right results and to allow establishing comparison between laboratories.

1. Introduction

Multiple sclerosis (MS) diagnosis is mostly clinical and relies on signs and symptoms attributable to focalized lesions in the brain white matter. Cerebrospinal fluid (CSF) is the closest fluid to the central nervous system and thus, the one that can mostly reflect the immunological changes that develop due to the progression of the disease. Importantly, laboratory findings can contribute to MS diagnosis by studying the CSF [1].

In the last McDonald criteria (2018) [2], the importance of performing the oligoclonal IgG bands (OCGB) assay is included for the diagnosis of MS and is strongly recommended in some situations: when clinical and magnetic resonance imaging (MRI) evidence is insufficient to support an MS diagnosis, when there is a presentation other than a typical clinically isolated syndrome (CIS), when clinical, imaging, or laboratory features are atypical of MS, and lastly, in populations in which MS is less common like children, older patients and non-white people [2].

Detection of intrathecal IgG synthesis is carried out through OCGB and IgG index to help the diagnosis of MS. Positive OCGB have been detected in a great number of CIS patients that progress to MS, being an independent predictive factor of MS conversion [3,4,5]. Doshi *et al.*

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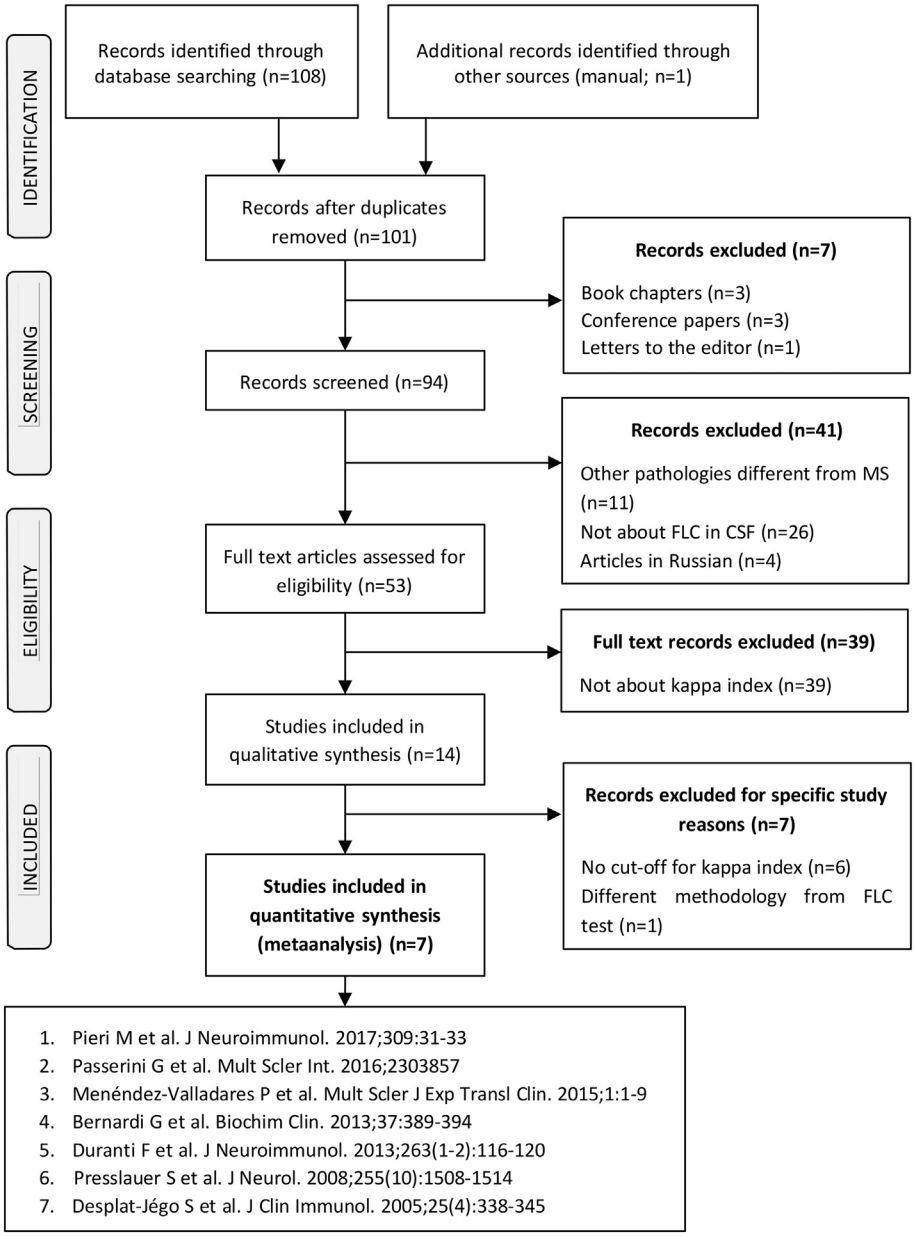

Fig. 1. PRISMA Flow diagram. Seven potential articles were included in the meta-analysis after removing studies, according to criteria described in the Flow Diagram, made from the search and selection of primary studies. The process was divided in four stages: identification, screening, eligibility and inclusion. The reasons for exclusion were indicated after the respective analysis in each stage.

demonstrate the importance of OCGB in MS diagnosis, observing positive OCGB in up to 90% of MS patients [5]. To quantify intrathecal IgG synthesis, diverse indices and formulas are used. One of the most common is the Tibbling-Link index or IgG index, observed to be increased in about 70-90% of MS patients. It is rarely increased in patients with negative OCGB. Unfortunately, IgG index has a low sensitivity, so it cannot replace the diagnostic value of OCGB. Nevertheless, its quantification is indispensable for performing the OCGB technique properly [6].

In the search for alternative biomarkers with high MS diagnostic and prognostic power, free light chains (FLC) in CSF have been determined. FLC in CSF have shown diagnostic sensitivity and specificity similar to OCGB [7-11].

In a healthy state, light chains of immunoglobulins are produced in excess over heavy chains. The level of unbound light chains, or FLC, in serum and CSF is low in healthy subjects. However, FLC levels appear to be altered in certain inflammatory diseases like MS [12]. This observation has been reported in various studies, in which high FLC kappa levels in CSF predict conversion to MS in patients with CIS [10,13,14]. The most common technique for quantifying FLC is nephelometry, which is a validate technique described in the literature [10].

Recently, the importance of the kappa index seems more relevant than the mere concentration of FLC kappa in CSF. Kappa index takes into account the blood-CSF barrier function, improving diagnostic accuracy of FLC kappa in CSF and reducing false positives.

In this study, we aimed to validate the clinical usefulness of the kappa index as a biomarker of MS conversion in CIS patients. A greater number of analysis will provide clinicians with greater security when using the kappa index to support a final diagnosis. In addition, we aimed at comparing the kappa index cut-off obtained previously with our patient's cohort by performing a meta-analysis with other similar studies.

2. Methods

2.1. Validation

A prospective longitudinal study was carried to validate the kappa index cut-off value of 10.62 obtained previously at our hospital [14]. All patients referred to the neurologist between August 2014 and September 2015 were included in the validation study. After a first clinical presentation, subjects were followed-up for at least two years.

Patients without a signed informed consent or under the age of 14 years were excluded from the study for being atypical cases of early MS.

All samples, provided by the Biobank of *Hospital Universitario Virgen Macarena* (Seville, Spain) [*Biobanco del Sistema Sanitario Público de Andalucía*], were collected and stored under similar conditions in agreement with published European guidelines for CSF collection and biobanking, proposed by the BioMS-EU consortium (Consortium for CSF Biomarker Research) [15]. According to that, samples were frozen at -80°C until analysis, and time delay between withdrawal, processing and freezing was less than 2 hours. This fact, ensures quality samples and optimal preanalytical conditions, avoiding freeze-thaw cycles.

The diagnosis of patients with clinically defined MS (CDMS) was performed by clinical evaluation and MRI, considering the McDonald criteria (2010), and supported by laboratory tests (OCGB).

During the diagnosis study of the patients, the levels of FLC kappa in CSF and serum were measured.

The FLC kappa level was quantified by endpoint nephelometry using the Human Kappa Freelite kit (The Binding Site Group Ltd) on the Siemens BNII analyzer. The measurement principle is based on the detection of the scattered light intensity. This method uses polyclonal antibodies coated onto polystyrene latex for FLC kappa. According to the manufacturer, the lower detection limit is 0.06 mg/L. The sample volume needed for each analysis is 300 μL. To obtain the kappa index, that takes into account the blood-CSF barrier function, the following formula was used: (CSF-FLC kappa * serum-albumin) / (serum-FLC kappa * CSF-albumin).

In this study, OCGB data was determined by isoelectric focusing (IEF) followed by transfer and IgG immunodetection by an alkaline phosphatase-labeled anti-IgG antibody, following the protocol described by Villar *et al.* [16]. OCGB results were reported as positive (≥ 2 bands in the CSF compared to the serum) [17-19] or negative.

2.2. Meta-analysis

Due to the differences found between different published studies related to kappa index in MS diagnosis, a random effects meta-analysis was assessed. The aim was to establish the actual state of knowledge for kappa index as conversion biomarker in MS. The review was carried using PRISMA guidelines [20]. The flow diagram is represented in Fig. 1. It maps out the number of records identified, included and excluded, and the reasons for exclusions.

We searched in Pubmed and SCOPUS from inception until September 1, 2017, using the search terms “free light chains” and “multiple sclerosis”. Studies were included if they fulfilled different criteria (showed in Fig. 1).

2.3. Statistical analysis

Statistical analysis was carried out using SPSS software v.20.0 (Armonk, NY: IBM Corp. 2011) and MedCalc v.11.4.2.0 (2010). We used Chi-square or Fisher-test for categorical variables and t-Student for quantitative variables for comparison between groups, and Kaplan-Meier analysis for survival curve comparison between defined groups (Log-rank test). Hazard ratio was calculated using univariate Cox regression analysis.

The Random effects model was applied with the DerSimonian and Laird estimator, using MetaDisc software (ES)® (1.1.1). Meta-analysis results are presented in forest plots separately for sensitivity, specificity and global random effects. With high between-study heterogeneity, the random effects model is the model of choice, rather than the fixed effect model [21]. (p-values < 0.05 are referred to as significant).

Cochran's Q-test was used to calculate between-group heterogeneity. The magnitude of heterogeneity was assessed by I2, which is an estimate of variability across studies based on heterogeneity rather than chance, ranging from 0 to 100%. Values above 25%, 50% and 75% correspond to low, moderate and high heterogeneity, respectively [22].

3. Results

3.1. Validation

A total of 334 patients were included in the validation study. The average age was 47 years (range: 15-89), and 58.7% were women.

During follow-up, 27.5% of the patients (92/334) converted to CDMS. Positive OCGB was found in 33.2% of the total (111/334), IgG index > 0.56 in 34.1% (114/334), and kappa index > 10.62 in 33.8% (113/334).

Using a kappa index cut-off of 10.62, CIS patients (N = 100) were divided in two groups: Group 1 included patients with high kappa index, above 10.62, and Group 2 included patients with low kappa index, under 10.62 (Table 1).

The percentage of women in Group 1 was greater compared to men (76.5% women), which was the group with greatest probability to convert to CDMS. In this group, 89.7% of the patients had positive OCGB, and 63.2% fulfilled MRI criteria and positive OCGB.

89.7% of patients from Group 1 presented IgG index > 0.56 while only 9.4% of patients from Group 2 presented an IgG index > 0.56.

A positive correlation between kappa index value and MRI criteria was found in CIS patients.

Most of the patients from Group 2 showed negative OCGB (87.5%), IgG index < 0.56 (90.63%), and did not fulfilled MRI criteria (78.13%).

A Kaplan-Meier analysis was performed to study the probability of conversion to MS in CIS patients according to a kappa index cut-off value of 10.62 (Fig. 2). Patients with high kappa index value (> 10.62), showed greater probability of conversion to MS ($p < 0.0001$).

In Group 1, 67.6% of the patients converted to MS during the period of the study, (minimum follow-up of two years), while only 12.5% of patients from Group 2 converted to MS (Table 1). In the univariate analysis, we obtained an HR of 6.02 (95% CI: 3.36- 10.79) for the kappa index > 10.62. The median time for MS conversion for Group 1 was 19 months with a standard error of 0.0652, which indicates the average speed at which MS conversion occurs in this group of patients.

Table 1
Laboratory and clinical data of CIS patients according to a kappa index cut-off of 10.62. Group 1: CIS patients with kappa index > 10.62; Group 2: CIS patients with kappa index < 10.62 [CIS: clinically isolated syndrome. CI: confidence interval. OCGB: oligoclonal IgG bands. BTC: Barkhof-Tintoré criteria. MRI: magnetic resonance imaging criteria].

KAPPA INDEX	*Group 1 > 10.62*	*Group 2 < 10.62*	*p value*
Patients (N)	68	32	-
Gender (female/male)	52/16	23/9	-
Age mean (95% CI)	35.21 (32.82-37.61)	37.82 (33.75-41.90)	0.243
OCGB (+/-)	61/7	4/28	< 0.0001
IgG index (> 0.56/ < 0.56)	61/7	3/29	< 0.0001
≥3 BCT (%)	46 (67.6)	7 (21.9)	< 0.0001
Fulfilled MRI and OCGB (yes/no)	43/25	3/29	< 0.0001
Conversion to MS (%)	46 (67.6)	4 (12.5)	< 0.0001

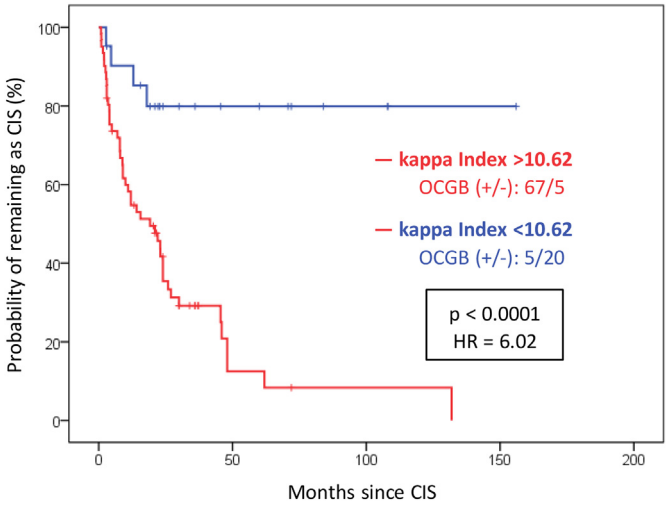

Fig. 2. Kaplan-Meier analysis for CIS patients according to the kappa index. The probability of remaining as CIS according to cut-off value for the kappa index is shown: kappa index > 10.62 (red line) and kappa index < 10.62 (blue line). The time is represented in months since CIS [CIS: clinically isolated syndrome. OCGB: oligoclonal IgG bands].

3.2. Meta-analysis

Search in Pubmed and SCOPUS using the keywords aforementioned generated 108 articles, and 1 additional article was provided by manual search. After eliminating duplicates, 101 articles remained. Seven potential articles were chosen according to the criteria described in the PRISMA Flow Diagram (Fig. 1).

The seven articles finally included in the meta-analysis (Pieri M, 2017; Passerini G, 2016; Menéndez-Valladares P, 2015; Bernardi G, 2013; Duranti F, 2013; Presslauer S, 2008; and Desplat-Jégo S, 2005) [8,11,14,23,24,25,26], included 300 MS cases from a total of 1155 adult patients. In these seven articles, in which 8 kappa index cut-off were defined, the following information was obtained: author names, year of publication, study population divided in groups, kappa index and OCGB in each group, preanalytical conditions, measuring equipment for FLC, kappa index cut-off, area under curve (AUC), sensitivity and specificity (Table 2).

In the seven articles included in the meta-analysis [8,11,14,23,24,25,26], two other groups with a diagnosis different from MS were also defined: non-inflammatory neurologic disease (NIND) and other inflammatory neurologic disease different from MS (IND). In four studies [8,14,24,26], a CIS group was also included. In the MS group, high median values of kappa index are observed in all studies, being highest median among the defined groups. In some cases, statistically significant differences were found between the MS group and the rest of groups: Desplat-Jégo S, 2005, $p < 0.005$ (MS vs NIND) [8], Presslauer S, 2008, $p < 0.001$ (MS vs control groups) [26], Duranti F, 2013 (*p*-value not specified) [11], Bernardi G, 2013, $p < 0.001$ (MS vs control group, in both laboratories) [25], Menéndez-Valladares P, 2015, $p < 0.0001$ (MS vs control groups) [14], Passerini G, 2016, $p < 0.001$ (MS vs control groups) [24], and Pieri M, 2017, $p < 0.001$ (MS vs control groups) [23].

Similarly, the studies that included CIS patients [8,14,24,26], observed greater kappa index values with regards to the respective control groups.

Desplat-Jégo *et al.* [8] using the same methodology as the one used in our study (same measuring equipment for FLC assay (BNII), and same reactive (Freelite)) but a higher kappa index cut-off was defined (kappa index = 20), obtained lower diagnostic accuracy values (sensitivity = 69.7% and specificity = 81.8%) [8] compared to our previous study (sensitivity = 93.1% and specificity = 95.7%) [14].

Presslauer *et al.*, using a kappa index of 5.9 obtained good diagnostic accuracy values (sensitivity = 96% and specificity = 86%) [26], and Bernardi *et al.* (lab 1) with a kappa index of 7.82 obtained a sensitivity of 95% and a specificity of 98% [25].

Using a similar cut-off to ours, Pieri *et al.* (kappa index = 12.3), obtained good diagnostic accuracy values (sensitivity = 93% and specificity = 100%) [23].

In two studies in which BNII equipment for FLC assay, and N Latex FLC assay were used [24,25], the cut-off values were similar between them, but much lower to those obtained with other equipment's and assays: Bernardi *et al.* (lab 2), kappa index = 2.72 [25], and Passerini *et al.*, kappa index = 2.43 [24].

Using the DerSimonian and Laird random effects model, with 95% confidence level, there was no significant statistical difference of

Table 2
Laboratory data of studies included in the meta-analysis. For each study the following parameters are indicated: number of total patients, number of MS patients, preanalytical conditions, measuring equipment for FLC assay [BNII™ (Siemens Healthcare Diagnostics Inc.), BN Prospec® (Siemens Healthcare Diagnostics Inc.), Delta (RADIM)], reactive kit for FLC [Freelite® (The Binding Site Group Ltd., Birmingham, UK), N Latex FLC (Siemens Healthcare Diagnostics Inc.)], Ac type (M: monoclonal. P: polyclonal), kappa index cut-off, area under curve, sensitivity, and specificity, as well as median values for kappa index in each group [MS: multiple sclerosis. FLC: free light chains. CSF: cerebrospinal fluid. CIS: clinically isolated syndrome. NIND: non-inflammatory neurologic disease. IND: other inflammatory neurologic disease different from MS].

References		Pieri M et al. 201,723	Passerini G et al. 201,624	Menéndez-Valladares P et al. 201,514	Bernardi G et al. 2013 (lab 1)25	Bernardi G et al. 2013 (lab 2)25	Duranti F et al. 201,311	Presslauer S et al. 200,826	Desplat- Jégo S et al. 20,058
Total patients (MS patients)		176 (71)	100 (34)	176 (29)	75 (24)	21 (16)	80 (23)	438 (70)	89 (33)
Preanalytical conditions		Rutine samples (−80 °C)	Rutine samples [−20 °C (CSF: 10 min 800 rpm; serum: 10 min 3000 rpm)]	Samples kept under Biobank conditions [−80 °C (10 min 2000 g)], stored < 2 h	Rutine samples (+4 °C)	Rutine samples (−70 °C)	Rutine samples	Rutine samples (−80 °C), stored < 2 h	Rutine samples (−80 °C)
Measuring equipment for FLC (reactive) (Ac)		BN Prospec (N Latex FLC) (M)	BN II (N Latex FLC) (M)	BN II (Freelite) (P)	Delta (Freelite) (M)	BN II (N latex FLC) (M)	BN Prospec (N Latex FLC) (M)	BN Prospec (Freelite) (M)	BN II (Freelite) (P)
kappa index cut- off		12.3	2.43	10.62	7.82	2.72	12	5.9	20
Area Under Curve		0.989	0.823	0.971	–	1	0.96	0.954	–
Sensitivity (%)		93	89.3	93.1	95	100	95	96	69.7
Specificity (%)		100	77.3	95.7	98	98	91	86	81.8
Kappa index (groups)	MS	41.67	22.4	88	52.3	19.4	41.07	65.74	41
	CIS	–	17.4	35.61	–	–	–	63.03	34
	NIND	2.56	1.9	3.37	2.9	1.7	2	(3.01;2.33;3.3;1. 34)	9
	IND	3.04	1.8	2.87	–	–	3.48	1.35	18.5

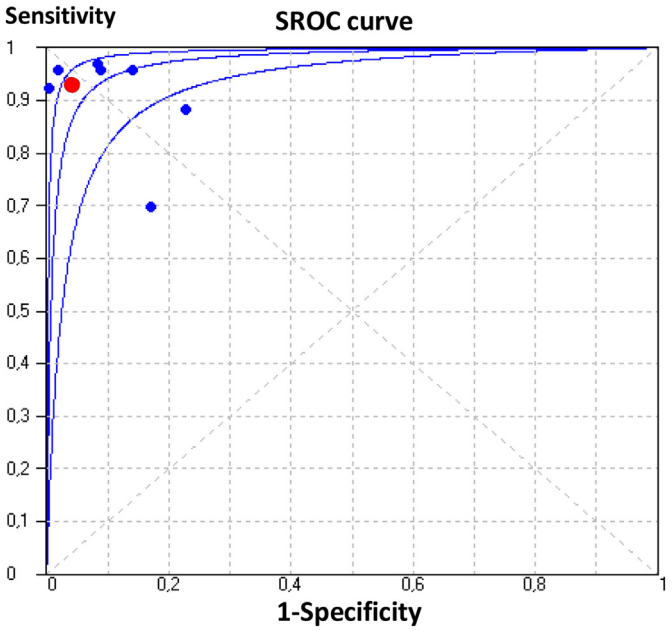

Fig. 3. SROC curve for the kappa index. Distribution of studies included in the meta-analysis (blue dots). The result from our previous study included in the meta-analysis is represented with a red dot. The interdependence relationship between sensitivity (ordinate axis) and specificity (abscissa axis) remains, which varies according to the cut-off threshold (blue central line). The confidence intervals for the cut-off threshold are represented with the upper and lower blue lines (SROC curve: summary receiver operating characteristic curve).

heterogeneity (r = -0.405, p = 0.320). That is, no significant different is observed between the eight kappa index cut-off included due to threshold effect as can be seen by the summary of the receiver operating characteristic (SROC) curve analysis (Fig. 3).

The SROC curve was constructed by combining estimates of sensitivity and specificity of the studies included in the meta-analysis. The calculated area under curve was 0.9719, revealing a high diagnostic performance for the kappa index as an MS diagnostic biomarker. The Q index = 0.9230 shows the point with the high sensitivity and specificity, and it is a global measure of the diagnostic test efficacy.

The global sensitivity obtained in the meta-analysis was 91% (95% CI: 88-94) with a range between 70% and 100% (Fig. 4A). The global specificity obtained in the meta- analysis was 89% (95% CI: 87-92) with a range between 77% and 100% (Fig. 4B).

Forest plots of sensitivities and specificities for the studies included in the meta-analysis exhibited heterogeneity: sensitivity (chi square = 19.70, p = 0.0063) (Fig. 4A); specificity (chi square = 45.76, $p < 0.00001$) (Fig. 4B).

Finally, the Odds Ratio (OR) as diagnostic test performance measurement is shown as a forest plot in Fig. 4C. The global OR value was 143.65 (95% CI: 39.19-526.50), which means that the kappa index has a good discriminating capacity. However, the heterogeneity among the studies was large with Cochran Q = 28.61 (p = 0.0002) with 7 degrees of freedom which is equivalent to an I2 of approximately 75.53%.

4. Discussion

In the validation study it is clear the existence of a positive correlation between intrathecal synthesis of IgG and intrathecal synthesis of FLC kappa. This conclusion comes from comparing the results of OCGB and IgG index in relation to the kappa index. The presence of positive OCGB together with a high kappa index (> 10.62) was observed in a high percentage of patients, as it is shown in Table 1.

In the study published by our group in 2015 [14], the kappa index median was greater in the MS group than in the CIS group, even though been superior in CIS patients respect to control group. In some of the articles included in the meta-analysis [8,24,26], the kappa index median of the CIS patients was similar to that found for MS patients. A possible explanation for this is that in these studies, CIS patients were described as CIS-MS, meaning that all CIS patients included converted into MS, and thus the similar kappa index value observed for CIS patients and MS patients. In our previous study [14], we included CIS patients with low- and high-risk of MS conversion.

The high heterogeneity found amongst the studies analyzed in the meta-analysis is most likely due to several factors, namely the use of different measuring equipment for FLC assay, the study design, sample size, and/or differences related to preanalytical conditions. A key point to the assay final result, is the correct sample processing in the preanalytical stage, in which samples are obtained and stored prior to processing. Routine samples were used in all articles analyzed. Nevertheless, for our studies, the samples used were stored under Biobank conditions to ensure the best estimation in obtaining kappa index cut-off. Samples were stored at -80°C in less than two hours after extraction. These conditions were also followed by Presslauer *et al.* [26]. To the best of our knowledge, our work is the only publication in which CSF and blood samples were handled in agreement with published European guidelines for CSF collection and biobanking, proposed by the BioMS-EU consortium. The compliance of these guidelines is fundamental to achieve the best possible preanalytical conditions.

The results obtained in the meta-analysis, i.e. global sensitivity of 91% (95% CI: 88-94) and global specificity of 89% (95% CI: 87-92) are consistent with recent reports. A meta-analysis, by Passerini *et al.* estimated a global sensitivity of 90% (95% CI: 82– 96), and a global specificity of 90% (95% CI: 81-95) [24]. They also conclude that the low comparability between studies is due to differences in the study design, population and in the threshold selection methods. In this study, neither diagnostic OR nor Cochran's Q test for heterogeneity were shown [24].

The diagnostic OR, which, in this case, represents the kappa index global effect related to the susceptibility of MS conversion, shows that the cut-off obtained in our previous study [14] is within the global range. This fact explains the good discriminatory capacity of the calculated kappa index cut-off in comparison with the rest of cohorts analyzed.

5. Conclusions

High FLC kappa levels in CSF, as well as a high kappa index, denote an increase in the probability of CIS to MS conversion. Therefore, this publication reinforces the validity of the kappa index as a diagnostic MS biomarker in the clinical context.

The diagnostic OR obtained in the meta-analysis shows the positive correlation between the diagnosis of MS and high kappa index values showing statistical significance for the diagnostic use of the kappa index biomarker.

In conclusion, the kappa index has shown the greatest clinical sensitivity and specificity as a support in MS diagnosis compared to OCGB and IgG index.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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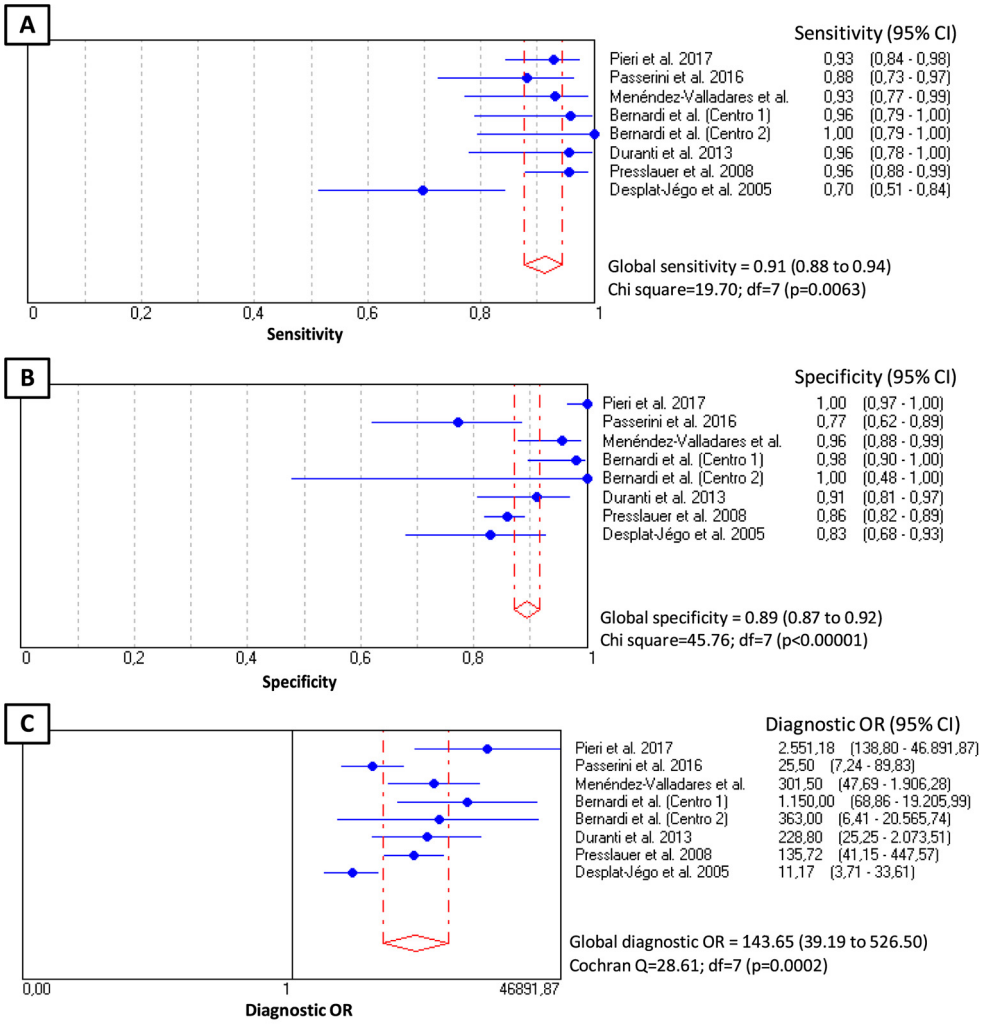

Fig. 4. A. Forest plot of sensitivities for the studies included in the meta-analysis; B. Forest plot of specificities for the studies included in the meta-analysis; C. Forest plot of meta-analysis global effect for the kappa index related to conversion susceptibility to MS (OR: Odds Ratio).

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