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Assessment of the reproducibility of oligoclonal IgM band detection for its application in daily clinical practice

M. Espiño [a,o], V. Abraira [b], R. Arroyo [c,o], L. Bau [d], C. Cámara [e], L. Campos-Ruiz [f,o], B. Casanova [g,o], C. Espejo [h,o], O. Fernández [i,o], A. García-Merino [f,o], M.I. García-Sánchez [j,o], M. Gómez [r], A. Gosis [o,s], G. Izquierdo [j,o], J. Meca [k,o], X. Montalban [h,o], F. Morandeira [q], J. Olascoaga [l,o], A. Prada [o,u], E. Quintana [m,o], Ll. Ramió-Torrentà [m,o], A. Rodríguez-Antigüedad [n,o], G. Salgado [t,o], J.L. Santiago [c,o], E. Sarasola [n,o], M. Simó-Castelló [g,o], J.C. Alvarez-Cermeño [o,p], L.M. Villar [a,o,*]

[a] Department of Immunology, Hospital Universitario Ramón y Cajal, Ctra de Colmenar Viejo km 9.100, 28034 Madrid, IRYCIS, Spain
[b] Department of Biostatistics, Hospital Universitario Ramón y Cajal, Ctra de Colmenar Viejo km 9.100, 28034 Madrid, IRYCIS, Spain
[c] Department of Neurology, Hospital Clínico San Carlos, Calle Profesor Martín Lagos, s/n, 28040 Madrid, Spain
[d] Department of Neurology, Hospital Universitari de Bellvitge, Av. Granvia s/n, Hospitalet de Llobregat, Barcelona, Spain
[e] Department of Immunology, Hospital San Pedro de Alcántara, Avenida Pablo Naranjo s/n, 10003 Cáceres, Spain
[f] Department of Neurology, Hospital Universitario Puerta de Hierro, Calle Manuel de Falla, 1, 28222 Majadahonda, Madrid, Spain
[g] Department of Neurology, Hospital Universitari La Fe, Avinguda Fernando Abril Martorell, 46026 Valencia, Spain
[h] Servei de Neurologia-Neuroimmunologia, Centre d'Esclerosi Múltiple de Catalunya, Vall d'Hebron Institut de Recerca, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Passeig Vall d'Hebron, 119-129, 08035 Barcelona, Spain
[i] Department of Neurology, Hospital Regional Universitario, Avda Carlos Haya, s/n, 29010 Málaga, Spain
[j] Department of Neurology, Hospital Universitario Virgen Macarena, Avd. Dr. Fedriani, 3, 41007 Sevilla, Spain
[k] Department of Neurology, Hospital Universitario Virgen de la Arrixaca, Ctra. Madrid-Cartagena, s/n, 30120 El Palmar, Murcia, Spain
[l] Department of Neurology, Hospital Universitario Donostia, P° Dr. Beguiristain, 107-111, 20014 San Sebastián, Spain
[m] Unitat de Neuroimmunologia i Esclerosi Múltiple, Hospital Universitari Dr. Josep Trueta, Institut d'Investigació Biomèdica de Girona (IDIBGI), Avenida França, s/n, 17007 Girona, Spain
[n] Department of Neurology, Hospital Universitario Basurto, Av de Montevideo, 18, 48013 Bilbao, Spain
[o] Red Española de Esclerosis Múltiple (REEM), Spain
[p] Department of Neurology, Hospital Universitario Ramón y Cajal, Ctra de Colmenar Viejo km 9.100, 28034 Madrid, IRYCIS, Spain
[q] Immunology, Hospital Universitari de Bellvitge, Av. Granvia s/n, Hospitalet de Llobregat, Barcelona, Spain
[r] Department of Neurology, Hospital San Pedro de Alcántara, Avenida Pablo Naranjo s/n, 10003 Cáceres, Spain
[s] Immunology, Hospital Regional Universitario, Avda Carlos Haya, s/n, 29010 Málaga, Spain
[t] Immunology, Hospital Universitario Virgen de la Arrixaca, Ctra. Madrid-Cartagena, s/n, 30120 El Palmar, Murcia, Spain
[u] Department of Neurology, Hospital Universitario Donostia, P° Dr. Beguiristain, 107-111, 20014 San Sebastián, Spain

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ABSTRACT

Background: The presence of oligoclonal IgM bands (OCMB) in cerebrospinal fluid (CSF) is an unfavourable prognostic marker in multiple sclerosis. There is no commercial test to investigate OCMB status. However, a sensitive and specific isoelectrofocusing (IEF) and western blot method was described. We aimed to study the inter-centre reproducibility of this technique, a necessary condition for a reliable test to be incorporated into clinical practice.
Methods: The presence of OCMB was analysed by IEF and western blot with prior reduction of pentameric IgM. We assayed the reproducibility of this test in a blinded multicentre study performed in 13 university hospitals. Paired-CSF and serum samples from 52 neurological patients were assayed at every centre.
Results: Global analysis rendered a concordance of 89.8% with a kappa value of 0.71.
Conclusion: These data indicate that OCMB detection by means of IEF and western blot with IgM reduction shows a good interlaboratory reproducibility and thus can be used in daily clinical setting.

Abbreviations: OCMB, oligoclonal IgM bands; CSF, cerebrospinal fluid; IEF, isoelectrofocusing; MS, multiple sclerosis.

* Corresponding author at: Servicio de Inmunología, Hospital Ramón y Cajal, Carretera de Colmenar km 9.100, 28034 Madrid, Spain. Tel.: +34 913368795; fax: +34 913368809.
E-mail address: luisamaria.villar@salud.madrid.org (L.M. Villar).

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1. Introduction

Biomarkers contribute to an early diagnosis and prognosis of different disorders and may identify optimal responders to different therapies [1–3]. However the applicability of a candidate biomarker is a complex procedure including validation of the discovery results in uni and multi-centre studies. The final challenge is to incorporate the biomarker use into clinical practice, since these techniques are often difficult to standardise. To demonstrate inter-centre reproducibility prior to generalize the use of every particular biomarker should be mandatory, even more in case of non-commercial techniques, since this will warrant accurate results.

The presence of oligoclonal IgM bands (OCMB) plays an important role as prognostic marker in multiple sclerosis (MS) [4,5]. When detected by means of isoelectrofusing (IEF) and immunoblotting [6] they identify MS patients with high risk of suffering an aggressive disease course in terms of relapse rate and disability progression. These results have been validated in different cohorts [7,8], but the inter-centre reproducibility of the assay has not been explored yet. We assessed it by means of a blind multicentre comparative study in 13 university hospitals whose immunologists received previous training in performing the technique.

2. Patients and methods

2.1. Study design

Thirteen Spanish hospitals entered the study. The study was approved by the ethical committee of every hospital. Each centre contributed to the study with 13 paired aliquots of 0.1 ml of cerebrospinal fluid (CSF) and serum from four patients with different neurological diseases. All specimens were taken for routine detection of OMCB. Samples were stored frozen at −80 °C since collection. They were sent in dry ice to the coordinator centre (Ramon y Cajal Hospital, Madrid) that distributed a set of aliquots of the 52 pairs of samples to every participant centre. Transportation was made again in dry ice. After performing the oligoclonal band study, all centres sent their results to the coordinator, who remitted global results to all participants.

2.2. Patients

Patients included in the study showed the following diagnoses: MS (44), nonspecific headaches [2], Guillain–Barre syndrome [1], progressive supranuclear palsy [1], neuroborreliosis [1], vasculitis [1], amyotrophic lateral sclerosis [1] and cavum carcinoma [1]. The percentages of positive samples do not correspond with the prevalence of OCMB in these disorders since participants were encouraged to include IgM positive cases in the study. The reference centre (Laboratory of Hospital Ramon y Cajal in Madrid, where the technique was developed) reported 30 positive cases in the MS group and other two within the remaining eight cases, which corresponded respectively to patients with neuroborreliosis and cavum cancer, a rare nasopharyngeal tumour closely associated with an infection by Epstein Barr virus.

2.3. Oligoclonal IgM detection

The presence of OCMB was analysed in paired CSF and serum samples by IEF and immunoblotting as previously described [6]. Briefly, samples were incubated for 30 min with 50 mmol dithiothreitol at pH 9.5 to reduce IgM. IEF was performed at pH 5 to 8 and an antihuman IgM labelled with alkaline phosphatase was used in the immunodetection.

We considered that a patient showed oligoclonal IgM bands when we detected two or more bands in CSF that were not present in paired serum sample. This included CSF-restricted oligoclonal pattern or bands present in the serum with additional ones in the CSF. We considered a patient lacked oligoclonal bands if presenting a polyclonal pattern or the same oligoclonal bands in the serum and CSF.

2.4. Statistical analyses

We studied concordance of OCMB results among different groups by two different methods. The first one consisted in the analysis of the percentage of agreement with a reference laboratory. We established the laboratory of Hospital Ramon y Cajal in Madrid, where the technique was developed, as the reference centre. In addition, we calculated the kappa index, what assesses the agreement beyond that expected by random: a kappa value of 1.0 indicates 100% concordance. A kappa value of 0.0 indicates no concordance at all. Values between 0.6 and 0.8 indicate a substantial level of concordance [9]. Standard error of kappa index was calculated with the Jackknife method.

3. Results

All laboratories but one completed the study. The remaining one only informed OCMB results of 49 (94%) patients and could not identify the pattern of the remaining three. The concordance of every laboratory with the reference lab is shown in Table 1. The overall percentage of agreement was 89.83 ± 1.84% (mean ± error standard of the mean [SEM]). 75% of the centres showed a concordance higher than 85%. In all cases it was higher than 75%. The most common causes of false negative results came from a short time for colour development that

Table 1
Oligoclonal IgM band results of patients included in the study.

Laboratory	OCMB positive results (n = 32)			OCMB negative results (n = 20)			Overall agreement (%)
Reference lab	32	0		20	0		NA
Lab. number	Matches	Mismatches	Agreement (%)	Matches	Mismatches	Agreement (%)	
2	29	3	90.6	20	0	100	94.2
3	32	0	100	20	0	100	100
4	31	1	96.9	17	3	85	92.3
5	29	3	90.6	18	2	90	90.4
6	32	0	100	16	4	80	92.3
7	25	7	78.1	19	1	95	86.5
8	27	5	84.4	19	1	95	88.5
9	25	7	78.1	20	0	100	86.5
10	30	2	93.7	18	2	90	92.3
11	26	6	81.2	18	2	90	84.6
12	24	8	75	20	0	100	84.6
13	23	6[a]	79.3[a]	14	6	70	75.5[a]

OCMB: oligoclonal IgM bands; NA: not applicable.
[a] This laboratory did not inform the results of three patients.

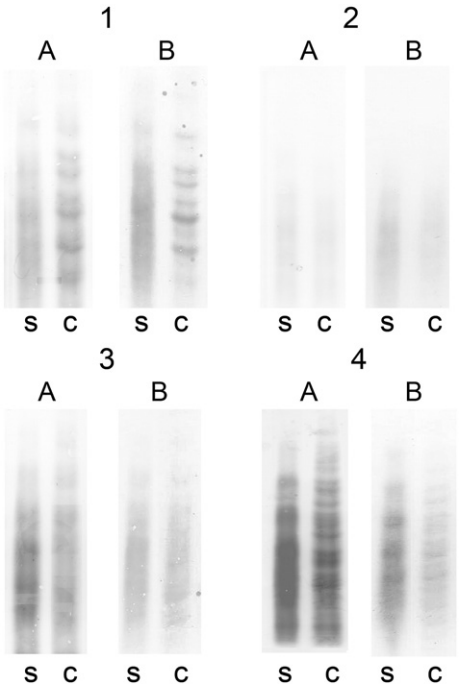

Fig. 1. Representative examples of concordant and discordant results obtained when exploring IgM patterns in the serum (s) and CSF (c). Results of reference centre (A) and other participant laboratories (B) are shown. 1 shows a positive pattern described by all laboratories in a patient with multiple sclerosis. 2 shows a negative pattern described by all laboratories in a patient with amyotrophic lateral sclerosis. 3 shows discordant results in a patient with vasculitis. Ten laboratories (A) reported a negative result (mirror pattern) other three (B) gave a positive result since they could not detect all serum bands. 4 shows discordant results obtained in other MS case. Nine laboratories reported a positive result (A). Other four (B) did not observe additional bands in the CSF and reported a negative (mirror) pattern.

prevented a clear visualization of the oligoclonal IgM pattern in some positive cases with low CSF IgM concentration. The most common reasons for false positive results were inadequate interpretation of mirror patterns. Fig. 1 shows representative examples of concordant and discordant positive and negative results.

The global analysis of the kappa index rendered a value of 0.71 (SEM: 0.053; 95% confidence interval: 0.608–0.822).

4. Discussion

Many molecules have been proposed as candidate biomarkers for MS diagnosis, prognosis and response to treatment. However, only a few have been integrated into routine clinical practice [10,11]. The most common reasons for this are the lack of validation, the complexity of the techniques or the low inter-assay reproducibility.

The detection of a biomarker must be accurate and as easy to carry out as possible. It is also important to demonstrate inter laboratory reproducibility to allow the comparison between different clinical centres. This is especially relevant in non-automatized techniques.

OCMB detection has proven to be an accurate prognostic biomarker in MS being these results validated in different cohorts [7,8]. Nevertheless, the lack of commercial kits for OCMB detection has limited the usefulness of this technique in clinical practice. However, a sensitive and specific IEF method for OCMB detection was described [6]. It is a simple manual technique that only requires certain laboratory skills. We have studied the reproducibility of this method in a multicentre study. Our results show a high rate of agreement between all the centres and the reference laboratory and a kappa index of 0.71, which indicates a substantial level of concordance [9].

In conclusion, these data show that OCMB detection performed in experienced laboratories by means of isoelectrofocusing and immunoblot gives reproducible inter-laboratory results and thus can be used in the clinical setting.

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