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Technical note

Utility of oligoclonal IgG band detection for MS diagnosis in daily clinical practice

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ABSTRACT

An early and accurate diagnosis of multiple sclerosis (MS) is very important, since it allows early treatment initiation, which reduces the activity of the disease. Oligoclonal IgG band (OCGB) detection is a good ancillary tool for MS diagnosis. However, it was argued that its usefulness was limited by the high interlaboratory variability. In the last years, different techniques for OCGB detection have appeared. We performed a blinded aleatorized multicenter study in 19 Spanish hospitals to assess the accuracy and reproducibility of OCGB detection in this new scenario. We studied cerebrospinal fluid (CSF) and serum samples from 114 neurological patients. Every hospital contributed to the study with triplicated pairs of CSF and

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serum samples of six patients and analyzed 18 different samples. Global analysis rendered a sensitivity of 92.1%, a specificity of 95.1% and a Kappa value of 0.81. This shows that current techniques for OCGB detection have good accuracy and a high interlaboratory reproducibility and thus, represent a good tool for MS diagnosis. When we analyzed separately the different techniques used for OCGB detection, the highest concordance was observed in western blot with alkaline phosphatase detection (kappa = 0.91). This indicates that high sensitivity techniques improve the reproducibility of this assay.

1. Introduction

MS is the most common demyelinating disease of the central nervous system in young adults. An early diagnosis of the disease is essential, since it has been described that early treatment improves patient outcome [1]. Since there are no diagnostic tests specific for the disease, different criteria have been established to contribute to early MS diagnosis [2,3]. These criteria include clinical and paraclinical data. Magnetic resonance imaging provides evidence of lesion dissemination in time and space and intrathecal IgG synthesis, demonstrated by the presence of oligoclonal IgG bands, prove the inflammatory origin of the lesions [3]. Oligoclonal IgG band (OCGB) detection has proven high sensitivity and specificity for MS diagnosis independently of magnetic resonance imaging data [4,5]. However, it has been objected that this technique shows high interlaboratory variability that limits its usefulness for MS diagnosis [2]. Nevertheless, the situation has changed in the last years because of the appearance of commercial kits and a high sensitivity method for OCGB detection [6]. We describe here the results of a blind multicentric study organized to check the reproducibility of OCGB detection in this new setting and assess their sensitivity and specificity for MS diagnosis.

2. Patients and methods

2.1. Study design

Nineteen Spanish hospitals entered this study organized by MS Spanish Network (REEM). The locations of the different participating centers are shown in Fig. 1. The study was approved by the ethical committees of every hospital. Each hospital contributed to the study with three paired aliquots of CSF and serum of six patients with different neurological diseases. Paired serum and CSF samples were obtained for routine detection of OGCB. They were stored frozen since collection. The time of storage ranged from a week to eight years. All centers stored their samples at −80 °C, with only one exception; in a center that sent their specimens less than a month after collection, samples were stored at −20 °C. Samples were sent in dry ice to the coordinator center (Hospital Ramon y Cajal, Madrid) that distributed them also in dry ice among the different participants. Sample size was calculated as previously described [7] considering a probable kappa of 0.8 ± 0.12. Distribution was randomized as described to avoid biases due to donor centers [8]. Each pair of samples was studied in three different centers and each center studied 18 pairs. The receptor laboratories were blind to patient diagnosis. Each center sent their oligoclonal band results to the coordinator, who remitted them to all participants. After this, each center notified their patient diagnoses to the coordinator center, where results were analyzed.

2.1.1. Patients

We studied 59 patients with MS and 55 with other neurological diseases (Table 1).

2.1.2. Oligoclonal IgG detection

The presence of OCGB was investigated by isoelectrofocusing and immunodetection in all centers. In five of them immunodetection was made by immunofixation with anti-IgG labeled with peroxidase (IFPO, Sebia commercial kit). In other six it was made by western blot with anti-IgG labeled with peroxidase (WBPO, Helena commercial kit) and finally, in the remaining eight it was made with anti-IgG labeled with alkaline phosphatase (WBAP) as previously described [6]. In all centers we considered a patient showed oligoclonal IgG bands when we detected two or more bands in CSF that were not present in paired serum sample.

2.1.3. Statistical analyses

Concordance of results between different groups was studied by kappa index (what assesses the agreement beyond that expected by random: a kappa value of 1.0 indicates 100%

Fig. 1. Location of the different centers participating in this study.

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

Pathology	Number of patients	Number of specimens (Pairs S/C)	Global results T: P/N	Results WBPO T: P/N	Results IFPO T: P/N	Results WBAP T: P/N
Multiple sclerosis	59	177	177:163/·14	44:37/7	52:49/3	77:73/4
Stroke	9	27	27: 0/27	12: 0/12	5: 0/12	10: 0/12
Myelitis	7	21	21: 1/20	7: 0/7	3: 1/2	11: 0/11
Normal pressure hydrocephalus	7	21	21: 0/21	8: 0/8	8: 0/8	5: 0/5
Guillain-Barré syndrome	6	18	18: 1/17	8: 0/8	2: 0/2	8: 1/7
Amyotrophic lateral sclerosis	3	9	9: 0/9	4: 0/4	3: 0/3	2: 0/2
Paraneoplastic syndrome	3	9	9: 0/9	2: 0/2	3: 0/3	4: 0/4
Pseudotumor cerebri	3	9	9: 0/9	2: 0/2	1: 0/1	6: 0/6
Autosomal recessive hereditary spastic paraplegia	3	9	9: 0/9	3: 0/3	3: 0/3	3: 0/3
Neuromyelitis optica	3	9	9: 0/9	2: 0/2	4: 0/4	3: 0/3
Optic neuritis	2	6	6: 0/6	4: 0/4	1: 0/1	1: 0/1
Epilepsy	1	3	3: 0/3		3: 0/3	
Glioblastoma multiforme	1	3	3: 0/3	3: 0/3		
Lewy body disease	1	3	3: 0/3	1: 0/1	1: 0/1	1: 0/1
Meningeal carcinomatosis	1	3	3: 0/3	2: 0/2		1: 0/1
Migraine	1	3	3: 0/3		1: 0/1	2: 0/2
Myelinolysis	1	3	3: 0/3	1: 0/1		2: 0/2
Neurowipple	1	3	3: 0/3	1: 0/1		2: 0/2
Vasculitis	1	3	3: 0/3	1: 0/1		1: 0/1
Pineal germ cell tumor	1	3	3: 3/0	1: 1/0	1: 1/0	1: 1/0

Pairs S/C: Pairs serum/ cerebrospinal fluid; T: P/N: Total samples: Positive/Negative; WBPO: Oligoclonal bands assayed by isoelectrofocusing and western blot with anti-human IgG labeled with peroxidase; IFPO: Oligoclonal bands assayed by isoelectrofocusing and immunofixation with anti-human IgG labeled with peroxidase; WBAP: Oligoclonal bands assayed by isoelectrofocusing and western blot with anti-human IgG labeled with alkaline phosphatase.

concordance. A kappa = 0.0 indicates no concordance at all). Standard error of kappa index was calculated with the Jacknife method [9].

To measure accuracy of the test for MS diagnosis, we considered as true positives, those positive results obtained in MS patients and as false positives, those obtained in other neurological diseases. Negative results obtained in non-MS patients were considered as true negatives and negative results obtained in MS were considered as false negatives. The following ratios were used:

$$\text{Sensitivity} = [TP / (TP + FN)] \times 100;$$

$$\text{Specificity} = [TN / (TN + FP)] \times 100$$

3. Results

Results of the samples analyzed with the three methods compared here are shown in Table 1. We first studied reproducibility of OCGB detection. Global analysis rendered a kappa index of 0.802 (95% Confidence interval: 0.716–0.892). The results of the laboratories grouped according to the technique used for oligoclonal band detection gave a kappa value of 0.77 for WBPO, 0.81 for IFPO and 0.91 for WBAP.

We next studied accuracy for MS diagnosis. Global analysis gave a high sensitivity (91.2%) and specificity (97.0%). When the different techniques were studied separately, laboratories using WBPO showed a sensitivity of $83.1 \pm 7.91\%$ (mean $\pm$ standard error) and a specificity of 98.6 ± 1.51. Those analyzing OCGB with IFPO had a sensitivity of 94.16 ± 3.64 and a specificity of 94.44 ± 3.64. Finally, laboratories using WBAP showed a sensitivity of 95.20 ± 2.51 and a specificity of 97.3 ± 1.79.

4. Discussion

It has been reported that positive CSF findings can be important to support the inflammatory nature of demyelinating lesions, to evaluate alternative diagnoses and to predict conversion to clinically definite MS after a clinically isolated syndrome suggestive of MS [3–5]. After a negative CSF value, extreme caution needs to be taken before making a diagnosis of MS [3]. However, the use of CSF in MS diagnosis was limited by the high variability of different "in-house" methods [10–13]. In the last years two different kits for oligoclonal IgG detection have been commercialised and a high sensitivity method described. To study the accuracy of these methods for MS diagnosis we conducted a multicenter study including specialised laboratories of MS units and general laboratories from 11 different towns in Spain. Our results show that all current techniques for oligoclonal IgG band detection have high sensitivity and specificity for accurate MS diagnosis. When studying reproducibility of the different techniques, we observed that although those based in IgG detection with peroxidase-labeled antibodies show acceptable values of kappa index, the best results are obtained with the high sensitivity technique based in IgG detection with antibodies labeled with alkaline phosphatase. In summary, oligoclonal band detection using widely available stardardized techniques and high sensitive methods provides a reliable tool for MS diagnosis. In addition, using high sensitivity technique warrants a high inter-assay reproducibility.

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