

STUDY OF OLIGOCLONAL BANDS RESTRICTED TO THE CEREBROSPINAL FLUID IN MULTIPLE SCLEROSIS PATIENTS IN THE CITY OF SÃO PAULO

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Abstract – The frequency of oligoclonal bands (OCB) restricted to the cerebrospinal fluid (CSF) from patients with multiple sclerosis (MS) varies widely in different populations. The objective of this study was to determine the frequency of these OCB in a group of MS patients in the city of São Paulo. Techniques used to detect OCB consisted of isoelectric focusing followed by immunoblotting. Oligoclonal bands were found in 49 (54.4%) out of 90 patients with clinically definite MS; in (31.2%) of the 16 patients with clinically isolated syndrome; in 7 (17.9%) of 39 patients with inflammatory disorders of the central nervous system (IDCNS), and in none of the individuals with no neurological condition (control group). The specificity of the method was 100% when compared to the control group and 82.1% when compared to the IDCNS group. These results suggest that the frequency of CSF OCB is much lower in Brazilian MS patients from São Paulo city than that reported in MS series from Caucasian populations.

KEY WORDS: multiple sclerosis, cerebrospinal fluid, oligoclonal bands, Brazilian population.

Estudo de bandas oligoclonais restritas ao líquido cefalorraquidiano em pacientes com esclerose múltipla na cidade de São Paulo

Resumo – A frequência da detecção de bandas oligoclonais (BOC) restritas ao líquido cefalorraquidiano (LCR) em pacientes com esclerose múltipla (EM) varia amplamente em diferentes populações. O objetivo deste estudo foi determinar a frequência destas BOC em pacientes com EM em amostra de população da cidade de São Paulo. A técnica utilizada para a detecção das BOC foi a focalização isoeletrica, seguida do imunoblotting. BOC foram detectadas: em 49 (54,4%) de 90 pacientes com EM clinicamente definida; em 5 (31,2%) de 16 pacientes com síndrome clínica isolada; em 7 (17,9%) de 30 pacientes com doenças inflamatórias do sistema nervoso central (DISNC); e em nenhum indivíduo sem doença neurológica. A especificidade do método foi 100% quando comparada ao grupo controle e 82,1% quando comparada ao grupo de DISNC. Estes resultados sugerem que a frequência de BOC no LCR é mais baixa em pacientes da cidade de São Paulo portadores de EM do que aquelas descritas em populações caucasianas.

PALAVRAS-CHAVE: esclerose múltipla, líquido cefalorraquidiano, bandas oligoclonais, população brasileira.

Multiple sclerosis (MS) is an inflammatory central nervous system (CNS) disorder, increasingly diagnosed in tropical countries. The anatomical substrate is the finding of inflammation, demyelination, axonal degeneration and gliosis. Although its etiology still remains a mystery, autoim-

mune mechanisms must play an essential role in its pathogenesis¹⁻⁴. MS diagnosis is still largely based upon clinical evidences. However, magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF) analysis are important tools for recognizing the topography, extent, phase of the le-

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sions, characterization of the local immune response and differential diagnosis with other neurological diseases^{2,5,6}.

CNS inflammation in MS can be estimated by intrathecal production of immunoglobulins (IgG). This production can be assessed by the finding of IgG bands in the CSF system without a clear recognition of the same bands in the serum, i.e. CSF oligoclonal bands (OCB)^{1,6}.

Different MS populations living in different countries appear to have different prevalence rates of CSF OCB. The prevalence rate is as high as 90 to 95% in Scandinavia⁷⁻⁹, and as low as 30 to 60% in China, Japan, Lebanon and India¹⁰⁻¹³. We describe the frequency of OCB in the CSF of a group of MS patients in the city of São Paulo, Brazil.

METHOD

The cohort comprised 164 patients seen at the Demyelinating Disorders Outpatient Clinics of the University of São Paulo Medical School. All patients or their legal guardians signed the informed consent agreement. The study was authorized by the Research Ethics Committee of the institution.

The patients' history, physical examination and analysis of the MRI were all done at the first visit, and then re-evaluated by a second researcher. Both researchers were neurologists with expertise in MS diagnosis. Diagnosis was based upon the international criteria of McDonald, revised in 2005¹⁴.

Serological tests (hemogram; erythrocyte sedimentation rate; urea and creatinine; transaminases; total and fractionated bilirubin; glycemia; calcium; free thyroxin; thyroid-stimulating hormone; C-reactive protein; anti-nuclear factor; latex test, fractions of the complement, anticardiolipin antibodies; cyanocobalamin and folate dosage; immunological reactions for syphilis, hepatitis B and C, HIV and HTLV) were requested to exclude diseases that could have differential diagnosis with MS.

No OCB investigation was made previously in CSF from these patients. So, the results of the CSF analysis were not used to establish the diagnosis or to include patients in this study.

We divided subjects into four different groups. Group 1 comprised 90 patients with clinically-definite multiple sclerosis (CDMS) according to McDonald's criteria¹⁴: 61 with relapsing remitting form (RR); 11 with secondary-progressive form (SP); 16 with primary-progressive form (PP); 2 with progressive relapsing form (PR). Group 2 comprised 16 individuals who presented a clinically isolated syndrome (CIS) indicative of a CNS first demyelinating event. Group 3 comprised 39 patients with a variety of chronic inflammatory disorders of the CNS. Group 4 comprised 19 subjects with neither neurological complaints nor infectious conditions, whose CSF sample was collected during anesthetic procedure for minor surgery.

Blood serum and CSF analysis were carried out simultaneously. The CSF analysis included the classical routine tests (cytomorphological profile; determination of total protein content; dosage of glucose) and immunological reactions for the infectious diseases more frequently observed in tropical countries^{2,3,6}.

The technique used to assess the OCB was isoelectric fo-

cusing (IF) in polyacrylamide gel (ETC – Elektrophorese – Technik Bahnhofstr). Each paired sample of CSF and blood serum was submitted to IF (CWP-400 Isolab Inc) always with a positive control. IF was transferred to a nitrocellulose membrane (Bio Agency), followed by immunoblotting using a primary antibody (Goat-anti-human IgG, Sigma) and a polyclonal rabbit anti-goat immunoglobulin (Dako Cytomation) as a secondary antibody. Subsequently, the reaction was developed using 3-amino-9-ethylcarbazole (Sigma). OCB were considered recognized when two or more bands were found in the CSF, but absent in the serum. All IF were examined by two specialists who were not aware to which group the samples belonged. In a case of discrepant or dubious interpretation of results, the sample was processed again.

Quantitative intrathecal immunoproduction of IgG was carried out in parallel. Concentrations of IgG and albumin both in the serum and CSF were measured by nephelometry. This allowed to determine: (1) the CSF/serum ratio for IgG or IgG quotient (IgG_Q) and the CSF/serum ratio for albumin or albumin quotient (A_Q); (2) IgG index (the ratio between IgG_Q and A_Q); (3) Reiber and Felgenhauer nomogram.

A_Q values were considered increased for values of $6,5 \times 10^{-3}$ or higher. A_Q over 8×10^{-3} in patients with more than 40 years are considered normal. IgG index was considered increased for values ≥ 0.8 .

RESULTS

Table 1 shows the demographic characteristics of the patients.

Out of the 90 patients with CDMS, 49 (54.4%) presented OCB restricted to the CSF. These OCB were detected in 21 out of 41 CDMS patients (51.2%) who were in use of immunomodulatory agents. Fig 1 shows the frequencies of positive CSF OCB according to the clinical course of MS (27 RR, 6 SP, 14 PP and 2 PR).

The sensitivity of OCB in patients with MS in our study was 54.4%. The specificity was 100% when patients without neurological disease were used as controls; 82.1% when patients with inflammatory diseases of the CNS were used as the control. These data, together with the accuracy and the positive and negative predictor values are shown in Table 2.

As shown in Fig 1, OCB were more frequent in progressive forms (PP, SP and PR) than in RR form of MS ($p=0.009$).

In the group of patients with CIS, positive CSF OCB were found in 5 (31.2%) out of 16 patients. There was no statistical difference between the frequency of OCB in patients with CIS when compared to patients with RR form of MS ($p=0.35$) (Fig 1).

OCB were found in 7 (17.9%) out of 39 patients with inflammatory diseases of the CNS. This frequency is much smaller than that observed for the pool of MS patients ($p=0.00002$) and for the subgroup of patients with RR form of MS ($p=0.001$).

No OCB were found in patients from the control group.

Table 1. Demographic characteristics of the studied population.

Groups	N	Age media	Gender F/M	Ethnical origin	
				European	African
MS	90	35.6±12.1	2.9/1	72%	18%
CIS	16	25.4± 8.2	2.2/1	81%	19%
ID	39	31.5±18.4	2.3/1	54%	46%
NNC	19	39.2±16.2	2.2/1	63%	37%

N: number; MS: multiple sclerosis; CIS: clinical isolated syndrome; ID: inflammatory disease of the central nervous system; NNC: no neurological complain.

Table 2. Indicators of significance in the detection of OCB for the diagnosis of multiple sclerosis, using the isoelectric focusing technique followed by immunoblotting.

OCB in multiple sclerosis	Control group (%)	ID group (%)
Sensitivity	54.4	54.4
Specificity	100	82.1
Accuracy	77.2	68.2
Positive predictor value	100	75.2
Negative predictor value	68.6	64.2

OCB: oligoclonal bands; control group: patients submitted to spinal tap for anesthesia; ID group: inflammatory diseases of the central nervous system.

Table 3. Frequency of restricted oligoclonal bands to the cerebrospinal fluid in patients with inflammatory diseases of the central nervous system.

	OCB+		
	n	n	%
Relapsing optic neuritis	2	2	100
HTLV myelopathy	2	1	50
Schistosomiasis	2	1	50
CNS vasculitis	15	3	20
Neuromyelitis optica	6	0	0
Relapsing myelitis	4	0	0
ADEM	3	0	0
Hepatitis B and C	3	0	0
Syphilis	1	0	0
Viral optic neuritis	1	0	0

OCB+: presence of oligoclonal bands by isoelectric focusing and immunoblotting; n: number of patients; CNS: central nervous system; ADEM: acute disseminated encephalomyelitis.

Table 3 shows the frequency of positive OCB in the CSF of patients with a variety of inflammatory diseases of the CNS. The frequencies of OCB in the CSF of MS patients in different populations worldwide are shown in Table 4.

The IgG index was abnormal in 42 of the 90 patients with CDMS (46.6%), with a median of 0.8, media of 1.02

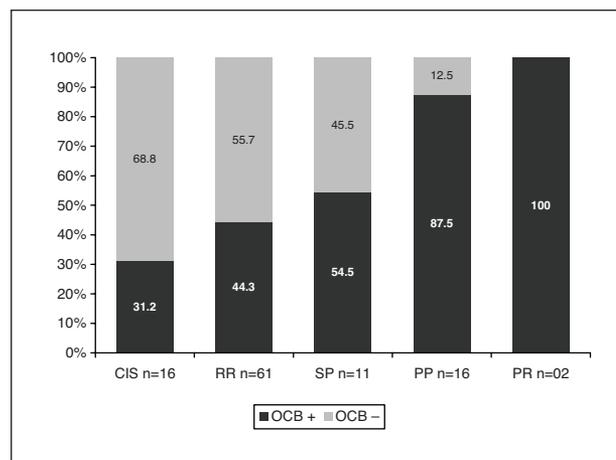


Fig 1. Correlation between the clinical forms of multiple sclerosis and analysis of cerebrospinal fluid. CIS: clinically isolated syndrome; RR: relapsing remitting; SP: secondary-progressive; PP: primary-progressive; PR: progressive relapsing; OCB: oligoclonal bands by isoelectric focusing and immunoblotting. No-association test for CIS and RR: p=0.35 and RR to progressive forms: p=0.009.

(SD 0.67). Sensitivity of the IgG index for the diagnosis of MS was 46.6%; specificity was 94.8% when patients without neurological disease were used as controls, and 64.2% when patients with inflammatory diseases of the CNS were used as controls.

There was concordance between the presence of OCB and increased IgG index in 37 patients with MS (41.1%). Detection of OCB and/or increased values of IgG index may allow to characterize the CSF immunoproduction of IgG in 57 out of 90 patients (63.3%).

In the group of patients with inflammatory diseases of the CNS, increased IgG index was found in 14 of 39 patients (35.8%).

In the control group, one of the 19 patients presented a slight increase in IgG index (5.2%).

Reiber and Felgenhauer nomogram for quantitative evaluation of immunoproduction is shown in Fig 2. The comparative study of qualitative (OCB) and quantitative IgG immunoproduction (IgG index) is shown in Fig 3.

The median cell count in the CSF was 5 cell/mm³.

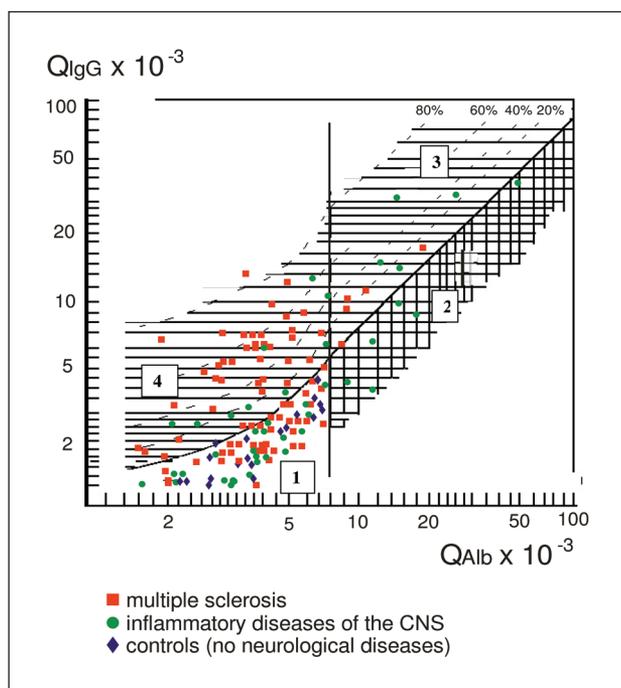


Fig 2. Quotient of immunoglobulin plotted against the quotient of albumin for the three groups of patients. QIgG: IgG quotient; QAlb: albumin quotient; region 1: normal; region 2: blood-brain barrier breakdown; region 3: blood-brain barrier breakdown associated with local synthesis; region 4: local synthesis.

Pleocytosis up to 30 cells/mm³ (mononuclear cells) was seen in 51.7% of the patients. In 98.1% of the patients with MS, the total protein content (mean: 31.3 mg/dl, SD: 7.2, median: 29 mg/dL) was below 72 mg/dl and the total cell count was below 30 cells/mm³. Glucose concentration was normal in all patients. Q_A was high in 14.1%, suggesting some degree of dysfunction of the blood-CSF barrier.

All immunological tests showed either normal results or slight alterations without clinical significance.

DISCUSSION

Our study showed that 54.4% of the patients with CDMS had OCB restricted to the CSF. The sensitivity of this finding was 100% when compared to patients without neurological diseases, and 82.1% when compared to patients with inflammatory diseases of the CNS.

The frequency of OCB found in this study is low in comparison with both the prevalence observed in European and North-America MS patients and other previous Brazilian studies which found a frequency of 85%¹⁷ and 91%¹⁸. However, this low rate is similar to that observed in other regions of the world (Table 4).

Three main hypotheses may be postulated to explain the lower results for OCB detection in our study: (a) there was a bias in clinical evaluation of the patients included; (b) there were problems in methodology for CSF analy-

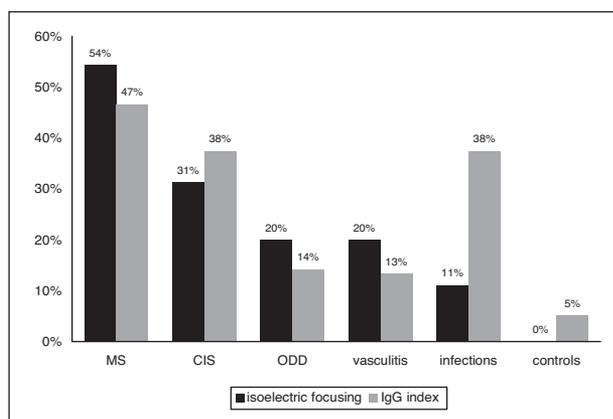


Fig 3. Comparison of the frequency of positive oligoclonal bands and IgG index ≥ 0.8 in the cerebrospinal fluid of patients with different central nervous system disorders and controls. MS: multiple sclerosis; CIS: clinically isolated syndrome; ODD: other demyelinating disease, include neuromyelitis optica, acute disseminated encephalomyelitis, relapsing myelitis and relapsing optic neuritis; controls: patients submitted to spinal tap for anesthesia.

sis of these patients; (c) OCB does occur in a smaller percentage in São Paulo city by unknown reasons.

As for the first hypothesis, a special and intensive care was taken to include only patients with MS clinical definite. The inclusion criteria strictly followed recommendations for MS diagnosis, widely discussed in the international panel of McDonald revised in 2005¹⁴. These patients were carefully and independently examined by two experienced neurologists, with expertise in MS diagnosis and treatment. Therefore, this hypothesis is highly unlikely to explain this finding.

As for the second hypothesis, some considerations must be made: (1) the results of CSF analysis were not used as an inclusion criteria; (2) our study was carried out in an academic reference laboratory with a very experienced research staff in CSF analysis, mainly in electrophoretic methods. The CSF sample was processed using high standards according to CSF Analysis Consensus of Freedman MS et al.⁶. This included the use of the most up-to-date internationally accepted technology and strict adherence to the criteria of reproducibility ensuring high levels of fidelity of the results; (3) the IF method followed by immunoblotting used in our patients is the most sensitive and suitable technique for detecting OCB when MS is suspected⁶. Nevertheless, the use of this technique is not a universal practice: according to the College of American Pathologists Survey¹⁶, only 10% of the 235 laboratories in the USA that conduct CSF analysis use this technique to detect OCB. This clearly shows that, unlike our group, in practice, the most suitable method for investigating OCB has been underutilized in many research centers, even in the most developed countries; (4) the analysis of paired CSF and serum electrophoresis was made by two skilled

Table 4. Frequency of oligoclonal bands restricted to the cerebrospinal fluid of patients with multiple sclerosis in different countries.

Country	Method	% OCB +			References
		MS	ID	Control	
Norway	IEF IF	100%		9%	Lunding J et al. 2000 ⁹
Slovenia	IEF IF	95%			Rot et al. 2008 ⁸
Norway	IEF IB	93%	67%	4%	Mygland A et al. 2007 ⁷
USA	IEF IB	90%		6%	Fortini AS et al. 2003 ²⁰
Canada	IEF IB	89%			Siritho S et al. 2009 ²⁶
Italy	IEF	89%			Annunziata P et al. 2006 ²⁷
Spain	IEF IB	87%	52%	17%	Falip M et al. 2001 ¹⁵
France	IEF	85%	21%	8%	Bourahoui A et al. 2004 ²⁸
Portugal	IEF IF	82%	40%	3%	Sá MJ et al. 2005 ¹⁹
Czech Rep.	IEF IB	81%		7%	Bednarova et al. 2005 ²⁹
Holland	IEF IF	76%			Koch M et al. 2007 ³⁰
France	IEF IF	76%		7%	Fromont A et al. 2005 ²¹
China	IEF SS	63%	47%	4%	Li B et al. 2007 ¹⁰
Japan	IEF IF SS	56%			Fukazawa et al. 1998 ¹¹
China	NR	40%			Lau KK et al. 2002 ²³
Lebanon	NR	40%			Yamout B et al. 2008 ¹²
Japan	IEF	33%			Tanaka K et al. 2005 ²⁴
India	NR	30%			Syal P et al. 1999 ¹³
Taiwan	IEF	7%	0%		Chang KH et al. 2006 ³¹
Brazil	IEF IB	54%	17%	0%	Present study

ID: inflammatory diseases of the central nervous system; IEF: isoelectric focusing; IB: immunoblotting; IF: immunofixation; SS: silver staining; NR: no reference.

researchers unaware of the diagnosis of the patients. So, the low frequency of OCB founded in this study may not be ascribed to problems in CSF analysis.

Apart from the relatively low sensitivity, the detection of OCB seemed to have a low specificity, mainly when a blind method of analysis was used^{7,8}. OCB have been described in other inflammatory diseases, their frequency of detection ranging from 25 to 80%^{5,7,19}, as well as in non-inflammatory diseases of the CNS (in up to 17% of patients¹⁵). However, there is no consensus about this low specificity of OCB in MS.^{9,20,21}

In the MS group, OCB were observed more frequently in progressive forms than in RR form. This may be explained by the continuous course of the disease and a consequent more effective exposition to immunological mechanisms in those forms of MS.

On the other hand, the frequency of OCB observed in patients with CIS is similar to that observed in patients with RR form of MS. This observation may give rise to two comments: (a) CIS is a true form of MS; (b) our clinical appraisal of patients included in this study was fully satisfactory.

The quantitative determination of immunoproduction of IgG must be made in addition to OCB search. This complementary method seems to improve the sensitivity of CSF diagnosis in MS, since 8.9% of patients with definite MS diagnosis presented increased IgG index without OCB in CSF. However, it remains to be explained why this difference occurs and what is the meaning of this finding.

The relatively low frequency of increased A_Q observed in CSF samples studied suggests that our MS patients do not often have impairment of the blood-brain-barrier (BBB) function.

The Reiber and Felgenhauer nomogram is a very interesting quantitative method to evaluate CSF intrathecal IgG production. It permits to recognize: (1) the patients in which no IgG immunoproduction nor BBB breakdown is happening (region 1 of Fig 2); (2) the occurrence of BBB breakdown, more usually seen in inflammatory diseases other than MS (region 2 of Fig 2); (3) the association of immunoproduction and BBB breakdown as it is shown in the region 3 of the Fig 2. This characteristic may be important in patients with MS, since it is probably the only complementary diagnostic method that allows recogni-

tion of the acute phase phenomena (BBB breakdown) taking place over an older chronic immunoproducing process. This information may be helpful for distinction when images seen at MRI may correspond to acute lesions or only to cicatricial demyelination; (4) the graphic representation of samples from patients with IgG immunoproduction is displayed in a similar way to that of the Link's IgG index analysis (region 4 of Fig 2).

Although CSF analysis in MS aims to characterize the local immune response in the CNS, a number of other features may differentiate MS from other diseases of the CNS, such as: (1) in MS, there is usually a high number of OCB; (2) in MS, the simultaneous presence of OCB in CSF and in the blood serum is uncommon, even if a high number of bands have been identified in the CSF; (3) there usually is a normal number of cell count or a slight pleocytosis, usually below 30 cells/mm³, with an absolute predominance of lymphomononuclear cells; (4) the total protein content is usually normal or slightly above normal, generally below 70 mg/dL; (5) the concentration of glucose should be within reference limits^{2-4,20}.

These data show the importance of the CSF analysis in all MS patients, mainly in those with initial clinical symptoms of demyelinating diseases. The detection of this CSF profile may be helpful to suggest the occurrence of an inflammatory process in the CNS, especially in patients whose clinical and imaging abnormalities are not clear-cut, thus providing the possibility of early diagnosis and treatment^{8,25}. This is more important in progressive forms of MS, mainly in the primary progressive form.

In conclusion, the expected results for the frequency of OCB in MS patients in Brazil cannot be estimated by simple extrapolation from the data obtained in European or North American populations. Possibly, variations in the clinical presentation and genetic susceptibility may determine the occurrence of significant variations^{11,22-24}.

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