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MULTIPLE SCLEROSIS IN BRAZIL

ANALYSIS OF CEREBROSPINAL FLUID BY STANDARD METHODS

MARZIA PUCCIONI-SOHLER, FABIOLA PASSERI*, CRISTIANE OLIVEIRA**, CARLOS OTÁVIO BRANDÃO*, REGINA PAPAIZ-ALVARENGA****

ABSTRACT - The demonstration of intrathecal IgG synthesis has been used as an important laboratory parameter to support the diagnosis of multiple sclerosis(MS). The Committee for European Concerted Action for Multiple Sclerosis has recommended a protocol for the assessment of intrathecal IgG synthesis. We applied this methodology to determine the cerebrospinal (CSF) profile of 128 Brazilian patients with MS. We detected hypercytosis lower than 35 cells/mm^3 in 97%, protein lower than 80mg/dl in 99%, normal blood-CSF barrier function in 76%, increased IgG local production around 53% and oligoclonal IgG bands by isoelectric focusing in 85% of the definite MS patients. The diagnostic accuracy of the quantitative analysis was lower than the qualitative. The detection of oligoclonal bands was especially important in the cases of normal quantitative assays of IgG. In addition, we found a lower frequency of inflammatory reaction in CSF in our MS cases, in comparison to some European studies.

KEY WORDS: multiple sclerosis, cerebrospinal fluid, oligoclonal bands, isoelectric focusing.

Esclerose múltipla no Brasil: análise do líquido cefalorraquidiano por métodos padronizados

RESUMO – A demonstração da síntese intratecal de IgG tem sido usada como importante parâmetro de apoio laboratorial ao diagnóstico de esclerose múltipla (EM). O Comitê Europeu de Ação para Esclerose Múltipla recomenda um protocolo para avaliação de síntese intratecal de IgG. Esta metodologia foi utilizada para determinar o perfil do líquido cefalorraquidiano (LCR) de 128 pacientes com o diagnóstico de esclerose múltipla. Verificou-se pleocitose menor do que 35 células/mm^3 em 97%, produção local de IgG por métodos quantitativos em 53% e presença de bandas oligoclonais em 85% dos casos de EM clinicamente definida. A análise qualitativa apresentou maior acurácia diagnóstica do que a avaliação quantitativa de IgG. A detecção de bandas oligoclonais foi especialmente importante nos casos de analise quantitativa de IgG. Observamos menor frequência de reação inflamatória no LCR, em relação aos estudos europeus.

PALAVRAS-CHAVE: esclerose múltipla, líquido cefalorraquidiano, bandas oligoclonais, focalização isoelétrica.

The majority of multiple sclerosis (MS) studies have reported differences in the geographic distribution[1]. It seems to be related with different aspects as: genetic and environmental factors[2]. The frequency and distribution of multiple sclerosis in the Brazilian population is unknown, but the disease is not rare in Brazil[3].

The role of cerebrospinal fluid (CSF) analysis has been well documented as an aid to the diagnosis of MS[4-6].The most important aspect of CSF study is to demonstrate the inflammatory

Laboratório Especializado de LCR (Neurolife) Rio de Janeiro, Projeto Atlântico Sul, Rio de Janeiro, Brazil: *Neurologista; **Estagiária; ***Coordenadora do Projeto Atlântico-Sul. Aceite: 21-agosto-1999.

Dra. Marzia Puccioni-Sohler - Rua Dezenove de Fevereiro 185/705 - 22280 030 Rio de Janeiro RJ - Brasil. Fax 55 21 295 1903. Email: sohler@netyet.com.br

origin of the disease in the central nervous system (CNS) through the detection of a local immune response[4]. The "Committee for European Concerted Action for Multiple Sclerosis", (CECAMS) suggested that the routine CSF analysis should include the evaluation of: cytology, blood-CSF barrier function and intrathecal synthesis of IgG[4]. These tests may be useful not only in supporting the diagnosis, but also in some cases to exclude MS[7-9].The combination of quantitative and qualitative methods of analysis should be performed for the intrathecal synthesis of total IgG[4]: The use of the CSF/serum quotient was considered to be of great value to determine the IgG synthezised in CNS, differentiating it from the fraction derived from blood[10]. The analysis of oligoclonal IgG bands by isoelectric focusing is the most sensitive test to detect intrathecal synthesis of IgG[11-13].It is considered essential to the routine examination of the CSF[4].The detection of the intrathecal synthesis of specific antibodies (against measles, rubella, varicella zoster) may also contribute to the diagnosis of MS [5,14].

The objective of this study was to standardize the CSF analysis in patients with MS in Brazil. This resulted in the delineating of CSF profile of Brazilian MS patients, using different tests for the evaluation of intrathecal IgG synthesis, based on pre-established reference values. The quantitative IgG evaluation included IgG index and IgG loc (locally synthezised)(mg/l)[8,9]. In addition, we also studied the IgG% and gamma globulin% of total protein in CSF [6,15]. These tests are also usually performed as a laboratory routine. Oligoclonal IgG bands were sought in both CSF and serum.

METHOD

Paired CSF and serum samples of 128 patients from different parts of Brazil, seen in Rio de Janeiro and registered in the South Atlantic Project, were collected during the period of March 1995 to June 1998. All the cases were examined by neurologists of the MS Brazilian Group[3]. The diagnosis was based on the Poser et al. criteria[1]: 103 were clinically definite and 25 clinically probable MS. The presence of rheumatologic and infectious diseases, and B12 deficiency were excluded by routine serological evaluation. All had negative serology for HIV-1 and HTLV-I.

CSF analysis

The CSF examination included white blood cells (WBC) count, glucose and protein analysis. Albumin and IgG concentrations were determined in serum and CSF by nephelometry, using the QM300 Protein Analysis System (Sanofi Diagnostics Pasteur Instruments, USA). The CSF-blood barrier function was based on the albumin quotient:

Q_{alb} = albumin$_{CSF}$/albumin$_{serum}$ (Reference Value $Q_{alb} < 8 \times 10^{-3}$).

The quantitative evaluation of intrathecal IgG synthesis was based on:

1. IgG index (Olsson and Pettersson[11]). An IgG index below 0.7 was considered normal.

· IgG index = Q_{IgG} / Q_{alb}

2. IgG (loc) (mg/l) (Reiber & Felgenhauer[9]). Reiber and Felgenhauer elaborated a diagram based on a hyperbolic function to determine the relation between the CSF/serum quotient and the individual CSF-blood barrier function.

· IgG (loc) (mg/l)= [QIgG - Q_{Lim} (IgG)]. IgG $_{serum}$

3. IgG% = CSF-IgG/total protein ratio (Fishman[15]). The normal ratio of the IgG and protein in CSF should be not higher than 15%.

4. Gamma globulin % fraction of the total protein in CSF by eletrophoresis on cellulose acetate with previous concentration. The upper normal limit was considered 14% of the total protein[15].

The qualitative method for the evaluation of local synthesis consisted in the detection of oligoclonal IgG bands by isoelectric focusing (IEF) of serum and CSF on polyacrilamide gel (Pharmacia) with silver staining according to a previously published method[12]. The presence of oligoclonal bands restricted or with additional bands in CSF and serum was considered as indicative of local synthesis of IgG, as recommended by the CECAMS[4].

Statistical analysis

The clinical and laboratory data of definite and probable MS groups were compared using the followling statistical studies: Student *t* test for mean values, chi-square or Fisher exact test for frequency and Mann-Whitney test for median. *P* values < 0.05 were considered statistically significant.

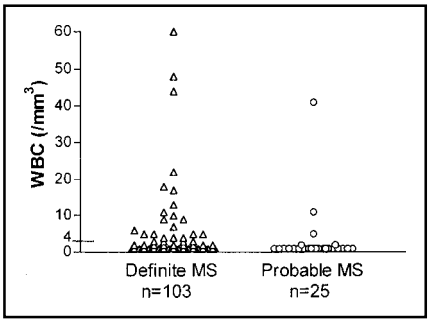

Fig 1. Cytology examination in CSF of 128 MS patients. WBC, White blood cells.

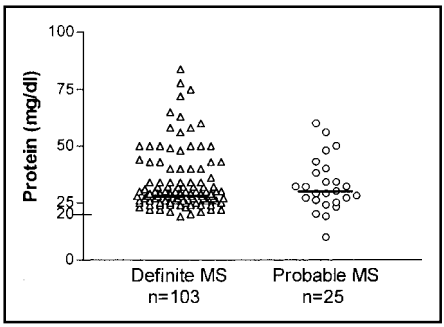

Fig 2. Protein analysis in CSF of 128 MS patients. The black line indicates the median. No difference was observed in both groups.

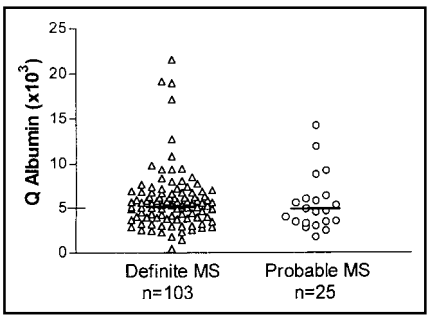

Fig 3. Albumin Quotient evaluation (Q Alb) in 128 MS patients. The black line indicates the median. No difference was observed in both groups. The Albumin Quotient was used as a parameter of blood-CSF barrier function. Q Alb higher than 8.0 x 10^{-3} was a parameter of Blood-CSF barrier dysfunction. The majority of the cases displayed a normal Albumin quotient in both groups.

RESULTS

Patient Characteristics

The group of patients included 89 women and 39 men, ranging in age from 14 to 62 years. The duration of symptoms was greater in the clinically definite cases [median age (min-max) 60 (4-348) months], than in the probable MS cases, 6.5 (0.4-204) months (p=0.007).There was a significant difference in the number of attacks between both groups of individuals: [median (min-max) 3(2-14)], for the definite and 1 (1-3), for the probable MS (p=0.0001).

CSF analysis

The CSF data of definite and probable MS patients are shown in Tables 1 and 2. There were no differences in the cytology, protein and albumin quotient between the groups (Figs 1, 2, 3). WBC counts higher than 35/mm^3 were observed in 3% (3/98) of the definite MS cases and 4% (1/25) of the probable. Levels of protein higher than 80 mg/dl were detected in only one case of definite MS (84 mg/dl).

The IgG Index and the IgG loc displayed higher sensitivity to detect the intrathecal synthesis of IgG, in comparison to IgG% and gamma globulin% of protein. The detection of oligoclonal IgG bands by isoelectric focusing was the most sensitive test to demonstrate intrathecal synthesis of IgG. Oligoclonal bands were detected in all cases with IgG index > or = 0.7 or IgG loc > or = 0.

A significantly lower frequency of the local IgG synthesis was observed in the group of probable MS (Tables 1 and 2).

DISCUSSION

We have determined the profile of the intrathecal humoral immune response of Brazilian MS patients, using a combination of quantitative and qualitative measures. The IgG index and IgG loc showed no significant differences to detect the intrathecal synthesis of IgG, considering that the majority of the patients had no blood-CSF barrier dysfunction. The IgG loc formula correlates the IgG and the albumin quotient, using a hiperbolic function[5,9,10]. It avoids the lost of sensitivity in cases of more severe CSF-blood barrier dysfunction, compared to the IgG index[9]. The % IgG protein is the simplest test. The % IgG protein and the

Table 1. CSF findings of 128 Brazilian MS patients.

CSF Findings	Definite MS (n=103)	Probable MS (n-25)	*p* value
WBC (/mm^3)	1 (1 - 60)	1 (1 - 41)	NS
Protein (mg/dl)	29 (19 - 84)	30 (10 - 60)	NS
Q Alb (x10^{-3})	5 (1.1 - 21.6)	5 (2 - 14)	NS
Gammaglobulin%	13 (5 - 35)	10 (4 - 34)	NS
% IgG Protein	13 (5.1 - 60.9)	11.2 (4.5 - 40.7))	NS
IgG Index	0.7 (0.3 -2.9)	0.5 (0.2 - 1.8)	NS

Values are shown as median (min-max); Q Alb (Albumin Quotient); a normal blood-CSF barrier function was indicated as Q Alb <= 8 x10^{-3}; normal IgG index <=0.7; the Mann-Whitney test was used to compare the median between both groups. NS`, not significant.

Table 2. Correlation between the MS Brazilian Groups and the frequency of the CSF findings.

CSF Findings	Definite MS	Probable MS	*p*
Oligoclonal IgG Band in CSF	62/73 (85%)	6/10 (60%)	NS
IgG (loc) >0	49/98 (50%)	4/21 (19%)	<0.05
IgG Index >=0.7	50/98 (51%)	4/21 (19%)	<0.05
% IgG protein >=15%	42/102 (41%)	7/25 (28%)	NS
Gammaglobulin% > 14	40/96 (42%)	3/24 (12.5%)	< 0.05
Q Alb >= 8 x10^{-3}	14/98 (14%)	4/21 (19%)	NS
Protein >40 mg/dl	23/103 (22%)	5/25 (20%)	NS
WBC > 4/ mm^3	17/103 (16.5%)	3/25 (12%)	NS

IgG (loc)>0 according to Reiber & Felgenhauer, 1987. IgG Index according to Tibbling et al.[8]. The frequencies were compared using the chi-square/ Fisher exact test.

eletrophoresis on cellulose acetate had a lower sensitivity in the IgG profile and cannot differentiate the IgG blood fraction from IgG synthezised in CNS in cases of blood-CSF barrier dysfunction or those with elevated concentration of IgG in serum.

According to other reports[4], the identification of oligoclonal bands by IEF was the most sensitive technique able to detect the intrathecal synthesis of IgG. The interpretation of the bands is occasionally difficult and depends on the observer's experience. In this sense, the IEF with immunodetection seems to be more objective, avoiding false-positive results[13]. The greater importance of this procedure was to demonstrate local IgG production not identified by quantitative methods. It was especially important in the probable MS cases. The high frequency of negative results for quantitative analysis of IgG in such group, may be related to the use of steroids. It decreases the IgG values but not so frequently the presence of oligoclonal bands[16]. An other reason for this observation may be related to the significant shorter duration of disease in the probable MS cases. Perhaps some of these cases are really not MS. It will be necessary follow up studies to the determine the evolution of these patients.

The significance of the presence of oligoclonal bands in MS patients is not clear. The pattern of absence or presence of these bands may persist during the lifetimes and is not related to the

disease activity[17]. Autopsy studies have demonstrated a relationship between the lack of oligoclonal bands in CSF and the absence of plasma cells in the meninges and plaques[17].

The present study demonstrated that 85% of the definite MS patients presented oligoclonal bands restricted to the CSF. Although CECAMS reported a sensitivity higher than 95% for oligoclonal bands in definite MS cases [4] but lower frequencies (40-60%) have been usually found in routine hospital laboratories[6]. The observation of lower IgG production in our MS cases may be related to the used method or may represent a characteristic of our population. Of special interest is also the finding of lower frequency of hypercytosis in our MS cases (16.5% in comparison to 50% of the clinically definite MS cases of the European study[4]). These data suggest that the inflammatory reaction in CSF of Brazilian MS cases seems to be less frequent than the other studies.

In conclusion, the evaluation of intrathecal IgG synthesis, using the CECAMS protocol was technically reproducible in Brazil. The combination of quantitative and qualitative procedures yields important results that aided in the diagnosis of MS[18].

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