Clinical Neurology and Neurosurgery 115 (2013) 2094–2098

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Prevalence of cerebrospinal fluid oligoclonal IgG bands in Greek patients with clinically isolated syndrome and multiple sclerosis

E. Andreadou[a,*], S. Chatzipanagiotou[b], V.C. Constantinides[a], A. Rombos[a], E. Stamboulis[a], C. Nicolaou[b]

[a] Department of Neurology, Athens National University, "Aeginition" Hospital, Athens, Greece
[b] Department of Medical Biopathology, Athens National University, "Aeginition" Hospital, Athens, Greece

ARTICLE INFO

Article history:
Received 31 January 2013
Received in revised form 27 June 2013
Accepted 21 July 2013
Available online 13 August 2013

Keywords:
Multiple sclerosis
Oligoclonal bands
Cerebrospinal fluid
Isoelectric focusing

ABSTRACT

Lower prevalence of cerebrospinal fluid oligoclonal IgG bands (IgG-OCBs) has been reported in multiple sclerosis (MS) patients from Southern Europe compared to other western countries.
Objectives: We aimed to determine the prevalence of CSF OCBs in Greek MS patients and to examine their relation with some selected clinical and demographical features.
Methods: Included patients fulfilled the 2005 McDonald criteria for definite MS (CDMS) or clinically isolated syndrome (CIS) and had a spinal tap performed between 2006 and 2010. Paired CSF and plasma samples were analyzed using isoelectric focusing followed by IgG-specific immunofixation. A pattern of two or more bands present only in the CSF was defined as positive. OCB status was correlated with age at disease onset, initial symptomatology, relapse rate, disease subtype, disease duration, medication, EDSS score and MSSS.
Results: Of the 231 included patients (53.2% with CDMS and 48.6% with CIS) 67.5% had OCBs. The prevalence of positive patterns did not differ between CIS and CDMS patients (67.6% vs. 67.5%, respectively). OCB-positive patients were younger than OCB-negative patients (35.2 ± 10.3 vs. 38.7 ± 11.8 years respectively, $p = 0.022$) and had more frequently cervical spinal cord lesions ($x^2 = 7.08$, $p = 0.008$). No difference was observed between the two subgroups in the other studied disease parameters.
Conclusion: Despite the lower frequency of positive IgG-OCB patterns in our patients, both subgroups were mostly similar with regard to their clinical and demographic characteristics suggesting that the OCB status lacks prognostic significance in MS.

1. Introduction

There is evidence that multiple sclerosis (MS) patients in southern European countries show positive cerebrospinal fluid (CSF) restricted oligoclonal IgG bands (IgG-OCBs) less frequently compared to MS patients from Northern Europe. According to previous reports, the prevalence of IgG-OCBs in CSF from MS patients in the Czech Republic is 81%, in Portugal 82.6%, in Spain 87.7% and in Turkey 85.7%, while in Scandinavian countries, the UK and Canada is more than 90% [1,2]. Moreover, it is unclear whether the CSF antibodies have prognostic significance in MS and whether OCB-negative patients constitute a different disease subtype. In literature the association of disease severity with the presence of CSF OCBs is controversial. Several researchers have reported that the prognosis of OCB-positive patients with MS is worse than that of OCB-negative patients [3–5]. Other investigators found no differences with respect to disease severity, disease course, or age at onset between OCB-positive and OCB-negative subgroups [6–8]. In a Turkish study, patients with CSF OCBs were found to have female predominance, better clinical course with less disability and better prognosis [2]. Female predominance in OCB-positive MS patients was also found by Nakashima and co-workers [6].

There are no data regarding the presence of OCBs in the CSF of MS patients from Greece. Thus, we aimed to determine the prevalence of IgG-OCBs in Greek MS patients and to investigate the correlation between the presence of OCBs and some selected clinical and demographic characteristics.

2. Methods

2.1. Patients

We included 231 patients. Included patients fulfilled the revised McDonald criteria [9] for definite MS or clinically isolated syndrome

* Corresponding author at: Department of Neurology, "Aeginition" Hospital, 74, Vas. Sophia's Avenue, 11528 Athens, Greece. Tel.: +3210 72 89 277; fax: +3210 5739900.
E-mail addresses: eandread@med.uoa.gr, elisandread@gmail.com (E. Andreadou).

0303-8467/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.clineuro.2013.07.026

(CIS) and had a spinal tap performed between 2006 and 2010. All patients were screened for other conditions that could mimic early MS, including connective tissue diseases, other autoimmune diseases, sarcoidosis or infections. The clinical data of all patients were reviewed retrospectively from the clinical notes and records of the MS clinic. The following parameters were recorded: sex, age at disease onset, age at lumbar puncture (LP), initial symptomatology, subtype of MS, disease duration from onset to the time of LP, annualized relapse rate and time to secondary progressive MS (SPMS). Disability was assessed using the Expanded Disability Status Scale (EDSS)[10] at the time of LP and also with the MS Severity Score (MSSS) [11].

2.2. Determination of the intrathecal IgG production

The CSF samples were obtained by LP with simultaneous serum sampling, and were stored at −80 °C.

The intrathecal IgG production was determined by calculation of the IgG-index in the CSF. First the total IgG fraction and the albumin concentrations were measured in serum and CSF, by means of a commercially available immunonephelometric assay using an automatic immunonephelometer (reagents and equipment provided by Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). For the evaluation of the blood-brain barrier permeability the albumin index (Q_{alb}) was calculated as the quotient of the albumin concentration in CSF to the concentration in serum multiplied by 10^3. Values >10 were considered as positive for a disruption of the blood-brain barrier and increased permeability. For the assessment of the intrathecal IgG production, the IgG-index was calculated as the quotient of the quotient IgG_{CSF}/IgG_{ser} to the quotient Alb_{CSF}/Alb_{ser}. Values ≥0.65 were considered as indicative for a local IgG production in CSF.

2.3. Immunoelectrophoretic detection of OCBs in CSF

Detection of the OCBs in the CSF was performed using a commercially available system including reagents and equipment (SEBIA, Evry Cedex, France). The methodology referred to the isoelectric separation of the CSF and serum proteins in an agarose gel in paired specimens of serum and unconcentrated CSF, after setting up equivalent IgG concentrations, through serum dilution. The assay was carried out in two stages: (1) isoelectric focusing on agarose gel to fractionate the proteins and (2) immunofixation with peroxidase labelled anti-IgG antiserum and subsequent incubation with the chromogenic substrate TTF3, to detect IgG oligoclonal bands and to demonstrate the difference, or lack of, in the distribution of IgG in the CSF and serum.

Paired serum and CSF samples were processed side-by-side and compared for the presence of any banding present in the CSF but not in the serum sample. A pattern of two or more bands present only in the CSF was evaluated as indicative for intrathecal IgG production (positive).

2.4. Statistics

Statistical analysis was performed with SPSS (version 17.0). All variables were checked for normality by the Shapiro-Wilks and for homogeneity of variances by the Levene test. Continuous variables were compared using independent samples *t*-test and categorical data using Chi-square test. Nonparametric tests (Mann–Whitney test) were used for comparison of albumin and IgG index, disease duration, EDSS, MSSS and annualized relapse rate, due to deviations from normal distribution. All analyses were two-tailed and the level of statistical significance was set to 5%.

3. Results

The clinical, demographic characteristics and laboratory findings of the included patients are summarized in Tables 1 and 2. Of the 231 patients, 53.2% had clinically definite MS (CDMS) and 46.8% CIS. The most common initial symptoms were sensory (32.5%), followed by brainstem–cerebellar (26.4%) and optic neuritis (22.5%). The female to male ratio was 2. Mean annualized relapse rate [for patients with relapsing–remitting MS (RRMS)] was 0.79 ± 0.63 and mean time to SPMS was 9.9 ± 6.5 years. A minority of patients were receiving treatment for MS at the time of CSF examination (11.8%).

From all the patients, 67.5% had positive OCBs and 66.7% had a positive IgG-index (≥0.65) (Table 3). OCB-positive patients were significantly younger than OCB-negative patients (35.2 ± 10.3 vs. 38.7 ± 11.8 years, respectively, $p = 0.022$). No differences were observed between OCB-positive and negative MS patients in initial symptomatology ($p = 0.761$), age at disease onset ($p = 0.097$), relapse rate ($p = 0.625$), disease subtype ($p = 0.384$), disease duration ($p = 0.661$), EDSS ($p = 0.149$) and MSSS ($p = 0.547$) scores. Furthermore all laboratory characteristics (MRI data and VEPs) were similar in the two patient groups, with the exception of more frequent positive cervical MRI (defined as the presence of ≥1 demyelinating lesion in the cervical spine) in OCB-positive patients ($x^2 = 7.08$, $p = 0.008$).

Elevated IgG-index correlated with younger age (34.6 ± 10.3 vs. 39.7 ± 11.4, $p = 0.001$) and age at disease onset (30.9 ± 9.4 vs. 34.4 ± 10.3, $p = 0.014$). IgG-index was more frequently elevated in MS patients with lesions in cervical MRI ($x^2 = 13.3$, $p = 0.0001$).

The same analyses were performed in the subset of patients with CDMS. The percentage of OCB-positive CDMS patients was unchanged (67.5%). Positive OCBs did not correlate with any of the demographic and disease characteristics, neither with positive cervical MRI ($p = 0.438$). Elevated IgG-index correlated with younger age (36.1 ± 10.9 vs. 42.4 ± 12.1, $p = 0.003$).

4. Discussion

In the present study, the incidence of OCB-positive patients fulfilling the diagnostic criteria for MS was found to be 67.5%. The prevalence of OCBs is higher than that reported in oriental populations [12] but lower than the frequency found in other Western countries, particularly in northern Europe and Canada, where have been reported rates of over 90% [1]. Moreover, the percentage of positive patterns is even lower than the values reported in other southern European and Mediterranean countries, where it ranges from 80% to 90% [2,4]. Interestingly, the prevalence of IgG-OCBs in our patient population is similar to that reported in MS patients from Western Australia, where it was found to be 66.4% [13]. In fact, we included a large proportion of patients with CIS. While it has been established that the OCB status (either positive or negative) does not alter during the course of MS, some CIS patients with initial absence of OCBs may later express OCBs [3]. Although a lumbar puncture was not repeated in our OCB-negative CIS patients, the prevalence of positive patterns did not differ between patients with CIS and those with CDMS (67.6 vs. 67.5%, respectively). Therefore, the inclusion of a high percentage of CIS patients in our study could not adequately explain this discrepancy. It has been suggested that the detection methods may play a role in the low prevalence of CSF OCBs. In the present study, OCB detection was performed by isoelectric separation in agarose gel, followed by ultrasensitive immunofixation, According to the International Consensus, this method is considered the “gold standard” for the detection of OCBs in the CSF [14,15]. However, in commercially available or in house prepared diagnostic kits, differences in the quality of the reagents and the technical performance conditions, can lead to differences