THE VALUE OF INTRATHECAL MRZ REACTION AND OLIGOCLONAL IgG BANDS FOR DISCRIMINATION BETWEEN THE PRIMARY PROGRESSIVE AND RELAPSING REMITTING COURSES OF MULTIPLE SCLEROSIS

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ABSTRACT

Generally, we diagnose two different courses of multiple sclerosis: relapsing remitting course (RR-MS) and primary progressive (PP-MS) course. Differences in pathogenesis, immunology, and prognosis are supposed between these entities. A reliable surrogate marker in cerebrospinal fluid and serum for these courses is still missing. The aims of our work were to investigate the frequency of MRZ reaction and oligoclonal IgG bands (IgG-OB) in RR-MS and PP-MS subgroups of patients and to evaluate its diagnostic significance. We examined 29 patients (n–29) with RR-MS and 10 patients with PP-MS (n–10). The intrathecal synthesis of specific antibodies (MRZ reaction) was evaluated in the form of antibody indices calculated according to the Reiber's formula. Oligoclonal IgG bands were detected by isoelectric focusing using a commercial SEBIA kit. The MRZ reaction was positive in 2/10 patients with PP-MS and in 11/29 patients with RR-MS. IgG-OB were positive in 1/10 patients with PP-MS and in 20/29 patients with RR-MS. The calculated sensitivities for MRZ reaction were 20 % in PP-MS and 37 % in RR-MS; for IgG-OB they were 10 % in PP-MS and 69 % in RR-MS. The different frequencies of both parameters in PP-MS and RR-MS support the hypothesis about different pathogenesis and can be used as reliable surrogate markers for differential diagnosis in the context of clinical settings.

ABBREVIATIONS USED

CSF – cerebrospinal fluid
MS – multiple sclerosis
IgG – immunoglobulin G
OB – oligoclonal bands
IEF – isoelectric focusing
MRI – magnetic resonance imaging

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory immune-mediated disease, probably of autoimmune origin. The diagnosis according to the International Panel on the Diagnosis of Multiple Sclerosis from 2005 revised the original McDonald's Diagnostic Criteria from 2001 [1]. The Criteria formally incorporated magnetic resonance imaging (MRI) into the well-established diagnostic workup that focuses on detailed neurological history and examination and a variety of paraclinical laboratory examinations. They laid particular emphasis on determining dissemination of lesions in time and space, incorporating different types of imaging criteria into the diagnostic scheme and assessing the value of cerebrospinal fluid analysis, particularly for the diagnosis of primary progressive multiple sclerosis. The incorporation of CSF (cerebrospinal fluid) findings into the McDonald's criteria has been supported by studies suggesting that CSF analysis increases diagnostic sensitivity, though at the cost of specificity. The Revised Criteria stated the belief that a positive CSF finding was preferably based on isoelectric focusing evidence of oligoclonal IgG bands with immunofixation demonstrating that bands that are different from those in the serum or an increased IgG index or both increase the "comfort level" for the diagnosis of MS in individuals with insidious progression of disease from the onset. However, such CSF findings are not specific and may be detected in patients with progressive myelopathies of other causes, especially those associated with infections [2, 3, 4]. Besides IgG-OB the MRZ reaction (intrathecal polyspecific immune response against neurotropic viruses: measles, rubella, varicella zoster) is a marker for differential diagnosis between MS and other demyelinating diseases of CNS because of its higher specificity for chronic autoimmune inflammation comparing oligoclonal IgG bands and the IgG index as mentioned above [5]. The specificity of MRZ to chronic inflammatory processes of autoimmune origin such as multiple sclerosis is in the range between 84 and 94 %. Combinations of M+R, M+Z or R+Z that are rarely seen in other diseases (e.g. acute infections) are clues to the presence of a chronic, especially autoimmune-type, disease. In other neurological diseases (neuroborreliosis, neurosyphilis, neurotuberculosis) the frequency of MRZ is below 1 % for the single species and far below 0.1 % for M+R+Z [6]. We previously published an evaluation of both the MRZ reaction and IgG-OB in a group of patients with OND (other neurological diseases) compared to the positivity of these parameters in a group of patients with MS [7].

The combined differential diagnostic value of both parameters for diagnosis between relapsing remitting (RR-MS) and primary progressive (PP-MS) courses has not been evaluated till now, in spite of supposed principal differences in the pathogenesis of both different MS courses. It is supposed that the RR-MS course is predominantly based on inflammation-demyelination and the PP-MS course on predominant neurodegeneration and less inflammation.

Our objectives were to assess the presence of MRZ reaction and IgG-OB in cohorts of patients with RR-MS and PP-MS and to evaluate their differential diagnostic potential between both courses.

MATERIALS AND METHODS

We evaluated the CSF and the serum for MRZ reaction and IgG-OB as the most specific and sensitive parameters of neuroinflammation in 29 patients (n–29) with RR-MS and 10 patients (n–10) with PP-MS.

The clinical diagnosis of multiple sclerosis was based on Diagnostic Criteria for Multiple Sclerosis from the International Panel published in Ann Neurol 2005; 58: 840–846 [1]. All patients fulfilled diagnostic criteria demonstrating dissemination of lesions in space and time including MRI (magnetic resonance imaging) positivity. None of these patients had a clinically isolated syndrome (CIS).

Serum and CSF samples were analysed in each patient.

ANALYTICAL PROCEDURES

Measles, rubella, and varicella zoster virus-specific IgG antibodies were detected both in the serum and CSF by sandwich enzyme immunoassay using commercial kits from Human, Germany (Measles-Virus Human ELISA IgG Antibody Test, Rubella-Virus Human ELISA IgG Antibody Test, Varicella-Zoster-Virus Human ELISA IgG Antibody Test). In this assay microtitre strip wells as a solid phase are coated with cell-culture derived Measles, Rubella, and VZV antigens. If the corresponding specific antibodies are present in a sample, they are bound to the antigens at the solid phase. After a washing step to remove unbound material, anti-human IgG peroxidase conjugate is added, which binds specifically to IgG class antibodies. After a second washing step to remove unbound conjugate, the enzyme-linked complexes are detected by incubation with a substrate solution. The subsequent development of a blue colour is changed into yellow by stopping the enzymatic reaction with sulphuric acid. Absorbances are measured at 450 nm using an ELISA microtitre plate reader.

Absorbances of serum and CSF samples were converted to arbitrary units (AU) in a log/log diagram based on a standard curve derived from seven serial dilutions of a positive standard serum. The highest standard concentration (approximately 2.0) was defined as 100 arbitrary units [5].

The specific antibody index (AI) was calculated according to the Reiber's formula: AI = Qspec /QIgG. Qspec is the ratio of

Table 1
Prevalence of MRZ reaction and IgG-OB in cohorts of patients with PP-MS and RR-MS

	MRZ reaction	IgG-OB
RR-MS (n–29)	11/29	20/29
PP-MS (n–10)	2/10	1/10

Table 2
Sensitivity of MRZ reaction and IgG-OB in cohorts of patients with PP-MS and RR-MS

	MRZ reaction (%)	IgG-OB
RR-MS (n–29)	37 %	69 %
PP-MS (n–10)	20 %	10 %

specific antiviral IgG antibodies in CSF and serum. QIgG is the ratio of total IgG antibodies in CSF and serum. The upper limit (Qlim) for IgG is Qlim = 0.93 *SQRT (Alb^2 + (6*10^{-6}) –1.7 *10^{-3}. If QIgG >Qlim, then AI = Qspec/Qlim. The values AI>1.4 are positive and indicate intrathecal synthesis of specific antibodies [8].

Oligoclonal IgG bands
Oligoclonal IgG bands in the serum and the CSF were detected by isoelectric focusing with subsequent immunoenzymatic staining using a commercial kit (SEBIA, France).

RESULTS

MRZ reaction was positive only in 2 of 10 (n–10) patients with primary progressive MS and IgG-OB were positive in 1 of 10 (n–10) patients with PP-MS.
MRZ reaction was positive in 11 of 29 patients (n–29) with RR-MS. IgG-OB were positive in 20 of 29 patients (n–29) with relapsing remitting course of MS.
The differences in positivity of MRZ reaction and IgG-OB between those cohorts of patients were statistically significant with a calculated sensitivity for MRZ reaction of 20 % in PP-MS and 37 % in RR-MS.
For IgG–OB the sensitivity was 10 % in PP-MS course and 69 % in RR–MS.

DISCUSSION

We found significantly less positivity in both parameters (MRZ and IgG-OB) in the primary progressive course of multiple sclerosis. It supports the widely accepted hypothesis about different pathogenesis and distinctive prognosis between both MS courses. The report exists about IgG-OB negative patients with relatively better prognosis [9]. True IgG-OB negative clinically definite multiple sclerosis occurs and, according to our results, is more common in the PP-MS course. From the short- and middle-time observational period this is in accord with the slower accumulating disability, i.e. temporarily and concomitantly better prognosis. MRI detected fewer lesions in patients with lower inflammatory disease activity, also reflected by the negativity of IgG-OB [11]. In the clinical setting the negativity of IgG-OB rather reflects the PP-MS course, but temporary evolution of IgG-OB positivity must be taken into account, especially in the case of clinically isolated syndrome (CIS), where the initial positivity is reported to range between 40 and 70 %, but when CSF examination is repeated after 1/2 to 1 year, the positivity of IgG-OB increases over 90 % [11]. One study described a significant delay in disability progression during the treatment with interferon-beta in the subgroup of MS patients with no IgG-OB detectable by IEF, compared to the patients with this CSF abnormality [12]. Whether IgG-OB negative MS patients have additional characteristic immune features remains to be settled. There is a need for concerted long-term follow-up studies of the subgroup of MS patients without CSF IgG OB regarding prognosis and immunological features.
As regards MRZ antibodies, 84–94 % of MS patients have intrathecal antibody synthesis against one, two or three of the measles, rubella, and varicella zoster viruses, and slightly more if herpes simplex is included. The frequencies and magnitudes of the antibody indices rise with increased total IgG synthesis [13]. Although much less frequent than the MRZ reaction, increased intrathecal synthesis of HSV antibodies (28% frequency in MS patients) is still higher than other antibody species in MS (e.g. intrathecal toxoplasma antibodies – 10 %, or intrathecal autoantibody synthesis against dsDNA – 20 %).
On the contrary, the MRZ reaction is strongly predictive of an autoimmune-type chronic inflammatory disease such as

multiple sclerosis even at the time of the first clinical symptoms. In the case of acute infections the specific antibody index has a higher diagnostic sensitivity than the oligoclonal IgG [14]. The rather lower sensitivity of MRZ reaction in our cohort of RR-MS patients could be explained by the smaller number of patients comparing numbers in relevant reports.
The relevance of the MRZ reaction is different from that of oligoclonal IgG for evaluating multiple sclerosis. The presence of IgG-OB in CSF has a lower specificity for MS and must be generally evaluated in the context of clinical setting and MRI findings [15]. The MRZ reaction has not been evaluated until now in the different courses of MS; we stated that the frequency is significantly lower in the PP-MS course, likely reflecting less pronounced inflammation in CNS as was similarly detected in IgG-OB.

CONCLUSION

If both parameters are taken together, we can conclude that the negativity of the MRZ reaction and IgG-OB does not exclude the possibility of diagnosing multiple sclerosis and rather has a better prognostic significance for both courses of MS and is much common in the PP-MS course. These significant differences in prevalence between the two courses can be used as an additional differential diagnostic marker.

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Electromyography laboratory

Stroke unit – nurse staff control centre

Inpatient clinic - nurse staff room

Outpatient clinic - nurse staff room