

## THE DIAGNOSTIC SIGNIFICANCE OF INTRATHECAL OCCURRENCE OF SELECTED ANTIVIRAL ANTIBODIES IN MULTIPLE SCLEROSIS

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### Abstract

Analysis of cerebrospinal fluid (CSF) represents an important role in the diagnosis of multiple sclerosis. Oligoclonal IgG bands, blood – CSF barrier, and cell count are normally examined. Intrathecal synthesis of specific IgG antibodies against measles, rubella, and varicella zoster viruses called MRZ reaction is a new diagnostic marker which is present in patients with chronic autoimmune diseases with the involvement of the nervous system, especially multiple sclerosis. The aims of our work were to investigate the frequency of intrathecal synthesis of specific antiviral antibodies and to determine a typical CSF profile in patients with multiple sclerosis. We examined a cohort of 65 patients: 50 patients had multiple sclerosis, 15 patients had other neurological diseases (OND). The intrathecal synthesis of specific antibodies was evaluated in the form of antibody indices calculated according to Reiber's formula. The condition of blood – CSF barrier was expressed by the ratio of albumin in CSF and serum. Oligoclonal IgG bands were detected by isoelectric focusing. Eighty-six per cent of multiple sclerosis patients had positive intrathecal synthesis of specific antibodies against one, two or three of the examined neurotropic viruses, 84 % of multiple sclerosis patients had positive oligoclonal IgG bands in CSF, intact blood – CSF barrier was detected in 70 % and pleocytosis in 36 % of patients with multiple sclerosis. MRZ reaction was negative in all patients with OND. Highly specific MRZ reaction improves the possibilities of CSF analysis in cases of chronic, inflammatory, and autoimmune diseases. The adaptation of MRZ reaction to CSF routine analysis increased considerably the significance of lumbar puncture for the diagnosis of multiple sclerosis, even at the time of first clinical symptoms.

### Key words

Multiple sclerosis, Intrathecal synthesis, MRZ reaction, Cerebrospinal fluid

### Abbreviations

CSF, cerebrospinal fluid; MS, multiple sclerosis; OND, other neurological diseases; AI, antibody index

## INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of autoimmune origin. It is the most common cause of neurological disability in young adults with the prevalence of about 60 cases per 100,000 inhabitants in the Czech Republic. The diagnosis according to Mc Donald's criteria is based on clinical pattern and course supported by magnetic resonance imaging, cerebrospinal fluid analysis, and electrophysiology (1).

Characteristic CSF parameters in multiple sclerosis include cytology, oligoclonal IgG bands, Q albumin as a marker of blood - CSF barrier function, and intrathecal synthesis of specific antiviral IgG antibodies called MRZ reaction.

MRZ reaction reflects the intrathecal synthesis of specific IgG antibodies against one, two or three of the measles (M), rubella (R), and varicella zoster (Z) viruses. It is expressed in the form of antibody indices (AI). The specific antibody index is the most sensitive inflammation parameter in CSF analysis (2). The positive MRZ reaction supports the diagnosis of a chronic disease like multiple sclerosis or autoimmune disease with involvement of the CNS, already at the time of first clinical symptoms (3). Eighty-four to 94 % MS patients have positive MRZ reaction. Combinations 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, in particular autoimmune-type disease. In other neurological diseases (neuroborreliosis, neurosyphilis, neurotuberculosis), the frequency of MRZ reaction is below 1 % for the single species and far below 0.1 % for M+R+Z (4).

The MRZ reaction, preferentially seen in the chronic autoimmune-type diseases, is not understood regarding its cause and pathophysiological meaning. There is no persistence of the antigens (e.g. measles virus) observed in the brain (5). Positive MRZ reaction is explained by the polyspecific, oligoclonal immune response of the self-organised immunological network (6).

Because of possible differential diagnostic difficulties in multiple sclerosis patients, especially at the onset of the disease, we decided to investigate selected CSF parameters and evaluate the typical CSF variables profile in multiple sclerosis.

## MATERIALS AND METHODS

### *Patients*

We investigated a cohort of 65 patients. The patients were treated at the Faculty Hospital Brno, Czech Republic, between 1999 and 2004. Fifty patients had multiple sclerosis, 15 patients had other neurological diseases (OND): bacterial meningitis (1), aseptic meningitis (5), sepsis (1), idiopathic facial nerve palsy (4), lumbar stenosis (1), low back pain (1), polyneuropathy (1), and neurasthenia (1). The diagnosis of multiple sclerosis was clinically defined and laboratory supported according to the internationally accepted Mc Donald's criteria. Serum and CSF sample pairs were analysed in each patient.

### *Analytical procedures*

#### INTRATHECAL SYNTHESIS OF SPECIFIC ANTIBODIES

Measles, rubella, and varicella zoster virus-specific IgG antibodies were detected both in 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 test microtitre strip wells as a solid phase are coated with cell culture derived Measles, Rubella or VZV antigens. If 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. 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 (7).

The specific antibody index (AI) was calculated according to Reiber's formula:  $AI = Q_{spec}/Q_{IgG}$ .  $Q_{spec}$  is the ratio of specific antiviral IgG antibodies in CSF and serum,  $Q_{IgG}$  is the ratio of total IgG antibodies in CSF and serum. The upper limit ( $Q_{lim}$ ) for IgG is  $Q_{lim} = 0.93 * \sqrt{Q_{alb}^2 + (6 * 10^{-6})} - 1.7 * 10^{-3}$ . If  $Q_{IgG} > Q_{lim}$ , then  $AI = Q_{spec}/Q_{lim}$ . The values  $AI > 1.4$  are positive and indicate intrathecal synthesis of specific antibodies (8).

#### ALBUMIN AND IgG CONCENTRATIONS

Albumin and total IgG concentrations in serum and CSF were measured by nephelometry.  $Q_{albumin}$  (albumin quotient,  $Q_{alb}$ ) is the ratio of albumin in CSF and serum and reflects the condition of blood-CSF barrier function.

#### OLIGOCLONAL IgG BANDS

Oligoclonal IgG bands in serum and CSF were detected by isoelectric focusing with subsequent immunoblot and staining. Two or more bands in CSF but not in serum were considered as positive.

#### CYTOLOGY

The cells in CSF were counted in a Fuchs - Rosenthal chamber. Pleocytosis was present in case the cell number exceeded 5 elements per 1  $\mu$ l. Differential cell count was evaluated from slides prepared by cytocentrifuge Cytospin - 2 (Shandon) followed by staining with May - Grünwald, Giemsa - Romanowski.

## RESULTS

### *Intrathecal synthesis*

In our cohort 86% MS patients had positive MRZ reaction, e.g. positive intrathecal synthesis against one, two or three of the M, R, Z viruses. Concerning single species, 68% had positive intrathecal synthesis against measles with AI in the range of 1.5-20.2, 40% had positive intrathecal synthesis against rubella with AI in the range of 1.5-51.0, and 36% had positive intrathecal synthesis against VZV with AI in the range of 1.5-10.6. We detected the following combinations of positive intrathecal synthesis: M+R in 14%, R+Z in 6%, M+Z in 12%, and M+R+Z in 14% MS patients. The results of intrathecal synthesis in our group of MS patients are summarised in *Tab. 1*.

The control group consisted of 15 patients with OND. The intrathecal synthesis of examined specific antiviral antibodies was negative in all patients with OND.

*Table 1*

Positive intrathecal synthesis of specific IgG antibodies against measles, rubella and varicella zoster viruses in multiple sclerosis

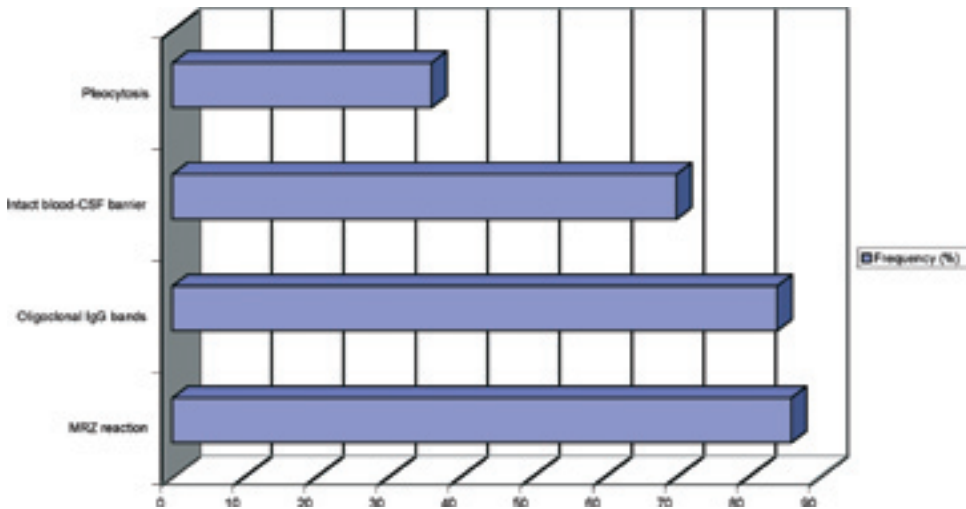
	Frequency (%)	AI median	AI max
Measles	68.0	2.5	20.2
Rubella	40.0	2.8	51.0
VZV	36.0	2.6	10.6
MRZ reaction	86.0	-	-

***Blood-CSF barrier function***

The condition of blood - CSF barrier is expressed by the ratio of albumin in CSF and serum (Q albumin). Elevated values of Q albumin indicate dysfunction of blood - CSF barrier. We detected elevated values of Q albumin in 30 % MS patients.

***Oligoclonal IgG bands***

Two or more oligoclonal IgG bands in CSF but not in serum are considered positive. We detected positive oligoclonal IgG bands in 84 % MS patients.



*Fig. 1*

Frequencies of characteristic CSF parameters in multiple sclerosis

### *Cytology*

Sixty-four per cent MS patients had normal cell count ( $<5$  elements/ $\mu$ l), 36 % MS patients had elevated CSF cell count ( $>5$  elements/ $\mu$ l), and only 4 % had values  $>40$  elements/ $\mu$ l. CSF findings are summarised in *Fig. 1*.

### DISCUSSION

Positive MRZ reaction is reported in 84–94 % of MS patients (3). We detected positive MRZ reaction in 86 % of MS patients. This result corresponds with previous findings. False positivity of MRZ reaction was excluded according to the results in the OND group. MRZ reaction improves the possibilities of CSF analysis, especially in cases of chronic, inflammatory, and autoimmune diseases. It is positive already at the time of the first clinical symptoms.

Positive oligoclonal IgG bands are reported in 95 % of MS patients. The frequency is however lower at the onset of the disease and increases during the course of the disease (9). We detected positive oligoclonal IgG bands in 84 % of MS patients. This lower number was caused by the fact that our cohort contained 5 patients at the time of the first clinical symptoms.

The albumin CSF/serum concentration quotient, Q albumin, is a widely accepted indicator of blood – CSF barrier function (10). Increased albumin concentrations in CSF must always be due to blood – CSF barrier dysfunction, because albumin originates exclusively from blood. Blood – CSF barrier expressed by Q albumin is mostly intact or slightly impaired in MS patients. Large values of Q albumin ( $>20 \times 10^{-3}$ ) are not consistent with the diagnosis of multiple sclerosis (4). In our cohort 70 % of MS patients had intact blood – CSF barrier. CSF cytology in MS patients presents a normal cell count or a slight pleocytosis usually not exceeding 40 elements/ $\mu$ l. A large cell count ( $>90$  elements/ $\mu$ l) does not correspond with the diagnosis of multiple sclerosis (4).

### CONCLUSIONS

A typical CSF profile in multiple sclerosis includes normal or slightly elevated cell count, a normal blood – CSF barrier, presence of oligoclonal IgG bands in 90–95 %, and positive MRZ reaction in 84–94 % of the patients. According to our data we confirmed that MRZ reaction is a sensitive diagnostic tool in chronic autoimmune diseases with the involvement of the nervous system. It increases considerably the significance of a lumbar puncture for the diagnosis of multiple sclerosis.

## DIAGNOSTICKÝ VÝZNAM PŘÍTOMNOSTI VYBRANÝCH PROTIVIROVÝCH PROTILÁTEK V LIKVORU PACIENTŮ S ROZTROUŠENOU SKLERÓZOU

### S o u h r n

Analýza mozkomíšního moku (likvor, CSF) patří mezi základní vyšetření v diagnostice roztroušené sklerózy mozkomíšni (sclerosis multiplex, SM). Běžně jsou stanoveny oligoklonální IgG pásy, stav hematolikvorové bariéry a cytologie. Intratekální syntéza specifických IgG protilátek proti viru spalniček (measles - M), zarděnek (rubella - R) a planých neštovic (varicella zoster - Z) označovaná jako MRZ reakce je přítomná u pacientů s chronickým autoimunitním onemocněním postihujícím nervový systém, především sclerosis multiplex. Cílem naší práce byla detekce intratekální syntézy specifických antivirových protilátek a určení typického likvorového profilu u pacientů se sclerosis multiplex. Vyšetřili jsme soubor 65 pacientů: 50 pacientů mělo sclerosis multiplex, 15 pacientů jiná neurologická onemocnění (OND). Intratekální syntéza specifických protilátek byla stanovena ve formě protilátkového indexu (antibody index, AI) podle Reiberova vzorce. Stav hematolikvorové bariéry byl vyjádřen poměrem albuminu v likvoru a séru, oligoklonální IgG pásy byly stanoveny metodou isoelektrické fokusace. Osmdesát šest procent pacientů se sclerosis multiplex mělo pozitivní intratekální syntézu specifických protilátek proti jednomu, dvěma nebo třem virům ze skupiny M, R, Z, 84 % pacientů se sclerosis multiplex mělo pozitivní oligoklonální IgG pásy v likvoru, u 70 % pacientů byla intaktní hematolikvorová bariéra a u 36 % pacientů byla přítomna pleocytóza. V kontrolní skupině pacientů nebyla MRZ reakce přítomna. Vysoce specifická MRZ reakce zlepšuje možnosti likvorové analýzy u chronických, zánětlivých, autoimunitních onemocnění. Zavedení MRZ reakce do rutinní likvorové analýzy zvýšilo význam lumbální punkce u sclerosis multiplex dokonce již v období prvních klinických příznaků.

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