Journal of Neuroimmunology 321 (2018) 66–71

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

The intrathecal polyspecific antiviral immune response (MRZ reaction): A potential cerebrospinal fluid marker for multiple sclerosis diagnosis

Sawsan Feki[a,*], Saba Gargouri[b], Sabrina Mejdoub[a], Mariem Dammak[c], Hend Hachicha[a], Olfa Hadiji[c], Lamia Feki[b], Adnen Hammami[b], Chokri Mhiri[c], Hela Karray[b], Hatem Masmoudi[a]

[a] Laboratory of Immunology, Habib Bourguiba University Hospital, Faculty of Medicine, University of Sfax, Tunisia
[b] Laboratory of Microbiology, Habib Bourguiba University Hospital, Faculty of Medicine, University of Sfax, Tunisia
[c] Department of Neurology, Habib Bourguiba University Hospital, Faculty of Medicine, University of Sfax, Tunisia

ARTICLE INFO

Keywords:
Multiple sclerosis
Measles
Rubella
Varicella zoster virus
Intrathecal humoral immune response
MRZ reaction

ABSTRACT

We tested the performance of MRZ-reaction, an intrathecal humoral immune response against-Measles (M), Rubella (R) and Varicella Zoster (Z) viruses, in multiple sclerosis (MS) diagnosis.

The MRZ-reaction was significantly more positive in MS than in non-MS group with a specificity of 91.9%. In MS group, the RZ-profile was the most prevalent and the R-specific antibody-index was correlated to the number of oligoclonal bands (OCB) in CSF. Interestingly, the MRZ-reaction was detected in 53% of OCB-negative-MS patients.

The MRZ-reaction seems to be a relevant CSF diagnostic marker of MS disease. The likely relation between its positivity and the vaccination status deserves to be investigated.

1. Introduction

In the cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients, the intrathecal synthesis (IS) of affinity-maturated immunoglobulin (Ig) G, which are thought to have a huge variety of specificities (Rostrom, 1982; Reiber et al., 2015; Pohl et al., 2010; Otto et al., 2011; Derfuss et al., 2001), is considered as a hallmark of the disease (Andersson et al., 1994; Disanto et al., 2012). In these conditions, many pathways for Ig production by antibody-secreting B cells within the central nervous system (CNS) have been described. (Meinl et al., 2006; Blauth et al., 2015). Meningeal structures consisting of ectopic follicles, called "Tertiary lymphoid organs", are reported to play a role in B cells traffic and differentiation in the CNS (Magliozzi et al., 2007; Bonnan, 2014). Indeed, it has been demonstrated that, unlike the CSF of healthy persons where CD19 B cells are nearly absent, B lymphocytes transmigrate across the blood-brain barrier (BBB) and accumulate in the CNS of MS patients (Cepok et al., 2001; Kuenz et al., 2008), establishing these cells as critical players in MS pathogenesis (Gredler and Reindl, 2012). In CSF of MS patients, B cells are mostly memory B cells or short-lived plasmablasts (Cepok et al., 2005; Kuenz et al., 2008; Cepok et al., 2006; Winges et al., 2007) (naïve B cells are not frequent in the CSF of MS patients (Cepok et al., 2005)). Ig production in this context could be in response or not to specific antigen stimulation leading to Ab-secreting cells inside the CNS. Under appropriate survival conditions (e.g. transformation by EBV infection (Holmøy and Vartdal, 2004), these short-lived antibody-secreting cells differentiate into long-lived plasma cells responsible for the chronic IS of IgG (Gredler and Reindl, 2012), which could be revealed by CSF isofocusing test (as IgG Oligoclonal bands (OCB)), IgG Index calculation and/or Reiber diagram interpretation (Reiber, 2016). These routine laboratory tests could be unable to resolve the problem of MS diagnosis in many clinically confusing cases (OCB-positive alternative diagnosis, OCB-negative MS). Recently, the detection of a polyspecific intrathecal humoral immune response against three common neurotropic viruses: Measles (M), Rubella (R) and Varicella Zoster (Z), called the MRZ reaction has gained a lot of interest in MS studies (Felgenhauer and Reiber, 1992; Reiber et al., 1998a, 1998b; Jarius et al., 2017). While its pathophysiological role remains not well-understood, this reaction is reported to be highly specific to disease diagnosis and it seems interesting in diagnosing OCB negative-MS patients (Stangel et al., 2013).

The aim of this study was to investigate the clinical relevance of MRZ reaction tested in different groups of MS and non-MS patients and to compare its diagnostic value to OCB detection.

* Corresponding author.
E-mail address: sawsanfeki@yahoo.fr (S. Feki).

https://doi.org/10.1016/j.jneuroim.2018.05.015
Received 7 February 2018; Received in revised form 5 May 2018; Accepted 26 May 2018

2. Patients and methods

2.1. Study population

Samples (couples of CSF/serum) of patients with suspicion of central nervous system (CNS) inflammatory disorder are routinely addressed to the Laboratory of Immunology (Habib Bourguiba University Hospital, Sfax, Tunisia) for biological investigation: CSF isofocusing test (Hydragel CSF isofocusing kit (Sebia®, France), determination of total IgG and albumin levels in sera and CSF by nephelemetry (BN Prospec, Siemens®, Germany) for *Tibbling and Link* index calculation using the formula: *[(IgG CSF/IgG serum)/(Albumin CSF/Albumin serum)]* and Reiber Diagram interpretation using a special software (Gillain et al., 2009).

In CSF isofocusing, a positive pattern (OCB+) is defined by the presence of at least two additional OCB in CSF. An IgG index ≥0.7 is considered as indicative of IS of total IgG. The Reibergram is used to assess the BBB function as well as the quantitative IS of total IgG.

In this study, 194 samples (97 couples of CSF/serum) were collected from 97 individuals addressed from the Neurology Department of Habib Bourguiba University Hospital: 60 patients with definite diagnosis of MS according to Mc Donald criteria (2010) (50 relapsing remitting and 10 progressive MS) including 45 OCB positive and 15 OCB negative patients, and 37 non-MS patients with other inflammatory conditions of the CNS (control group) (Tables 1 and 2).

OCB positive patients were classified according to the number of additional bands in their CSF in comparison with serum (patients with 2; 3 to 5; and > 5 additional bands).

The study was approved by the local Ethical Committee.

2.2. Determination of MRZ reaction

Measles (M), Rubella (R) and Varicella (Z) viruses specific-IgG levels were measured using a commercially available enzyme-linked immunosorbent assay for virus antibody determination in CSF and serum (Euroimmun®, Germany) according to the manufacturer's instructions. The IS of IgG to M, R and Z viruses was detected by calculation of the corresponding virus-specific antibody index (AI) according to Reiber's formula: $AI = QIgG[spec]/QIgG[total]$ (if QIgG[total] < reference range (Qlim)) and $AI = QIgG[spec]/Qlim$ (if QIgG[total] > Qlim). AI values ≥1.5 were considered to be indicative of IS of IgG against the corresponding pathogen and MRZ reaction was considered positive if at least two AI were ≥ 1.5. When virus specific-IgG level was undetectable in CSF, the AI could not be calculated, and was graded as 0.6 (the lowest limit of normal range).

Table 1
Clinical and radiological features of enrolled multiple sclerosis (MS) and non-MS patients.

	MS group	Non-MS group
Size	60	37
Age (years) [Median (range)]	34 (16–67)	42.5 (19–74)
Gender	50 F/10H	24 F/13H
Diagnosis	MS	9 Neuro-Behçet's 6 Neuromyelitis optica 5 Sarcoidosis 4 Neuro-Sjogren's Syndrome 3 Neuro-lupus 6 Vasculitis 4 Autoimmune encephalitis
Clinical form		
Relapsing remitting	50	–
Progressive	10	
Optical Neuritis	28 (46.6%)	14 (37.8%)
MRI		
Cerebral Abnormalities	60 (100%)	25(67.5%)
Myelitis	36 (60%)	15 (40.5%)
EDSS Mean (range; SD)	2.67 (0–7; ± 2)	–

MRI: Magnetic resonance imaging; EDSS: Expanded Disability Status Score

Table 2
Immunological features of enrolled multiple sclerosis (MS) and non-MS patients.

	MS group	Non-MS group
Total IgG index		
< 0,7	23 (38.4%)	27 (73%)
≥0,7	37 (61.6%)	10 (27%)
Mean (range)	1.17 (0.4–4.22)	0.71 (0.42–3.96)
Reiber diagram		
-Blood brain barrier dysfunction	14(23.3%)	15 (40.5%)
-IgG intrathecal synthesis	36 (60%)	10 (27%)
CSF Isofocusing		
OCB (+)	45 (75%)	12 (32.4%)
OCB (−)	15 (25%)	25 (67.6%)

OCB: oligoclonal bands.

2.3. Statistical analysis

We calculated the sensitivity of a test as [true positives/(true positives +false negatives)] and the specificity as [true negatives/(true negatives + false positives)].

The positive predictive value (PPV) was defined as [true positives/(true positives+ false positives)] and the negative predictive value (NPV) as [true negatives/(true negatives+ false negatives)].

Quantitative variables were expressed with median and range values. Statistical significance of differences in these variables between groups of patients was assessed by Mann-Whitney *U* test. Qualitative variables were tested using Fisher's exact test (two-tailed). Spearman's test was used to evaluate correlations. A p value of < 0.05 was defined as statistically significant. All calculations were performed with SPSS 20.0 software.

3. Results

3.1. Anti-viral specific antibodies prevalence in the study population

Overall, detectable levels of specific IgG to M, R and Z viruses in serum and CSF were observed in 84.5% (82/97) and 75.2% (73/97), 95.9% (93/97) and 94.9% (92/97), 96.9% (94/97) and 93.8% (91/97), respectively. Of note, all patients with no detectable specific IgG against Measles in serum (15/97) were aged < 35 years.

3.2. MRZ reaction in MS Group vs non-MS Group

Each of the 3 specific AI of M, R and Z viruses was found significantly more frequently positive in the MS group (58.3%, 65% and 63.3%, respectively) than in the non-MS group (24.3%, 16.2% and 10.8%, respectively) (Fig. 1).

As expected, the positivity of MRZ reaction in MS patients was significantly much more frequent than in the control group (65% Vs 8.1%; $p < .001$) (Fig. 1).

Interestingly, the RZ reaction was the most prevalent profile found among bispecific positive reactions in MS patients (12/19) and the most constant dual combination of anti-viral reaction (32/39 MRZ reaction positive-MS patients). The RZ reaction was also strongly associated ~~to~~ with the disease (53,3% versus 8.1%, $p < .001$). Conversely, the most prevalent single reaction was observed with Measles, which was found in 58.3% of cases (14/24 patients with single reaction) (Fig. 2), with nearly the same frequencies in both MS and non-MS groups (57.1% and 60%, respectively).

Regarding the performance of the MRZ reaction for MS diagnosis,