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# What does an isolated cerebrospinal fluid band mean: a tertiary centre experience

## Co oznacza obecność pojedynczego prążka w płynie mózgowo-rdzeniowym? Doświadczenie nabyte w oparciu o badania materiału zebranego w ośrodku medycznym trzeciego stopnia referencyjności

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Abstract

**Introduction:** The presence of oligoclonal bands in cerebrospinal fluid of multiple sclerosis patients is now well established to support the clinical diagnosis. On the other hand, a single band response can represent the initial stage of an oligoclonal response, before the other antibody clones become visible. **Method:** The aim of the current study was to evaluate the presence of an isolated cerebrospinal fluid single immunoglobulin band, and to analyse the clinical and radiological diagnosis of the samples with a single immunoglobulin band. In this study, 3524 cerebrospinal fluid samples were re-examined using agarose gel isoelectric focusing, and ones with an isolated cerebrospinal fluid immunoglobulin band were detected. **Results:** A single band in cerebrospinal fluid was detected in 1.4% samples. A clinically isolated syndrome was diagnosed in 27.5%of them, relapsing remitting multiple sclerosis in 49%, secondary progressive multiple sclerosis in 11.8%, and radiologically isolated syndrome in 2%. No primary progressive multiple sclerosis patient was found. All Barkhoff criteria were met in 90.1% of them. The remaining were diagnosed with other inflammatory neurological diseases (9.8%). **Conclusion:** The presence of an isolated cerebrospinal fluid monoclonal immunoglobulin band is rare. Although most of the samples were diagnosed as multiple sclerosis according to both clinical and paraclinical (magnetic resonance imaging) parameters, they had only a single immunoglobulin band in cerebrospinal fluid. Not only oligoclonal bands, but also an isolated cerebrospinal fluid single band might be a cornerstone for the diagnosis of multiple sclerosis at least for some patients.

**Key words:** cerebrospinal fluid, single band, multiple sclerosis

Streszczenie

**Wprowadzenie:** Obecność prążków oligoklonalnych w płynie mózgowo-rdzeniowym chorych na stwardnienie rozsiane jest obecnie ogólnie przyjętym kryterium wspierającym rozpoznanie kliniczne. Z drugiej strony obecność pojedynczego prążka może świadczyć o początkowym stadium obecności prążków oligoklonalnych, zanim pojawią się inne klony przeciwciał. **Metoda:** Celem niniejszej pracy była ocena występowania pojedynczego prążka immunoglobuliny w płynie mózgowo-rdzeniowym oraz analiza rozpoznania klinicznego i radiologicznego u pacjentów, u których stwierdzono pojedynczy prążek. Ponownemu badaniu za pomocą ogniskowania izoelektrycznego na żelu agarozowym poddano 3524 próbki płynu mózgowo-rdzeniowego oraz wyłoniono te, w których obecny był pojedynczy prążek immunoglobuliny. **Wyniki:** Obecność pojedynczego prążka w płynie mózgowo-rdzeniowym została stwierdzona w 1,4% próbek. Izolowany zespół objawów klinicznych rozpoznano u 27,5% pacjentów, postać rzutowo-nawracającą stwardnienia rozsianego – u 49%, postać wtórnie przewlekłą – u 11,8%, a zespół objawów radiologicznych – u 2% badanych. U żadnego pacjenta nie stwierdzono postaci pierwotnie postępującej stwardnienia rozsianego. Wszystkie kryteria Barkhoffa były spełnione u 90,1% badanych. U pozostałych pacjentów (9,8%) rozpoznano inne choroby neurologiczne o podłożu zapalnym. **Wnioski:** Występowanie pojedynczego prążka immunoglobuliny monoklonalnej w płynie mózgowo-rdzeniowym jest rzadkim zjawiskiem. Mimo iż w większości badanych próbek rozpoznano stwardnienie rozsiane na podstawie zarówno kryteriów klinicznych, jak i paraklinicznych

(obrazowanie rezonansem magnetycznym), w płynie rdzeniowym tychże pacjentów występowały jedynie pojedyncze prążki immunoglobuliny. Nie tylko prążki oligoklonalne, ale również pojedyncze prążki obecne w płynie mózgowo-rdzeniowym mogą stanowić podstawę rozpoznania stwardnienia rozsianego przynajmniej u niektórych chorych.

**Słowa kluczowe:** płyn mózgowo-rdzeniowy, pojedynczy prążek oligoklonalny, stwardnienie rozsiane

## INTRODUCTION

The presence of immunoglobulins (Ig) in cerebrospinal fluid (CSF) has become an established laboratory test for the detection of inflammatory conditions of the central nervous system (CNS) involving the humoral immune system. A selective increase in CSF IgG can be measured by quantitative analysis of intrathecal IgG or by qualitative detection of CSF-restricted oligoclonal bands (OCBs) (Kostulas, 1985; Link, 1991; Link and Huang, 2006; Reiber, 1991). Two or more oligoclonal IgG bands detected by separation of CSF proteins while not in corresponding serum reflect a local B-cell response in central nervous demonstrable system (CNS) inflammation. Diagnosis of multiple sclerosis (MS) should be based on clinical and magnetic resonance imaging (MRI) features, but as other CNS disorders may cause similar symptoms, it is necessary to perform additional tests, such as evoked potentials and vasculitic tests, together with an analysis of the CSF (Polman *et al.*, 2005).

The analysis of CSF is especially important not only for the diagnosis of MS but also the understanding of pathogenesis of some inflammatory central or periferic nervous system diseases. Although the percentage of positive OCBs (more than two bands in CSF only) and elevated quantitative indices in patients with MS in Europe and America is usually over 90% and 80%, respectively (Andersson *et al.*, 1994; Arata and Leonardi, 1988; Tourtellotte *et al.*, 1988), in Asian countries the frequencies of OCBs or increased IgG index in patients with MS were reported to be much lower (about 50%) (Kira *et al.*, 1996; Li *et al.*, 2007; Nakashima *et al.*, 1999) than those in Europe and America. In our country, the percentage of positive OCBs in patients with clinically definite MS is 83% (Idiman *et al.*, 2009). Zeman *et al.* suggested that clinically definite MS with negative OCBs is rare and should be diagnosed with caution (Zeman *et al.*, 1993; Zeman *et al.*, 1996). Although oligoclonal response is "non-specific," it is helpful as a diagnostic aid, provided other known causes of local synthesis of oligoclonal bands have been excluded. An oligoclonal response represents an immunologic response to a specific antigen or set of antigens, and is found in other inflammatory and infectious diseases affecting the CNS, although these can be differentiated from MS using additional CSF and/or clinical findings (Davies *et al.*, 2003; Freedman *et al.*, 2005).

There are five classic patterns of CSF and serum staining with isoelectric focusing on agarose gels with immunoblotting: Pattern 1, no bands in CSF and serum sample, is considered negative; Pattern 2, oligoclonal IgG bands in CSF, not in the serum sample, indicative of intrathecal IgG synthesis, definitively shows specific bands present only in the CSF but not the serum sample; Pattern 3, oligoclonal bands in CSF (like Pattern 2) and additional identical oligoclonal bands in CSF and the serum sample, stil indicative of intrathecal IgG synthesis; Pattern 4, identical oligoclonal bands in CSF and the serum sample, illustrative of a systemic not intrathecal immune reaction, with a leaky or normal or abnormal blood–CSF barrier and oligoclonal bands passively transferred in the CSF, can be seen in conditions such as the Guillain–Barré syndrome; and Pattern 5, monoclonal bands in CSF and the serum sample; this is the pattern seen owing to the presence of a paraprotein (monoclonal IgG component) (Freedman *et al.*, 2005).

Finally, Freedman *et al.* (2005) discussed a single band in the CSF but not in the serum, indicating the presence of an intrathecal monoclone, that was usually considered a negative or normal CSF study.

There is some evidence that a second lumbar puncture (after an interval of at least 6 months) should be considered in patients with a single OCB, because those patients who convert to a "full" OCB pattern are more likely to be subsequently diagnosed with MS. However, in a small subset of patients with MS only a single immunoglobulin (Ig) band, confined to the CSF, is identified (Bass *et al.*, 1988; Ben-Hur *et al.*, 1996). In this study, we aimed to evaluate the presence of an isolated CSF Ig band, and to analyse the clinical and radiological diagnosis of the samples with a single Ig band.

## MATERIAL AND METHOD

We re-examined 3524 consecutive CSF samples of patients who had undergone lumbar puncture for diagnostic purposes in 2005–2013 at Neuroimmunology Laboratory of Dokuz Eylul University Hospital. All diagnostic lumbar punctions were conducted prior to corticosteroid and immunosuppressive treatment. OCB status was determined by agarose gel isoelectric focusing (IEF) with immunoblotting. The method is based upon techniques originally described by Keir *et al.* (1990) and Andersson *et al.* (1994). The samples with an isolated sharp CSF Ig band but no equivalent band in serum were detected by a blinded neurologist. Since it is a rare finding, to avoid a technical problem, as a principle of our neuroimmunology laboratory, samples that came out with a sharp monoclonal band were studied once again to confirm or not confirm the finding.

## Statistics

Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS version 17.0). Demographic characteristics of the patients were analyzed using descriptive statistics. Descriptive statistics are reported as means ± standard deviations or percentages. Between-group differences were tested with Kruskal–Wallis test due to the distributional characteristics. Alpha <0.05 was considered statistically significant.

## RESULTS

An isolated single band in CSF was detected in 51 out of 3524 patients (1.4%; 28 female, 23 male). Clinically isolated syndrome (CIS) was diagnosed in 27.5% of them, relapsing remitting multiple sclerosis (RRMS) according to McDonald (2005) criteria in 49%, secondary progressive MS (SPMS) in 11.8%, and radiologically isolated syndrome (RIS) in 2%. No primary progressive MS (PPMS) patient was found.

There was one patient diagnosed with RIS. The patient with RIS was admitted into our hospital because of severe, frequent, hemicranial headache. She had no neurologic findings, but revealed multiple periventricular T2 lesions that showed gadolonium (Gd) enhancement on cranial MRI. Her spinal cord MRI was normal. Lumbar puncture was conducted upon her admission to our hospital. In the follow-up period (12 months) she underwent normal neurologic examination. One year later, both her cranial and spinal MRI were repeated, and multiple periventricular lesions without Gd enhancement were found. CSF examination was not repeated.

In patients with CIS, lumbar puncture was conducted within 1 month during the first clinical episode prior to corticosteroid therapy. The demographic and CSF features of the patients with demyelinating diseases, RIS, and other inflammatory neurologic diseases (the mean age, disease duration, the mean EDSS scores, the duration of follow-up, and the mean CSF IGG index) are shown in Tab. 1.

In the patients with CIS, the mean CSF IgG index was higher than in the patients with definite multiple sclerosis ($p < 0.05$). The mean age, the mean disease duration and the mean EDSS score of MS patients excluding CIS and RIS patients were 38.35 ± 11.01 (18–57) years, 78.6 ± 69.3 (11–264) months and 3.1 ± 1.9 (0–7.5), respectively, and 90.1% of them met the Barkhoff criteria.

The remaining patients were diagnosed with other inflammatory neurological diseases (OIND) (9.8%) (one patient with chronic inflammatory demyelinating polyneuropathy, one patient with neuromyelitis optica, one patient with paraneoplastic syndrome, two patients with acute disseminated encephalomyelitis). Some of our patients with OIND were found to have higher IgG index than some of the MS patients. Since CSF index is an indicator of the relative amount of CSF IgG as compared with serum, those patients with OIND seemed to reveal more IgG production (Tab. 1).

## DISCUSSION

The presence of an isolated CSF single band is rare. In this study, 3524 CSF examples were studied, and a single band in CSF was detected in 1.4% of them. Interestingly, most of the patients were diagnosed with MS according to both clinical and paraclinical (MRI) criteria. Those patients had long disease duration and high mean EDSS scores, but had only a single Ig band in CSF. In fact, a single CSF band is an indication for repeating a CSF analysis unless other criteria clearly point to a diagnosis of MS, and for considering an alternative diagnosis. In none of our cases lumbar puncture and CSF examination were repeated, owing to their convincing clinical and other paraclinical findings. Furthermore, we determined OCB status by isoelectric focusing and IgG-specific immunofixation which has sensitivity of 88–100% and specificity of 94% for MS (Kostulas *et al.*, 1987; Olsson *et al.*, 1984; Petzold, 2013).

One group who studied OCB status with agarose gel electrophoresis found that in 0.55% of their samples a single band was found, and seven out of 20 single band patients

| *n* (%) | The mean age (year) | The mean follow-up duration (months) | The mean EDSS score | The mean CSF IgG index |
|---|---|---|---|---|
| **CIS (*n* = 14)** | 28.2 ± 7.9 (16–40) | 8.57 ± 20.5 (0.75–72) | 1.6 ± 0.59 (0–2.5) | 0.83 ± 0.41 (0–2) |
| **RRMS (*n* = 25)** | 36.8 ± 10.1 (18–57) | 74.9 ± 71.2 (11–264) | 2.5 ± 1.6 (0–7.5) | 0.75 ± 0.46 (0–2) |
| **SPMS (*n* = 6)** | 44.6 ± 13.0 (22–57) | 107.3 ± 53.5 (48–152) | 5.5 ± 1.3 (3.5–6.5) | 0.65 ± 0.57 (0–1.1) |
| **RIS (*n* = 1)** | 27 | 12 | - | 0.61 |
| **OIND (*n* = 5)** | 41.6 ± 11.4 (31–55) | 115 ± 100.5 (0.5–240) | - | 0.54 ± 0.35 (0–1) |

*Tab. 1. The mean age, the mean follow-up duration, the mean EDSS score and the mean CSF IgG index values of the patients*



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had clinically definite MS with affective disorder comorbidity (Ben-Hur *et al.*, 1996). A second study using IEF found only three out of 1490 CSF samples to have a single band. Two of these had lymphoma or lymphomatoid granulomatosis within the nervous system (McCombe *et al.*, 1991). None of our single band patients revealed a lymphocytic malignancy. A third study using IEF repeated lumbar puncture after 14 to 505 days from the initial one, and found that nine out of 31 single band patients converted to an oligoclonal pattern, 15 retained the same monoclonal pattern, and seven converted to a normal CSF IEF profile. The authors reported that only one out of the 15 patients who on follow-up CSF examination were found to have a persisting intrathecal monoclonal band, had a presentation that was thought to be due to demyelination. This study suggested that the isolated CSF monoclonal band patern in CIS patients was a part of the early evolution of an intrathecal oligoclonal response in CNS (Davies *et al.*, 2003).

OCB positivity has been defined as two or more bands present in CSF but absent in plasma at the same point in time for MS diagnosis. Since this single band pattern is between no band and two bands, some authors have called this band type a borderline pattern. It is reported to be connected to the maturation of the humoral immune response, a process requiring time with interindividual differences due to genetic modulation. From the initial production of IgG with a wide range of affinity, this response could lead to the selection of a few plasma cell clones that secrete high-affinity IgG. Thus, some patients with MS could be on the way to the maturation of the immune response, yielding the borderline pattern (Franciotta *et al.*, 2005; Link and Huang, 2006). The monoclone simply represents the dominant clone in an evolving oligoclonal response.

In order to increase the specificity of the diagnosis, and to minimize the number of false diagnoses with MS, the International Committee has recommended the use of both clinical and paraclinical criteria, the latter involving information obtained from magnetic resonance imaging, evoked potentials, and CSF analysis (McDonald *et al.*, 2001). To maximize the benefit of CSF as a diagnostic paraclinical test, the most sensitive method should be used (Freedman *et al.*, 2005). When the clinical suspicion is high, and the test comes back negative for local synthesis of OCBs, this should be considered an "alert" to the clinician to reassess the case (Zeman *et al.*, 1993; Zeman *et al.*, 1996). Nevertheless, some patients tested with the most sensitive methods could reveal an isolated single band in CSF, suggesting intrathecal Ig production. This finding might be considered as a part of the early evolution of an intrathecal oligoclonal response, or represent an intrathecal paraprotein. Our study suggested that not only OCBs of more than two in CSF, but also an isolated single CSF band might be a predictive value for the diagnosis of MS, at least for some patients. However, the patients with an isolated single band need very careful consideration. The finding of a single band confined to the CSF may hint a disease other than multiple sclerosis.

### Conflict of interest

*The authors do not report any financial or personal connections with other persons or organizations which might negatively affect the content of this publication and/or claim authorship rights to this publication.*

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