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# Intrathecal oligoclonal IgG bands are infrequently found in neuro-Behçet's disease

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## ABSTRACT

**Objectives.** Oligoclonal bands (OCB) of immunoglobulins (IgG) in the cerebrospinal fluid (CSF) provides an evidence for the humoral response and have been screened in the CSF and serum of patients revealing 5 different patterns. In this study, patients with Behçet's disease (BD) are screened in a larger sample to potentially provide information about the possible role of CSF oligoclonal immunoglobulins in the diagnosis of this disease.

**Methods.** Paired CSF and serum samples from 121 consecutive BD patients with neurological complaints (43 women and 78 men) were included in this study. Parenchymal NBD was diagnosed in 74 patients, and 22 patients had cerebral venous sinus thrombosis (CVST); of the remaining patients, 18 had primary headache disorders not directly associated with BD, and 7 had a cerebrovascular event. OCB of IgG were detected by isoelectric focusing on agarose and immunoblotting of matched serum and CSF sample pairs. Intrathecal production of IgG only is considered positive (Pattern 2 or 3).

**Results.** In the whole group, only 8 patients had OCB in the CSF showing pattern 2. All these positive cases had parenchymal neuro-BD (10.8% positive and 78.4% negative in parenchymal neuro-BD group). All other groups were negative.

**Conclusion.** The rare presence of oligoclonal IgG bands in CSF can be utilised as another laboratory finding in the diagnosis of NBD.

## Introduction

Abnormalities of cerebrospinal fluid (CSF), particularly with respect to locally synthesised immunoglobulin G (IgG), have been associated with a range of neurological conditions and demonstrated qualitatively by oligo-

clonal banding (OCB). With isoelectric focusing (IEF) and immunoblotting methods, five patterns of OCB can be obtained: 1 identifies normal, whereas 4 and 5 reflect systemic IgG production. Only pattern 2, with IgG bands in the CSF not matched in the serum, and pattern 3, with some of the bands leaked from the CSF to the serum, represent intrathecal IgG production (1). Neurological involvement in Behçet's disease (BD) is present in 5–10% of the patients in Turkish cohorts. Among the neuro-BD (NBD) patients, the majority (80%) develop parenchymal involvement, while in about 20% raised intracranial pressure due to cerebral venous sinus thrombosis (CVST) is the main clinical manifestation (2).

Although parenchymal NBD and multiple sclerosis (MS) usually have distinct clinical and radiological features, due to the similarities in disease course such as exacerbations and remissions, and some neuroradiological findings, these can occasionally be confused with each other (3, 4). Although not specific for MS, 95% of patients present an oligoclonal distribution of IgG bands exclusively in the CSF (pattern 2 or 3). The sensitivity (94.5%) and specificity (84.1%) of OCB in MS is relatively high (5, 6).

In NBD, previous studies reported that pleocytosis, elevated total protein and immunoglobulins can be detected in the CSF (7, 8). If the pleocytosis is presented with a granulocytic predominance, parenchymal NBD is more probable (9). The presence of oligoclonal Ig in CSF has also been evaluated in NBD and IgA and IgM type of oligoclonal bands have been reported to be related to disease activity, whereas 38% had shown IgG bands in the CSF (3). However, five typical OCB patterns were not differentiated in this small pioneer study. In the present study, we have

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evaluated the presence of OCB in consecutive BD patients with neurological complaints considering the patterns as well.

**Material and methods**

*Patients*

A total of 139 paired CSF and serum samples from 121 consecutive BD patients (F/M: 43/78) were included. The median age of the patients was 34.0 years (range 13–64). All patients fulfilled the diagnostic criteria of the International Study Group for BD (10). When patients were sub-grouped (2), parenchymal NBD was diagnosed in 74 and CVST in 22 patients. Among the remaining, 7 had arterial stroke and 18 had primary headache disorders (migraine or tension type headache), who were not considered to have NBD. At the time of the lumbar puncture, 36.4% of the NB patients received an immunomodulatory treatment. This study was approved by the Institutional Ethics Committee.

All CSF samples were obtained for diagnostic purposes after receiving consent and paired CSF, and serum samples were analysed in parallel. For detection of OCB, IEF and immunoblotting was performed on matched serum and CSF sample pairs, as described (11). Two or more pairs from different time points were screened from 13 patients. The neat CSF and diluted serum samples were run on an agarose gel with synthetic ampholytes (Pharmalytes, pH 3–10 and 8–10.5, Serva GmbH). IEF was performed in 1% IEF agarose, followed by passive protein transfer onto nitrocellulose membrane. Immunodetection of IgG was achieved by double antibody staining with horseradish peroxidase. The patterns were interpreted qualitatively by comparing the serum and the CSF. Positivity for OCB (pattern 2 and 3) was compared with the Fisher's exact test.

**Results**

Among 121 consecutive BD patients enrolled, the majority had parenchymal NBD (77.1%). Only in 8 patients of this group, OCB were detected in the CSF samples, only resulting in pattern 2 (10.8% of parenchymal NBD).

**Table I.** The distribution in BD patients according to disease subgroups.

	n.	OCB pattern				
		1	2	3	4	5
Parenchymal NB	74	58	8	0	8	0
CVST	22	19	0	0	3	0
BD-stroke	7	7				
BD-HA	18	18				

OCB: oligoclonal bands; CVST: cerebral venous sinus thrombosis; BD-stroke: BD patients with stroke; BD-HA: BD with headache.

In one of these patients the IgG bands disappeared at the next CSF examination performed 10 months later. In two of these patients we have detected an accompanying systemic monoclonal reaction revealing a mixed pattern of 2 and 5. In 58 out of 74 (78.4%) patients in parenchymal NBD group, no IgG bands were detected (pattern 1). In this group, 2 consecutive spinal tap sample pairs were available from 5 different patients with both determinations similarly negative. Only in 2 patients we have detected a single IgG band in the CSF. As this finding did not meet the definition of a positive OCB ( $\geq 2$  bands in the CSF not matched in the serum) (12), they were considered negative as well. In one NBD patient, 4 different sample pairs were collected and retested within a period of 5.5 years. In these samples a single band was detected two times with alternating presence. In further 8 patients identical band patterns were detected in both the serum and CSF samples compatible with pattern 4 as systemic response, rather than intrathecal antibody production (1, 13). When analysed according to the treatment, the distribution of OCB in treated and untreated patients was not significantly different (5/31 vs. 2/29,  $p > 0.05$ ). Among the parenchymal NBD patients with positive OCB, 3 had a clinical and radiological picture like MS, 4 had a secondary progressive type brainstem NBD, and 1 had a single brainstem attack, but was lost to follow-up. On the other hand, two of the OCB negative parenchymal NBD cases had MS-like clinical presentation, and 21 had a progressive course. OCB positivity was significantly higher in the group with MS-like presentation ( $p = 0.025$ ). However, clinical course (progressive

vs. non-progressive) did not have any significant relationship with the OCB status.

In this cohort, none of the 22 patients with CVST was positive for OCB (pattern 2 or 3). Only three patients had a systemic IgG response evidenced by pattern 4 (18.8%). In those patients a systemic BD exacerbation had been observed clinically.

In the remaining BD groups with primary headache (BD-HA), as well as concomitant cerebrovascular accidents (BD-stroke), no oligoclonal IgG were shown in 25 patients (Table I).

**Discussion**

The main finding of this study is the rare intrathecal IgG production in NBD. Considering the high incidence of OCB in MS, this finding may contribute to the differential diagnosis of these diseases, when a clinical suspicion is present (6). Our laboratory's OCB positivity rate in patients with definite MS is 89% (Korkmaz *et al.*; unpublished data), whereas only 10.8% of parenchymal NBD patients were positive. In those 8 patients with OCB no specific features were shown compared to the negatives, except a clinical and radiological picture resembling MS in three only. Although, in the OCB negative group, there were also MS-like cases, the ratio was significantly smaller, suggesting that OCB positive cases might represent cases in the disease spectrum between MS and BD, or MS cases who also have BD. A previous report has shown oligoclonal IgG positivity in 5 out of only 13 NBD patients (3). Another retrospective study of 12 BD patients with neurological involvement found local synthesis of OCB in one and serum leak (pattern 3) in another patient at

some stage of the disease. The present data extend these findings. With the consideration that "the neurologist asks whether OCB are detectable in the CSF while absent in the patient's serum as a consequence of an abnormal B-cell response within the CNS (*i.e.* types 2 and 3), or whether no OCB are detectable in CSF, indicating that there is no evidence for this type of abnormal immune response within the CNS" (12), the answer is negative for the great majority of NBD patients.

Even in the few patients with IgG presence in the CNS, a persisting antibody production was not evident. In one of the patients with a pattern 2 OCB, these bands disappeared 10 months later, which is very unusual for MS. Moreover, a single CSF IgG band was detected in 2 other parenchymal NBD patients that did not persist in one of them. The significance of intrathecal synthesis of a single band is uncertain. Monoclonal IgG has also been reported in some MS patients with progression to oligoclonal pattern in the follow-up (33.3%), but some of them also reverted to normal CSF or continued to exhibit only the monoclonal band (13). Certain CSF findings can help to distinguish between the different inflammatory CNS diseases. The most common abnormality of CSF in NBD is a mild pleocytosis and elevated protein levels (14). A potentially relevant finding for differentiating NBD from other chronic inflammatory diseases is the occurrence of a granulocytic pleocytosis (15). Elevated protein and/or pleocytosis in CSF has also been associated with a poorer prognosis (16).

Another finding in NBD implicated the systemic response in a subgroup of patients presenting with pattern 4. Eight of the patients showed this pattern, persistent in 2, which is rather a significant proportion of our cohort. OCB identical in CSF and serum tended to result from systemic infections without primary neurological involvement. In a previous evaluation of 1874 samples,

neoplasia and peripheral neuropathies accounted for over 50% of the diagnoses and infections and systemic inflammatory disorders for 32% for pattern 4 findings (17). However, we do not have supporting data on NBD patients with pattern 4.

As previously demonstrated (18, 19), antibodies in CNS are not prominent in NBD. The introduction of immunomodulatory treatment in NBD may have influenced the presence IgG in those patients with antibodies in the CSF. Although steroid treatment has been shown to improve the barrier function in some patients, no significant association with changes of OCB pattern was demonstrated in NBD (17). Our results are in accordance with this conclusion as well.

As a conclusion, the absence of OCB in CSF can be utilised as another laboratory finding supportive of NBD, in addition to the CSF pleocytosis, which suggests a more inflammatory process in NBD.

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