

Cerebrospinal-Fluid Profile in Neuroborreliosis and Its Diagnostic Significance

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ABSTRACT. Selected cerebrospinal-fluid (CSF) parameters (intrathecal synthesis of *Borrelia*-specific antibodies, oligoclonal IgG bands, CSF-to-serum quotient of albumin as a marker of blood–CSF barrier function and cytology) and typical CSF profile in neuroborreliosis were evaluated with the aim of elucidating possible clinical and laboratory similarities of neuroborreliosis (NB) and other neurological diseases (OND). From the cohort of 58 patients (38 diagnosed for NB, 20 with OND) NB patients had positive *Borrelia*-specific IgG antibodies in 97 % and positive *Borrelia*-specific IgM antibodies in 55 %; oligoclonal IgG bands were detected in 55 %. The blood–CSF barrier was impaired in 89 %, positive cytology was detected in 97 % of the NB patients. Evaluation of specific intrathecal synthesis improves CSF diagnosis of NB, therefore, a combined CSF analysis has to be considered along with the clinical picture and medical history when formulating the diagnosis of NB.

Abbreviations

Ab	antibody, antibodies	Ig	immunoglobulin
AI	specific Ab index	Ins	intrathecal synthesis
Alb	albumin	NB	neuroborreliosis
Bs	<i>Borrelia</i> -specific	OND	other neurological diseases
CNS	central nervous system	Q	CSF/S quotient (CSF-to-serum quotient; e.g., Q_{Alb} , of Alb)
CSF	cerebrospinal fluid	Q_{IgG}	ratio of total IgG in CSF and serum
EIA	enzyme immunoassay	Q_{IgM}	ratio of total IgM in CSF and serum
ELISA	enzyme-linked immunosorbent assay	Q_{lim}	limiting IgG or IgM quotient (see the text)
EUCALB	European Union Concerted Action on Lyme Borreliosis	Q_{spc}	ratio of Bs Ab (IgG or IgM) in CSF and serum (related to Q_{lim} ; see the text)

Borreliosis is an inflammatory disease caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato complex transmitted to humans by *Ixodes* ticks (Schwarzová and Čížnár 2004). The spirochetes can spread locally in the skin or invade other organs. The mechanism of attack and clinical symptoms may be variable and usually depend on the subtype of borrelia (Duniewicz *et al.* 1999; Vrethem *et al.* 2002; Severinová *et al.* 2005; Vancová *et al.* 2005). *B. burgdorferi* sensu stricto typically causes arthritis, *B. garinii* mainly causes neurological symptoms whereas *B. afzelii* is responsible mainly for skin symptoms. NB is a frequent manifestation of disseminated borreliosis and may develop either in the 2nd or the 3rd stage of the disease (Oschmann *et al.* 1998). Czechia is an area of borreliosis endemicity with an estimated incidence of 61 cases per 100 000 inhabitants (Janovská 2001). Neurological involvement can affect both the peripheral and the CNS; involvement of the nervous system may result in a wide spectrum of clinical symptoms including headache, meningitis, cranial neuritis, mono and polyradiculitis with motor and sensory dysfunctions (Tumani *et al.* 1995). In Europe, meningopolyradiculoneuritis (Bannwarth's syndrome) represents the most common manifestation of acute NB, with the facial nerve being affected much more frequently than other cranial nerves. Clinical symptoms affecting the CNS are rarely observed and then mostly in chronic courses (Kaiser 1998).

In clinical practice the diagnosis of borreliosis is based on the epidemiological history, clinical findings, serological investigations (*cf.* Rudenko *et al.* 2005) and, in NB, the finding of mononuclear pleocytosis in CSF (Berglund *et al.* 2002; Sobek *et al.* 1998, 2001). However, Bs-IgM and Bs-IgG tests are positive in 3 and 10 %, respectively, healthy individuals in Czechia (Janovská 2001). Patients may also remain seropositive years after adequate antibiotic treatment (Hammers-Berggren *et al.* 1994; Kaiser 1994). Isolated absolute CSF values of specific antibodies detected by immunoenzymic methods can be misleading since blood–CSF barrier impairment may be the possible origin of an increased CSF value. Also specific techniques like Western blot or immunospot assay still have the barrier-related evaluation problem (Reiber and Lange

1991). According to EUCALB, it is necessary to demonstrate intrathecal production of specific antibodies for NB diagnosis. Supporting laboratory parameters include oligoclonal bands in CSF, intrathecal total IgM and IgG synthesis and mononuclear pleocytosis in CSF (Robertson *et al.* 2000; Adam *et al.* 2001, 2003a,b; Sobek *et al.* 2002).

The neurological symptoms are not specific for NB but may occur in many OND. This makes the clinical diagnosis sometimes difficult. Due to possible clinical and laboratory similarities with OND we decided to investigate selected CSF parameters and evaluate the typical CSF variables profile in NB.

MATERIAL AND METHODS

Patients. A cohort of 58 patients ($n = 58$; treated at the *Faculty Hospital Brno* in 2002–2005) was investigated; thirty-eight of them suffered from NB. The control group consisted of 20 patients with OND: bacterial meningitis ($n = 1$), aseptic meningitis (5), sepsis (1), idiopathic facial nerve paresis (3), lumbar stenosis (1), low back pain (1), polyneuropathy (1), neurasthenia (1), neurodegenerative disorder (1), herpes simplex encephalitis (2), and zoster meningitis (3).

The diagnosis of NB was based on epidemiological history, characteristic clinical findings and serological positivity supported by mononuclear pleocytosis. Clinical symptoms included facial nerve paresis, abducens nerve paresis, Bannwarth's syndrome, meningitis, radiculoneuropathy, low back pain. Serum and CSF sample pairs were collected from each patient and analyzed immediately or stored at $-20\text{ }^{\circ}\text{C}$.

Borrelia-specific IgG and IgM were detected by sandwich EIA using a commercial kit from *Test-Line, Clinical Diagnostics Ltd.* (Czechia) (EIA *Borrelia garinii* IgG, IgM; in this test microtiter strip wells are coated with sonicated total, flagellin and M310 antigens against *B. garinii*). If corresponding specific Ab are present in a sample, they are bound to the antigens at the solid phase during the first incubation. After removing unbound material by washing, anti-human peroxidase conjugate is added, followed by the 2nd incubation. After a 2nd washing step (to remove unbound conjugate), the enzyme-linked complexes are detected by incubation with a substrate solution. Subsequent development (blue color changes during the enzymic reaction to yellow) was stopped by sulfuric acid. Absorbance A_{450} was measured in ELISA microtiter plate reader. All reagents were part of the commercial kit.

Alb, IgG and IgM concentrations in serum and CSF were measured nephelometrically. The ratio of Alb in CSF and serum (Q_{Alb}) reflects the conditions of blood–CSF barrier function. Increased values of Q_{Alb} indicate dysfunction of this barrier because Alb originates exclusively from blood (Sobek *et al.* 2003; Táborský *et al.* 2003; Reiber and Peter 2004).

Intrathecal synthesis. Among many numeric and graphic approaches for quantitation of Ins, we refer to a nonlinear concept with a hyperbolic function for the discrimination between brain- and blood-derived protein fractions in CSF according to Reiber *et al.* (1991). Absorbance of serum and CSF samples detected by EIA were converted to arbitrary units (AU) in a log–log diagram based on a standard curve derived from 7 serial dilutions of a positive standard serum with A 0.05–2.0; the highest standard concentration was defined as 100 AU (Tumani *et al.* 1995).

Portion of specific Ab in CSF that are synthesized intrathecally was expressed in the form of specific Ab index (AI) calculated according to Reiber's formula:

$$\begin{aligned} \text{AI}_{\text{IgG}} &= Q_{\text{spc(IgG)}}/Q_{\text{IgG}} \\ \text{AI}_{\text{IgM}} &= Q_{\text{spc(IgM)}}/Q_{\text{IgM}} \end{aligned}$$

where Q_{spc} is the ratio of Bs IgG or IgM in CSF and serum. Q_{spc} has to be related to the limiting IgG or IgM quotient (Q_{lim}) according to the formulae:

$$\begin{aligned} Q_{\text{lim(IgG)}} &= 0.93 \times [Q_{\text{Alb}}^2 + (6 \times 10^{-6})]^{1/2} - 1.7 \times 10^{-3} \\ Q_{\text{lim(IgM)}} &= 0.67 \times [Q_{\text{Alb}}^2 + (120 \times 10^{-6})]^{1/2} - 7.1 \times 10^{-3} \end{aligned}$$

If $Q_{\text{IgG}} > Q_{\text{lim(IgG)}}$, then $\text{AI}_{\text{IgG}} = Q_{\text{spc(IgG)}}/Q_{\text{lim(IgG)}}$. Likewise, $Q_{\text{lim(IgM)}}$ must be used for calculation of IgM AI. Values $\text{AI} > 1.4$ are positive and indicate Ins of specific Ab (Reiber and Lange 1991).

Oligoclonal IgG bands in serum and CSF were detected by isoelectric focusing with subsequent immunoblot and staining. Two or more bands in CSF or in both serum and CSF were considered as positive (Štourač 2000).

Cytology. The cells in CSF were counted in a Fuchs–Rosenthal chamber. Pleocytosis was present if the cell concentration was $>5/\mu\text{L}$ (Zeman *et al.* 2000, 2001). Differential cell count was evaluated from slides prepared by Cytospin-2 (*Shandon Ltd*, UK) followed by May–Grünwald, Giemsa–Romanowski staining.

Statistical analysis. Pearson's χ^2 -test and Fisher's exact test were used.

RESULTS

Intrathecal synthesis. In the NB subgroup 97 % patients had a positive Ins of Bs-IgG with AI in the range of 1.5–17.4 (Table I); 55 % of NB patients had positive Ins of Bs-IgM (AI in the range 1.6–59.9). One OND patient had a positive Ins of Bs-IgG with AI = 2.5.

Positive *oligoclonal IgG bands* were detected in 55 % NB patients and 10 % OND patients.

Blood–CSF barrier function (condition) was expressed by Q_{Alb} ; its elevated values were detected in 89 % NB patients and 60 % OND patients.

Cytology. In the NB subgroup 97 % patients had an elevated cell concentration ($>5/\mu\text{L}$), 76 % NB patients had values $>40/\mu\text{L}$; the highest value found was $443/\mu\text{L}$. In the OND subgroup 30 % patients had a normal cell concentration.

The frequency of examined CSF parameters in NB is summarized in Table II.

Table I. Borrelia-specific antibody indices (Bs-IgG AI, Bs-IgM AI) in neuroborreliosis^a

Characteristic	Bs-IgG AI	Bs-IgM AI
AI median	3.7	5.8
AI mean	5.0	12.9
AI maximum	17.4	59.9

^aStatistical analysis using Pearson's χ^2 -test and Fisher's exact test revealed statistically significant differences ($p < 0.001$) in Ins positivity between NB and OND groups; for abbreviations *see* p. 599.

Table II. Frequency (%) of characteristic CSF parameters in neuroborreliosis^a

Parameter	%
Bs-IgG AI	97
Bs-IgM AI	55
Oligoclonal IgG bands	55
Elevated Q_{Alb}	89
Pleocytosis	97

^aFor abbreviations *see* p. 599.

DISCUSSION

The main signs of an acute, active disease in CNS are increased CSF cell count and increased Q_{Alb} . The presence or absence of an intrathecal humoral immune response cannot be used as a unique sign of acute disease. There are three different interpretations of Ins of Ab: (1) acute disease with reaction to a specific antigen, (2) post-acute decreasing Ab synthesis, (3) part of a polyspecific immune response seen in chronic inflammatory diseases (Reiber 1998; Štourač *et al.* 2001); we detected a positive Ins of Bs-IgG in 97 and of Bs-IgM in 55 % NB patients. CSF response in the early acute phase of NB is characterized by mononuclear pleocytosis with the initial absence of humoral immune response (Felgenhauer 1998). However, at the time of manifestation of clinical symptoms and, thus at the time of lumbar puncture, Ins of specific Ab is already present. After antibiotic treatment, CSF cell count and blood–CSF-barrier function return to normal values (Felgenhauer 1998). A slow, long-lasting decay of Ins of Ab, sometimes detectable 10–15 years after sufficient treatment was reported in NB by Reiber (1996). This was the case of 2 patients in our NB subgroup who had a positive Ins of IgG_{spc} and a normal cell count. Both were treated with antibiotics already before the lumbar puncture was performed.

Oligoclonal IgG bands are reported in 63 % NB patients (Reiber 1996; Lodin 2003; Reiber *et al.* 2003). In our cohort, 55 % NB patients had positive oligoclonal IgG bands either in CSF or both in serum and CSF.

The blood–CSF barrier in NB is disturbed in almost all cases (Tumani *et al.* 1995); we detected elevated values of Q_{Alb} in 89 % NB patients which is in consent with the above observation.

Cellular immune response in NB is characterized by an elevated cell count and also by the presence of plasma cells, activated lymphocytes, neutrophils and total cell count usually exceeding 90 elements per μL (Fig. 1).

Concerning the OND subgroup, one patient with a positive Ins of Bs-IgG was diagnosed for purulent meningitis with neutrophil pleocytosis in CSF and other CSF data typical of purulent meningitis; this patient did not fulfil the diagnostic criteria for NB. Tumani *et al.* (1995) reported that false-positive AI results may be obtained in cases of bacterial CNS infection but only in cases of Ins of Ab to a common antigen. Positive Ins of anti-borrelia Ab in this patient was due to a polyspecific immune response accompanying an inflammatory process in the brain. Positive oligoclonal IgG bands, elevated cell count and increased Q_{Alb} corresponded with respective diagnoses in the OND subgroup.

Our results show that the typical CSF profile in NB can be characterized by mononuclear pleocytosis, damaged blood–CSF barrier, Ins of Bs Ab and high percentage of oligoclonal IgG bands.

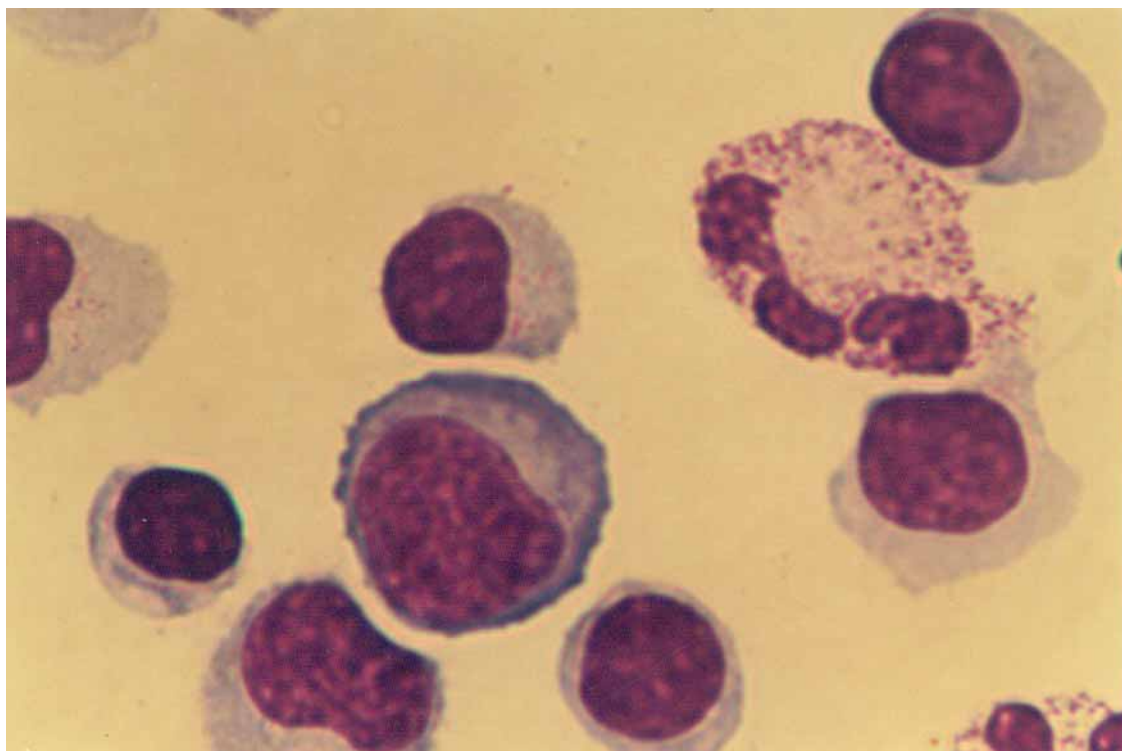


Fig. 1. Cellular immune response in neuroborreliosis; pleocytosis with the presence of plasma cells, activated lymphocytes and neutrophils; magnification $\times 1500$.

NB is a frequent manifestation of disseminated borreliosis. The diagnosis is based on epidemiological history, clinical findings, serological investigation and cerebrospinal fluid parameters. Since the blood–CSF barrier is frequently impaired, it is necessary to demonstrate intrathecal production of specific antibodies instead of isolated absorbance values. Evaluation of specific Ins improves the CSF diagnosis in NB if compared with absorbances of single specific antibodies in CSF. However, the detection of positive Ins of specific Ab cannot serve as the only indicator of acute NB. Evaluating NB status, the results of other CSF parameters must also be considered. Therefore, a combined CSF analysis must be included along with the clinical picture and relevant medical history when formulating the diagnosis of NB.

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