Cerebrospinal-Fluid Profile in Neuroborreliosis and Its Diagnostic Significance

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Received 23 February 2006
Revised version 25 July 2006

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

*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; Tábor-ský 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 (Tumaní et al. 1995).

Portion of specific Ab in CSF that are synthetized intrathecially was expressed in the form of specific Ab index (AI) calculated according to Reiber’s formula:

\[
\text{AI}_{IgG} = \frac{Q_{\text{spc(IgG)}}}{Q_{IgG}} \\
\text{AI}_{IgM} = \frac{Q_{\text{spc(IgM)}}}{Q_{IgM}}
\]

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 formulæ:

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

If Q_{IgG} > Q_{\text{lim(IgG)}} then AI_{IgG} = Q_{\text{spc(IgG)}}/Q_{\text{lim(IgG)}}. Likewise, Q_{\text{lim(IgM)}} must be used for calculation of IgM AI. Values 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/μL (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 χ²-test and Fisher’s exact test were used.