Suspected Early Lyme Neuroborreliosis in Patients With Erythema Migrans

Katarina Ogrinc,1 Stanka Lotrič-Furlan,1 Vera Maraspin,1 Lara Lusa,2 Tjaša Cerar,3 Eva Ružič-Sablijić,3 and Franc Strle1

1Department of Infectious Diseases, University Medical Center Ljubljana, 2Institute for Biostatistics and Medical Informatics, Medical Faculty, and 3Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Slovenia

(See the Editorial Commentary by Wormser and Halperin on pages 510–2.)

Background. Our objective was to obtain data on patients with erythema migrans (EM) who have symptoms/signs suggesting nervous system involvement and to compare epidemiologic, clinical, and microbiologic findings in patients with and without cerebrospinal fluid (CSF) pleocytosis.

Methods. Adult patients with EM and suspected early Lyme neuroborreliosis were included in this study.

Results. Of 161 patients, 31 (19%) had elevated and 130 (81%) had normal CSF cell counts. In contrast to patients with normal CSF cell counts, those with pleocytosis (1) more often reported radicular pain and more often presented with meningeal signs but less frequently complained of malaise; (2) had larger EM skin lesions despite similar duration; (3) more commonly had Borrelia garinii isolated from EM skin lesions (odds ratio for pleocytosis was 31 times higher in patients with established B. garinii skin infection compared to patients with other Borrelia species isolated from their EM skin lesion) and from CSF; and (4) more frequently fulfilled microbiologic criteria for established borrelial infection of the central nervous system. The positive predictive value of pleocytosis for microbiologically proven borrelial infection of the central nervous system (defined by isolation of Borrelia from CSF and/or demonstration of intrathecal synthesis of borrelial antibodies) was 67.9%, whereas normal CSF white cell counts ruled out Lyme neuroborreliosis with a predictive value of 91.9%.

Conclusions. Comparison of European patients with EM who had symptoms/signs suggesting early Lyme neuroborreliosis revealed several differences in the clinical presentation and in microbiologic test results according to CSF findings.

Keywords. Lyme borreliosis; Lyme neuroborreliosis; erythema migrans; Borrelia burgdorferi sensu lato; cerebrospinal fluid examination.

Lyme borreliosis is the most common tick-transmitted disease in Slovenia, with >250 new cases per 100 000 inhabitants registered in 2010 [1]. It is caused by Borrelia burgdorferi sensu lato (s.l.) [2]. The illness most often affects skin, nervous system (NS), joints, and heart. The most frequent manifestation is erythema migrans (EM) [3]. The aim of the present study was to obtain data on patients with EM who had signs/symptoms suggesting nervous system involvement, to assess which of the signs/symptoms are the most reliable indicators of cerebrospinal fluid (CSF) pleocytosis, and to determine the value of pleocytosis for demonstration of microbiologically proven Lyme neuroborreliosis (LNB) by comparing the findings in patients with pleocytosis and those with normal CSF cell count. The evaluation was done from the perspective of a primary care provider.

PATIENTS AND METHODS

The study approach was approved by the Medical Ethics Committee of the Ministry of Health of the Republic of Slovenia (35/0806).
Patients
All consecutive adult patients seen at our Lyme borreliosis outpatient clinic between October 2005 and March 2011 who had EM and signs and/or symptoms suggesting NS involvement of moderate to severe intensity and who consented to lumbar puncture were included in this prospective study. The symptoms/signs suggesting NS involvement were headache, vertigo, disturbances of sleep, memory or concentration disorders, radicular pain, paresthesias, neck stiffness, and peripheral facial palsy. At least 1 of the symptoms/signs was required to qualify for inclusion in the study. EM was defined according to criteria reported previously [3, 4].

Patients were classified into 2 groups according to CSF findings. Those with pleocytosis (CSF cell count >5 × 10⁶/L) were interpreted as having clinically evident LNB. The second group comprised patients who had normal CSF cell counts and thus did not satisfy one of the essential criteria for LNB [4–7]. Epidemiologic, clinical, and microbiologic characteristics of the 2 groups were assessed and compared.

Clinical Evaluation
The history was taken at the first visit, and patients underwent clinical examination. Demographic, epidemiologic, and clinical data were obtained using a structured questionnaire. After responding to open questions, patients were specifically asked about the presence or absence of certain symptoms—newly onset or preexisting symptoms that were recently aggravated (headache, vertigo, disturbances of sleep, memory or concentration disorders, radicular pain, paresthesias, nausea, vomiting, malaise, fatigue, neck pain, lower back pain, arthralgia, myalgia)—and to score the intensity of each present symptom from 1 to 10. Precise description of the evolution of EM and accompanying symptoms, previous antibiotic treatment, and information on tick bites were also of special interest. In clinical examination, particular attention was paid to EM and signs of neurologic involvement.

Laboratory Evaluation
Basic blood analysis included erythrocyte sedimentation rate, concentration of C-reactive protein, a complete blood cell count, liver and kidney function tests, and concentrations of electrolytes and glucose. CSF was tested for cell count, protein level, and glucose level. In case of a traumatic lumbar puncture, the white cell count was adjusted (ie, for every 1000 × 10⁶/L red blood cells, 1 × 10⁶/L white blood cells were subtracted). Immunoglobulin classes G (IgG) and M (IgM) and albumin levels were determined in serum and CSF. Basic urinalysis and electrocardiography were performed in all patients.

Serologic Evaluation
Antibodies to *B. burgdorferi* s.l. in serum and CSF were determined by an indirect chemiluminescence immunoassay (LIAISON, Diasorin, Italy) using recombinant antigens OspC and VlsE for IgM, and VlsE for IgG antibody detection. Results were graded according to the manufacturer’s instructions.

On the basis of LIAISON results, intrathecal synthesis of borrelial antibodies was determined using the approach described by Reiber and Peter [8]: Antibody index values >1.4 were interpreted as indicating production of intrathecal borreliant antibody.

Intrathecal synthesis was also determined using the IDEA Lyme Neuroborreliosis kit (DakoCytomation, Cambridgeshire, UK) with *B. afzelii* flagellin antigen. According to the manufacturer’s instructions, an index of ≥0.3 indicates intrathecal synthesis of specific borreliant antibodies.

In all patients, serum IgM and IgG antibodies to tick-borne encephalitis virus (TBEV) were determined using Enzygnost Anti-TBEV (IgM, IgG) test (Dade Behring Marburg GmbH, Marburg, Germany) performed according to the manufacturer’s instructions.

Cultivation and Typing of *B. burgdorferi* s.l.
Modified Kelly-Pettenkofer medium (MKP) was used for cultivation of *B. burgdorferi* s.l. from blood, CSF, and skin specimens as described elsewhere [9–11]. Blood (9 mL) and CSF (1 mL) samples were obtained from all patients; the majority of patients consented to skin biopsy, which was performed at the border of EM. CSF and skin samples (2.5 × 2 × 2 mm) were inoculated directly into tubes containing 7 mL MKP. Blood samples were sent to the microbiology laboratory where they were centrifuged, and 1-mL samples of plasma were inoculated into tubes with 7 mL of MKP. All samples were cultivated at 33°C and examined weekly by dark-field microscopy for the presence of spirochetes [11, 12] (up to 9 weeks for skin and CSF specimens, 12 weeks for blood specimens). Isolates were identified to species/strain level using pulsed-field gel electrophoresis after *MluI* restriction of genomic DNA or by polymerase chain reaction–based restriction fragment-length polymorphism of the intergenic region [11–14].

Definition of Microbiologically Proven LNB
Patients who had *B. burgdorferi* s.l. isolated from CSF and/or intrathecal synthesis of borreliant antibodies were interpreted as having microbiologically proven LNB.

Statistical Methods
Data were summarized as medians with interquartile range (IQR) for numerical variables and as frequencies and percentages for categorical variables. The association between the presence of pleocytosis and clinical and demographic characteristics of patients was assessed with univariate logistic regression models (univariate analyses). To examine the joint effect of patients’ characteristics on the presence of pleocytosis, we...
developed a parsimonious multiple logistic regression model using backward variable selection based on Akaike information criterion (adjusted analysis). In all models, age and seasonal occurrence were modeled using restricted cubic splines [15]; the other numerical variables were modeled linearly on the logit scale. To evaluate the predictive performance of the multivariable model, we evaluated its area under the receiver operating characteristic curve (AUC). The optimism of the AUC estimate was evaluated by using 200 bootstrap samples [16] and testing them on the original sample. Results are presented as unadjusted and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) and P values.

The association between the presence of symptoms and pleocytosis was assessed using the χ² test with the Yates continuity correction; to control for false positives, the P values were adjusted using a multivariate permutation procedure [17].

The positive and negative predictive values of pleocytosis as an indicator of LNB (and their 95% exact binomial CIs) were calculated assuming the microbiologically proven LNB as the gold standard. R statistical language was used for the analyses [18].

RESULTS

During a 5.5-year-period, 2751 patients with EM were referred to our institution. A total of 161 (5.9%) patients, 66 (41%) males and 95 (59%) females, aged 49 years (IQR, 38–57 years), who had at least 1 sign and/or symptom of moderate to severe intensity suggesting NS involvement and who consented to lumbar puncture, were included in the study (Figure 1). Of the 161 patients, 31 (19.3%) had pleocytosis and 130 (80.7%) had normal CSF cell counts. Basic demographic, epidemiologic, and clinical data are shown in Table 1; the frequency and intensity of the symptoms are depicted in Figure 2; and CSF analysis, B. burgdorferi s.l. culture, and serologic results are shown in Table 2. Patients with pleocytosis more often reported radicular pain, more frequently had meningeal signs and peripheral facial palsy, and had larger EM skin lesions at the initial visit (Table 1 and Figure 3A). The probability of pleocytosis decreased with age up to about 50 years of age, and steeply increased afterward; moreover, it increased from May till August and decreased afterward (Table 1).

Radicular pain (adjusted OR [AOR] = 7.4 [95% CI, 1.8–29.4]; P = .005), meningeal signs (AOR = 8.0 [95% CI, 1.6–40.8]; P = .01), peripheral facial palsy (AOR = 9.0 [95% CI, 1.3–60.2]; P = .02), size of EM (AOR = 1.6 for 10-cm difference [95% CI, 1.2–2.2]; P = .003), and seasonal occurrence were statistically significantly associated with pleocytosis on multivariable analysis (Table 1).

Skin samples were cultured from 139 patients and were positive in 55 of 116 (47.4%) patients without previous antibiotic treatment and in 0 of 23 patients who had received antibiotic therapy for EM before skin biopsy was performed (P < .001).

Among the skin isolates, B. afzelii predominated (41/55 isolates [74.5%]) over B. garinii (12/55 isolates [21.8%]) and B. burgdorferi sensu stricto (s.s) and Borrelia spielmanii (1 isolate each [1.8%]). Borrelia garinii was more often isolated from the skin of patients with CSF pleocytosis (9/12 [75%]) than from those without (3/43 [7%]), whereas B. afzelii was more frequently cultured from skin specimens of patients with normal CSF cell counts (38/43 [88.4%]) than from those with pleocytosis (3/12 [25%]). Odds for pleocytosis were 31 times higher in patients with B. garinii skin infection in comparison to patients with other Borrelia species isolated from their EM skin lesion (OR = 30.8 [95% CI, 5.8–162]; P < .001), and 14 times higher in comparison to patients with negative skin culture result (OR = 14.3 [95% CI, 3.5–58.2]; P < .001).

CSF cultures were positive in 6 of 127 (4.7%) patients who had not received antibiotics and from 0 of 34 patients who had received antibiotics before CSF examination (P = .34). The corresponding findings for blood cultures were 4 of 127 (3.1%) and 0 of 34 (P = .58).

In 8 patients, B. burgdorferi s.l. was cultured from >1 specimen. Isolates obtained from different sites in an individual patient were identical B. burgdorferi s.l. species.
The positive and negative predictive values of pleocytosis for microbiologically proven LNB were 67.9% (of 28 microbiologically positive patients, 19 had pleocytosis [95% CI, 48%–84%]) and 91.9% (of 123 microbiologically negative patients, 113 had normal CSF cell counts [95% CI, 86%–96%]), respectively.

Within the group of patients with pleocytosis, *Borrelia* central NS infection was more often established in those with higher cell counts (Figure 3C).

**DISCUSSION**

EM is the most frequent manifestation of Lyme borreliosis. Sometimes, particularly in patients not (properly) treated for EM, *Borrelia* species may disseminate from the infected skin to other tissues or organs [2, 19]. In some patients, symptoms and/or signs suggesting NS involvement develop, such as headache, vertigo, disturbances of sleep, memory or concentration disorders, radicular pain, neck stiffness, and peripheral facial palsy. In such patients, CSF examination is required to substantiate the diagnosis of LNB, for which—according to the Infectious Diseases Society of America and the European clinical definitions [4–7]—pleocytosis is an essential criterion. All our patients had early Lyme borreliosis indicated by EM and had symptoms and/or signs suggesting NS involvement: 31 patients with pleocytosis were interpreted as having a clinical diagnosis of LNB, and 130 had normal CSF white cell counts.

Our results indicate that the majority of patients with EM and “neurologic” symptoms of moderate to severe intensity had actually no infection/inflammation of central NS; their symptoms could be explained by a cytokine-mediated toxic metabolic effect [20].

Comparison of demographic, epidemiologic, and clinical data of the 2 groups (Table 1, Figure 2) revealed several differences. A rather unexpected finding was that patients with

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Elevated CSF Cell Count (n = 31)</th>
<th>Normal CSF Cell Count (n = 130)</th>
<th>Unadjusted OR (95% CI)</th>
<th>P Value</th>
<th>Adjusted ORa (Backward Variable Selection)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex (%)</td>
<td>16 (51.6)</td>
<td>50 (38.5)</td>
<td>1.7 (.8–3.7)</td>
<td>.18</td>
<td></td>
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</tr>
<tr>
<td>Ageb,c</td>
<td>54 (31–65)</td>
<td>49 (39–55)</td>
<td>2.7</td>
<td>.006</td>
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<td>20 vs 40</td>
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<td>60 vs 40</td>
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<tr>
<td>Seasonalityb</td>
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<td>July vs March</td>
<td>16 (51.6)</td>
<td>49 (39–55)</td>
<td>2.7</td>
<td>.006</td>
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<td>July vs October</td>
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<tr>
<td>Annual No. of tick bitesc</td>
<td>3 (1–5)</td>
<td>2 (0–5)</td>
<td>1.0 (.9–1.1)</td>
<td>.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple EM (%)</td>
<td>9 (29)</td>
<td>30 (23.1)</td>
<td>1.4 (.6–3.3)</td>
<td>.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of EM, d,e</td>
<td>15 (5–30)</td>
<td>13 (5–26)</td>
<td>1.0 (.9–1.1)</td>
<td>.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic therapy for EM (%)d</td>
<td>4 (12.9)</td>
<td>30 (23.1)</td>
<td>0.5 (.2–1.5)</td>
<td>.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of neurologic symptoms, d,e</td>
<td>10 (5–17)</td>
<td>10 (4–30)</td>
<td>1 (1–1)</td>
<td>.49</td>
<td></td>
<td></td>
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<tr>
<td>Fever (&gt;38°C)d</td>
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<td></td>
<td></td>
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<tr>
<td>Radicular paind</td>
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<td></td>
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<tr>
<td>Headache</td>
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<td>Ringlike EMe</td>
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<tr>
<td>Largest diameter of EM, cmf</td>
<td>30 (13–37)</td>
<td>14 (8–25)</td>
<td>1.5 (.2–1.9)</td>
<td>.48</td>
<td></td>
<td>.005</td>
</tr>
<tr>
<td>Meningeal signse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.01</td>
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<tr>
<td>Peripheral facial palsye</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.02</td>
</tr>
</tbody>
</table>

Data are median (interquartile range) or frequency (percentage). Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; EM, erythema migrans; OR, odds ratio.

a The estimated area under the curve (AUC) was 0.85, and its optimism-corrected AUC was 0.72.

b Model using restricted cubic splines; estimated OR comparing different values are reported for descriptive purposes only.

c Median (interquartile range).

d In the course of the illness up to presentation.

e Findings at presentation.

f The OR compares the odds of 2 patients whose EM diameter differs by 10 cm.
normal CSF cell count reported a larger number of symptoms compared to patients with elevated CSF cell count (mean, 5.2 vs 4.3; \( P = .12 \), Welsh \( t \) test) and that the majority of symptoms that we actively searched for were more frequently present in patients with normal CSF cell counts than in those with pleocytosis (Figure 2); however, the association was statistically significant only for malaise. In comparison to patients with normal CSF cell counts, those with pleocytosis more often reported radicular pain, sleep disturbances, and lower back pain, and more often had meningeal signs and peripheral facial palsy (Table 1, Figure 2). Because radiculoneuritis, meningitis, and cranial neuritis represent a classic triad of early LNB [2, 21], the finding of a higher ratio of those symptoms/signs in patients with elevated CSF cell count was expected, whereas comparable frequencies of headache in the 2 groups were not, but this can be explained by the rather small number of patients with pleocytosis. It is well known that the complete triad is not present in each individual patient, but in the present study the

![Figure 2. Frequency and intensity of symptoms in patients with and without pleocytosis. Normal cerebrospinal fluid (CSF), CSF white cell count ≤5 \times 10^6/L; abnormal CSF, CSF white cell count >5 \times 10^6/L. Intensity of symptoms: white (1) is the lowest, black (10) is the highest intensity. Abbreviation: CSF, cerebrospinal fluid.](cid:2013:57 (15 August) • 505)
proportions of these symptoms/signs were lower than expected for European patients with early LNB [22–26]. However, the criterion for inclusion in our study was the presence of EM associated with symptoms/signs potentially indicating LNB; thus, selection of our patients was not primarily on the basis of neurologic involvement manifested by signs typical for LNB. Our approach was more from the standpoint of the primary care physician who decides whether a patient with typical EM and

Table 2. Cerebrospinal Fluid (CSF), Serology, and *Borrelia burgdorferi* sensu lato Culture Results in Patients With Pleocytosis and Those With Normal CSF White Cell Counts

<table>
<thead>
<tr>
<th>Culture</th>
<th>Elevated CSF Cell Count (n = 31)</th>
<th>Normal CSF Cell Count (n = 130)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF Cell count, x10⁶/L ⁹</td>
<td>65⁶c (6–682)</td>
<td>1 [0–5]</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Elevated, &gt;5 x 10⁶/L (%)</td>
<td>31 (100)</td>
<td>0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Protein concentration, g/L ⁹</td>
<td>0.52 (0.20–1.84)</td>
<td>0.37 (0.22–0.72)</td>
<td>.01</td>
</tr>
<tr>
<td>Elevated, &gt;0.45 g/L (%)</td>
<td>17 (54.8)</td>
<td>31 (23.8)</td>
<td>.01</td>
</tr>
<tr>
<td>Glucose concentration, mmol/L ⁹</td>
<td>2.8 (1.9–4.0)</td>
<td>2.9 (1.6–7.0)</td>
<td>.06</td>
</tr>
<tr>
<td>Albumin quotient ³⁵, ³⁶ ⁹</td>
<td>0.0075 (0.0024–0.0618)</td>
<td>0.0050 (0.0022–0.0118)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Elevated, &gt;0.0074 (%)</td>
<td>17/21 (54.8)</td>
<td>21/129 (16.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IgG quotient ³⁵, ³⁶ ⁹</td>
<td>0.0037 (0.0011–0.0304)</td>
<td>0.0023 (0.0010–0.0053)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Elevated, &gt;0.0035 (%)</td>
<td>19/31 (61.3)</td>
<td>20/129 (15.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Borrelial serology (LIAISON) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive serum IgM</td>
<td>15/23 (65.2)</td>
<td>42/115 (36.5)</td>
<td>.02</td>
</tr>
<tr>
<td>Positive serum IgG</td>
<td>17/23 (73.9)</td>
<td>62/115 (53.9)</td>
<td>.12</td>
</tr>
<tr>
<td>Positive serum IgM and/or IgG</td>
<td>21/23 (91.3)</td>
<td>78/115 (67.8)</td>
<td>.04</td>
</tr>
<tr>
<td>Positive CSF IgM</td>
<td>12/22 (54.5)</td>
<td>5/115 (4.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive CSF IgG</td>
<td>11/22 (50)</td>
<td>13/115 (11.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive CSF IgM and/or IgG</td>
<td>14/22 (63.6)</td>
<td>17/115 (14.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Intrathecal synthesis present (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM Reiber (LIAISON)</td>
<td>8/21 (38.1)</td>
<td>3/115 (2.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IgG Reiber (LIAISON)</td>
<td>12/21 (57.1)</td>
<td>6/115 (5.2)⁹</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IgM DAKO</td>
<td>5/27 (18.5)</td>
<td>0/100</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IgG DAKO</td>
<td>9/27 (33.3)</td>
<td>0/100</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IgM Reiber and/or Dako</td>
<td>11/29 (37.9)</td>
<td>3/123 (2.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IgG Reiber and/or Dako</td>
<td>16/29 (55.2)</td>
<td>6/123 (4.9)⁹</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Borrelial culture results (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive blood culture</td>
<td>1⁴/31 (3.2)</td>
<td>3⁴/130 (2.3)</td>
<td>.58</td>
</tr>
<tr>
<td>Positive CSF culture</td>
<td>5⁴/31 (16.1)</td>
<td>1⁴/130 (0.8)</td>
<td>.01</td>
</tr>
<tr>
<td>Positive skin culture</td>
<td>12/28 (42.9)</td>
<td>43/111 (38.7)</td>
<td>.86</td>
</tr>
<tr>
<td>Positive skin culture without previous</td>
<td>12/26 (46.2)</td>
<td>43/90 (47.8)</td>
<td>.94</td>
</tr>
</tbody>
</table>

Quantitative data were analyzed using the Kruskal-Wallis test and qualitative data with Yates’ corrected χ² test or 2-tailed Fisher exact test.

Abbreviations: CSF, cerebrospinal fluid; IgG, immunoglobulin G; IgM, immunoglobulin M.

⁹ Median (range).

One patient had >1000 (1707) x 10⁶/L erythrocytes in the CSF; his adjusted CSF white cell count was 72 x 10⁶/L instead of 74 x 10⁶/L.

One patient had concomitant tick-borne encephalitis (TBE) virus infection with proven intrathecal synthesis of IgG antibodies to TBE virus, but not to *Borrelia*; she was excluded from calculation of positive predictive value of pleocytosis for microbiologically proven Lyme neuroborreliosis (LNB).

Albumin (IgG) quotient is a quotient between CSF and serum albumin (IgG) concentration.

In 1 patient, intrathecal synthesis of borrelial IgG antibodies could have resulted from a previous borrelial infection (untreated erythema migrans 6 months prior to another erythema migrans and suspected LNB).

All blood isolates were identified as *Borrelia afzelii*.

Four CSF isolates were identified as *Borrelia garinii*, 1 as *B. afzelii*.

The CSF isolate was identified as *B. afzelii*.

Nine isolates were identified as *B. garinii*, 3 as *B. afzelii*.

Thirty-eight isolates were identified as *B. afzelii*, 3 as *B. garinii*, 1 as *Borrelia burgdorferi* sensu stricto, 1 as *Borrelia spielmanii*.
additional symptoms/signs suggesting NS involvement has LNB, rather than from the standpoint of a neurologist who is more usually involved in the management of preselected patients.

Borrelial serum IgM and/or IgG antibodies were present in 71.7% (99/138) of patients. The others were seronegative or had borderline values of specific antibodies (Table 2), probably due to a relatively short duration of illness (median, 7 days vs 14 days in seropositive patients, \( P < .001 \)) and/or amelioration of the serologic response as a result of antecedent antibiotic treatment of EM received by 25.6% of seronegative patients compared to 17.2% of seropositive patients (\( P = .37 \)), even though the dose/duration of treatment was not adequate in all patients (data not shown). Borrelial serum antibodies were more often positive in patients with pleocytosis than in those with normal CSF cell counts, but the difference was significant only for IgM antibodies. As expected, in CSF both IgM and IgG antibodies were more frequently positive in patients with pleocytosis. The same was also valid for intrathecal synthesis of borrelial antibodies calculated on the basis of LIAISON results as well as with the DAKO test system and for calculations based on positive result with at least 1 of the 2 testing systems (37.9% vs 2.4% for IgM, \( P < .001 \); 55.2% vs 4.9% for IgG, \( P < .001 \)). The discrepancies between the results obtained with the 2 approaches have been described previously [27]. In 1 patient without pleocytosis, intrathecal synthesis of borrelial antibodies could have resulted from a previous borrelial infection (Table 2).

*Borrelia* species were isolated from CSF in 5 of 31 (16.1%) patients with pleocytosis, which is a slightly higher isolation rate than reported previously in patients with early LNB [22, 28]. Four of the isolates were typed as *B. garinii* (3 of 4 patients had Banwarth syndrome, 1 had peripheral facial palsy and symptoms of meningitis) and 1 as *B. afzelii* (a patient with EM and peripheral facial palsy without symptoms/signs of meningitis and with CSF cell count 59 \times 10^6/L). In the group of 130 patients without pleocytosis, *Borrelia* species were isolated from CSF in only 1 patient (who had multiple EM and systemic symptoms); the isolate was *B. afzelii*. The results corroborate previous findings that *B. garinii* is the main causative agent of LNB in Europe and that it causes what is appreciated as typical early Lyme neuroborreliosis (Bannwarth syndrome), whereas the large majority of patients with *B. afzelii* isolated from CSF do not fulfill European criteria for LNB [21, 23].

The size of EM and isolation of *B. garinii* from EM skin lesion were identified as independent predictors of pleocytosis. Although *B. afzelii* predominated among skin isolates (74.5%), followed by *B. garinii* (21.8%) and *B. burgdorferi* s.s. and *B. spielmanii* (1.8% each), in patients with pleocytosis, *B. garinii* was more often isolated from skin specimens than from patients with normal CSF cell counts (9/12 vs 3/43; \( P < .001 \)). The chances for pleocytosis were 31 times higher in patients with *B. garinii* isolated from skin than in patients with other skin isolates. Although the duration was comparable, the longest diameter of EM was bigger in patients with EM skin lesion caused by *B. garinii* than by *B. afzelii* (34 cm vs 15 cm, \( P = .003 \)), which is an expected finding as *B. garinii* is known to cause a faster expansion of EM [29]. This is also the reason why patients with pleocytosis (where the predominating etiologic agent is *B. garinii*) had bigger EM skin lesions at presentation than did patients with normal CSF cell counts (30 cm vs 14 cm, \( P = .003 \); Figure 3) despite comparable duration of their lesions.

Borrelial central NS involvement was ascertained either by isolation of the causative agent from CSF or by demonstration of intrathecal borrelial antibody production in 19 of 28 (67.9%) patients with pleocytosis but in only 10 of 123 (8.1%) patients with normal CSF cell counts (\( P < .001 \)). Thus, in European patients with EM and symptoms/signs suggesting early LNB,
pleocytosis indicates (microbiologically proven) LNB with a positive predictive value of 67.9% (95% CI, 48%–84%), whereas the absence of pleocytosis rules out the LNB with a predictive value of 91.9% (95% CI, 86%–96%); if a patient without pleocytosis in whom intrathecal borrelial antibody synthesis could have been a result of past borrelial infection was excluded, the latter value would be 92.6%.

The study had some limitations, including relatively small number of patients with pleocytosis, heterogeneity of approaches used for determination of intrathecal synthesis of borrelial antibodies, and incomplete testing results due to the lack of serum and/or CSF samples in some patients.

**CONCLUSIONS**

Comparison of European patients with EM and symptoms and/or signs suggesting early LNB demonstrated that common symptoms such as headache, malaise, fatigue, and disturbances of memory or concentration are not specific findings indicating borrelial infection of the NS. However, the comparison revealed several differences in the clinical course and in microbiologic test results according to CSF findings. In contrast to patients with normal CSF cell counts, those with pleocytosis (1) more often reported radicular pain and more often presented with meningeval signs but less frequently complained of malaise; (2) had larger EM skin lesions despite similar duration; (3) more commonly had *B. garinii* isolated from EM skin lesions (the OR for pleocytosis was 31 times higher in patients with established *B. garinii* skin infection in comparison to patients with other skin isolates) and from CSF; and (4) more frequently fulfilled microbiologic criteria for borrelial infection of the central NS. The positive predictive value of pleocytosis for microbiologically proven borrelial infection of the central NS was 67.9%, whereas normal CSF white cell counts ruled out LNB with a predictive value of 91.9%.

**Notes**

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