MAJOR ARTICLE

Comparison of Findings for Patients with *Borrelia garinii* and *Borrelia afzelii* Isolated from Cerebrospinal Fluid

F. Strle,[1] E. Ružić-Sabljić,[2] J. Cimperman,[1] S. Lotrič-Furlan,[1] and V. Maraspin[1]

[1]Department of Infectious Diseases, University Medical Centre Ljubljana, and [2]Institute of Microbiology and Immunology, University of Ljubljana, Slovenia

Background. The most common cause of Lyme neuroborreliosis in Europe is *Borrelia garinii,* followed by *Borrelia afzelii.* However, no series describing patients with culture-confirmed cases of Lyme neuroborreliosis have been published, and no comparison of findings for patients with *B. garinii* and *B. afzelii* isolated from cerebrospinal fluid (CSF) has been reported.

Methods. All adult patients identified at a single medical center during a 10-year period who had borreliae isolated from CSF and typed as *B. garinii* or *B. afzelii* (using large DNA fragment patterns obtained with the *Mlu*I restriction endonuclease and separated with pulsed-field gel electrophoresis) were included.

Results. A comparison of 23 patients who had *B. garinii* isolated from CSF with 10 patients who had *B. afzelii* isolated from CSF revealed that a reliable clinical diagnosis of Lyme neuroborreliosis (before obtaining a CSF culture and intrathecal borrelial antibody production result) was established more frequently in the *B. garinii* group than in the *B. afzelii* group (19 of 23 patients vs. 1 of 10 patients). Patients in the *B. garinii* group reported radicular pains and expressed meningeal signs more often, but reported dizziness less often (occurrences of several other symptoms and/or signs were comparable). Lymphocytic pleocytosis, as well as several other CSF abnormalities, were frequent among patients with *B. garinii* isolated from CSF but were rare among patients in the *B. afzelii* group.

Conclusions. Patients with *B. garinii* isolated from their CSF have a distinct clinical presentation, compared with patients with *B. afzelii. B. garinii* causes what, in Europe, is appreciated as typical early Lyme neuroborreliosis (Bannwarth syndrome), whereas the clinical features associated with *B. afzelii* are much less specific and more difficult to diagnose.

Lyme borreliosis is the most common tick-transmitted illness in the Northern Hemisphere [1, 2]. It is caused by *Borrelia burgdorferi* sensu lato [3]. Human disease is associated with at least 3 *Borrelia* species: *B. burgdorferi* sensu stricto *(B. burgdorferi), Borrelia afzelii,* and *Borrelia garinii.* All isolates from North American patients have been members of the genomic group *B. burgdorferi,* whereas European isolates have included at least 2 additional genospecies: *B. afzelii* and *B. garinii* [4]. Although probably all 3 genospecies can cause all major manifestations, there are indications that infection with different *B. burgdorferi* sensu lato genospecies results in distinct clinical manifestations of Lyme borreliosis, most probably as a consequence of their distinct organotropism. For example, in Europe, *B. afzelii* is mostly associated with skin manifestations, such as erythema migrans (EM) and acrodermatitis chronica atrophicans, whereas *B. garinii* is the main cause of Lyme neuroborreliosis [1, 5–8]. Differences in geographical distribution of genospecies may explain the distinctions between the clinical picture of Lyme borreliosis in Europe and in North America [1, 2, 9]. Although data indicating the association of different *B. burgdorferi* sensu lato genospecies with distinct clinical manifestations of Lyme borreliosis are relatively abundant and obvious [1, 2, 7–9], comparisons of the characteristics of individual clinical manifestations caused by different *Borrelia* genospecies are quite scarce. In

Received 20 January 2006; accepted 28 May 2006; electronically published 8 August 2006.

Reprints or correspondence: Dr. Franc Strle, University Medical Centre Ljubljana, Dept. of Infectious Diseases, Japljeva 2, 1525 Ljubljana, Slovenia (franc.strle@kclj.si).

Clinical Infectious Diseases 2006; 43:704–10

1058-4838/2006/4306-0007$15.00

fact, information is limited to the reports concerning patients with EM [10–12]. Comparison of culture-confirmed EM caused by *B. afzelii* with EM caused by *B. burgdorferi* showed differences in epidemiological, clinical, and laboratory findings, suggesting 2 distinct syndromes that are caused by different agents [10]. Two other studies have demonstrated several differences between EM due to *B. afzelii* and *B. garinii* [11, 12].

The primary aim of the present study was to further assess the idea that the characteristics of individual clinical manifestations of Lyme borreliosis depend on the species of *B. burgdorferi* sensu lato; by comparison of findings for adult patients with either *B. afzelii* or *B. garinii* isolated from CSF, we tested the hypothesis that the features of Lyme neuroborreliosis in the 2 groups differ.

PATIENTS AND METHODS

Patients. The clinical part of the study was performed at the Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, from 1995 through 2004. The study approach was approved by the medical ethics committee at the Ministry of Health of the Republic of Slovenia. We have been using an identical approach in treating patients with potential Lyme neuroborreliosis for >15 years. In short, for patients with likely Lyme borreliosis who exhibit clinical signs or symptoms suggestive of CNS involvement, lumbar puncture is performed, and CSF samples are examined, including the assessment of intrathecal borrelial antibody production and the culture of CSF samples for the presence of borrelia. Patients with heterogeneous clinical presentations are approached, including patients who exhibit obvious signs of Lyme neuroborreliosis and patients with vague clinical indications of CNS involvement; for some of these patients, the primary aim of the evaluation is to exclude Lyme neuroborreliosis. Before borrelia culture and borrelia intrathecal antibody production results are received, patients are classified according to clinical and basic laboratory information as having "evident" Lyme neuroborreliosis, "highly probable" Lyme neuroborreliosis, or "possible" Lyme neuroborreliosis. A provisional (i.e., working) clinical diagnosis of evident Lyme neuroborreliosis is made for patients with lymphocytic pleocytosis and EM verified during the last 4 months before neurologic involvement. A provisional clinical diagnosis of highly probable Lyme neuroborreliosis is made for patients with pleocytosis associated with radiculoneuritis and/or peripheral facial palsy but without information on EM. Patients with symptoms that potentially indicate CNS involvement but who do not fulfill simple clinical criteria for evident or highly probable Lyme neuroborreliosis are provisionally interpreted as having possible Lyme neuroborreliosis. The first 2 groups are categorized irrespective of borrelia serum antibody findings, whereas the representatives of the third group are required to be seropositive for borrelia or to have EM.

A subset of patients with CSF culture results positive for borrelia (i.e., all adult patients with *B. burgdorferi* sensu lato isolated from CSF samples and typed as either *B. garinii* or *B. afzelii* using large DNA fragment patterns) qualified for enrollment in the present study. Patients were divided into 2 groups according to the etiology; findings for patients in *B. garinii* group were compared with findings obtained for patients in the *B. afzelii* group.

Methods. For each patient, serum and CSF specimens were collected. Routine blood tests and CSF investigations were performed to determine cell counts, protein concentration, albumin levels, immunoglobulin levels, and glucose levels. CSF WBC count $> 5 \times 10^6$ cells/L was considered to be abnormal and suggestive of meningitis.

Serum and CSF IgM and IgG antibodies to *B. burgdorferi* sensu lato were determined by an indirect immunofluorescent test. A local isolate of *B. afzelii* was used as an antigen. Serum antibody titers of $\geqslant$1:256 and CSF antibody titers $\geqslant$8 were considered to be positive on the basis of the results for the control group from the same geographic region [13]. The presence of intrathecal borrelial antibody production was ascertained using capture EIA (Dako).

For each patient, a 1-mL sample of CSF, obtained by lumbar puncture, was immediately inoculated into modified Kelly-Pettenkofer medium [14, 15] and promptly transported to the laboratory. For several patients, skin biopsy samples and 5-mL samples of blood were also obtained for culture. Tubes were incubated at 33°C and were examined weekly for 9 weeks for the presence of spirochetes by dark-field microscopy [14]. Isolated strains were subcultured 2–4 times. Total genomic DNA was extracted by the gel insert method, as described elsewhere [7]. For species identification, genomic DNA was digested with 30 U of *Mlu*I restriction enzyme, electrophoresed for 24 h in 1% agarose gel with a pulsed time ramped from 1 to 40 s, and stained with ethidium bromide [7]. *Mlu*I large restriction fragment patterns (LRFP) were used to identify species, according to Belfaiza et al. [16] and Picken et al. [17]. Intraspecies variation in LRFP profiles (Mla for *B. afzelii* and Mlg for *B. garinii*) was indicated by type number [16, 17].

Differences in the quantitative data were analyzed by the Kruskal-Wallis test, and differences in the qualitative data were analyzed by the Yates corrected χ^2 test or 2-tailed Fisher's exact test with use of Epi Info software, version 6.04 (Centers for Disease Control and Prevention); $P < .05$ was considered to indicate statistical significance.

RESULTS

Of 485 patients who consented to participate in the study and who had CSF specimens cultured for the presence of borreliae, 48 (9.9%) had positive culture results. In 12 (25%) of these patients, borreliae did not grow well enough to enable LRFP

analysis. Of the remaining 36 patients, 3 had isolates that were typed as *B. burgdorferi*, and 33 had isolates that were typed as either *B. afzelii* or *B. garinii*. These 33 patients were included in the present comparison; for patients who fulfilled the criteria for a provisional diagnosis of evident or highly probable Lyme neuroborreliosis, the isolation rate was 20 (11.6%) of 173, whereas in the remaining patients, the isolation rate was 13 (4.2%) of 312 ($P = .004$).

Twenty-three patients with *B. garinii* (subtypes Mlg2–14, Mlg3–4, Mlg4–4, and Mlg7–1) and 10 patients with *B. afzelii* (Mla1) isolated from CSF samples were analyzed. A provisional (i.e., working) clinical diagnosis (made before obtaining CSF culture and intrathecal borrelial antibody production results) of evident or highly probable Lyme neuroborreliosis was established for 19 (83%) of 23 patients in the *B. garinii* group, but for only 1 (10%) of 10 patients with *B. afzelii* isolated from CSF. The 4 patients with *B. garinii* infection (who had normal routine CSF findings and, thus, did not fulfill the criteria for a working clinical diagnosis of evident or highly probable Lyme neuroborreliosis) exhibited clinical indications that could have pointed to Lyme neuroborreliosis. One patient presented to the hospital with headache and peripheral facial palsy (with durations of 1 month and 2 days, respectively). The other patient had had solitary EM 21 months before examination in our department; in spite of treatment with doxycycline administered at a dosage of 100 mg twice per day for 14 days, EM was followed, after several weeks, by malaise and fatigue, mild headache, dizziness, paraesthesias, sleepiness, and arthralgias. The third and the fourth patient, both of whom had normal routine CSF findings but positive CSF culture results, had had EM (solitary EM and radicular pains for 1 patient and multiple EM and short-lasting headache for 1 patient) and had CSF samples examined 2–3 months earlier; in both patients, lymphocytic pleocytosis and elevated protein concentrations had been established at that time, but neither patient had received a diagnosis of Lyme neuroborreliosis from their physicians. In the *B. afzelii* group, clinical indications of Lyme neuroborreliosis were less obvious: 8 of 10 patients had normal routine CSF findings, and only 1 patient fulfilled clinical criteria for highly probable Lyme neuroborreliosis. Duration of symptoms indicating potential CNS involvement for ⩽6 months before CSF samples were examined (an arbitrary time limit for distinction between early and late Lyme neuroborreliosis) was reported more often in the *B. garinii* group than in the *B. afzelii* group (in 22 of 23 patients vs. in 3 of 10 patients). Thirteen (57%) of 23 patients in the *B. garinii* group and 5 (50%) of 10 patients in the *B. afzelii* group reported having had typical EM. Nevertheless, at the time that CSF samples were examined, the lesion was still visible for 8 (35%) of 23 patients in the *B. garinii* group, compared with 0 of 10 patients in the *B. afzelii* group.

Table 1. Demographic and clinical characteristics for 23 patients with *Borrelia garinii* and 10 patients with *Borrelia afzelii* isolated from CSF samples.

Variable	*B. garinii* group ($n = 23$)	*B. afzelii* group ($n = 10$)	*P*
Sex, F/M	6/17	6/4	.11
Age, median years (range)	48 (18–72)	48 (27–62)	.95
Symptoms of LB			
Median duration of symptoms (range)	26 days (7 days to 22 months)	9 months (4 days to 7 years)	.02
No. (%) of patients with duration of symptoms ⩽6 months	22 (96)	3 (30)	<.001
Symptoms indicating potential CNS involvement			
Median duration of symptoms (range)	19 days (1 day to 21 months)	7.5 months (4 days to 6 years)	.07
No. (%) of patients with duration of symptoms ⩽6 months	22 (96)	3 (30)	<.001
Working clinical diagnosis			
Evident NB	11 (48)	0 (0)	.01
Highly probable NB	8 (35)	1 (10)	.30
Possible NB	4 (17)	9 (90)	<.001
EM	13 (57)[a]	5 (50)[b]	1.00
EM visible at examination	8 (35)[c]	0 (0)	.07
ACA	0 (0)	2 (20)	.09

NOTE. Data are no. (%) of patients, unless otherwise indicated. ACA, acrodermatitis chronica atrophicans; EM, erythema migrans; LB, Lyme borreliosis; NB, Lyme neuroborreliosis.

[a] Includes 11 patients with solitary and 2 patients with multiple skin lesions.
[b] Includes 4 patients with solitary and 1 patient with multiple skin lesions.
[c] Includes 7 patients with solitary and 1 patient with multiple skin lesions.

Table 2. Symptoms and signs reported for 23 patients with *Borrelia garinii* and 10 patients with *Borrelia afzelii* isolated from CSF samples.

Symptom or sign	No. (%) of patients		
	B. garinii group (n = 23)	*B. afzelii* group (n = 10)	*P*
Fatigue	19 (83)	8 (80)	1.00
Malaise	18 (78)	8 (80)	1.00
Sleepiness	12 (52)	3 (30)	.28
Memory disturbances	3 (13)	4 (40)	.16
Concentration disturbances	4 (17)	5 (50)	.09
Paresthesia	9 (39)	6 (60)	.45
Dizziness	5 (22)	6 (60)	.05
Nausea	6 (26)	3 (30)	1.00
Vomiting	1 (4)	3 (30)	.07
Pains	23 (100)	9 (90)	.30
Headache	16 (70)	9 (90)	.38
Typical radicular pain[a]	15 (65)	0 (0)	<.001
Cervical/lumbar pain[b]	13 (57)	4 (40)	.46
Arthralgias	8 (35)	7 (70)	.13
Myalgias	8 (35)	4 (40)	1.00
Temperature >38°C	8 (35)	1 (10)	.22
Meningeal signs	14 (61)	1 (10)	.009
Peripheral facial palsy	8 (35)[c]	1 (10)	.22
Arthritis	1 (4)	1 (10)	.52
Heart involvement[d]	1 (4)	0 (0)	1.00

[a] Severe, burning, tearing, migrating pain with characteristic exacerbations at night; at the site involved by pain, the skin may be dysestetic and hyperpathic.
[b] Pain that does not have characteristics of radicular pain.
[c] Includes 5 patients with unilateral peripheral facial palsy and 3 patients with bilateral peripheral facial palsy.
[d] Transitory atrioventricular conduction disturbances (atrioventricular blocks I and II).

The basic demographic and clinical characteristics for patients for whom *B. burgdorferi* sensu lato was isolated from CSF samples are presented in table 1.

A comparison of the frequency of individual clinical signs and symptoms in the 2 groups revealed that patients in the *B. garinii* group reported radicular pains and expressed meningeal signs more often than did patients in the *B. afzelii* group, whereas dizziness was found more frequently among patients in the *B. afzelii* group than among patients in the *B. garinii* group; the frequencies of occurrence for several other symptoms and signs were comparable (table 2).

Pleocytosis with a predominance of lymphocytes was established for 19 (83%) of 23 patients with *B. garinii* isolated from CSF samples, whereas in the *B. afzelii* group, all but 2 patients had $\leqslant 5 \times 10^6$ cells/L in CSF samples. Also, protein concentrations in CSF samples were more frequently elevated and were higher for patients in the *B. garinii* group, compared with patients in the *B. afzelii* group. In addition, 2 of 4 patients in the *B. garinii* group who had normal routine CSF findings but positive CSF culture results had had lymphocytic pleocytosis and elevated protein concentrations at examination 2–3 months earlier. Significant differences between the 2 groups were also found for several other CSF parameters (table 3). Laboratory evidence of borrelial infection for patients with borreliae isolated from CSF is shown in table 4. The strains, isolated from skin and blood samples, were congruent with the corresponding isolates from CSF samples with respect to species and LRFP subtype.

DISCUSSION

Lyme neuroborreliosis may appear during the first few weeks or months after infection or late in the course of Lyme borreliosis. Early Lyme neuroborreliosis, which is better defined and much more frequent than late Lyme neuroborreliosis [1, 2, 9, 18, 19], typically comprises lymphocytic meningitis, involvement of cranial nerves, and involvement of peripheral nerves [20]. Clinical diagnosis is straightforward when the triad is complete or when 1 or more manifestations of the triad are associated with the presence of or a reliable history of EM [1, 2, 9, 18, 19]. Generally, the most prominent clinical symptom is pain caused by radiculoneuritis. Radicular pain is seen more often in European than in American patients and is more frequent and pronounced in adults than in children [1, 2, 9, 21–23]. The initial clinical report of what, 60 years later, was classified as early Lyme neuroborreliosis dates to 1922 [24]. Since the etiology of Lyme borreliosis was determined, several groups of patients with Lyme neuroborreliosis have been described; however, no series of patients with culture-confirmed Lyme neuroborreliosis has been published. Here, we present a group of 33 patients with either *B. garinii* or *B. afzelii* isolated from CSF samples.

The diagnosis of early Lyme neuroborreliosis is, as a rule, based on clinical characteristics, the presence of lymphocytic pleocytosis, and demonstration of CNS borrelial infection, as evidenced by seroconversion, intrathecal borrelial antibody production, isolation of borreliae from CSF samples, or demonstration of borrelial DNA in CSF samples [18, 19, 25]. In practice, seroconversion is rarely found to be a useful criterion (in our study, seroconversion was established in 4 of 33 patients), because at the time of the appearance of neurological signs, the majority of patients are seropositive. In addition, seroconversion confirms recent borrelial infection, but it does not confirm CNS involvement. The main limitations of PCR for the demonstration of borrelial DNA in CSF samples are low sensitivity, the possibility of false-positive findings, and the fact that the procedure has not been standardized [19, 25]. Isolation of the etiological agent from patient samples is the

Table 3. Laboratory and clinical findings for 23 patients with *Borrelia garinii* and 10 patients with *Borrelia afzelii* isolated from CSF samples.

Variable	*B. garinii* group ($n = 23$)	*B. afzelii* group ($n = 10$)	*P*
Duration of illness at time of lumbar puncture,[a] median duration (range)	19 days (1 day to 21 months)	7.5 months (4 days to 6 years)	.07
Leukocyte count			
Median value $\times 10^6$ cells/L (range)	176 (1–991)	2 (0–213)	.001
No. (%) of patients with $>5 \times 10^6$ cells/L	19 (83)	2 (20)	.001
Lymphocyte count			
Median value $\times 10^6$ lymphocytes/L	155 (1–934)	1.5 (0–160)	<.001
No. (%) of patients with $>5 \times 10^6$ cells/L	19 (83)	2 (20)	.001
IgG level			
Median mg/L (range)	48 (11.0–518)	30 (19–55)	.009
No. (%) of patients with >34 mg/L	18 (78)	2 (20)	.005
IgM level			
Median mg/L (range)	2.1 (0.2–44.1)	0.3 (0.1–9.9)	.03
Proportion (%) of patients with >0.7 mg/L	18/22 (82)	2/8 (25)	.007
Protein concentration			
Median g/L (range)	0.77 (0.23–3.1)	0.31 (0.20–0.80)	<.001
No. (%) of patients with >0.45 g/L	20 (87)	2 (20)	<.001
Albumin concentration			
Median mg/L (range)	308 (93–1723)	205 (128–432)	.03
Proportion (%) of patients with >350 mg/L	12/22 (55)	2 (20)	.12
Albumin quotient[b]			
Mean value (range)	0.007 (0.002–0.043)	0.005 (0.003–0.012)	.06
Proportion (%) of patients with >0.0074	12/22 (55)	2 (20)	.12
IgG quotient[c]			
Mean value (range)	0.004 (0.001–0.051)	0.002 (0.001–0.007)	.01
No. (%) of patients with >0.0035	15 (65)	2 (20)	.02

NOTE. This table reports CSF findings other than intrathecal borrelia antibody production.

[a] Duration from the onset of symptoms indicating potential CNS involvement to the time of CSF examination.

[b] Defined as CSF albumin concentration/serum albumin concentration.

[c] Defined as CSF IgG concentration/serum IgG concentration.

most reliable method for the diagnosis of borrelial infection, and isolation of the etiological agent from CSF samples is the most reliable method for the diagnosis of Lyme neuroborreliosis. This method also provides live microorganisms, which can be further characterized. However, isolation from CSF samples is a markedly low-yield procedure, and results are obtainable only after several weeks [1, 9, 14, 25]. In everyday clinical practice in Europe, the demonstration of intrathecally synthesized borrelial antibodies has, as a rule, been used for the establishment of the diagnosis of Lyme neuroborreliosis. The problem with this diagnostic approach is insensitivity during the first few weeks of CNS involvement and long persistence; intrathecal borrelial antibody production can be detected for several months or years, even after appropriate therapy with antibiotics [18, 19, 25]. Our study gave us the opportunity to assess the sensitivity of intrathecal borrelial antibody production in a group of patients for whom the etiologic agent had been isolated from CSF samples. We were able to demonstrate intrathecal borrelial antibody production in only 14 (42%) of 33 patients, including 13 (59%) of 23 from the *B. garinii* group and 1 (10%) of 10 from the *B. afzelii* group. Short duration of the illness could have been the explanation for the absence of intrathecal borrelial antibody production in several patients in *B. garinii* group (7 of 10 patients without intrathecal borrelial antibody production had a duration of symptoms indicating potential CNS involvement of $\leqslant$14 days; 6 of these 7 patients presented with peripheral facial palsy); however, such an explanation would be plausible for only 2 patients in the *B. afzelii* group. Although isolation of borrelia from CSF samples is acknowledged to confirm CNS involvement, such a finding does not automatically confirm that positive culture results are indeed representative of the laboratory and clinical findings for most patients with Lyme neuroborreliosis.

Information on the predominant role of *B. garinii* as the etiological agent in European patients with Lyme neuroborreliosis has been based on typing results for borrelia isolated

Table 4. Laboratory evidence of borrelial infection for 23 patients with *Borrelia garinii* and 10 patients with *Borrelia afzelii* isolated from CSF samples.

Findings	No. (%) of patients		
	B. garinii group (n = 23)	*B. afzelii* group (n = 10)	P
Serum antibodies[a]			
IgM	3 (13)	2 (20)	.63
IgG	18 (78)	8 (80)	1.00
Seroconversion[b]			
IgM	1 (4)	0 (0)	
IgG	3 (13)	1 (10)	1.00
Intrathecal borrelial antibody production			
IgM	2/21 (10)	0/5 (0)	1.00
IgG	13 (57)	1 (10)	.02
Isolation of borreliae			
From EM skin lesion	5/8 (63)	0/0 (0)	
From ACA skin lesion	0/0 (0)	1/2 (50)	
From blood	1/5 (20)	0/1 (0)	

NOTE. ACA, acrodermatitis chronica atrophicans; EM, erythema migrans.

[a] Presence of positive borrelial antibody titers at the time that the CSF sample was obtained.

[b] Conversion from negative to positive result or at least a 4-fold elevation in antibody titers.

from CSF samples obtained from patients with Lyme neuroborreliosis [7, 8, 15, 26, 27], on demonstration of genetic material distinct to borrelia species in CSF samples [28, 29], and on species-specific serological responses [26, 30]. With each of these approaches, the principal species found in patients with Lyme neuroborreliosis was *B. garinii,* followed by *B. afzelii* (table 5). However, the designs of some of the cited studies do not allow very precise conclusions with respect to the proportion of the etiologic agents because of several potential biases in the collection of isolates and in the selection of isolates for typing. The findings of our study are based on consecutive patients registered at a single center during a 10-year period for whom borreliae were isolated from CSF samples and, therefore, represent a reliable ratio of *B. garinii* isolates to *B. afzelii* isolates (70% vs. 30%). However, by using this approach, we excluded not only a few patients with *B. burgdorferi* isolated from CSF samples but also a substantial number of patients with positive CSF culture results from whom we were not able to harvest enough borreliae to perform typing with LRFP (which requires a high density of borreliae in culture).

The majority of our patients with positive CSF culture results exhibited evident or highly probable Lyme neuroborreliosis, but for several patients, clinical indications of CNS involvement were quite vague, and the isolation of borreliae from CSF samples was rather surprising. A comparison of the findings for the *B. garinii* and for the *B. afzelii* group revealed that the large majority of patients with positive CSF culture results who did not exhibit clinical features characteristic of European Lyme neuroborreliosis [18, 19] were in the *B. afzelii* group, whereas typical cases were nearly exclusively limited to the *B. garinii* group. Almost all patients from the latter group had either EM and lymphocytic pleocytosis or lymphocytic pleocytosis associated with radiculoneuritis and/or peripheral facial palsy, and they, therefore, complied with provisional clinical diagnosis of evident or highly probable Lyme neuroborreliosis, as defined in the present study. Moreover, among our patients with *B. garinii* isolated from CSF samples, the proportions of patients with severe radicular pain, peripheral facial palsy, and expressed meningeal signs were in accordance with the reported European findings for adult patients with Lyme neuroborreliosis [22, 23, 31], as were almost all other clinical and CSF findings shown in tables 2 and 3. On the contrary, findings for the *B. afzelii* group were quite different: only 1 of 10 patients fulfilled the aforementioned simple clinical criteria for Lyme neuroborreliosis, none reported radicular pains, and only 2 (20%) had peripheral facial palsy. Some of the differences between the 2 groups could be explained, to a certain extent, as the result of longer durations of illness for patients in the *B. afzelii* group; however, for several patients, such an explanation is most probably not applicable. For example, it is highly unlikely that a patient with severe radicular pains would not try to find medical help and eventually receive a diagnosis of Lyme neuroborreliosis or that such a patient would forget severe pains or facial palsy but would remember several other much less-prominent symptoms. Therefore, the results of the present study indicate pro-

Table 5. The etiology of Lyme neuroborreliosis for 196 European patients according to species of *Borrelia burgdorferi* sensu lato.

Mode of detection, study	Pathogen				Total
	Borrelia garinii	*Borrelia afzelii*	*Borrelia burgdorferi*	Other	
Isolation from CSF					
Busch et al. [8]	21	10	4	1[a]	36
Peter et al. [26]	3	…	…	…	3
Ružić-Sabljić et al. [15]	25	14	1	…	40
Ornstein et. al. [27]	5	1	…	…	6
Orstein et al. [29]	26	8	1	…	35
Subtotal	80	33	6	1	120 (61)
PCR of CSF samples					
Lebech et al. [28]	11	1	…	1[a]	13
Ornstein et al. [29]	4	1	2	…	7
Subtotal	15	2	2	1	20 (10)
Serological testing					
Peter et al. [26]	18	2	3	5[b]	28
Ryffel et al. [30]	16	7	2	3[c]	28
Subtotal	34	9	5	8	56 (29)
Total	129 (66)	44 (22)	13 (7)	10 (5)	196 (100)

NOTE. Data are no. (%) of patients with the specified pathogen. Data shown for Ružić-Sabljić et al. [15] refer to patients seen in Slovenia.

[a] *B. garinii* and *B. afzelii.*

[b] Inconclusive.

[c] *Borrelia valaisiana.*

nounced differences between the *B. garinii* group and the *B. afzelii* group. In contrast to the *B. garinii* group, the large majority of the *B. afzelii* group did not fulfill European criteria for Lyme neuroborreliosis [18, 19]. The findings of the present study might indicate that, although *B. afzelii* is able to pass through the blood-brain barrier, it has restricted capability to initiate a substantial inflammation of the CNS. The significance of this genospecies in Lyme neuroborreliosis remains to be elucidated.

Patients with *B. garinii* isolated from CSF samples have a distinct clinical presentation, compared with that of patients with *B. afzelii* infection. *B. garinii* causes what, in Europe, is diagnosed as typical early Lyme neuroborreliosis (i.e., painful meningoradiculoneuritis or Bannwarth syndrome), whereas the clinical features of CNS involvement associated with *B. afzelii* are much less specific and more difficult to diagnose.

Acknowledgments

Financial support. The Ministry of Science and Technology of the Republic of Slovenia (grants J3-4005 and P3-0296 to F.S.).

Potential conflicts of interest. All authors: no conflicts.

References

1. Stanek G, Strle F. Lyme borreliosis. Lancet **2003**; 362:1639–47.
2. Steere AC. Lyme disease. N Engl J Med **2001**; 345:115–25.
3. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease: a tick-borne spirochetosis? Science **1982**; 216:1317–9.
4. Baranton G, Postic D, Saint Girons I, et al. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int J Syst Bacteriol **1992**; 42:378–83.
5. van Dam AP, Kuiper H, Vos K, et al. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. Clin Infect Dis **1993**; 17:708–17.
6. Balmelli T, Piffaretti JC. Association between different clinical manifestations of Lyme diseases and different species of *Borrelia burgdorferi* sensu lato. Res Microbiol **1995**; 146:329–40.
7. Ružić-Sabljić E, Maraspin V, Lotrič-Furlan S, et al. Characterization of *Borrelia burgdorferi* sensu lato strains isolated from human material in Slovenia. Wien Klin Wochenschr **2002**; 114:544–50.
8. Busch U, Hizo-Teufel C, Fingerle V, Nitschko H, Wilske B, Preac-Mursic V. Three species of *Borrelia burgdorferi* sensu lato (*B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*) identified from cerebrospinal fluid isolates by pulsed-field gel electrophoresis and PCR. J Clin Microbiol **1996**; 34:1072–8.
9. Nadelman RB, Wormser GP. Lyme borreliosis. Lancet **1998**; 352: 557–65.
10. Strle F, Nadelman RB, Cimperman J, et al. Comparison of culture-confirmed erythema migrans caused by *Borrelia burgdorferi* sensu stricto in New York state and *Borrelia afzelii* in Slovenia. Ann Intern Med **1999**; 130:32–6.
11. Carlsson SA, Granlund H, Jansson C, Nyman D, Wahlberg P. Characteristics of erythema migrans in *Borrelia afzelii* and *Borrelia garinii* infections. Scand J Infect Dis **2003**; 35:31–3.
12. Logar M, Ružić-Sabljić E, Maraspin V, et al. Comparison of erythema migrans caused by *Borrelia afzelii* and *Borrelia garinii.* Infection **2004**; 32:15–9.
13. Ružić-Sabljić E, Maraspin V, Cimperman J, Lotrič-Furlan S, Strle F. Evaluation of immunofluorescence test (IFT) and immuno (Western) blot (WB) test in patients with erythema migrans. Wien Klin Wochenschr **2002**; 114:586–90.
14. Preac-Mursic V, Wilske B, Schierz G. European *Borrelia burgdorferi* isolated from humans and ticks: culture conditions and antibiotic susceptibility. Zentralbl Bakteriol Mikrobiol Hyg [A] **1986**; 263:112–8.
15. Ružić-Sabljić E, Lotrič-Furlan S, Maraspin V, Cimperman J, Pleterski-Rigler D, Strle F. Analysis of *Borrelia burgdorferi* sensu lato isolated from cerebrospinal fluid. APMIS **2001**; 109:707–13.
16. Belfaiza J, Postic D, Bellenger E, Baranton G, Saint Girons I. Genomic fingerprinting of *Borrelia burgdorferi* sensu lato by pulsed-field gel electrophoresis. J Clin Microbiol **1993**; 31:2873–7.
17. Picken RN, Cheng Y, Strle F, et al. Molecular characterization of *Borrelia burgdorferi* sensu lato from Slovenia revealing significant differences between tick and human isolates. Eur J Clin Microbiol Infect Dis **1996**; 15:313–23.
18. Stanek G, O'Connell S, Cimmino M, et al. European Union Concerted Action on Risk Assessment in Lyme Borreliosis: clinical case definitions for Lyme borreliosis. Wien Klin Wochenschr **1996**; 108:741–7.
19. Brouqui P, Bacellar F, Baranton G, et al. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin Microbiol Infect **2004**; 10:1108–32.
20. Pachner AR, Steere AC. The triad of neurologic manifestations of Lyme disease: meningitis, cranial neuritis, and radiculoneuritis. Neurology **1985**; 35:47–53.
21. Christen HJ, Hanefeld F. Neurologic complications of erythema-migrans-disease in childhood: clinical aspects. Zentralbl Bakteriol Mikrobiol Hyg [A] **1986**; 263:337–42.
22. Stiernstedt G, Gustafsson R, Karlsson M, Svenungsson B, Sköldenberg B. Clinical manifestations and diagnosis of neuroborreliosis. Ann N Y Acad Sci **1988**; 539:46–55.
23. Pfister HW, Kristoferitsch W, Meier C. Early neurological involvement (Bannwarth's syndrome). In: Weber K, Burgdorfer W, eds. Aspects of Lyme borreliosis. Berlin, Germany: Springer-Verlag, **1993**:152–67.
24. Garin C, Bujadoux R. Paralise par les tiques. J Med Lyon **1922**; 71: 765–7.
25. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. Clin Microbiol Rev **2005**; 18:484–509.
26. Peter O, Bretz AG, Postic D, Dayer E. Association of distinct species of *Borrelia burgdorferi* sensu lato with neuroborreliosis in Switzerland. Clin Microbiol Infect **1997**; 3:423–31.
27. Ornstein K, Berglund J, Nilsson I, Norrby R, Bergstrom S. Characterization of Lyme borreliosis isolates from patients with erythema migrans and neuroborreliosis in southern Sweden. J Clin Microbiol **2001**; 39:1294–8.
28. Lebech AM, Hansen K, Rutledge BJ, Kolbert CP, Rys PN, Pershing DH. Diagnostic detection and direct genotyping of *Borrelia burgdorferi* by polymerase chain reaction in cerebrospinal fluid in Lyme neuroborreliosis. Mol Diagn **1998**; 3:131–41.
29. Ornstein K, Berglund J, Bergstrom S, Norrby R, Barbour AG. Three major Lyme *Borrelia* genospecies *(Borrelia burgdorferi* sensu stricto, *B. afzelii* and *B. garinii)* identified by PCR in cerebrospinal fluid from patients with neuroborreliosis in Sweden. Scand J Infect Dis **2002**; 34: 341–6.
30. Ryffel K, Peter O, Rutti B, Suard A, Dayer E. Scored antibody reactivity determined by immunoblotting shows an association between clinical manifestations and presence of *Borrelia burgdorferi* sensu stricto, *B. garinii, B. afzelii,* and *B. valaisiana* in humans. J Clin Microbiol **1999**;37: 4086–92.
31. Borg R, Dotevall L, Hagberg L, et al. Intravenous ceftriaxone compared with oral doxycycline for the treatment of Lyme neuroborreliosis. Scand J Infect Dis **2005**; 37:449–54.