◆ ORIGINAL ARTICLE ◆

Borrelia burgdorferi detected by culture and PCR in clinical relapse of disseminated Lyme borreliosis

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A total of 165 patients with disseminated Lyme borreliosis (diagnosed in 1990–94, all seropositive except one culture-positive patient) were followed after antibiotic treatment, and 32 of them were regarded as having a clinically defined treatment failure. Of the 165 patients, 136 were tested by polymerase chain reaction (PCR) during the follow-up. PCR was positive from the plasma of 14 patients 0–30 months after discontinuation of the treatment, and 12 of these patients had a clinical relapse. In addition, *Borrelia burgdorferi* was cultured from the blood of three patients during the follow-up. All three patients belonged to the group with relapse, and two of them were also PCR positive. This report focuses on the 13 patients with clinical relapse and culture or PCR positivity. Eight of the patients had culture or PCR-proven initial diagnosis, the diagnosis of the remaining five patients was based on positive serology only. All 13 patients were primarily treated for more than 3 months with intravenous and/or oral antibiotics (11 of them received intravenous ceftriaxone, nine for 2 weeks, one for 3 weeks and one for 7 weeks, followed by oral antibiotics). The treatment caused only temporary relief in the symptoms of the patients. All but one of them had negative PCR results immediately after the first treatment. The patients were retreated usually with intravenous ceftriaxone for 4–6 weeks. None of them was PCR positive after the retreatment. The response to retreatment was considered good in nine patients. We conclude that the treatment of Lyme borreliosis with appropriate antibiotics for even more than 3 months may not always eradicate the spirochete. By using PCR, it is possible to avoid unnecessary retreatment of patients with 'post-Lyme syndrome' and those with 'serological scars' remaining detectable for months or years after infection.

Key words: Borrelia burgdorferi; cultivation; Lyme disease; polymerase chain reaction; therapy of Lyme borreliosis; treatment failure.

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Introduction

Published cases of Lyme borreliosis (LB) patients with culture-proven treatment failure have been rather few (1–4). In addition to culture, polymerase chain reaction (PCR) has shown borrelial DNA in chronic disease after antibiotic treatment (5–7). Based on the assumption that spirochetal DNA would not persist in the body very long after the eradication of living spirochetes (6), PCR-based detection of *Borrelia burgdorferi* has been used as an indicator of ongoing infection in patients with relapse of the disease, and as predictor of responsiveness to treatment (6–10).

The differentiation of a treatment failure from 'post-Lyme syndrome' is difficult, and these two conditions have been poorly defined. However, it is generally agreed that a true treatment failure means ongoing infection, ie the presence of live *B. burgdorferi* in the body of the patient. In contrast, 'post-Lyme

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syndrome' consists of residual symptoms lasting for several months after therapy without evidence of the presence of the spirochete. Some symptoms of 'post-Lyme syndrome' may arise via immunological mechanisms after the infection (6, 8).

Opinions of clinicians on the optimum duration of antibiotic treatment in disseminated LB are divided. Most published recommendations favour intravenous antibiotics for 2–4 weeks or, alternatively, oral therapy for 4 weeks. However, in practice clinicians frequently use antibiotic courses longer than officially recommended. According to a recent questionnaire study, about half of the clinicians working in highly endemic areas in the USA prefer to use treatments lasting for 3 or more months for patients with chronic LB (11). However, there is no convincing evidence showing that long-term treatment could prevent treatment failures.

In this study, we report on a group of 13 patients with disseminated LB ('second or third stage'), and with culture or PCR-proven treatment failure after antibiotic treatment which lasted for more than 3 months.

Patients and methods

Patients and treatments

A total of 165 adult patients with disseminated LB were diagnosed at the Turku University Central Hospital in 1990–94 following case definition for LB developed by the Centers for Disease Control (CDC) (12, 13). A vast majority of our patients were treated with intravenous ceftriaxone for 2 weeks, followed by oral amoxicillin for 14 weeks. Ten per cent of all patients were treated for more than 18 weeks with antibiotics. Eight patients were lost from the follow-up. Thirty-two (19.4%) of the patients had a clinically defined treatment failure (reappearance of at least one of the original manifestations, or at least one new major manifestation of LB). The median duration of antibiotic treatment in the remaining patients was the same (16 weeks) as in the patients with treatment failure and culture or PCR positivity. Of the 165 patients, 136 were tested by PCR after the treatment (1–5 specimens/patient studied), and the plasma samples of 14 (10.3%) of them were positive. Of the 14 patients, 12 had a treatment failure and the remaining two patients were asymptomatic. *B. burgdorferi* was cultured from the blood of three patients during the follow-up. All three patients belonged to the treatment failure group, and two of them were PCR positive. All 13 culture or PCR-positive patients with treatment failure and most of the remaining patients were seen by one of the authors. The mean age of the 13 patients at diagnosis was 46.2 years (range 25–63 years), and all lived in an area where LB is endemic. Eight of the patients had culture or PCR-proven initial diagnosis; the diagnosis of the remaining five patients was based on positive serology only.

Measurement of borrelia antibodies

Immunoglobulin (Ig)M and IgG antibodies against sonicated *B. burgdorferi* were measured by an in-house ELISA using *B. burgdorferi* (ATCC 35210) as antigen (14). All steps were carried out automatically by an Auto-EIA II instrument (Labsystems, Helsinki, Finland). Serum samples were tested at a dilution of 1:100. The results were expressed as relative ELISA units. Seropositivity was determined by comparing antibody results from test serum samples with those of 110 healthy controls. The cut-off value for weakly positive results was the mean +2 SD of the controls.

Culture of B. burgdorferi

Biopsy, cerebrospinal fluid (CSF) or blood specimens were inoculated into tubes containing BSK-II medium and incubated at 30 °C. The tubes were examined macroscopically twice weekly and passaged once weekly for at least 2 months. Dark-field microscopy was carried out if the colour of the culture medium indicated growth. The final identification of the cultured spirochetes was based on the PCR.

Extraction of DNA for the PCR

1 ml of plasma, serum, CSF or minced biopsy specimen was centrifuged (13000 rpm, 10 min, Heraeus Biofuge® 17RS, Heraeus Sepatech, Osterode, Germany), 800 µL of the supernatant was removed, and the remaining 200 µL was mixed with 300 µL of SDS solution (0.1 M NaOH, 2 M NaCl and 0.5% sodium dodecyl sulphate). After incubation at 80 °C for 15 min, 200 µL of 0.1 M Tris-HCl (pH 8) was added. After SDS treatments, DNA was extracted with phenol-chloroform, precipitated with ethanol and finally dissolved in Tris-EDTA buffer (15).

PCR

The pre- and post-PCR procedures were carried out by separate technicians and in physically separated rooms. A 5-µL volume of extracted DNA was added into the reaction tube. The specific target chosen for the PCR was a DNA fragment from the flagellin gene sequence of *B. burgdorferi*. The pretreatment samples were run with primers prB31/41–4 and prB31/41–5, resulting in a 730-bp PCR product (16). The PCR with all specimens obtained from patient 11 and the brain tissue specimens of patient 8 was run in two steps, first with external primers prB31/41–4 and prB31/41–5 and then with nested primers WK1 and WK2, resulting in a 290-bp fragment (17). The PCR with specimens obtained after treatment was carried

out as described earlier with primers WK1 and FL7 resulting in a 497-bp PCR product (17–19). Each PCR run included a positive control containing DNA extracted from a reference strain (B31) of *B. burgdorferi sensu stricto* (ATCC 35210). Hybridization of the amplified products was intermittently used to prove the positive results of the PCR. In each case result of the hybridization was always positive. Further, every sixth tube of each run was used as a negative control subjected to all above sample treatments. Negative controls remained negative in each run.

Circulating immune complexes

Serum concentration of circulating immune complexes (CICs) was measured by using platelet ^{125}I-labelled staphylococcal protein A test (PIPA) (20). In brief, stored pooled human platelets were used and the IgG binding of CICs of IgG class to the Fc receptors of test platelets was measured by radioiodinated protein A. The test was interpreted as positive if the level of CICs was 3 SD units or more above normal material (3–7 SD units borderline; 8 ≥ SD units moderately or strongly positive).

Results

As can be calculated from the numbers presented in the Patients an Methods section, the PCR or culture positivity rate was 40.6% among the patients with clinical relapse (13 of 32) and 1.9% among the rest (2 of 104) of the patients ($P < 0.01$). *B. burgdorferi* was cultivated from the blood in 9.4% of the patients with treatment failure (3 of 32) and from none of the rest of the patients.

The mean age was 46.2 years among the 13 patients with culture or PCR-proven treatment failure and 43.4 years among the remaining 152 patients. The female:male ratio did not differ significantly between these two groups, as it was 1.6 among patients with failure and 1.3 in the other group. The vast majority of the patients in both groups had a high risk of exposure to tick bites because they lived permanently or spent most of the summer time in rural environment. All 165 patients, except one culture-positive patient, had either IgM or IgG antibodies against *B. burgdorferi* before or after the treatment. The median duration of the antibiotic treatment was 16 weeks in both the patients with culture or PCR-proven treatment failure and the rest of the patients. The antibiotics used were similar in both groups. The most commonly employed treatment was intravenous ceftriaxone for 2 weeks followed by oral amoxicillin (500 mg tid) plus probenecid (500 mg tid), or as an alternative treatment doxycycline (100 mg bid) if there was allergy to penicillins.

Table 1 shows the pretreatment symptoms and signs of the 13 patients with clinical relapse and culture or PCR positivity. The symptoms and signs at

Table 1. Pretreatment symptoms and signs, and brain magnetic resonance imiging (MRI) findings of 13 patients with clinical relapse of disseminated Lyme borreliosis and culture or polymerase chain reaction (PCR) positivity.

	Patient No Sex (M or F) and age (yrs)												
Symptom or brain MRI finding	1 F40	2 F61	3 F63	4 M53	5 M25	6 F46	7 F57	8 M42	9 M45	10 F52	11 F36	12 F35	13 M45
History of erythema migrans	+	+	−	+	+	−	−	−	−	−	−	−	+
Arthritis	−	−	−	−	−	−	+	−	−	−	−	−	−
Arthralgia	−	+	−	+	+	+	+	−	+	−	+	−	+
Myalgia or fibromyalgia	+	+	−	−	+	+	+	−	+	−	+	−	+
Headache	−	−	−	−	+	+	+	−	+	+	−	−	−
Dizziness	−	−	−	−	−	+	+	−	+	+	+	−	+
Meningitis	−	−	−	−	+	−	−	−	+	+	−	−	−
Radiculitis or neuritis	−	+	+	−	−	−	−	−	−	−	−	−	−
Neuropathy	+	+	+	−	−	−	−	−	+	−	+	−	−
Carpal tunnel syndrome	+	+	−	−	−	−	−	−	−	−	−	−	−
Diplopia	−	−	−	−	−	−	−	−	+	−	−	−	−
Epilepsy	−	−	−	−	−	−	−	+	−	−	−	−	−
Encephalitis	−	−	+	−	−	−	−	+	−	+	−	−	−
Transient hemiparesis	−	−	+	−	−	−	−	−	−	−	−	−	−
Febris	+	+	+	−	+	−	−	−	−	+	−	+	−
Hepatopathy	+	−	−	−	−	−	−	−	−	−	−	+	−
Chorioretinitis or uveitis	−	−	−	−	−	−	−	−	−	−	−	+	−
Pleuritis or pericarditis	−	−	−	−	−	+	−	+	−	−	−	−	−
Vasculitis (proven by biopsy)	+	−	−	−	−	−	−	+	−	+	−	−	−
Abnormal brain MRI	+	nd	+	nd	nd	+	−	+	−	+	−	nd	nd

nd, data not available.

the detection of relapse were usually the same as prior to the first treatment (data not shown). None of the 13 patients had erythema migrans after the first treatment and before the laboratory detection of probable treatment failure. Before the first treatment, only three patients recalled previous tick bites. However, deer fly bites with resulting erythema migrans or fever were reported by two patients. History of erythema migrans as well as other signs or symptoms of the disease are presented in Table 1.

Abnormal brain magnetic resonance imaging (MRI) findings were obtained in five (63%) of eight patients studied. One of them (patient 3) also had cervical myelopathy demonstrated by MRI (multifocal large lesions in the white matter in the brain and in the cervical spinal cord) (Fig 1). The MRI findings were suggestive of encephalitis or demyelination in three patients (patients 3, 8 and 10), whereas the other two patients (patients 1 and 6) had multiple small, unspecific infarct-like lesions in the white matter (Fig 2). The case report of patient 1 before detection of the central nervous system (CNS) findings has been previously published (3). Detailed case reports, showing also MR images, of patients 8 and 10 have been published elsewhere (21). Large areas of demyelination in periventricular white matter were detected histologically and by MRI in patient 10, from whom *B. burgdorferi* was cultured in a CSF specimen. The disease had a fatal outcome in this patient. Patient 8 was a 40-year-old man presenting with epileptic seizures, and MRI-detected multifocal lesions, which disappeared after repeated courses of antibiotics. The brain biopsy specimen obtained from this patient showed vasculitis, and DNA of *B. burgdorferi* was found by PCR in this specimen. Two patients were also included in a study comparing two treatment regimens for disseminated LB (22).

All patients with neurological symptoms were carefully examined together with a neurologist, and all of them had normal CSF IgG:albumin ratio. Results of other tests pertinent to exclude multiple sclerosis, neurosarcoidosis and other neurological disorders were also normal. None of the patients with vasculitis fulfilled the criteria for systemic lupus erythematosus (SLE) or could be diagnosed with any other autoimmune disorder.

Table 2 shows the antibiotics given before the laboratory detection of persistent infection. The mean

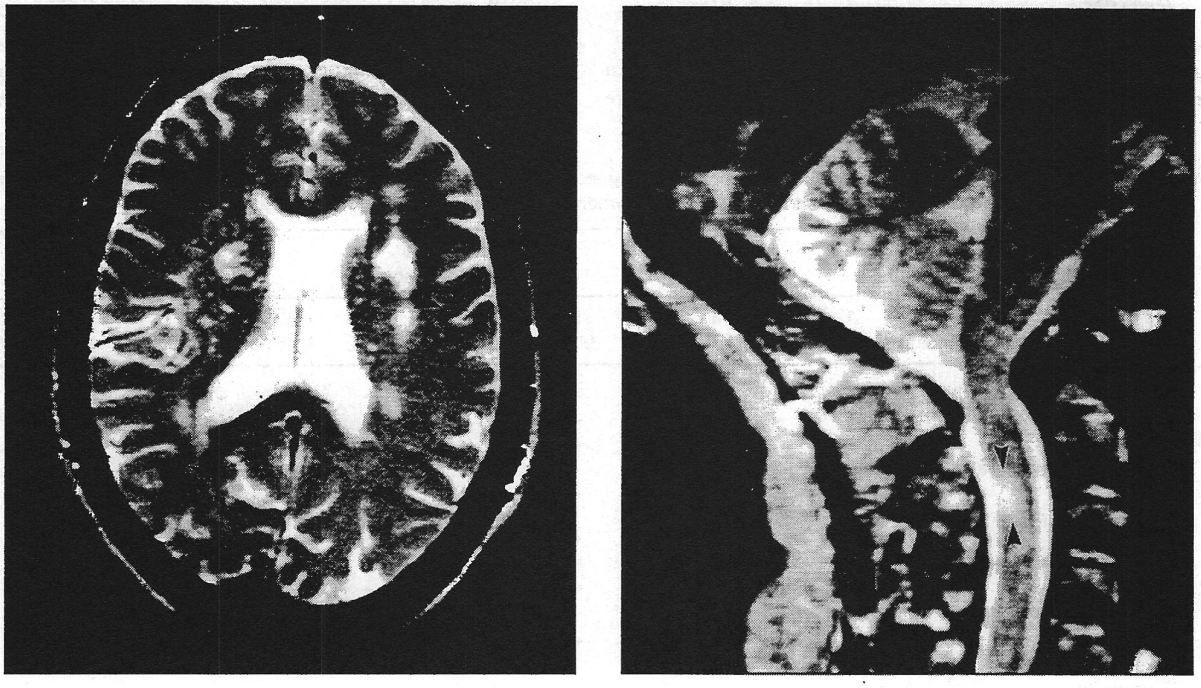

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Figure 1. Magnetic resonance images (MRI) of a 62-year-old patient (patient 3) with cervical myelopathy. The figure shows large cerebral and intramedullary white matter lesions on T2-weighted MRI (not enhanced with contrast medium, Siemens Magnetom 1.5 Tesla) of the brain **(a)** and cervical spinal cord **(b)** of the patient with slowly progressive sensory and motor radicular symptoms as well as visual symptoms after a fly bite followed by high fever for 1 week. DNA of *Borrelia burgdorferi* was detected in her cerebrospinal fluid (CSF) specimen by polymerase chain reaction (PCR). The symptoms temporarily improved after antibiotic therapy but relapsed and disappeared again after a new antibiotic therapy. Serum antibodies against *B. burgdorferi* converted from positive to negative, and PCR for *B. burgdorferi* was negative in the CSF and plasma specimens after the antibiotic treatments.

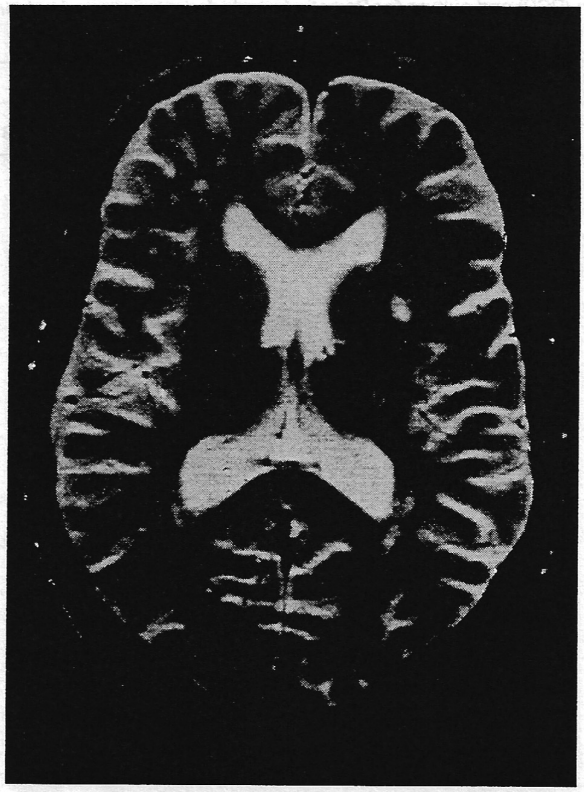

Figure 2. Magnetic resonance image (MRI) of the brain of a 42-year-old patient (patient 1) showing infarct-like lesions in the white matter. The image was taken by T2-weighted MRI, no enhancement with contrast medium (Siemens Magnetom 1.5 Tesla). The number and size of the lesions shown by MRI were the same after long treatments with intravenous and oral antibiotics as 2 years earlier, shortly after *Borrelia burgdorferi* was cultured from the patient's blood sample during a 4-week fever episode. Also the cerebrospinal fluid and skin (with panniculitis) cultures were positive for *B. burgdorferi* earlier in the course of her disease.

duration of intravenous and/or oral antibiotic treatment was 23 weeks (range 14–47 weeks). However, only one patient received antibiotics intravenously for more than 3 weeks before the laboratory detection of treatment failure (patient 8). For retreatment, the patients received ceftriaxone 2 g daily for 4–6 weeks, followed by oral antibiotics in three patients (data not shown). The response to retreatment was considered good in nine of the 13 patients (four of them became symptomless), and poor in four patients. None of the 13 patients was PCR positive after retreatment.

Table 3 shows antibody levels, PCR and culture for *B. burgdorferi* as well as circulating immune complexes. Five (42%) of the 12 patients who were seropositive before the first treatment were seronegative at the time of laboratory detection of the treatment failure. The seronegative but culture-positive patient remained seronegative after treatment. IgM seroreactivity turned negative more often (five of six cases) than the IgG seroreactivity (three of nine cases). The HLA genotypes of 10 of the 13 patients were analysed. Four patients were HLA DR2 and three were HLA DR4 positive. One patient had both HLA DR2 and DR4 alleles (Table 3).

Discussion

In this study, the symptoms of nearly 20% of patients with disseminated LB reappeared after antibiotic therapy, which was continued for even more than 3 months. This clinical relapse rate is slightly below the level reported earlier among patients treated with shorter courses of antibiotics (7, 9, 13, 23). However, at least two studies have shown better clinical outcome of the disease with a shorter duration of antibiotic treatment than the present study. In one of the trials (24), outcomes of neuroborreliosis patients were excellent without any treatment failures. In the other

Table 2. Antibiotic treatment given to 13 patients with disseminated Lyme borreliosis before laboratory detection of continuous infection.

Antibiotic treatment (duration in weeks)	Patient No												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Ceftriaxone iv 2g/day	2	2	2		2	2	2	7	2	3	2		2
Cefixime 200 mg every 8 h				14				14					
Penicillin G iv 4 million IU every 4 h	1												
Doxicycline 100 mg every 12 h	14							17		24			
Amoxicillin 500 mg every 8 h		14	14		14	14	14	3	14	1	1		14
Azithromycin 250 mg/day								3			2		
Rifampin 600 mg/day								3					
Cefuroxime axetil 500 mg every 12 h											14		
Cefadroxil 500 mg every 8 h												17	
Total duration	17	16	16	14	16	16	16	47	16	28	19	17	16

Table 3. Antibody levels against and polymerase chain reaction (PCR) and culture for *Borrelia burgdorferi*, as well as circulating immune complexes (CICs) and HLA DR2 and DR4 alleles of patients with clinical relapse and culture or PCR positivity.

	Patient No												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Before first treatment													
IgM antibodies	+	+	+	−	−	+	−	+	−	−	+	−	−
IgG antibodies	−	+	+	+	+	+	+	−	+	−	−	+	+
CIC			+	−			−			+	+	−	−
Plasma PCR			−		−		+	−			−	−	−
CSF PCR	+		+		−			−	+	+	+		−
Biopsy PCR	+			−	−			+					
Blood culture			−		−		+	−			−	−	+
CSF culture	+		−		−			−	+	+	−		−
Biopsy culture	+			−	−			−					
Interval between the end of first treatment and the laboratory detection of treatment failure (weeks)	32	130	40	86	43	22	34	44	22	4	32	0	60
At the time of laboratory detection of treatment failure													
IgM antibodies	+	−	−	−	−	−	−	−	−	−	−	−	−
IgG antibodies	−	−	−	+	+	+	+	−	+	−	−	−	+
CIC		−	+	−	+	+	−	+	−		+		−
Plasma PCR	+	+	+	+	+	+	+	+	+	+	+	+	−
CSF PCR		−			−	−		−	−				−
Biopsy PCR										+			−
Blood culture	+				+	−				−			+
HLA DR2	+	−	+	−			−	+	−		+	−	−
HLA DR4	−	+	−	−			−	+	+		−	−	−

CSF, cerebrospinal fluid; Ig, immunoglobulin.

trial (25), conducted among patients with acute disseminated Lyme disease (mainly multiple erythema migrans), the rates of clinical cure were 85% and 88% in patients receiving ceftriaxone and doxycycline, respectively. However, the patients in the ceftriaxone group reported persistent symptoms at the last follow-up visit more frequently (27% of the patients) than what would be expected from the clinical cure rates defined by the investigators. In a third study the patients with chronic LB received tetracycline for 1–11 months (mean 4 months), and the treatment failed in 10% of the patients (26).

The clinical outcome of patients with *B. burgdorferi* infection after antibiotic therapy is difficult to assess and standardize. It is therefore not feasible to compare clinical studies on treatment efficacy in patients with disseminated LB. Differences between cure rates in various geographical locations may occur and be caused by genetic factors and variations in the infecting organisms (subspecies of *B. burgdorferi sensu lato*). We were able to identify the genospecies of *B. burgdorferi sensu lato* in only one of the three culture-positive treatment failure cases, and this was *B. burgdorferi sensu stricto*. In the remaining two cases the PCR products were too weak to be sequenced. However, we are aware of only this patient ever infected with *B. burgdorferi sensu stricto* in Finland. It seems that even in the ticks *B. burgdorferi sensu stricto* is rare in Finland compared with *B. garinii* and *B. afzelii* (27). In the present study, culture or PCR-based evidence for the presence of live spirochetes was obtained in more than 40% of the patients with relapsed disease. We consider these 13 cases to have true treatment failure and not 'post-Lyme syndrome'. This observation supports the earlier reports on cases of LB patients with culture or PCR-proven treatment failures (1–5, 7). True treatment failures may result from inadequate absorption or penetration of the drug to privileged sites where the organisms reside (8), from the theoretically possible resistance of the spirochete to antibiotics (1, 2, 28–30), from intracellular survival of the organism (31–34), or from insufficient duration of therapy.

Clinicians' opinions on the optimum duration of antibiotic treatment in late LB are strongly divided. Most published recommendations favour intravenous antibiotics for 2–4 weeks or, alternatively, oral therapy for 4 weeks. It is now clear that all patients will not

recover with treatments obeying these recommendations (1–4, 25). However, there is no convincing evidence showing that substantially better results could be achieved by lengthening the treatment beyond the recommended duration. Our patients were primarily treated for a period of more than 3 months, which usually included 2 weeks of intravenous antibiotics. Still, their infection relapsed. None of the 13 patients retreated for 4–6 weeks with intravenous antibiotics remained PCR positive. The clinical response to retreatment was considered good in two-thirds of the patients. In LB, the treatment response often becomes noticeable only gradually during several months after the cessation of the antibiotic treatment. Therefore, it is not practical to decide on the length of the antibiotic treatment during its course in most cases. Our results suggest that patients who do not respond favourably to the initial therapy should be followed up and repeatedly tested by PCR and culture and retreated if any of these tests is positive when several months have elapsed since the first treatment. However, randomized double-blind studies with careful and long follow-ups are needed to find out the most effective regimens and sufficient durations of antibiotic treatments for disseminated LB.

Nine (69.2%) of the 13 patients with clinical relapse and culture or PCR positivity originally had multiorgan manifestations of the disease. Usually the symptoms and signs were of multiorgan nature also at the detection of relapse. Multiorgan manifestations at the time of diagnosis were almost as common in the remaining part of our clinical material (95 of 152 patients; 62.5%) as in the subgroup reported here. This indicates that the outcome of infection in patients with multiorgan manifestations is, at least in our patients receiving fairly long antibiotic treatment, as good as in patients without multiorgan symptoms. It seems possible that the initial clinical manifestations were more severe in the group with treatment failure and culture or PCR positivity, because the duration of the antibiotic treatment (before the laboratory confirmation of the treatment failure), as judged clinically, was more often longer than 18 weeks in the subgroup reported here than in the rest of the patients. The duration of pretreatment disease did not differ between the two groups having usually lasted at least for 2 months.

Reinfection of the patients could not be excluded. However, it is probable that reinfected patients would have developed symptoms compatible with the first stage of LB. None of our patients developed erythema migrans during the follow-up period, and their symptoms were similar to those before the first antibiotic treatment.

Three of the 13 patients had only IgM antibodies against *B. burgdorferi*, and one culture-positive patient was seronegative despite the disseminated stage of the disease. The reason for the lack of IgG antibodies, or of both IgM and IgG antibodies, was not restriction of the infection to privileged sites, as all these patients had a multiorgan disease. We have previously shown that patients with late LB with live spirochetes or borrelial DNA in their body fluids may have low or negative serum borrelia antibody levels (35). Low or negative antibody levels may result from formation of CICs (36). Six (46%) of our 13 patients had CICs either before the first treatment or at the time of laboratory detection of treatment failure. Antibody levels decreased or changed to seronegative in six (46%) of our 13 patients after the first treatment. However, this did not guarantee a successful eradication of the spirochete, as shown by PCR and culture.

Six (60%) of the 10 tested patients with treatment failure had either HLA DR2 or HLA DR4 alleles or both. Frequencies of HLA DR2 and HLA DR4 alleles in a Finnish control population have been reported to be 25.5% and 29.8%, respectively (37). HLA DR4 and, to a lesser extent, HLA DR2 have been associated with the development of chronic arthritis in LB (38). Our results suggest that the chronic symptoms are not necessarily of autoimmune origin but may be connected to the insufficient ability of the host to eradicate the spirochete, even when supported with appropriate long-term antibiotic treatment.

We conclude that the treatment of disseminated LB with appropriate antibiotics for even longer than 3 months may not always eradicate the spirochete as these 13 patients with treatment failure and culture or PCR positivity were treated with antibiotics for a long time (mean 23 weeks, median 16 weeks). However, it remains unresolved whether the prognosis of patients with disseminated LB could be improved by a long initial treatment. The patients not responding to initial therapy should be followed by repeated PCR and culture, and retreated if any of these tests turns positive. PCR offers a practical means to differentiate patients with 'post-Lyme syndrome' or with 'serological scars' from those who definitely need retreatment (39). Randomized studies with long follow-ups are warranted to find out the most successful regimens and adequate durations of antibiotic treatments for disseminated LB.

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