Subacute Multiple-Site Osteomyelitis Caused by *Borrelia burgdorferi*

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In a pediatric case of severe multiple-site osteomyelitis caused by *Borrelia burgdorferi*, the presence of spirochetes in a bone lesion was documented both by culture and by the polymerase chain reaction (PCR). Positive PCR results were also obtained with culture fluid yielding spirochetal growth and with acute-phase serum. Although the disease evidently was a late manifestation of Lyme borreliosis, antibodies to *B. burgdorferi* were low in titer and were restricted to the IgM class. The distribution of osteomyelitic lesions in multiple bones and the positive PCR results obtained with serum argue for hematogenous spread of the spirochetes. Before the specific diagnosis was established, the patient received several potent antimicrobial drugs, without a favorable outcome. In contrast, therapy with ceftriaxone led to a rapid cure that persisted thereafter. We conclude that infection due to *B. burgdorferi* must be considered a possible cause of subacute pediatric osteomyelitis.

Pediatric osteomyelitis most frequently involves a single bone; polyosteal infections are rare. *Staphylococcus aureus* and streptococci are the etiologic agents most often detected [1, 2]. However, in the majority cases, the pathogen remains unidentified [1, 3].

Oligoarthritis is one of the most common signs in the later stages of Lyme borreliosis [4, 5]. In only a few cases have septic arthritis and osteomyelitis been reported, and only once has *Borrelia burgdorferi* been demonstrated by staining of the bone [6]. Culture of *B. burgdorferi* from bone has not been reported previously. In this article we report a case of subacute osteomyelitis due to *B. burgdorferi* affecting multiple sites in an 8-year-old girl. In this case the presence of the spirochetes in bone was documented both by culture and by the polymerase chain reaction (PCR).

Methods

Assay for antibodies to B. burgdorferi. Sera from our patient were tested in three laboratories by means of five ELISA systems and western blotting (table 1). The basic tests were conducted in two ELISA systems in our laboratory. One system assessed IgM and IgG antibodies to sonicated borrelial antigen [7]. Interpretation of the results was based on a comparison with the results obtained with sera from 110 healthy blood donors. The second ELISA measured IgM and IgG antibodies to purified native 41-kD flagellin of *B. burgdorferi* (Lyme Borreliosis ELISA Kit, 2nd Generation; Dako, Copenhagen). In this instance the results were interpreted as stipulated by the manufacturer.

The serum sample collected on 11 March 1992 was analyzed at the Institute of Medical Microbiology and Hygiene in Freiburg, Germany; the two ELISAs conducted in that laboratory measured antibodies to the 14-kD tryptic peptide of *B. burgdorferi* flagellin [8] and antibodies to a mixture of outer surface proteins of *B. burgdorferi*, respectively. Finally, the Central Microbiological Laboratory in Stockholm analyzed five serum samples in an IgM-capture ELISA, with sonicated whole-cell *B. burgdorferi* as antigen [9]. In addition, the latter laboratory performed western blots for IgM and IgG antibodies in these sera [9].

Cultivation of B. burgdorferi. One bone-biopsy specimen was inoculated into modified Kelly's medium [4] and incubated at 30°C. Culture tubes were inspected with dark-field microscopy for the presence of spirochetes. Tubes with positive findings were studied by PCR.

Amplification by PCR. Nested PCR was used to amplify a *B. burgdorferi*–specific segment of a gene coding for 41-kD endoflagellin. The external primers and the first PCR procedure were the same as those described by Wallich et al. [10]. The internal primers for nested PCR were the same as those used by Krüger and Pulz for single-step PCR [11].

PCR products were detected by gel electrophoresis on 1.5% agarose with ethidium bromide staining. Each PCR run included a positive control containing DNA extracted [12] from a reference strain of *B. burgdorferi* (ATCC 35210). In addition, at least one negative control was subjected to all of

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Table 1. Various laboratory results in a case of subacute multiple-site osteomyelitis due to *Borrelia burgdorferi* in an 8-year-old girl.

Test	Result* on indicated month/day (1992)												
	2/5	2/10	2/12	2/20	3/2	3/11	3/17	3/25	4/9	4/22	5/13	6/17	8/31
Bone culture			+										
PCR													
Bone			+										
Serum	+	+			+					−		−	−
ELISA													
B. burgdorferi sonicate†													
IgM	+	+		+	+	++	+	+	−		−	−	−
IgG	−	−		−	−	−	−	−	−		−	−	−
41-kD flagellin§													
IgM	++					++							++
IgG	−					+							−
14-kD tryptic peptide of flagellin‖													
IgM						++							
IgG						+							
Mixture of outer surface proteins‖													
IgM						+							
IgG						−							
IgM-capture‡#	±		−			±				+			+
Western blot‡													
IgM	p41			p41		−				p41			p41
IgG	p41, p31			p41, p31		−				p41			p41, p31
Assay for total serum immunoglobulins**													
IgM	6.07					4.63							2.56
IgA	3.95					0.85							1.85
IgG	17.9					11.3							13.7

* Results for culture, PCR, and ELISA are negative (−), borderline (±), weakly positive (+), and moderately positive (++). Results for western blot are antigens detected. Results for immunoglobulin assays are levels (g/L).

† Whole-cell borrelial sonicate prepared in-house and analyzed at our laboratory.

‡ Analyzed at the Central Microbiological Laboratory in Stockholm.

§ Purified native 41-kD flagellin of *B. burgdorferi* (Lyme Borreliosis ELISA Kit, 2nd Generation; Dako, Copenhagen); analyzed at our laboratory.

‖ Analyzed at the Institute of Medical Microbiology and Hygiene in Freiburg, Gemany.

IgM-capture ELISA with sonicated whole-cell *B. burgdorferi* as antigen.

** Normal ranges: IgM, 0.5–2.5 g/L; IgA, 0.4–2.5 g/L; and IgG, 6.5–15.1 g/L.

the procedures used for the treatment and testing of samples. DNA for PCR was extracted [12] from sera and from bone obtained by biopsy.

Case Report

History. On 26 January 1992 an 8-year-old girl with fever and a 3-week history of pain in the left ankle was admitted to Turku University Central Hospital. The patient's history was elicited and indicated that both her grandfather and his sister had rheumatoid arthritis. The girl had sustained a complicated fracture of the right leg in 1988; no infections were associated with this injury, which was followed by a complete recovery.

The patient lived in southwestern Finland, where Lyme borreliosis is endemic; the tick season begins in April and lasts until October or November. However, no tick bites or erythema migrans–like skin lesions were documented. An eczematous lesion that responded only moderately to local treatment with corticosteroids had developed on the skin of the right palm in December 1991. At the beginning of January 1992, the child began limping because of pain in her left ankle. On January 23 she became febrile, with a temperature of 39°C.

Status at admission. When the patient was admitted, her left ankle and knee were warm but not swollen. The tenderest point was in the left medial malleolar region. In addition, the left knee and the lower part of the left femur were painful. Except for the eczematous area of the right hand, no possible primary foci of septic infection were detected.

Roentgenographic and scintigraphic observations. Roentgenography revealed multiple osteomyelitic lesions in both distal tibiae and in the left femoral metaphysis (figure 1). In addition, two small lesions were detected in the distal metaphysis of the right femur. The skeletal structure in the left ankle and foot was osteoporotic. Other skeletal structures

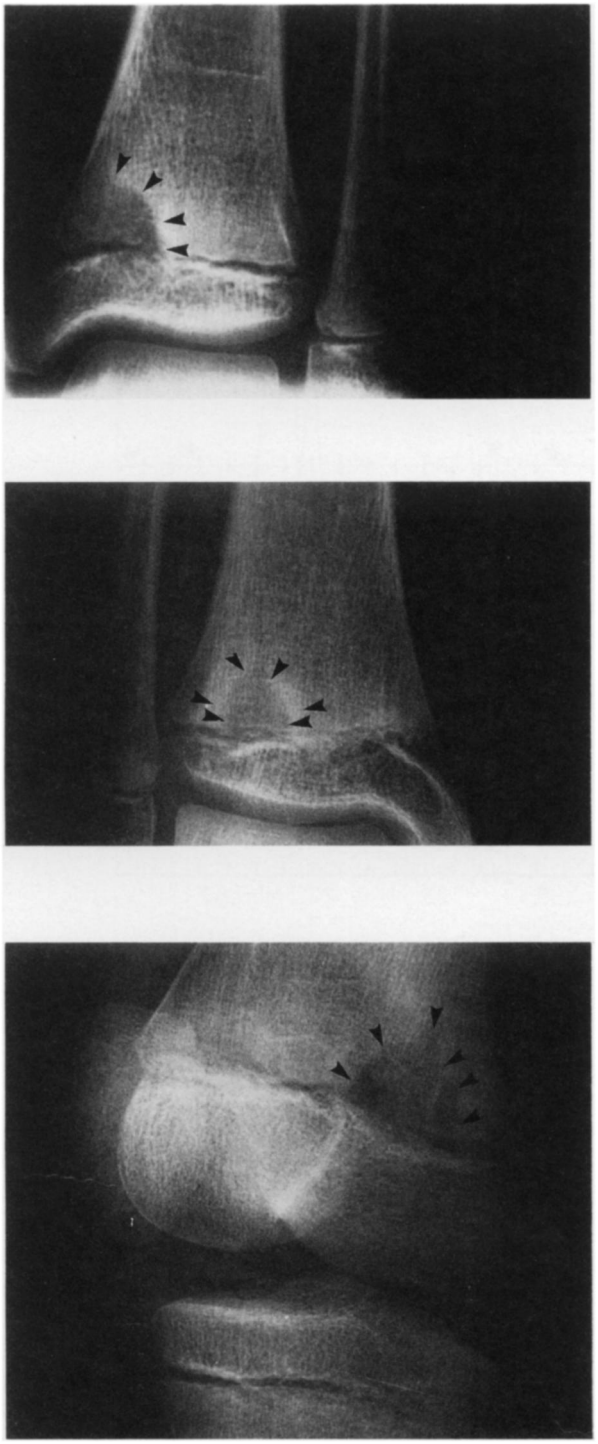

Figure 1. Osteomyelitic lesions (*arrows*) due to *Borrelia burgdorferi* infection in an 8-year-old girl. *Top:* A sharply restricted area of destruction 1.5 cm in diameter and a periosteal reaction in the metaphysis near the epiphyseal line of the left tibia. *Middle:* A focus of destruction with a sclerotic margin in the right distal tibia. *Bottom:* A focus of destruction with a sclerotic margin in the lower third of the left femoral metaphysis.

were normal. Three-phase bone scanning and scintigraphy with technetium-labeled leukocytes showed increased uptake in the left knee and ankle regions and slightly increased uptake in the right ankle. The bone scan also revealed increased uptake in the area of the left sacroiliac joint.

Laboratory results. At the time of admission, the level of C-reactive protein (CRP) in serum was 80 mg/L (normal, <10 mg/L); by 5 days later, the value had risen to 195 mg/L (figure 2). At admission, the hematocrit was 31%, the white blood cell count was 12.1×10^9/L, and the erythrocyte sedimentation rate (ESR) was 131 mm/h. Serum immunoglobulin levels were elevated; the IgM level remained above normal during the follow-up period of 8 months (table 1). Serum protein electrophoresis revealed elevated levels of the α fraction, the β_2 fraction (representing acute-phase proteins), and the γ fraction but did not detect oligoclonal bands. On 10 February the serum level of IgM rheumatoid factor was measured at 13.6 ELISA units (EU)/mL (normal, <10 EU/mL); thereafter, the level was repeatedly normal. Other laboratory results were within the normal range.

Serological results. An elevated serum level of antistreptodornase was detected on one occasion (5 February); the titer was 600 U/mL (negative, <400 U/mL). Assays for antistreptolysin and antistaphylolysin gave negative results, as did serological tests for syphilis. Weakly or moderately positive ELISA reactions were detected in at least one serum sample for IgM antibodies to sonic extract, native flagellin, 14-kD tryptic peptide of flagellin, and outer surface proteins of *B. burgdorferi* (table 1). Moreover, weakly positive results for IgG antibody to native flagellin and 14-kD tryptic peptide of flagellin were obtained for one serum sample. IgM-capture ELISA gave a borderline or weakly positive result on four occasions. The bands obtained by western blotting of five sera are listed in table 1.

Biopsies and bacterial cultures. On 26 January open biopsies of the left distal tibial and distal femoral foci yielded small amounts of purulent discharge. Bacterial, mycobacterial, and fungal cultures of these specimens were negative, as were gram staining, staining for mycobacteria, and direct microscopy for fungi. These first specimens were not cultured for *B. burgdorferi.*

On 12 February new samples were taken at biopsy from the distal femur and distal tibia. One biopsy specimen was inoculated into modified Kelly's medium, and the rest were inoculated into conventional culture media for the recovery of bacteria, mycobacteria, and fungi. After a 2-week incubation period, dark-field microscopy revealed spirochetes in the Kelly's medium. Only a few of the spirochetes moved rapidly and had an intact spiral morphology (figure 3, *top*); the rest of the organisms moved slowly and had a distorted spiral structure (figure 3, *bottom*). No spirochetes were detected on further passage. Although *Staphylococcus epidermidis* and *Corynebacterium* species grew in the Kelly's medium, culture of the bone samples on conventional media

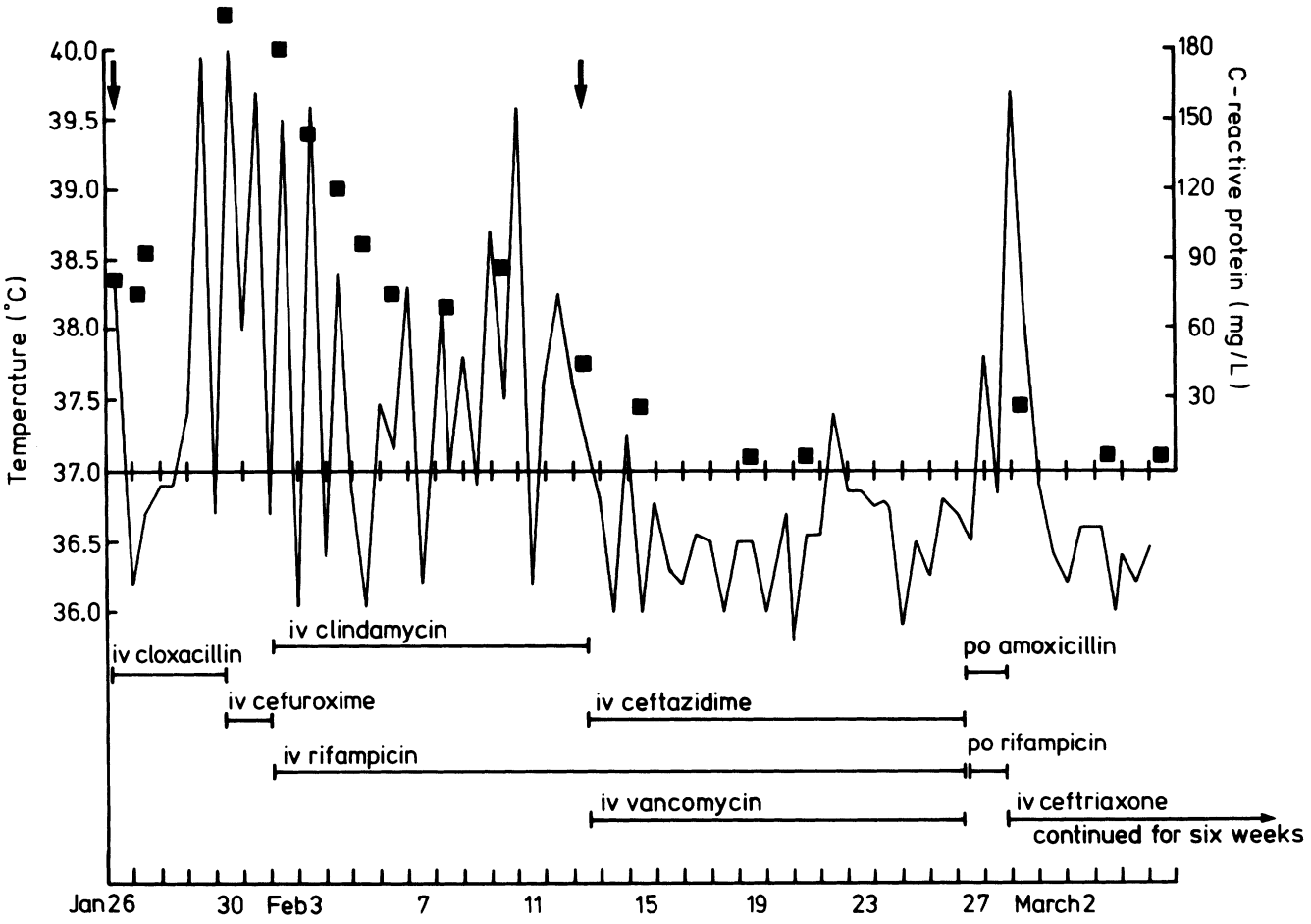

Figure 2. Body temperature and C-reactive protein (■) profiles in relation to dates of bone biopsies (*arrows*) and antibiotic therapy in a case of osteomyelitis due to *Borrelia burgdorferi* in an 8-year-old girl.

yielded no growth of these bacteria. Thus, the organisms were considered contaminants.

Both at admission and on repeated occasions thereafter, cultures of blood and urine on conventional media were negative.

PCR results. *B. burgdorferi* DNA was detected by PCR in the bone-biopsy specimens collected on 12 February and in the primary cultures inoculated with these specimens. Moreover, borrelial DNA was detected in the patient's first, second, and fifth serum samples but not in sera collected later during and after treatment with ceftriaxone (table 1).

Bone marrow histology and leukocyte function. Moderate eosinophilia and a slight excess of plasma cells were observed in bone marrow. Otherwise, the cytology of the bone marrow appeared normal.

Excess numbers of neutrophils with rough granules and reactive thrombocytosis were evident in the peripheral blood. The results of the leukocyte chemiluminescence test and the chemotactic response were normal. The phagocytic capability of leukocytes was clearly reduced, whereas their intracellular killing ability was normal. Phagocytic function returned to normal after antimicrobial treatment.

Treatment and outcome. Therapy with cloxacillin (600 mg iv every 6 hours) was instituted empirically on 26 January, continued for 5 days, and then followed by therapy with cefuroxime (750 mg iv every 6 hours) for 2 days. Since this treatment had no effect on the patient's symptoms, it was replaced with a combination of clindamycin (250 mg iv every 6 hours) and rifampin (250 mg iv every 12 hours). However, the patient's septic fever persisted, and, after a brief favorable response, her clinical status, ESR, and CRP values worsened (figure 2).

On 12 February therapy with ceftazidime (1,250 mg iv every 8 hours), vancomycin (250 mg iv every 6 hours), and rifampin (250 mg iv every 12 hours) was begun. Within 2 days the patient's temperature returned to normal.

On 26 February, parenteral antimicrobial treatment was discontinued and peroral treatment with amoxicillin (500 mg every 8 hours) and rifampin (450 mg daily) was instituted. Within 1 day, the patient again developed fever (temperature, 39.7°C), and her CRP value increased slightly.

On 27 February spirochetal growth from the patient's bone-biopsy specimen was reported. Treatment with ceftriaxone (1 g iv every 12 hours) was instituted and continued

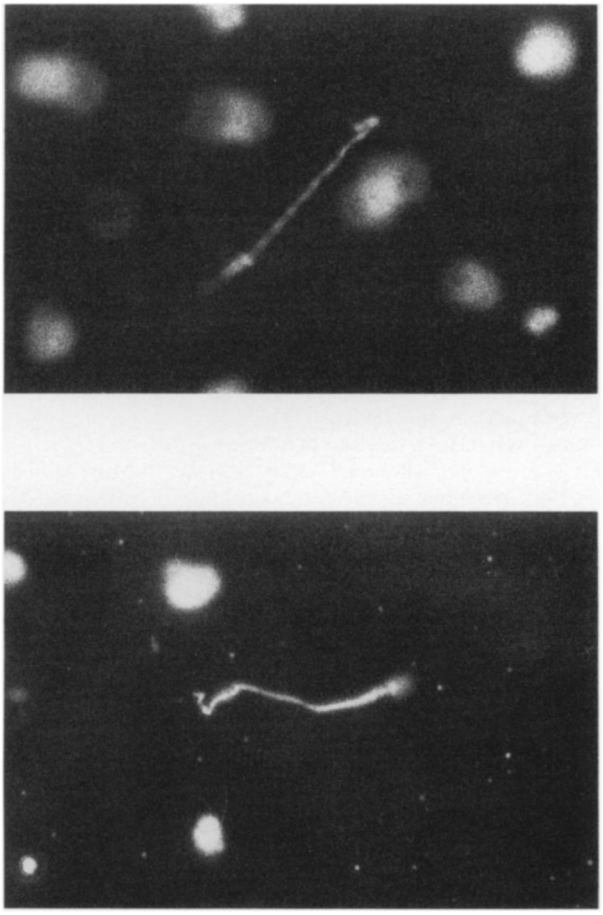

Figure 3. Dark-field microscopy of a bone-biopsy culture containing *Borrelia burgdorferi* (magnification, × 3,000). Only a few organisms had a spiral morphology and moved rapidly like spirochetes (*top*); the rest had lost their spiral structure and moved slowly (*bottom*).

for 6 weeks. The fever disappeared and the CRP value returned to normal within 3 days after the initiation of ceftriaxone therapy. Furthermore, the eczematous lesion on the patient's hand disappeared after 2 weeks of treatment with this drug. Within 1 month of the start of ceftriaxone therapy, the general condition of the patient had returned to normal. She was able to walk normally, although she felt moderate pain in the left foot when walking on tiptoe.

After the discontinuation of ceftriaxone treatment, peroral cefadroxil (500 mg every 8 hours) was administered for 12 weeks. At the time of this report, the patient's CRP and ESR values are normal, and no symptoms have reappeared.

Discussion

The case described herein demonstrates that *B. burgdorferi* can cause severe multifocal osteomyelitis. The pathogen was isolated from culture, and its DNA was detected directly in osteomyelitic lesions. Although peripheral blood was not cultured for borreliae, the positive PCR results obtained for the sera collected during the period of septic fever strongly suggest systemic infection.

The distribution of osteomyelitic lesions at multiple sites in the bones argues for hematogenous spread of *B. burgdorferi*. This patient's osteomyelitis developed in January; her primary infection obviously had commenced several months earlier. Spirochetemia is common in the early stage of Lyme borreliosis [13]. However, it may also occur during later stages, and the disease can follow a relapsing fever–like course [4, 13–17].

Hematogenous osteomyelitis is the most common form of bone infection in children [2]. It most frequently involves the highly vascular metaphyses of rapidly growing long bones [1–3]. Usually—but not always [3]—metaphyseal osteomyelitic lesions are restricted to the epiphyseal line. In our patient, the lesion in the left ankle traversed to the epiphysis. This observation suggests that borreliae can invade and destroy the growth cartilage.

Our patient had areas of destruction in several bones, including one lesion associated with a periosteal reaction. She also had osteoporosis in the bones of the left ankle and foot. Cortical and marginal bone destruction as well as osteoporosis associated with Lyme arthritis have been reported previously [18].

Although our patient had late-stage Lyme borreliosis, her serological response consisted mainly of IgM antibodies. In a study of a patient with culture-confirmed borreliosis [16], we documented a persistent IgM antibody response without a concomitant rise in the titer of IgG antibody. This observation emphasizes the point that an isolated, persistent IgM response cannot be deemed insignificant in a patient with suspected Lyme borreliosis. Our results also suggest that cases may be missed if only a limited panel of serological tests is used diagnostically.

Although the latex method yielded negative results, our patient had a low level of reactivity in an ELISA for IgM rheumatoid factor. Earlier studies have documented low levels of this factor in the serum of certain patients with Lyme disease and have correlated its presence with disease activity, with total serum titers of IgM, and with titers of IgM antibody to *B. burgdorferi*. However, absorption studies have proven that the ELISA for IgM rheumatoid factor and the ELISA for antispirochetal IgM measure different antibody populations [19]. In the case described herein, the value for total serum IgM was highest in the first sample tested, while that for IgM antibody to *B. burgdorferi* was highest more than 1 month thereafter. We suggest that total serum IgM was a marker of disease activity in our patient.

The borrelial spirochetes isolated from our patient's bone-biopsy specimen did not grow on further passage; moreover, the spiral morphology of most of the organisms in primary culture was damaged, and their motility was weak. Antimicrobial treatment may have impaired the viability of the spirochetes and damaged them structurally [20].

Before a specific diagnosis was made, the patient received several rather potent antimicrobial drugs. Only the combination that included ceftazidime had a beneficial effect. However, this regimen did not eradicate the borreliae, whose persistence was evidenced by the reappearance of symptoms immediately after a transition to peroral treatment with amoxicillin and rifampin. In contrast, cure rapidly followed the initiation of ceftriaxone therapy and persisted during a prolonged course of treatment with this drug and during and after a subsequent 3-month course of peroral cefadroxil.

We conclude that *B. burgdorferi* infection must be considered a possible cause of subacute pediatric osteomyelitis. The spirochetes apparently enter the bone via the hematogenous route and cause multiple lesions in the highly vascularized metaphyses of long bones. The lesions may also traverse to epiphyses. Definitive diagnosis should be based on cultivation of spirochetes from bone lesions. New gene-amplification technology offers advantages in the diagnosis of this disease.

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