Detection of *Borrelia burgdorferi* by polymerase chain reaction in synovial membrane, but not in synovial fluid from patients with persisting Lyme arthritis after antibiotic therapy

Susanne Priem, Gerd R Burmester, Thomas Kamradt, Karsten Wölbart, Michael G Rittig, Andreas Krause

Abstract

**Objectives**—To identify possible sites of bacterial persistence in patients with treatment resistant Lyme arthritis. It was determined whether *Borrelia burgdorferi* DNA may be detectable by polymerase chain reaction (PCR) in synovial membrane (SM) when PCR results from synovial fluid (SF) had become negative after antibiotic therapy.

**Methods**—Paired SF and SM specimens and urine samples from four patients with ongoing or recurring Lyme arthritis despite previous antibiotic therapy were investigated. A PCR for the detection of *B. burgdorferi* DNA was carried out using primer sets specific for the *ospA* gene and a *p66* gene of *B. burgdorferi*.

**Results**—In all four cases, PCR with either primer set was negative in SF and urine, but was positive with at least one primer pair in the SM specimens. In all patients arthritis completely resolved after additional antibiotic treatment.

**Conclusions**—These data suggest that in patients with treatment resistant Lyme arthritis negative PCR results in SF after antibiotic therapy do not rule out the intraarticular persistence of *B. burgdorferi* DNA. Therefore, in these patients both SF and SM should be analysed for borrelial DNA by PCR as positive results in SM are strongly suggestive of ongoing infection.

(*Ann Rheum Dis* 1998;57:118–121)

*Borrelia burgdorferi*, the causative agent of Lyme borreliosis, is difficult to detect microscopically or by culture because of the paucity of spirochetes at lesional sites and the long generation period of the borreliae. Therefore, the diagnosis of Lyme disease is usually made clinically and supported by the results of serological tests.1–3

In Lyme arthritis, only about one third of European patients remember having had a tick bite or typical clinical features of early Lyme disease like *erythema migrans*.1 Moreover, Lyme arthritis frequently mimics other rheumatological diseases. Serology is sometimes difficult to interpret especially in chronic cases because many patients show an isolated IgG seropositivity that does not discriminate between a past or present infection.1–5

The diagnostic situation can be even more difficult in patients with ongoing or recurring symptoms after antibiotic therapy. In these patients it may be impossible to definitely attribute clinical symptoms to a persisting infection with *B. burgdorferi*. The polymerase chain reaction (PCR) has recently been shown to sensitively and specifically detect *B. burgdorferi* DNA in various clinical specimens including synovial fluid (SF) and synovial membrane (SM). In untreated Lyme arthritis patients, PCR in SF reaches a sensitivity of about 85% and may even exceed 90% by analysing SF and urine specimens in parallel.4–7 Although a positive PCR only demonstrates the presence of bacterial DNA and not of live organism, it is quite suggestive of an active infection in patients with arthritic symptoms. Therefore, PCR is increasingly used to support the diagnosis of Lyme arthritis. Its role in monitoring the efficacy of antibiotic therapy, however, has not been evaluated yet. After antibiotic therapy, PCR in SF usually becomes negative, but it is not clear if this always indicates the eradication of the spirochetes.8 We therefore investigated paired SF/SM samples from patients with treatment resistant Lyme arthritis by PCR.

**Methods**

**PATIENTS**

Four patients with treatment resistant Lyme arthritis were investigated. After referral to our clinic Lyme borreliosis was diagnosed based on the following criteria: all patients lived in an area that is highly endemic for Lyme disease, all patients had a typical monarthrosis or oligoarthritis involving the knees, no clinical or serological evidence for other inflammatory rheumatological diseases including rheumatoid arthritis, reactive arthritis or collagen vascular disease could be found, and all patients had a positive serological test for Lyme arthritis according to the CDC criteria.9 Table I gives the patients’ characteristics.

Patient 1, a 50 year old male patient had first been seen by an orthopaedic specialist because of an acute onset arthritis of the left knee. Serological tests had revealed a high IgG antibody titre against *B. burgdorferi* and the diagnosis of Lyme arthritis was made. Doxycycline, 200 mg/day, and diclofenac were prescribed and an intraarticular injection of dexamethasone had been performed. Arthritis persisted with rapidly recurring joint effusions and the
Table 1 Clinical, demographic, and serological characteristics of the patients studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)/sex</th>
<th>Duration of arthritis (months)</th>
<th>ELISA</th>
<th>Immunoblot (RDa)</th>
<th>Previous antibody treatment*</th>
<th>Previous corticoid treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50/male</td>
<td>3</td>
<td>IgM neg</td>
<td>IgM: ND</td>
<td>ceftriaxone, 2 g for 28 days</td>
<td>intraarticular</td>
</tr>
<tr>
<td>2</td>
<td>51/female</td>
<td>2</td>
<td>IgM neg</td>
<td>IgM: ND†</td>
<td>ceftriaxone, 2 g for 21 days</td>
<td>none</td>
</tr>
<tr>
<td>3</td>
<td>28/female</td>
<td>9</td>
<td>IgG pos</td>
<td>IgG: 93, 72, 66, 62, 49, 41, 39, 34, 31, 25</td>
<td>doxycycline, 200 mg for 35 days</td>
<td>intraarticular</td>
</tr>
<tr>
<td>4</td>
<td>43/female</td>
<td>7</td>
<td>IgM pos</td>
<td>IgM: 66, 41, 39, 31, 25</td>
<td>ceftriaxone, 2 g for 14 days</td>
<td>systemic</td>
</tr>
</tbody>
</table>

*Antibiotic treatment given before analysis of synovial tissue. †ND: not done.

A 43 year old woman, patient 4, presented in our outpatient clinic complaining of a bilateral gonarthritis of seven months duration. ELISA and immunoblot showed a high IgG antibody titre against *B. burgdorferi* with several specific bands, and the *B. burgdorferi* PCR in urine and SF gave positive results. The patient was treated with ceftriaxone, 2 g daily for two weeks without any improvement. A few weeks later the patient was seen in another hospital with an acute polyarthritis involving both wrists and several metacarpophalangeal joints and a deterioration of the right gonarthritis. Treatment with doxycycline, 200 mg daily for 30 days, and prednisolone 10–20 mg per day was started. Again, there was no sufficient response and the patient was referred to our clinic. A closed needle arthrocentesis with synovial biopsy of the right knee was performed.

**PCR**

PCR was performed as described previously. In brief, 1–10 ml of SF or urine were centrifuged at 350 g for 20 minutes at 10°C. The pellet obtained was washed with 0.9% saline, cells were pelleted again, and DNA was extracted from the pellet by alkaline lysis. SM samples were disrupted mechanically, followed by a freeze and thaw procedure. The pieces obtained were subjected to alkaline lysis. Boiled *B. burgdorferi* sensu stricto (strain LW2) were used as positive control (external amplification control). Negative controls with water and SF were also run in every determination.

Nested PCR was performed with two primer sets (TIB Molbiol, Berlin, Germany) targeting *B. burgdorferi* specific gene sequences. One primer set (ospA primer) was specific for a portion of the plasmid located *ospA* gene (5′ outer primer GGG AAT AGG TCT AAT ATT AGC C; 3′ outer primer CAC TAA TTT TTA AAG TGG AAG T; 3′ nested primer GCA AAA TGT TAG CAG CCT TGA T; 3′ nested primer CTG TGT ATT CAA GTC TGG C; length of amplicons: 665 and 392 bp; EMBL accession number X66065). The second primer set (p66 primer) targeted a sequence of the...
a chromosomal gene encoding a 66 kDa protein (5' outer primer CGA AGA TAC TAA ATC TGT; 3' outer primer GAT CAA ATA TTT CAG CTT; 5' nested primer TGC AGA AAC ACC TTT TGA AT; 3' nested primer AAT CAG TTC CCA TTT GCA; length of amplicons 371 and 236 bp; EMBL accession number M58429).\(^{11,12}\)

As an internal amplification control, a primer pair specific for the pyruvate dehydrogenase gene (\textit{PDH} primer) was used (5' GGT ATG GAT GAG GAG CTG GA; 3' CAG CCC TCG ACT AAC CTT GT; length of the amplicon 185 bp; EMBL accession number J03576).\(^{13}\)

The PCR reaction mixture (total volume 25 µl) contained 2.5 µl of the isolated DNA, 2.5 µl 10-fold PCR buffer (Perkin Elmer Cetus, Norwalk, CT; final concentration 10 mM TRIS-HCl, 50 mM KCl, 1.5 mM MgCl\(_2\), 0.001% (w/v) gelatine), 0.3 µM of each primer, 200 µM of each nucleotide (Boehringer Mannheim, Mannheim, Germany), and 0.8 U of Taq polymerase (Ampli Taq, Perkin Elmer Cetus). The outer PCR was carried out with a total of 40 cycles: DNA was denatured at 94°C for one minute, primers annealed at 42°C for one minute, and extended at 72°C for one minute. Nested PCR was performed with 2.5 µl of the outer amplicons as template. Twenty five cycles were carried out with the temperature profile described above. Amplicons were visualised on a 3% agarose gel stained with ethidium bromide and photodocumented using a gel documentation system (Appligene imager, Appligene Oncor, Heidelberg, Germany).

### Results

Patients were evaluated 8 to 10 weeks after antibiotic treatment had been completed. At the time of investigation, all four patients were still seropositive and had an active arthritis despite previous antibiotic therapies (table 1).

Urine samples were collected and within one week SF and SM specimens were obtained in parallel by arthrocentesis with needle biopsy, arthroscopic biopsy or arthroscopic synovec-tomy, respectively. In none of the SF and urine samples could \textit{B burgdorferi} DNA be detected by PCR. In contrast, in SM samples \textit{B burgdorferi} PCR was positive (fig 1). SM samples of patient 1 and 2 showed positive PCR results with the \textit{ospA} primer while the SM specimen of patient 3 was PCR positive with the \textit{p66} primer only. SM samples from patient 4 were PCR positive with both primer sets (table 2). Additionally, in the material obtained by bursectomy from patient 1 \textit{B burgdorferi} could be detected by PCR with the \textit{ospA} primer.

Because of the persisting arthritis and these PCR findings, all patients were treated again with antibiotics. Patients 1, 2, and 4 received cefotaxime, 2 g three times per day, for three weeks, followed by a six week course with oral doxycycline or minocycline (200 mg/daily), respectively. Patient 3 was treated with imipenem, 1.0 g three times per day, for two weeks and, subsequently, with 200 mg doxycycline per day for six weeks. In all four patients arthritis completely subsided within four to six months and did not recur after a median observation period of 18 months.

![Figure 1 Detection of (A) \textit{ospA} and (B) \textit{p66} gene sequences of \textit{B burgdorferi} by PCR in SF and SM from patient 4. Nested PCR products were visualised on a 3% agarose gel stained with ethidium bromide. At presentation in our clinic, PCR was positive in SF (lane 3). After antibiotic therapy, PCR became negative in SF (lane 5) but was positive in SM (lane 7). Lane M: molecular weight markers, lane 1: positive control (\textit{B burgdorferi sensu stricto, strain LW2}), lanes 3, 4, 6: negative control (water lane).](http://ard.bmj.com/)

### Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>PCR in synovial before antibiotic treatment</th>
<th>PCR after antibiotic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCR in urine</td>
<td>PCR in SF</td>
</tr>
<tr>
<td></td>
<td>\textit{ospA} primer</td>
<td>\textit{p66} primer</td>
</tr>
<tr>
<td>1</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>4</td>
<td>positive</td>
<td>positive</td>
</tr>
</tbody>
</table>
Discussion

Previous studies have demonstrated that PCR is a valuable tool in the diagnosis of Lyme borreliosis. However, only little is known about the value of PCR analysis in monitoring the disease course and in evaluating the efficacy of treatment. Further treatment decisions are easily made in patients with ongoing arthritis and positive PCR or patients with good clinical reponse to antibiotic therapy and negative PCR results. The situation is much more complicated, however, if PCR in SF has become negative after antibiotic treatment despite persistence of arthritis. While on the one hand in these patients arthritides may be mediated by infection induced immunopathology, the lack of borrelial DNA in SF on the other hand does not necessarily rule out the intraarticular persistence of spirochetes, for example, in SM.

In this study we describe four patients with gonarthritides who were PCR positive for B burgdorferi DNA in SF at presentation in our clinic. After repeated oral and parenteral antibiotic therapy PCR became negative in both SF and urine although arthritides were resistant to treatment in these patients. However, in all four patients borrelial DNA could readily be detected in SM specimens indicating persistence of bacterial DNA. As in all patients arthritis resolved after additional antibiotic treatment PCR results were suggestive of microbial persistence.

These results extend those of a recent study by Jaulhac et al who were the first ones to describe the detection of B burgdorferi DNA in SM samples by PCR. Among the 12 patients investigated in that study, three had been treated with antibiotics previously. In two of these patients PCR was positive in SM only, but negative in SF. However, while the sensitivity of PCR was 83% in SM, sensitivity of the PCR in SF was only 50% even in untreated patients making the protocol described more suitable for SM analysis. In our study we used an optimised PCR protocol that detects B burgdorferi DNA in over 80% of patients with Lyme arthritis by analysing SF alone and in over 90% by analysing paired SF and urine samples. Despite this high sensitivity, PCR was negative in both SF and urine samples in our patients who had been treated with antibiotics.

Although positive PCR results do not prove infection by living bacteria, persistence of DNA raises the possibility of persisting active infection. The mechanisms by which B burgdorferi may have escaped the antibiotic episode remains to be elucidated. It has been shown previously by us and others that B burgdorferi evades into specific sites that are only incompletely accessible to antibiotics and the immune system. Moreover, evidence emerged from in vitro studies that the spirochetes may persist within synovial fibroblasts. This prompted us to treat our patients with parenteral cephalosporins to achieve high tissue concentrations, followed by treatment with doxycycline or minocycline that acts on intracellular microorganisms. In all four patients this therapy was able to cure the arthritis. Controlled studies will be necessary to further evaluate this therapeutic approach in otherwise treatment resistant infections.

Taken together, this study shows that in patients with ongoing or recurring Lyme arthritis after antibiotic therapy a negative B burgdorferi PCR in SF or urine does not necessarily exclude a persisting infection. In these patients, SM biopsy specimens should be obtained for PCR analysis, which is much more sensitive than SF-PCR to detect intraarticular persistence of the bacterial DNA. This is important because PCR positive patients are likely to suffer from ongoing infection and therefore may benefit from additional antibiotic therapy.

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