

# Detection of *Borrelia burgdorferi* DNA in urine of patients with ocular Lyme borreliosis

Uwe Pleyer, Susanne Priem, Lars Bergmann, Gerd Burmester, Christian Hartmann, Andreas Krause

## Abstract

**Aim**—To evaluate the diagnostic value of the polymerase chain reaction (PCR) to detect *Borrelia burgdorferi* DNA in patients with ocular Lyme borreliosis.

**Methods**—Of 256 consecutive uveitis patients six selected individuals with clinical evidence for Lyme borreliosis and 30 patients with non-Lyme uveitis were enrolled. Lyme serology was performed by ELISA and western blotting. Urine samples were examined by an optimised nested polymerase chain reaction (PCR) protocol.

**Results**—Only four of six uveitis patients suspected for Lyme borreliosis were ELISA positive, while all six subjects showed a positive western blot. *B burgdorferi* PCR was positive in all of these six patients. Whereas two of the 30 controls had a positive Lyme serology, *B burgdorferi* DNA was not detectable by PCR in any sample from these patients.

**Conclusions**—PCR for the detection of *B burgdorferi* DNA in urine of uveitis patients is a valuable tool to support the diagnosis of ocular Lyme borreliosis. Moreover, these patients often show a weak humoral immune response which may more sensitively be detected by immunoblotting.

(Br J Ophthalmol 2001;85:552-555)

Lyme borreliosis is a systemic infectious disease caused by the tick borne spirochaete *Borrelia burgdorferi* sensu lato.<sup>1</sup> If not treated in early phases it may persist causing dermatitis, arthritis, and neuritis. Ocular involvement is less frequently (<5%) reported<sup>2</sup> but may lead to persistent visual impairment. Therefore, early diagnosis and treatment are of clinical importance.<sup>3</sup> However, the clinical diagnosis is often difficult since the majority of patients do not recall a tick bite or an erythema migrans.<sup>4</sup>

Serology is of limited value, because antibody titres are usually not detectable before 4-6 weeks after infection and may never become positive.<sup>5,6</sup> Contrarily, positive Lyme serology may just indicate a past borrelial infection without proof of actual disease.

Since detection of *B burgdorferi* is insensitive, polymerase chain reaction (PCR) is increasingly being used to support the diagnosis. Various studies were able to demonstrate borrelial DNA in clinical specimens with a sensitivity between 20% and 100%, depending on disease stage, organ manifestation, and samples analysed.<sup>7,8</sup> Interestingly, urine PCR seems to be of particular value.<sup>9-14</sup> Recently, we were able to establish an optimised PCR protocol which allows the detection of *B burgdorferi* DNA in urine of 45% of patients with neuroborreliosis and 79% of patients with Lyme arthritis.<sup>9</sup> Since diagnostic difficulties still exist in ocular Lyme disease and PCR has not been systematically employed so far, we aimed to evaluate its diagnostic value.

## Patients and methods

We studied 256 consecutive uveitis patients from areas that are highly endemic for Lyme borreliosis.<sup>15</sup> The diagnosis of uveitis was based on clinical characteristics according to the criteria of the IUSG.<sup>16</sup> Detailed ophthalmic examination included biomicroscopy, three mirror contact lens examination of the eye, and fluorescein angiography in patients with retinitis or choroiditis. Patients were evaluated according to our in-house diagnostic standard for uveitis including complete history and physical examination in all patients and, depending on the clinical findings, neurological examination, x ray of the chest and the sacroiliac joints, determination of HLA-B27, measurement of angiotensin converting enzyme activity, serological tests for the detection of infections with *Treponema pallidum*, *Toxoplasma gondii*, herpes simplex, herpes zoster,

Department of Ophthalmology, Charité, Humboldt University, Campus Virchow Hospital, Augustenburger Platz 1, D-13353 Berlin, Germany  
U Pleyer  
L Bergmann  
C Hartmann

Department of Medicine, Rheumatology and Clinical Immunology, Charité, Humboldt University, Campus Charité Mitte, Schumannstrasse 20/21, D-10098 Berlin  
S Priem  
G Burmester  
A Krause

Correspondence to: Dr Uwe Pleyer  
uwe.pleyer@charite.de

Accepted for publication 13 December 2000

Table 1 Oligonucleotide primer sequences (from 5' to 3')

Target gene	Primer	Sequence	Base no	Amplification length (bp)	EMBL accession no
ospA	Outer primer 1	ggg aat agg tct aat att agc c	18-39	665	X66065
	Outer primer 2	Cac taa ttg tta aag tgg aag t	660-682		
	Nested primer 1	Gca aaa tgt tag cag cct tga t	54-75	392	
	Nested primer 2	Ctg tgt att caa gtc tgg ttc c	423-444		
p66	Outer primer 1	cga aga tac taa atc tgt	9-26	371	M58429
	Outer primer 2	gat caa ata ttt cag ctt	362-379		
	Nested primer 1	tgc aga aac acc ttt tga at	30-49	236	
	Nested primer 2	aat cag tcc cca ttt gca	671-690		
PDH	primer 1	ggt atg gat gag gag ctg ga	142-161	185	J03576
	primer 2	cag ccc tcg act aac ctt gt	218-237		

Table 2 Clinical data of patients with ocular Lyme borreliosis

Patient, initials, age (years), sex	Ocular manifestation	Systemic symptoms	Tick bite Erythema migrans	Steroid treatment before dx.	Treatment treatment-response	Visual acuity before/after treatment
1 RK 48, M	Bilateral uveitis posterior (>10 years), secondary (steroid) glaucoma, relapse	Arthritis, myalgia	+	+	2 weeks ceftriaxone IV, 4 weeks doxycycline PO recurrence -> 4 weeks doxycycline PO slow improvement	RE: 20/32 20/20 LE: 20/40 20/25
2 RHF 54, F	Bilateral uveitis posterior, (>3 years), relapse	Arthritis, fatigue	+	+	2 weeks ceftriaxone IV, 4 weeks doxycycline PO recurrence -> 4 weeks. Minocycline PO slow improvement	RE: 20/125 20/50 LE: 20/80 20/50 RE: 20/80 20/50 LE: 20/80 20/32
3 SR 59, F	Bilateral uveitis intermedia, (>5 years)	Arthritis	+	-	2 weeks ceftriaxone IV, 4 weeks doxycycline PO	RE: 20/25 20/20 LE: 20/25 20/25
4 UN 53, F	Bilateral uveitis posterior (>3 years)	Arthritis, peripheral neuropathy	+	+	3 weeks clindamycin PO, 2 weeks ceftriaxone IV slow improvement	RE: 20/32 20/25 LE: 20/50 20/32
5 FH 37, M	Bilateral uveitis posterior	Hepatitis	+	+	3 weeks ceftriaxone IV, 4 weeks doxycycline PO slow improvement	RE: 20/32 20/20 LE: 20/32 20/20
6 KE 76, F	Bilateral uveitis anterior	Headache	+	+	4 weeks doxycycline PO	RE: 20/64 20/50 LE: 20/64 20/32

IV = intravenous; PO = by mouth.

human immunodeficiency virus, Epstein-Barr virus, and cytomegalovirus were performed. Six selected patients with clinical evidence for Lyme borreliosis were enrolled in the study. All patients reported to have had a tick bite and an erythema migrans and complained about constitutional symptoms. Five patients had arthralgias and one patient suffered from headache and fatigue. Thirty patients with various forms of uveitis not related to Lyme borreliosis served as controls.

Lyme serology was carried out using full antigen ELISA (Seramun Diagnostics, Dolgenbrod, Germany).<sup>9</sup> Western blotting (DPC Biermann, Bad Nauheim, Germany) was done in patients with positive or indeterminate ELISA results and in patients with clinical symptoms suggestive of Lyme borreliosis but negative ELISA. Nested PCR was performed

as described previously<sup>9</sup> using two primer sets (TIB Molbiol, Berlin, Germany) targeting the plasmid located *ospA* gene (*ospA* primer) and a chromosomal gene encoding a 66 kD protein (*p66* primer) (Table 1).

## Results

The median duration of ocular disease was 6 months and even exceeded 3 years in four patients (Table 2). In all patients uveitis was present bilaterally, classified as posterior uveitis in four patients, two patients each showed perivasculitis and choroidal lesions (Figs 1 and 2). Anterior uveitis and intermediate uveitis were diagnosed once. In all patients extraocular manifestations of Lyme borreliosis could be observed including arthritis (n=4), cranial nerve palsy, peripheral neuropathy (n=1), and

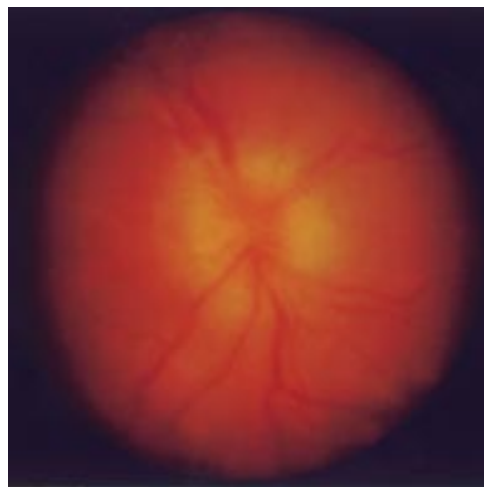


Figure 1 Fundus photograph. Patient 1 presenting with bilateral posterior uveitis, arthritis, and myalgia. Left eye at initial presentation (VA 20/40) with optic disc swelling, choroiditis, and vitritis. Seroblots were positive for immunoglobulin G and *B burgdorferi* DNA (*p66*) was detectable in urine of this patient.

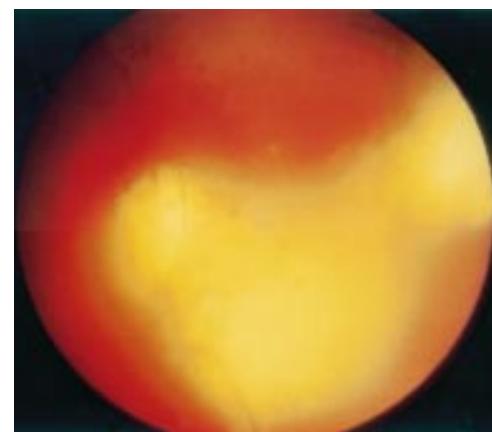


Figure 2 Patient 2 presenting with recurrent bilateral posterior uveitis and arthritis. Left eye at initial presentation (VA 20/80) with papillitis, optic disc swelling, and multiple choroidal lesions. A positive Lyme ELISA for IgG and western blots for IgM and IgG were detected. Urine PCR disclosed *B burgdorferi* DNA (*ospA*) that became negative following the initial treatment course. She developed recurrent uveitis, became PCR positive again during relapse of inflammation and eventually became stable after a second treatment course.

Table 3 Laboratory findings of patients with ocular Lyme borreliosis

Patient, age (years), sex	Lyme serology ELISA	Lyme seroblot (kDa)		Urine PCR before treatment	Urine PCR after treatment
1 RK 48, M	IgM negative IgG negative	IgM IgG	ND 66, 62, 41, 37, 18	positive for p66	negative
2 RHF 54, F	IgM negative IgG positive	IgM IgG	41, 39, 31, 21 72, 62, 41, 29, 18	positive for ospA	negative, after recurrence positive again for ospA
3 SR 59, F	IgM negative IgG positive	IgM IgG	ND 93, 72, 60, 41, 39, 34, 30, 25, 18	positive for p66	negative
4 UN 53, F	IgM negative IgG positive	IgM IgG	ND (93), 72, 66, 62, 60, 48, 34, 31, 29, 18	positive for ospA	negative
5 FH 37, M	IgM negative IgG negative	IgM IgG	ND 66, 60, 41, 39, 31	positive for p66	negative
6 KE 76, F	IgM negative IgG positive	IgM IgG	ND 66, 62, 60, 41, 39, 37, 34, 29, 28, 21, 18	positive for p66	negative

ND = not done.

hepatitis (n=1). ELISA was positive in four patients while two patients were repeatedly seronegative. However, western blots were positive<sup>17</sup> in all patients (Table 3). In the control group only 2/30 patients (7%) showed positive ELISA and/or western blot results.

*B burgdorferi* PCR was positive in all urine samples from patients with ocular Lyme borreliosis. In contrast, in none of the 30 urine specimens from controls could *B burgdorferi* DNA be detected. After antibiotic treatment PCR became negative in all patients. Marked improvement of the ocular manifestation was seen in our patients with anterior uveitis, and a more protracted response was observed in all individuals with posterior manifestation. Uveitis relapsed in two of these cases. Both had received systemic corticosteroids which may have contributed to treatment failure. One patient temporarily became PCR positive again during relapse. After a second treatment course, a stable reduction of intraocular inflammation was achieved.

### Discussion

The results of the present investigation showed that in six uveitis patients with a history and clinical signs of Lyme disease *B burgdorferi* DNA could be detected in urine samples. Since we only analysed highly selected patients and not all patients with findings compatible with Lyme borreliosis our results do not allow us to calculate the sensitivity of urine PCR in the diagnosis of Lyme uveitis. However, PCR was negative in all 30 patients with none-Lyme uveitis giving a specificity of 100%.

Although a positive PCR does not prove an infection with viable organisms, the positive results in our patients with clinical manifestations that resolved after antibiotic therapy strongly suggest spirochaetal persistence. In view of the current discussion about persistent infection versus infection induced immunopathology as the main mechanisms of chronic Lyme borreliosis our results point towards a direct infectious cause of ocular Lyme disease.<sup>18</sup> In one patient affected by a recurrent episode of bilateral chorioretinitis, urine PCR became positive again during relapse. The second treatment course not only improved the

clinical manifestation of uveitis, but also led to a negative urine PCR on repeated tests. This may indicate that PCR might be of value in following the infectious course of the disease. Adequate negative controls were run with each test to rule out false positive results. In addition, we already proved by sequencing of the PCR products that with our protocol *B burgdorferi* DNA is amplified exclusively.

Serological analysis in our PCR positive patients revealed that only four patients had a positive Lyme serology as determined by ELISA. In contrast, IgG immunoblots were positive in all six patients. It is well known that serological reactions may vary considerably and that a negative serology does not exclude the infection.<sup>6, 19, 20</sup> Possible explanations for this phenomenon include the invasion of spirochaetes into immunologically privileged sites.<sup>20, 21</sup> Several investigators have reported impaired immune responses in uveitis patients with confirmed diagnosis of Lyme borreliosis.<sup>3, 21</sup> Moreover, most of our patients had received immunosuppressive therapy such as corticosteroids that probably led to negative or weak immune response<sup>9</sup> and delayed diagnosis even for years.

Taken together, Lyme borreliosis has to be considered as a (rare) cause of intraocular inflammation even with negative ELISA findings. Additional laboratory tests, in particular immunoblots, are indicated in suspicious individuals—for example, in patients with extraocular manifestations compatible with Lyme disease. Nested PCR appears to be a valuable tool to identify patients with long standing disease. Moreover, PCR may also help to monitor the efficacy of anti-infectious therapy in patients with intraocular borrelial infections. However, the clinical value of this diagnostic tool, especially its sensitivity, still needs to be determined in subsequent studies.

Supported by DFG (Pl 150/10-1), (Kr 997/2-1), and BMBF (01 KI 9503).

- 1 Van Dam A, Kuiper PH, 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.
- 2 Rosenbaum JT, Rahn DW. Prevalence of Lyme disease among patients with uveitis. *Am J Ophthalmol* 1991;112:462–3.

- 3 Karma A, Seppälä I, Mikkilä H, *et al.* Diagnosis and clinical characteristics of ocular Lyme borreliosis. *Am J Ophthalmol* 1995;119:127–35.
- 4 Rahn DW, Malawista SE. Lyme disease. Recommendations for diagnosis and treatment. *Ann Intern Med* 1991;114:472–81.
- 5 Krause A, Burmester GR, Rensing A, *et al.* Cellular immune reactivity to recombinant OspA and flagellin from *Borrelia burgdorferi* in patients with Lyme borreliosis. Complexity of humoral and cellular immune responses. *J Clin Invest* 1992;90:1077–84.
- 6 Dattwyler RJ, Volkman DJ, Luft BJ, *et al.* Dissociation of specific T- and B-lymphocyte response to *Borrelia burgdorferi*. *N Engl J Med* 1988;319:1441–6.
- 7 Jaulhac B, Nicolini P, Piemont Y, *et al.* Detection of *Borrelia burgdorferi* in cerebrospinal fluid of patients with Lyme borreliosis. *N Engl J Med* 1991;324:1440–5.
- 8 Mouritsen CL, Wittwe CT, Litwin CM, *et al.* Polymerase chain reaction detection of Lyme disease. Correlation with clinical manifestations and serologic responses. *Am J Clin Pathol* 1996;105:647–54.
- 9 Priem S, Rittig MG, Kamradt T, *et al.* An optimized PCR leads to rapid and highly sensitive detection of *Borrelia burgdorferi* in patients with Lyme borreliosis. *J Clin Microbiol* 1997;35:685–90.
- 10 Schwan TG. The urinary bladder, a consistent source of *Borrelia burgdorferi* in experimentally infected white-footed mice (*Peromyscus leucopus*). *J Clin Microbiol* 1988;26:893–4.
- 11 Magnarelli LA, Anderson JF, Stafford III KC. Detection of *Borrelia burgdorferi* in urine of *Peromyscus leucopus* by inhibition enzyme-linked immunosorbent assay. *J Clin Microbiol* 1994;32:777–82.
- 12 Lebech AM, Hansen K. Detection of *Borrelia burgdorferi* DNA in urine samples and cerebrospinal fluid samples from patients with early and late lyme borreliosis by polymerase chain reaction. *J Clin Microbiol* 1992;30:1646–53.
- 13 McGuire BS, Chandler FW, Felz MW, *et al.* Detection of *Borrelia burgdorferi* in human blood and urine using the polymerase chain reaction. *Pathobiology* 1992;60:163–7.
- 14 Schmidt BL. PCR in laboratory diagnosis of human *Borrelia burgdorferi* infections. *Clin Microbiol Rev* 1997;10:185–201.
- 15 Kolmel HW, Neumann P, Schneider T, *et al.* Prevalence of antibodies to *Borrelia burgdorferi* in serum and cerebrospinal fluid samples from patients with neurological disorders in Berlin. *Int J Med Microbiol Virol Parasitol Infect Dis* 1992;277:504–11.
- 16 Bloch-Michel E, Nussenblatt RB. International uveitis study group recommendations for the evaluation of intraocular inflammatory disease. *Am J Ophthalmol* 1987;103:234–5.
- 17 Hauser U, Lehnert G, Lobentzner R, *et al.* Interpretation criteriae for standardized western blots for three european species of *Borrelia burgdorferi* sensu latu. *J Clin Microbiol* 1997;35:1433–44.
- 18 Gross DM, Forsthuber T, Tary-Lehmann M, *et al.* Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis. *Science* 1998;281:703–6.
- 19 Krause A, Brade V, Schoerner C, *et al.* T cell proliferation induced by *Borrelia burgdorferi* in patients with lyme borreliosis. *Arthritis Rheum* 1991;34:393–402.
- 20 Häupl T, Hahn G, Ritting M, *et al.* Persistence of *Borrelia burgdorferi* in ligamentous tissue from a patient with chronic lyme borreliosis. *Arthritis Rheum* 1993;36:1621–6.
- 21 Preac-Mursic V, Pfister HW, Spiegel H, *et al.* First isolation of *Borrelia burgdorferi* from iris biopsy. *J Clin Neuro-Ophthalmol* 1993;13:155–61.