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.

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Lyme borreliosis is a systemic infectious disease caused by the tick borne spirochaete *Borrelia burgdorferi* sensu lato.1 If not treated in early phases it may persist causing dermatitis, arthritis, and neuritis. Ocular involvement is less frequently (<5%) reported2 but may lead to persistent visual impairment. Therefore, early diagnosis and treatment are of clinical importance.3 However, the clinical diagnosis of Lyme arthritis is often difficult since the majority of patients do not recall a tick bite or an erythema migrans.4 Serology is of limited value, because antibody titres are usually not detectable before 4–6 weeks after infection and may never become positive.5–6 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.7–8 Interestingly, urine PCR seems to be of particular value.9–10 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.9 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.11 The diagnosis of uveitis was based on clinical characteristics according to the criteria of the IUSG.12 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, a 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,
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, Dölgenbrod, Germany). 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 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 extracellular manifestations of Lyme borreliosis could be observed including arthritis (n=4), cranial nerve palsy, peripheral neuropathy (n=1), and

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

IV = intravenous; PO = by mouth.
hepatitis (n=1). ELISA was positive in four patients while two patients were repeatedly seronegative. However, western blots were positive\(^2\) 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 samples from patients with ocular Lyme borreliosis our results point towards a direct infectious cause of ocular Lyme disease. 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 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.

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### Table 3 Laboratory findings of patients with ocular Lyme borreliosis

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

ND = not done.
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