Short Communication

Molecular Diagnosis of a Bilateral Panuveitis due to *Borrelia burgdorferi* Sensu Lato by Cerebral Spinal Fluid Analysis

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SUMMARY: The present paper describes a case of bilateral panuveitis due to *Borrelia burgdorferi* sensu lato diagnosed by a PCR approach using cerebral spinal fluid. Since the culture of *B. burgdorferi* takes a long time to grow and the accuracy of serological tests is doubtful in patients, the PCR method of amplifying a *B. burgdorferi* flagellin could be suitable to make a positive diagnosis in a case of atypical clinical history of Lyme disease.

Lyme borreliosis is an infectious disease caused by a tick-borne spirochete, *Borrelia burgdorferi* sensu lato. This bacterium presents various clinical features, classically including dermatologic, rheumatologic, cardiac, and neurologic manifestations. Ophthalmic manifestations of the illness remain a rare feature but are probably underestimated due to the difficulties of diagnosis (1-7). The present paper describes a polymerase chain reaction (PCR)-based diagnosis of bilateral panuveitis associated with retinitis pigmentosa-like features due to *B. burgdorferi* sensu lato.

A 47-year-old Algerian female, living in France for 20 years, was hospitalized in the ophthalmic ward of our hospital in France for a decrease in visual acuity that she had been experiencing for the past 6 weeks. Physical ocular examination showed a mild granulomatous panuveitis with retinitis pigmentosa combined with nyctalopia, which she had been experiencing for the past 6 weeks. A chest X-ray showed no abnormalities. Complete hemogram was normal, but the erythrocyte sedimentation rate (ESR) was 30 mm at the first hour and the C-reactive protein (CRP) was normal, but the erythrocyte sedimentation rate (ESR) was 30 mm at the first hour and the C-reactive protein (CRP) was 17 mg/l. The serum presented a hypergammaglobulinemia at 58.2 mg/ml without oligoclonal bands. A PCR assay, amplifying a 200-bp segment of the gene coding for *B. burgdorferi* flagellin, was performed on the CSF (8). Briefly, DNA was released by heating at 95°C for 15 min and PCR amplification was performed using the following primers: forward 5’-GATGATGCTGCTGGCATGGGAGTT and reverse 5’-CTGTCTGCATCTGAATATGTGCC TCTGG-3’. The result of this amplification was positive (Figure 1), XbaI and DraI were cut once, each giving 2 fragments: 67 and 133 bp (XbaI) and 81 and 119 bp (DraI). On the other hand, SmalI did not cut the amplicon, as expected. To validate the assay, negative and positive controls (DNA extracted from the *B. burgdorferi* strain B31) were analyzed at the same time. On the 200 bp, a fragment of 105 bp was sequenced and compared with the NCBI GenBank database by using the BLAST algorithm, giving us 96% similarity with both *B. afzelii* and *B. burgdorferi* (NCBI Blast = CP000395.1 and X63413.1). Thus, it was not possible in the complex *B. burgdorferi* sensu lato to choose between *B.

Fig. 1. Molecular evidence of *B. burgdorferi*. Agarose gel of PCR product from the patient’s cerebrospinal fluid (CSF) with use of *B. burgdorferi* flagellin DNA primers. Lanes 1 and 6, 100-bp ladder; lane 2, patient’s CSF amplicon at 200 bp restricted by SmalI; lane 3, restriction products by XbaI; lane 4, restriction products by DraI; lane 5, positive control.
afzelii and burgdorferi sensu stricto. The antimicrobial treatment by ceftriaxone (2 g/day for 3 weeks) slightly improved the visual acuity of the patient and decreased the inflammatory response.

Conventional methods remain inadequate for the diagnosis of many cerebral nervous system and ophthalmic infections. Although *B. burgdorferi* can be detected by culture, the sensitivity of this technique is low, approaching less than 5% for CSF, which could be an interesting sample for the exploration of ocular manifestations (5). Moreover, the culture of *B. burgdorferi* takes a long time to grow and is often limited to specialized laboratories (2). The serological tests (ELISA and Western blot) are the alternatives for a retrospective diagnosis. However, these tests may vary from one laboratory to another, depending especially on the use of different antigens extracted from various species and strains of *B. burgdorferi* (9,10). The ELISA test used in this case was positive but was not confirmed by the immunoblot, leaving doubt about the diagnosis. Recent studies performed in Europe on different sera give us recommendations on interpretation criteria, when only 2 or 3 positive bands are detected among 7-8 predefined bands (9). If we applied these recommendations to our patient, they would reveal a positive blot, but this conclusion is overestimated because the serological kit used does not permit this interpretation. Thus, molecular techniques might be used as an alternative diagnostic approach in the ocular manifestations of Lyme disease. The positive PCR result obtained in the CSF, combined with the negative results of the other etiological studies, lead us to a positive diagnosis of a *B. burgdorferi* uveitis. This case looks very much like the one described by Karma et al. (3) with retinitis pigmentosa and cerebral demyelination, where the serological antibodies in serum and CSF were negative at first examination, whereas the PCR was positive in the vitreous and CSF. The same sample of serum and CSF tested in another laboratory yielded positive titers, showing the ambiguity of the serological results (10) and the usefulness of genomic amplification. This PCR approach was also an alternative method using DNA amplification in urine to diagnose ocular Lyme borreliosis as described Pleyer et al. (11) in 6 patients. When clinical suspicion tends towards a late stage of Lyme disease and the serological tests are doubtful, the PCR method could be suitable for making by a positive diagnosis, as this method also amplifies dead bacteria. Nevertheless, this sophisticated method cannot currently be used as a screening test when serological results are negative (10,12).

REFERENCES