Tick-host-Borrelia interaction
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Chapter 6

A case of meningoencephalitis by the relapsing fever spirochaete Borrelia miyamotoi in Europe.


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On April 1 2012, a 70-year-old patient came to our clinic reporting slow cognitive processing, memory deficits, and a disturbed gait, all of which had gradually developed over several months and progressed during the last few weeks before the patient’s initial visit. He did not report fever, and he had not been outside the country for several years. He had recently been treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone), polychemotherapy, and rituximab (last dose on Aug 2, 2011) for a stage 4 diffuse large B cell lymphoma. His medical history also included Pneumocystis jirovecii pneumonia, unexplained chronic diarrhoea, a splenectomy, extensive tick exposure, and two tick bites in the summer and fall before onset of symptoms. On neurological examination there was a distinct bradyphrenia, and on cognitive assessment, the patient scored 26 of 30 points on the mini mental state examination. Vital signs were normal and body temperature was 36.4°C. Cranial MRI showed no abnormalities, but two lumbar punctures showed cerebrospinal fluid pleocytosis with raised protein values. The cause of this chronic meningitis was not identified by wide-ranging microbiological, pathological, and haematological diagnostic testing (appendix). A C6-immunofluorescence assay for Borrelia burgdorferi in serum, but not in cerebrospinal fluid, was weakly positive (index 1.8). However, a B burgdorferi IgG and IgM immunoblot were non-conclusive and negative, respectively. A B burgdorferi sl qPCR in cerebrospinal fluid was negative. Nonetheless, because of the absence of an alternative diagnosis and the progression of symptoms, on April 17, 2012, the patient was treated for a possible Lyme neuroborreliosis with once daily 2000 mg ceftriaxone intravenously for 2 weeks. During several weeks the patient fully recovered. At his last visit to the outpatient clinic in May, 2013, the patient did not have residual symptoms. Supported by the recent evidence of the presence of B miyamotoi in Ixodes ricinus ticks across Europe\(^1,2\), and the relation in time of the patient’s symptoms with the tick bites, and his immunocompromised status, we retrospectively considered B miyamotoi as the causative agent. We identified motile spirochaetes in stored pre-treatment cerebrospinal fluid by dark-field microscopy (appendix). Additionally, a 16S rDNA pan-relapsing fever Borrelia quantitative (q)PCR and a qPCR targeting the B miyamotoi flagellin gene was positive in two separate pre-treatment cerebrospinal fluid samples and one pre-treatment blood sample (appendix). Notably, 2.2% of 352 I ricinus nymphal ticks from the vicinity of the patient’s recreational house in the dunes of Zandvoort, the Netherlands, proved to be positive for B miyamotoi by qPCR (appendix). Amplification and sequencing of the glpQ and p66 genes confirmed B miyamotoi as the causative agent and showed 100% identical sequences in ticks and the patient’s clinical samples (appendix). We were unable to culture the spiro-chaetes in modified Barbour-Stoenner-Kelly medium from stored blood and cerebrospinal...
fluid samples. Finally, ELISA and Western blot did not show anti-GlpQ antibodies in blood and CSF.

Relapsing fever is caused by various *Borrelia* species, which are predominantly transmitted by soft ticks. However, relapsing fever *Borrelia* species have also been identified in hard ticks, including *B. miyamotoi* in *Ixodes* ticks. *B. miyamotoi* infection has been associated with systemic complaints, including malaise and fever, in case series. Recently, in the USA, *B. miyamotoi* was shown to be able to cause meningoencephalitis in an immunocompromised patient. Physicians worldwide managing immunocompromised patients from *Ixodes* tick-endemic areas with a meningoencephalitis should consider *B. miyamotoi* as a potential causative agent and should be aware that regular diagnostic tests for *B. burgdorferi* will most probably overlook this diagnosis. Whether *B. miyamotoi* is also able to cause neurological symptoms in non-immunocompromised patients requires further investigation.
REFERENCES


SUPPLEMENTARY APPENDIX

![Supplementary Figure 1](image)

Figure 1. Amplified and sequenced fragments of the *B. miyamotoi* glpQ (AB824730 and AB824855) and P66 (AB824731 and AB824856) genes from cerebrospinal fluid of our patient and five ticks from the Netherlands, including ticks from the vicinity of the patient’s recreational house in the Dunes, were compared to each other, and to known *B. miyamotoi* sequences in Genbank obtained from both ticks, animals and humans, by pairwise alignment using the Unweighted Pair-Group Method using Arithmetic Averages. The degree of homology is depicted in a dendogram.
**Figure 2.** *B. miyamotoi* in the patient’s cerebrospinal fluid. Dark field microscopic images (magnification x400) of *Borrelia miyamotoi* in the patient’s CSF (after being stored for one year in -80°C).

### Online supplement 1. Diagnostic work-up

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<th>Imaging</th>
<th>Cranial MRI</th>
<th>MRI spinal cord</th>
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<tr>
<td></td>
<td>No intracerebral lymphoma localisations</td>
<td>Increased leptomeningeal intensity of the cauda equina roots</td>
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<th>Cell count</th>
<th>Mononuclear cells</th>
<th>Protein level</th>
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<tr>
<td>03-04-2012</td>
<td>1164/3 uL</td>
<td>60%</td>
<td>4.86 g/L</td>
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<td>12-04-2012</td>
<td>354/3 uL</td>
<td>83%</td>
<td>6.55 g/L</td>
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<tr>
<td>03-05-2012 (post-treatment)</td>
<td>162/3 uL</td>
<td>100%</td>
<td>2.12 g/L</td>
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<table>
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<tr>
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<th>Bacteria</th>
<th>Mycobacteria</th>
<th>Viruses</th>
<th>Serology</th>
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<tr>
<td></td>
<td>Routine cultures negative; <em>B. burgdorferi</em> PCR negative; <em>Tropheryma whipplei</em> PCR negative</td>
<td>Ziehl-Neelsen negative; mycobacterial culture negative; <em>M. avium</em></td>
<td>HSV, VZV, CMV, EBV, entrovirus, JC virus, and</td>
<td><em>B. burgdorferi</em> C6 EIA (IgM/IgG) negative; TPHA</td>
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<tr>
<th>Pathology CSF</th>
<th>Microscopy</th>
<th>Immunohistochemistry</th>
<th>Molecular analysis</th>
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<tr>
<td></td>
<td>Cellular CSF with high numbers of granulocytes and plasma cells</td>
<td>Plasmacytosis with a relative abundance of kappa positive plasma cells</td>
<td>No evidence of a relapse of the original B cell lymphoma</td>
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METHODS

B. miyamotoi-specific qPCR
A B. miyamotoi-specific qPCR was performed using the iQ Multiplex Powermix PCR reagent kit, which contains iTaq DNA polymerase (Bio-Rad Laboratories, Hercules, USA), in a LightCycler 480 Real-Time PCR System (F. Hoffmann-La Roche, Basel, Switzerland). Optimal reaction conditions in a final volume of 20µl were iQ multiplex Powermix, 200 nM forward (5'-AGA AGG TGC TCA AGC AG-3') and reverse (5'-TCGATCTTTGAAAGTGACATAT-3') primers each, 200 nM probe (5'-Atto647N-AGC ACA ACA GGA GGG AGT TCA AGC-BHQ2-3'), and 3 to 8 µl of template DNA. Cycling conditions included an initial activation of the iTaq DNA polymerase at 95°C for 5 min, followed by 60 cycles of a 5 s denaturation at 95°C followed by a 35 s annealing-extension step at 60°C (Ramp rate 2.2 °C/s and a single point measurement at 60 °C) and a cooling-cycle of 37 °C for 20 s. Analysis was performed using the second derivative calculations for crossing point values. Curves were assessed visually. Samples with a positive score were assumed being positive for Borrelia miyamotoi. The specificity of primers and probes was tested with samples containing DNA from the following microorganisms: Borrelia burgdorferi senso lato, Rickettsia helvetica, Anaplasma phagocytophilum, Neoehrlichia mikurensis, and Babesia spp. Correct sizes of DNA fragments of qPCR-amplicons were confirmed on a bioanalyzer (Agilent Technologies, Palo Alto, CA). For each run positive and negative controls and blank samples were included. In order to prevent contamination, the PCR proceedings were performed in three separate rooms, of which the reagent setup and sample addition rooms were kept at positive pressure, whereas the DNA extraction room was kept at negative pressure. All rooms had airlocks. Of the 352 Ixodes ricinus nymphs tested for B. miyamotoi eight lysates were positive by qPCR. The three DNA extracts of the patient’s material, two CSF and one EDTA-blood sample, were also positive by qPCR. The Cp-values (Ct 35, 36 and 39) were lower than the highest serial dilution of the positive control (Ct 41).

Molecular identification/typing of B. miyamotoi
From five qPCR-positive tick samples and one representative clinical sample the presence of B. miyamotoi was confirmed by PCR amplification and sequencing of a 920bp fragment of the glycerophosphodiester phosphodiesterase (glpQ) gene. The PCR was performed with the HotStarTaq master mix (Qiagen, Venlo, The Netherlands) using forward (5’-atgggttcaaacaaaaagtcacc-3’) and reverse primers (5’-cattactgtgtcagtaaaatctgtaataccatctac-3’) under the following conditions: 15 min 94°C, then cycles of 20 s 94°C, 30 s 70°C, 30 s 72°C lowering the annealing
temperature 1°C each cycle till reaching 60°C, then 40 cycles at this annealing temperature and a final extension step of 7 min at 72°C. The P66 gene was amplified under identical conditions with different forward (5’-gatactaaattaatccaaatcg-3’) and reverse (5’-ggaatgagctactatgg-3’) primers. PCR amplicons were sequenced using BigDye Terminator Cycle sequencing Ready Reaction kit (Perkin Elmer, Applied Biosystems). All sequences were confirmed by sequencing both strands. The collected sequences were assembled, edited, and analysed with BioNumerics version 6.5 (Applied Maths NV, Sint-Martens-Latem, Belgium). Sequences were compared to B. miyamotoi glpQ sequences deposited in Genbank, by pairwise alignment using Unweighted Pair Group Method with Arithmetic Mean. The DNA sequence of the glpQ-fragment amplified from the patient was identical to the ones derived from the (Dutch) Ixodes ricinus lysates, but different from the B. miyamotoi-sequences derived from Ixodes persulcatus, and I. scapularis, and from the human isolate (De1) derived from Russia (West Siberia). The DNA-sequence of the P66 fragment of the patient was identical to the ones derived from five Dutch and one Swedish Ixodes ricinus lysate, but different from the B. miyamotoi-sequences derived from Ixodes persulcatus, and I. scapularis, and from the human isolate (De1) derived from Russia (West Siberia).

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