Chronic neuroborreliosis by *B. garinii:* an unusual case presenting with epilepsy and multifocal brain MRI lesions

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SUMMARY

Late/chronic Lyme neuroborreliosis (LNB) represents a challenging entity whose diagnosis requires a combination of clinical and laboratory findings, surrounded by much controversy. Here we describe a patient who had a peculiar form of late LNB with CNS lesions shown by magnetic resonance imaging (MRI), and epileptic seizures, etiologically diagnosed by conventional and molecular methods. The current case provides evidence that patients presenting with epileptic seizures and MRI-detected multifocal lesions, particularly when a facial palsy has also occurred, should raise the suspicion of LNB, as this diagnosis has important implications for treatment and prognosis.

KEY WORDS: Neuroborreliosis, seizures, real-time PCR.

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INTRODUCTION

Lyme borreliosis (LB) is a worldwide multisystem infection transmitted to humans by the bite of an infected tick of the genus Ixodes (Stanek *et al*., 2012). *Borrelia burgdorferi sensu lato*, the causative bacterium of LB, is genetically divergent and has been divided into several species or genomic groups. *B. burgdorferi sensu stricto*, *B. garinii*, and *B. afzelii* have been cultured from patients and are thought to be responsible for human Lyme borreliosis. Borrelia genospecies seem to be associated with distinct clinical syndromes (Floris *et al*., 2007), and the dominating species in Europe are *B. garinii* and *B. afzelii*.

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Lyme borreliosis involves the nervous system (neuroborreliosis) in about 15% of patients, and the most common manifestations of European Lyme neuroborreliosis are painful radiculopathy and cranial neuropathy (especially of the facial nerve) with pleocytosis in the cerebrospinal fluid (CSF), while other neurological manifestations are rare (Pfister *et al*., 2006). Regardless of the type of neurological manifestations, however, exclusion of Lyme neuroborreliosis as a trigger of disease is recommended in selected patients with demyelinating disease, hemiparesis, or epilepsy (Oksi *et al*., 1996). Here we describe a patient who had a peculiar form of chronic neuroborreliosis with central nervous system (CNS) lesions shown by magnetic resonance imaging (MRI) and epileptic seizures.

CASE REPORT

A 26-year-old man had previously been healthy, apart from a right facial nerve paralysis that oc-

curred two years before and resolved spontaneously. On March 10, 2013 he had a generalized seizure and was admitted to the hospital. Another seizure occurred on the day of admission. He recalled no tick bites, erythema migrans, or history of fatigue, myalgia, or arthralgia. Neurological examination was normal apart from a minimal evidence of the previous right facial nerve palsy. Extensive blood tests were normal, as were electroencephalography, electromyography and cortical visual and somatosensory evoked potentials.

Brain MRI revealed hyperintense lesions in T2 and FLAIR sequences, with a bright appearance in diffusion-weighted images, not enhancing after gadolinium administration, and involving the right temporal cortex, the left temporal pole, insula and hippocampus, as well as the periventricular white matter, semi-oval centers and corpus callosum (Figure 1). There was no evidence of contrast enhancement or restricted diffusion. Brain MRI angiography and transcranial Doppler studies were normal. A whole body 18F-FDG PET/CT examination study was unremarkable. The cerebrospinal fluid (CSF) revealed 2 white blood cells/ µl with normal glucose and increased total protein concentrations (0.544 g/l, normal range: 0.18 to 0.43), and absence of oligoclonal bands. Cytological analysis was negative for malignant cells. CSF gram stain and bacterial cultures were all negative. In addition, Brucella, Rickettsia, CMV, EBV and HCV serology were all negative.

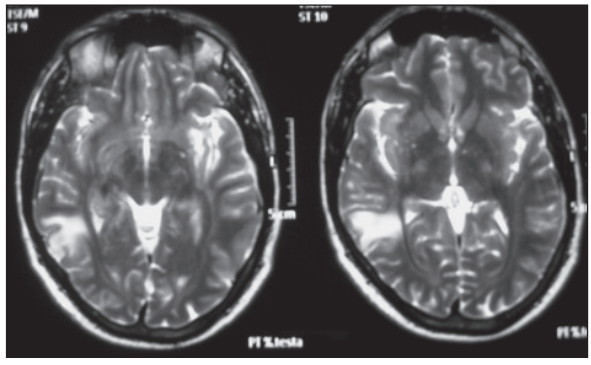

FIGURE 1 - *Cranial MRI showing a high T2 signal in the brain subcortical area and white matter of the right temporal lobe, as signs of inflammation and/or ischemia. Smaller lesions were found by MRI in the basal ganglia, thalamus and lamina interna.*

An ELFA kit, "VIDAS Lyme Screen" (bioMerieux, Marcy L'Étoile, France), was used as a screening test for Borrelia serology and a serum sample of our patient was found weakly positive for total Ig anti-*Borrelia burgdorferi* B31 strain whole cell sonicates. Then a confirmation Western blot test (recomLine Borrelia burgdorferi IgM and IgG immunoblots, Mi-

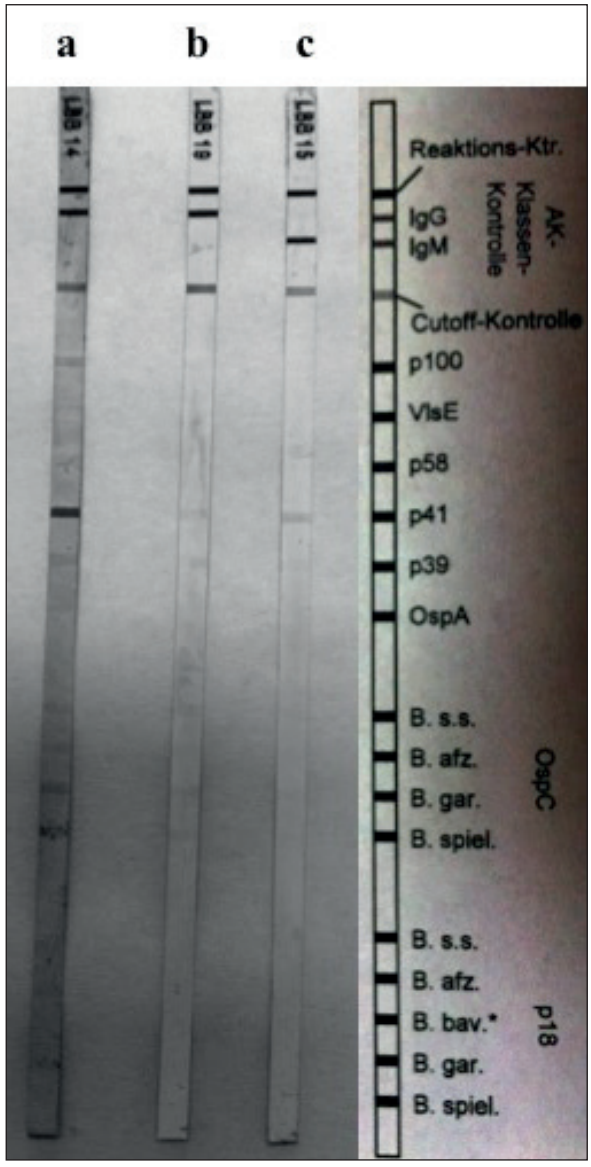

FIGURE 2 - *Western blot analysis of serum IgG (a), CSF IgG (b) and serum IgM (c) of the studied patient. The samples tested in strip (a) and in strip (b) were brought to the same concentration of total IgG (see text for details).*

krogen, Neuried, Germany) was carried out. Before Western blot test, both serum and CSF samples were diluted in order to bring them to the same concentration of total IgG (15 mg/l).
In Western blot analysis band p41, shared by all *Spirochetaceae*, was very intense and clear-cut in serum IgG (strip a, LBB14), but it appeared with a lower intensity in serum IgM (strip c, LBB15) and in CSF IgG (strip b, LBB19). Moreover p100 in serum IgG (strip a, LBB14) was an obvious band, while it was very weak in CSF IgG (strip b, LBB19) and in in serum IgM (strip c, LBB15). Band p39 was clear-cut, but exhibited low intensity in both serum and CSF samples. OspC of *B. garinii* was a clear-cut band with a low intensity in serum IgG (strip a, LBB14). With the exception of p41 in serum IgG (strip a, LBB14), all the remaining bands observed were below the intensity of the cut-off/control band (Figure 2). Positivity to antibodies against p100 and p39 are reported to be strongly suggestive of late Borreliosis (Gruber, 2013). Regarding the serology of Spirochetaceae, RPR, TPHA, anti-treponemal ELISA for

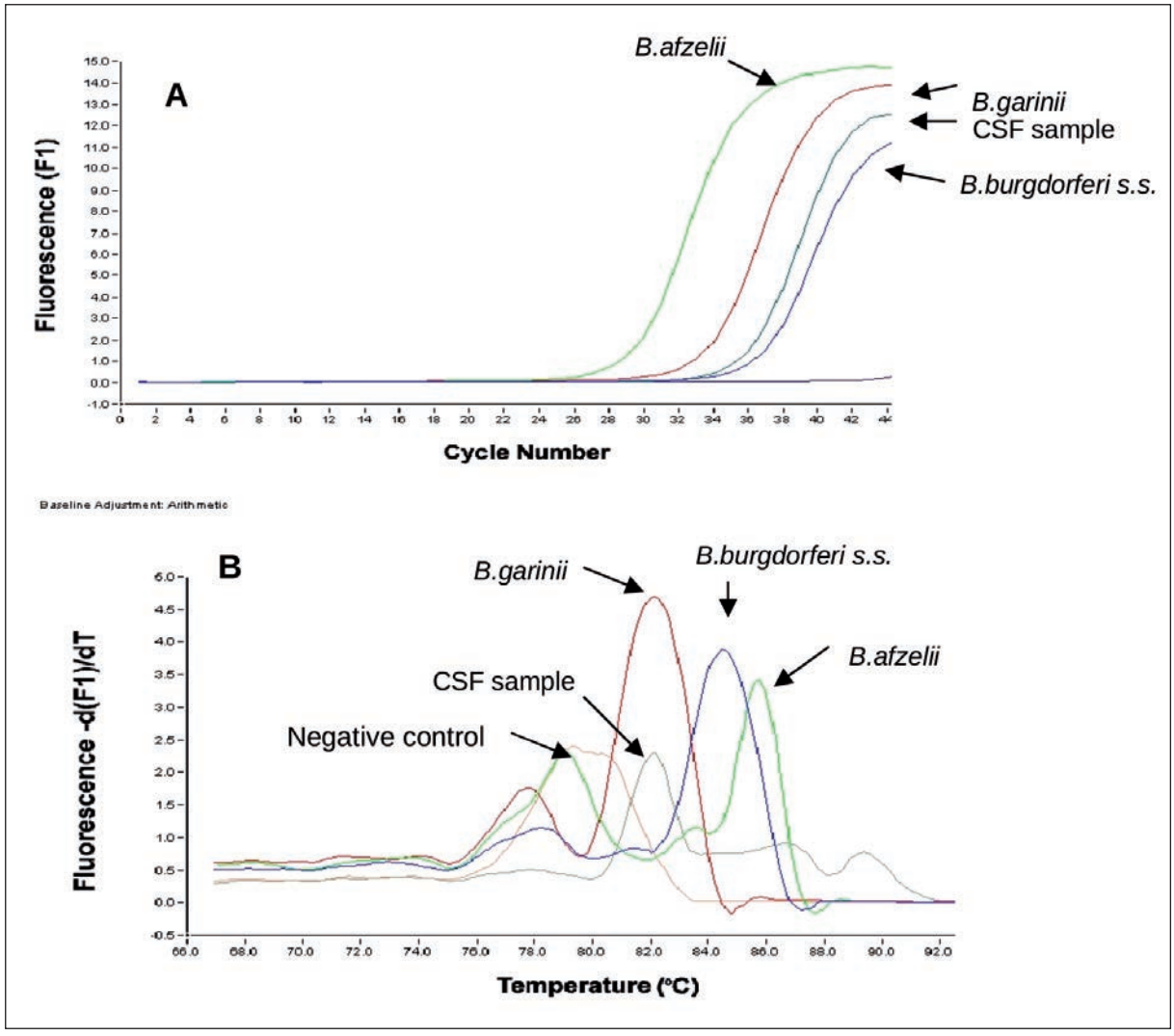

FIGURE 3 - *Amplification curves (A) and melting curve analysis (B) of B. burgdorferi s.s., B. afzelii, B. garinii and the CSF sample under investigation for the recA gene. The Tm values were: 84.49°C for B. burgdorferi s.s., 85.37 °C for B. afzelii, 82.08 °C for B. garinii and 82.07°C for the CSF sample. The negative control (CSF negative sample) melted at a temperature of 79.65°C.*

IgG and IgM and Western blot for IgG and IgM were carried out in parallel, but were all negative. In addition, the complement fixation test for anti-Leptospira total Ig was performed with a negative result.

Real-time PCR assay
A volume of 500 µl of CSF specimens was centrifuged (8000 x g for 10 min) and the pellet was suspended in 100 µl QIAamp elution buffer and DNA extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The LightCycler system (Roche Diagnostics, Italy) with LightCycler FastStart DNA Master SYBR Green I was used for amplification and real-time detection. For genus and species-specific real-time PCR assay, the forward primer nTM17.F 5'-GTCGATCTATTGTATTAGATGAGGCTCTCG-3'and reverse primer nTM17.R 5'-GCCAAAGTTCTGCAACATTAACACCTAAAG-3' (J. Pietila *et al.*, 2000; J.J Lazarus *et al*., 2012.) were used to amplify a 222 bp portion of the *recA* gene.
The PCR mixture contained 2 µl of extracted DNA, 0.5 µl of each primer, 1.6 µl of MgCl2, 2 µl of SYBR Green and 13.4 µl of H_2O for PCR supplied by the kit. The amplification PCR conditions were: denaturation at 95°C for 10 min, followed by 40 cycles consisting of 95°C for 10s, 59°C for 8s and 72°C for 11s. After amplification, melting curve analysis was carried out by evaluating a *Tm* (melting temperature) of 84.49±0.13°C for *B. burgdorferi s.s.,* 84.86±0.58 for *B. afzelii* and 82.27±0.38 for *B. garinii.* The unspecific products, even primer-dimers, produced when little or no template is present, melted at temperatures below 80°C.
As shown in Figure 3, the real-time PCR for *recA* gene and the melting curve (Tm 82.07°C) of the CSF sample of our patient indicate that an isolate of the species *B. garinii* was the etiological agent of the investigated case.
The clinical features of our patient in combination with serology and real-time PCR results allowed the diagnosis of chronic/persistent neuroborreliosis (Ljøstad & Mygland, 2013). The patient was treated with 200 mg oral doxycycline (200 mg per day for 21 days). Follow-up brain MRI performed 2 months later showed a substantial reduction of the changes. He remains in full clinical remission with no clinical manifestation throughout his 4-month follow-up period.

DISCUSSION

PCR findings along with serology indicate that an isolate of the species *B. garinii* was the etiological agent of the investigated case. Chronic neuroborreliosis represents a challenging entity whose diagnosis requires a combination of clinical and laboratory findings, still surrounded by much controversy but with important implications for treatment and prognosis (Ljøstad & Mygland, 2013). The clinical picture of our patient is of interest for clinicians, as it did not conform to the classic clinical manifestations of chronic neuroborreliosis that consist of progressive encephalomyelitis, stroke-like symptoms due to vasculitis, radiculitis, or mononeuritis multiplex (Ljøstad & Mygland, 2013). Particularly noteworthy in this regard was the occurrence in our patient of facial nerve palsy two years prior to the onset of epilepsy. In this way, the current case provides evidence that patients presenting with epileptic seizures and MRI-detected multifocal lesions, particularly when a facial palsy has also occurred, should raise the suspicion of Lyme borreliosis as a trigger of the disease, as this diagnosis has important implications for treatment and prognosis.
In addition to the pathophysiology, unusual aspects of our case are the cranial MRI scans that showed ischemic lesions in the vascular territory of the middle cerebral artery. Serology and molecular techniques confirmed infection with *B. garinii*. Therefore we believe that the vascular pathology in this patient is a manifestation of a "cerebrovascular course" of neuroborreliosis (Wilke *et al*. 2000). Vasculitis has for a long time been postulated as the mechanism of nervous system injury in Borrelia infection, and case reports of several patients have been published (Back *et al*., 2013).
Covert and/or obvious neuroborreliosis was finally suggested as an infectious "primum movens" of several neurodegenerative diseases. *B. burgdorferi* has been reported (Crowley *et al*., 2013) to extract cholesterol from the plasma membrane of eukaryotic cells and that prokary-

otic cholesterol-glycolipids can be transferred to epithelial cell membranes by two mechanisms:

1) a contact-dependent mechanism through direct attachment;
2) a contact-independent method through released (outer membrane vesicles) OMV.

The *B. burgdorferi* membrane is unique in that it contains lipid rafts, with cholesterol and cholesterol-glycolipids with physical properties that are similar to those of eukaryotic membranes. Transfer of antigenic cholesterol-glycolipids could play a major role in the pathogenesis of the spirochetoses (Crowley *et al.*, 2013). An autoimmune mechanism suspected for many neurodegenerative diseases (Pagani *et al.*, 2011; Czirr *et al.*, 2012) might eventually find a mechanistic explanation in Borrelia-derived antigenic cholesterol-glycolipids included in host cell membranes.

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