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Title: A Twist On Lyme: The Challenge of Diagnosing European Lyme Neuroborreliosis

Running Title: Diagnosing European Lyme Neuroborreliosis

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Abstract

Lyme neuroborreliosis is a tick-borne illness with central and peripheral nervous system manifestations. Clinical features and methods for accurate diagnosis differ across world regions owing to different causative *Borrelia* species. The importance of these distinctions is highlighted by a 12 year old Canadian girl who acquired Lyme neuroborreliosis in Europe.

Keywords: Lyme Disease, Lyme neuroborreliosis, Borrelia, Diagnosis, European

Case Report

A 12-year-old girl was admitted to our hospital on August 28, 2007, with a ten-day history of mid-scapular back pain and 24 hours of unilateral facial weakness two weeks after returning from a one-month vacation in rural France. She had been horseback riding, but did not recall any insect or tick bites. Ambulation, bowel and bladder function were normal. There was no recent history of fatigue, myalgia, or arthralgia, and the family did not recall a preceding rash.

On examination, forward neck flexion elicited L'Hermitte's symptom (radiating discomfort with forward neck flexion indicative of cervical spine pathology such as inflammation). A right-sided lower motor neuron facial nerve palsy and bilateral increased lower extremity tone were noted. Muscle power was normal in all muscle groups tested. Deep tendon reflexes were increased at the knees and ankles and the left plantar response was extensor. There was reduced sensation to pinprick and temperature between the T4 and T6 sensory levels.

MRI of the spine revealed increased T2-weighted signal in the spinal cord, mild spinal cord swelling and diffuse gadolinium enhancement of the spinal meninges and proximal nerve roots (Figure 1). Brain MRI was normal. The peripheral white blood cell (WBC) count and serum erythrocyte sedimentation rate were normal. Cerebrospinal fluid (CSF) protein was elevated (1.25 g/L, normal 0.15-0.40 g/L) and CSF glucose was reduced (1.6 mmol/L, normal 2.1-3.6 mmol/L). CSF WBC count was 424 x 10^6 (87% lymphocytes) with zero red blood cells. Cytological analysis was negative for malignant cells. CSF bacterial cultures were negative. CSF polymerase chain reaction (PCR) studies were negative for varicella zoster virus, human herpes viruses 6-8, West Nile virus,

herpes simplex viruses 1 and 2, cytomegalovirus, Epstein Barr virus, enterovirus, and *Mycoplasma pneumoniae*. Oligoclonal bands were present in the CSF, but not serum. CSF PCR using probes targeting *Borrelia burgdorferi* 23S rRNA genes was negative. Testing for intrathecal antibodies was not possible due to an insufficient amount of CSF.

Borrelia serology obtained 10 days after admission was positive by ELISA assay using two different commercial kits, namely the Immunetics™ C6 ELISA (antigen is the C6 peptide of the VlsE protein) and the Diagnostic Automation™ IgG & IgM ELISA (*Borrelia burgdorferi* B31 strain whole cell sonicates) (Table 1). Confirmatory Western blot testing (MarDx™) was negative for both anti-*Borrelia* IgG and IgM according to the manufacturer's and CDC criteria for interpretation. The only band present on the MarDx IgG blots was p41. The results from IgM blots were less consistent and blots had either p41 or no detectable bands present.

The clinical features of a lower motor neuron facial nerve palsy, meningoradiculitis, CSF pleocytosis, positive serology by ELISA, and recent travel to an endemic area led to the presumptive diagnosis of transverse myelitis due to Lyme neuroborreliosis. Intravenous ceftriaxone was administered for 28 days starting on the day of presentation to hospital. The back pain and L'Hermitte's symptom resolved within 48 hours. Facial weakness remained marked after two weeks of antibiotic therapy, leading to treatment with seven days of oral prednisone. Near complete recovery of facial expression occurred within 12 weeks of presentation.

In order to reconcile the indeterminate initial laboratory investigations for Lyme borreliosis with the patient's clinical symptoms and response to antimicrobial therapy, further serologic analysis was performed (Table 1). The original serum sample taken the

day of admission showed a positive screening ELISA result, and negative North American IgM and IgG Western blots (i.e., MarDx). Serum samples taken 17 days, 6 weeks and 3 months after disease onset showed the same results. Given our child's history of European travel, IgM and IgG Western blots using the European assay were performed using Trinity Biotech EU-Lyme IgM and EU Lyme + VlsE IgG Western Blot systems, respectively. These test systems incorporate low passage antigens of *Borrelia afzelii* "PKO" and *Borrelia garinii* which appear to be exclusive to Europe and Japan.. Banding patterns are interpreted on a modified MiQ 12 2000 interpretive criteria which requires the presence of two or more bands (i.e., p17, p39, p41 and *B. afzelii* (PKO) or *B. garinii* 22 kD OspC) to be considered positive. The European IgM Western blot was found to be positive beginning one week after admission (17 days after onset of symptoms, 7 days after onset of treatment) and persisted for 3 months after presentation. These samples produced bands corresponding to the p41 and *B. garinii* 22 kD OspC regions. IgG antibodies remained undetectable on European Western blot when serum was tested three months post-presentation.

We highlight the challenge of diagnosing European Lyme neuroborreliosis in a Canadian child. Lyme neuroborreliosis is a systemic *Borrelia* infection with neurological involvement. With rare exceptions, the causative species in North America is *Borrelia burgdorferi sensu stricto*. In Europe, at least three species may be responsible including *Borrelia burgdorferi* and, more commonly, *Borrelia garinii* or *Borrelia afzelii*. Transmission to humans typically occurs through the bite of an infected *Ixodes* species of tick (e.g. *Ixodes scapularis* in North America and *Ixodes ricinus* in Europe). Though

human infection can occur throughout the year, most cases occur during early summer months when the nymphal stage is most active (3).

Recognizing the symptoms of Lyme borreliosis is essential for prompt diagnosis and treatment. North American Lyme borreliosis generally manifests itself in three distinct clinical stages (reviewed in (20)). Well-characterized neurological symptoms attributable to Lyme borreliosis include a primarily lymphocytic meningitis with or without painful cranial neuritis or polyradiculitis, encephalomyelitis, and peripheral neuropathy (11). Importantly, the clinical features of European Lyme borreliosis are different from North American disease (13, 17, 20). Erythema migrans is often slower spreading and appears less intensely inflamed in European cases, and so may be less readily recalled by patients. The most common presentation of European Lyme neuroborreliosis is the triad of Banworth's syndrome (lymphocytic meningitis, cranial neuropathy, and painful radiculitis) rather than aseptic meningitis, which is seen more commonly in North American disease. Additionally, if left untreated, infections caused by European *Borrelia* genospecies are more likely to progress to chronic low-grade encephalitis. The most common clinical presentation of Lyme neuroborreliosis in children is peripheral facial nerve palsy, occurring in up to 71% of patients, followed by aseptic meningitis (7, 18). Transverse myelitis, other cranial neuropathies, and ataxia have been rarely reported in children (4, 14-16, 19). Non-specific symptoms such as fatigue, headache, and myalgias are common, and neurological examination was normal in 21% of children in one Dutch study (7).

Although the clinical features of our patient were highly suggestive of Lyme neuroborreliosis, investigations using diagnostic methods optimized for North American

126 *B. burgdorferi sensu stricto* were largely negative. Specific testing for an immune
127 response to European strains of the organism was suggestive but not conclusive for an
128 acute infection. While our patient did have serum anti-*Borrelia* IgM antibodies detectable
129 by Western blotting, she did not subsequently develop IgG seropositivity by this
130 procedure. The VlsE C6 peptide used in the Immunetics ELISA is a conserved sequence
131 found in *Borrelia burgdorferi* and the European genospecies *B. afzelii* and *B. garinii*,
132 which provides an extremely *Borrelia*-specific assay. Positive results in a C6 ELISA
133 often precede the development of a positive IgG Western blot (presence of five or more
134 significant bands) which appears to be the case for this patient. While the absence of
135 detectable Western blot IgG antibodies is quite surprising given the extent of neurological
136 involvement at the time of presentation, the lack of Western blot IgG antibody response
137 after treatment is not. Studies have clearly demonstrated the negative impact of
138 antimicrobial treatment on the production and subsequent detection of Western blot IgG
139 antibodies (1). Alternatively, isolated elevations in anti-*Borrelia* IgM serum antibodies
140 are present in up to 20% of children with other neurological diagnoses including viral
141 meningitis and headache (5).

142 There is no gold-standard diagnostic test for Lyme neuroborreliosis. Direct
143 culture of *Borrelia* species and PCR are of low sensitivity, therefore laboratory diagnosis
144 instead relies on the detection of anti-*Borrelia* antibodies. In North America, testing
145 follows a two-step algorithm (8, 9). Serum samples are screened for antibodies using an
146 ELISA assay, a relatively sensitive, but not specific test. Confirmatory testing is then
147 performed using Western blotting, which is specific, but not sensitive. The sensitivity of
148 the two step approach is well-described to increase in later stages of the disease for both

European (22) and North American (2, 21) acquired borreliosis. While sensitivity may be less than 40% in cases of acute Stage 1 Lyme disease, both retrospective (2) and prospective (21) studies from New England have found the sensitivity of the two step approach to be 85% to 100% in cases of Stage 2 acute neuroborreliosis. It has been noted that European *Borrelia* strains induce variable host antibody responses leading to reduced reliability of serum Western blot analysis (10, 12). For the diagnosis of European Lyme neuroborreliosis, examination of the ratio of intrathecal to serum antibodies may be a more sensitive test (5, 6). In this case, diagnostic testing was initiated in accordance with Canadian Public Health Laboratory Network guidelines, which recommend consideration of CSF PCR, and not intrathecal antibody testing, in patients with neurological symptoms (8). Although determination of CSF to serum antibody index is a more sensitive test for Lyme neuroborreliosis acquired in Europe, no residual CSF remained for this analysis.

Lyme neuroborreliosis should be considered in the differential diagnosis of new neurological symptoms in children and adults with histories of travel to Lyme-endemic areas both within and outside of North America. The geographic site of potential exposure must be disclosed to the diagnostic laboratory so that the appropriate assays may be employed. Timely recognition and treatment are imperative in order to facilitate recovery and to prevent long-term sequelae.

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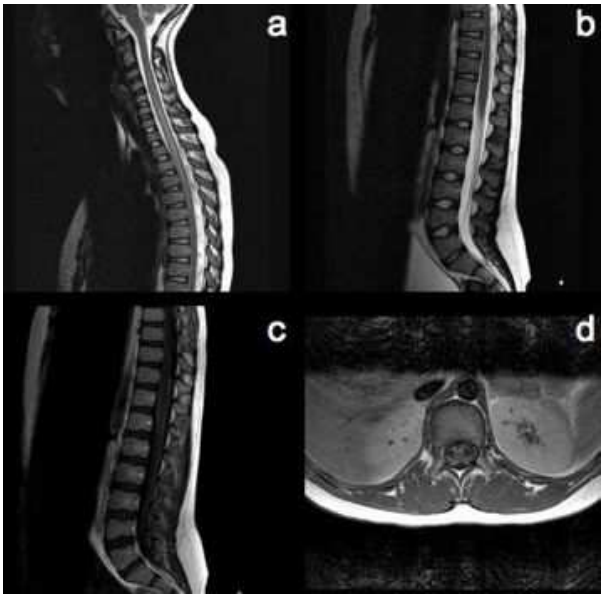

Figure 1:

MRI of the spine shows high T2 signal within the spinal cord (a), spinal cord swelling (b), and gadolinium enhancement of the meninges (c) and nerve roots (d).

Table 1 Summary of laboratory diagnostic testing

Date	Sample	C6 ELISA (Immunetics™)	IgM & IgG ELISA (Diagnostic Automation™)	Western blot IgM (MarDx™)	Western blot IgG (MarDx™)	European Western blot IgM (Trinity Biotech EU)	European Western blot IgG (Trinity Biotech EU)	PCR
2007.08.28	CSF	NP	NP	NP	NP	NP	NP	Negative
2007.08.29	Serum	Reactive	Reactive	Negative	Negative	NSQ	Negative	NP
2007.09.04	Serum	Reactive	Reactive	Negative	Negative	Positive	Negative	NP
2007.09.26	Serum	Reactive	Reactive	Negative	Negative	Positive	Negative	NP
2007.11.28	Serum	Reactive	Reactive	Negative	Negative	Positive	Negative	NP

NSQ: not sufficient quantity
NP: not performed