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Are There Undiagnosed TBE-, Herpes- or Enteroviral Infections among Children Being Evaluated for Lyme Neuroborreliosis?

Barbro H. Skogman, Pia Forsberg, Sirkka Vene, Britt Akerlind

Center for Clinical Research, Falun, Sweden
Email: barbro.hedinskogman@ltdalarna.se, pia.forsberg@liu.se, sirkka.vene@smi.se, britt.akerlind@lio.se

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Abstract

Lyme neuroborreliosis (LNB) in children is a challenging diagnosis based on clinical manifestations and laboratory findings. The aim of this study was to investigate whether herpes simplex virus (HSV) 1 or 2, varicella zoster virus (VZV), enterovirus or tick-borne encephalitis virus (TBEV) could be identified in cerebrospinal fluid (CSF) or serum from children being evaluated for LNB, in order to elucidate whether such infectious diseases may be missed by the clinician. Methods: Ninety-nine pediatric patients (n = 99) were retrospectively included from a previous study on LNB in southeast of Sweden. They had been diagnosed as "Possible LNB" or "Not determined" due to negative *Borrelia* antibody index in CSF. Routine polymerase chain reaction (PCR) methods were used for detection of herpes viral RNA or enteroviral DNA in CSF. An ELISA assay was used for detection of anti-TBEV antibodies (IgM and IgG) in serum. Results: One patient showed elevated anti-TBEV IgM and IgG antibodies in serum, indicating a current TBE infection. No positive PCR reactions for HSV 1 or 2, VZV or enterovirus were detected in CSF from any of the patients. In conclusion, our results suggest that undiagnosed herpes- or enteroviral infections are unlikely to explain CNS symptoms in children being evaluated for LNB, whereas missed TBE infections may occur. TBEV serology should be included when evaluating children for LNB in TBE endemic areas.

Keywords

Enterovirus, Herpes Simplex Virus, Lyme Neuroborreliosis, Varicella Zoster Virus, Tick-Borne Encephalitis

1. Introduction

Lyme Borreliosis (LB) is caused by the spirochete *Borrelia burgdorferi* and is the most common tick-borne in-

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fection in both Europe and the USA [1] [2]. The infection may give rise to different symptoms by affecting organs such as the skin, joints, heart muscle or nervous system [3]-[5]. Neurological signs and symptoms are not specific and the diagnosis Lyme Neuroborreliosis (LNB) needs laboratory confirmation [6] [7]. According to European guidelines, both pleocytosis in CSF (>5 × 10^6 mononuclear cells /L) and intrathecally produced *Borrelia* specific antibodies (*i.e.* positive antibody index, AI) are needed to confirm the LNB diagnosis [7]. Among children with neurological symptoms suggestive for LNB, many cases do not meet these criteria for confirmed LNB [8]-[11]. Patients may receive antibiotic treatment on vague grounds and investigation for other neurotropic agents are seldom performed unless the patient show specific manifestations of viral infection such as skin rash, vesicles, diarrea or distinct signs of viral meningitis/encephalitis.

Acute facial nerve palsy or subacute meningitis are major neurological manifestations in LNB [12] [13]. However, facial nerve palsy may also be associated with viral infection, such as an acute or reactivated herpes simplex virus (HSV) infection or a reactivated varicella zoster virus (VZV), even when detectable vesicles or other clinical manifestations of viral infection are absent [14]-[18]. Antiviral therapy and/or corticosteroids have been shown to improve the prognosis in adult patients with idiopathic facial nerve palsy [19], but studies in children are few and not conclusive [20] [21]. As for children with meningeal signs and symptoms, clinical manifestations associated with enteroviral infection are not always easily distinguishable from LNB and the distribution of season is similar [22]. Furthermore, tick-borne encephalitis (TBE) is a tick-borne infectious disease that peaks during summer season with clinical features that may mimic LNB and may be unspecific in smaller children [4] [23]-[25]. For these reasons, TBE-, herpes- and enteroviral infections may be deceptive for the clinician.

The aim of this study was to investigate whether HSV 1 or 2, VZV, enterovirus or TBEV could be identified in CSF or serum from children being evaluated for LNB, in order to elucidate whether such infectious diseases may be missed by the clinician.

2. Material and Methods

2.1. Study Population

Children with neurological symptoms suggestive for LNB were subjects in this retrospective study from a high endemic area in southeast Sweden. Patients had taken part in a previous prospective study on LNB and had been diagnosed as “Confirmed LNB”, “Possible LNB” or “Not determined” based on laboratory findings [9]. Out of 177 consecutive patients, 72 children had been diagnosed as “Confirmed LNB” with pleocytosis in CSF and intrathecally produced *Borrelia* specific antibodies in CSF (positive AI), following the European case definition [6] [7]. These patients were not included in our present study since the aim was to focus on children not meeting the criteria for “Confirmed LNB”. Furthermore, 6 children had to be excluded due to missing clinical data (n = 1), insufficient patient samples (n = 3) or other diagnosis such as demyelisation disease (n = 1) and sarcoidosis (n = 1). These excluded patients (n = 6) did not differ in age or gender compared to the study population (n = 99). Children with distinct symptoms of aseptic meningitis were not included.

Thus, 99 children were enrolled in our retrospective study. Out of these children, 44 patients had been diagnosed as “Possible LNB” with pleocytosis in CSF but no *Borrelia* specific antibodies in CSF (negative AI). Furthermore, 55 patients with no pleocytosis in CSF and no *Borrelia* antibodies in CSF (negative AI) had been diagnosed as “Not determined”.

Demographic and clinical characteristics of the study population are shown in **Table 1**. No patient had had any skin rash, vesicles, diarrhea or distinct signs of viral meningitis/encephalitis at admission. None of the patients in the study had been treated for a previous LNB. Data on TBE vaccination status was unfortunately not available.

2.2. Laboratory Assays

For *Borrelia* diagnostics, a flagella-based ELISA assay was used to detect *Borrelia* specific anti-IgM and anti-IgG antibodies in serum and CSF (DAKO, Glostrup, Denmark) [26] [27]. Cut-off levels in serum were set as recommended by the manufacturer. In CSF, an antibody index (AI) based upon the optical density (OD) formula was calculated, as recommended, to ensure measure of intrathecal antibody production. $OD_{CSF}/OD_{serum} \times (OD_{CSF} - OD_{serum})$. The test was considered positive when the AI was ≥ 0.3 [27]. Immunoblot was not used as confirmatory test for LNB.

Table 1. Demographic and clinical characteristics of patients (n = 99).

	"Possible LNB" (n = 44)	"Not determined" (n = 55)
Age, years, median (range)	7 (1 - 18)	12 (2 - 18)
Male, n (%)	20 (45)	26 (47)
Known tick bite, n (%)	26 (59)	31 (56)
Duration of symptoms, n (%)		
<1 week	26 (59)	21 (38)
1 - 4 weeks	16 (36)	15 (27)
1 - 2 months	2 (5)	8 (15)
>2 months	0 (0)	11 (20)
Major clinical manifestations, n (%)[a]		
Facial nerve palsy	34 (77)	20 (36)
Headache	26 (59)	40 (73)
Meningitis, subacute	20 (45)	6 (11)
EM and/or lymfocytoma	23 (52)	3 (5)
Laboratory findings, n (%)		
Pleocytosis in CSF, median (range)[b]	68 (5 - 575)	1 (0 - 4)
Anti-*Borrelia* antibodies in CSF, n (%)[c]	0 (0)	0 (0)
Anti-*Borrelia* antibodies in serum, n (%)[d]		
Anti-IgM	12 (27)	6 (11)
Anti-IgG	3 (7)	2 (4)
Anti-IgM and IgG	9 (20)	6 (11)
Antibiotic treatment, n (%)		
Ceftriaxone, iv	21 (48)	0 (0)
Doxycycline, po	23 (52)	3 (5)

[a]Patients may have several clinical manifestations. [b]Pleocytosis: >5 × 10^6 mononuclear cells /L in CSF. [c]Intrathecally produced *Borrelia* specific IgG or IgM antibodies. [d]Elevated anti-*Borrelia* antibody titers in serum, as recommended by the manufacturer. LNB = Lyme Neuroborreliosis, CSF = cerebrospinal fluid, EM = erythema migrans, Ig = immunoglobulin, iv = intravenous, po = peroral.

A commercial routine ELISA assay (Immunozym kit, Germany) was used on serum samples for TBEV serology [28] [29]. Anti-IgM antibodies for detection of a current TBE infection and anti-IgG antibodies for detection of a previous TBE infection or vaccination. Cut-off levels were set as recommended by the manufacturers. If anti-TBEV antibody titers were elevated, a neutralization test was conducted to confirm the TBE diagnosis [30].

For viral PCR investigations, a commercial total nucleic acid kit was used for the extraction of viral deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) from CSF samples (Roche MagnaPure Compact instrument). HSV 1 and 2 and VZV were analysed in a routine real-time PCR whereas enteroviral analyses were carried out with a routine semi-nested PCR [31]-[33]. All serum and CSF samples had been frozen and stored at −70˚C for 2 - 4 years before viral analyses were performed.

2.3. Statistical Analyses

SPSS soft wear, version 15.0, was used for statistical calculations. Mann Whitney U test and Fishers exact test

were used when comparing demographic data between excluded (n = 6) and included (n = 99) patients. Levels of significance were determined as $p < 0.05$. Written informed consent was obtained from all children and parents/guardians—The study was approved by the regional Ethical Committee at Linköping University (Dnr 03-546)

3. Results

One patient was identified with elevated anti-TBEV IgM and IgG antibody titers in serum, indicating a current TBE infection (**Table 2**). This patient was a 17-year-old girl who had reported headache, fatigue, fever and loss of appetite with duration of 2 - 4 weeks. She showed no abnormalities in neurological examination. The lumbar puncture at admission showed mononuclear pleocytosis in CSF (24×10^6 mononuclear cells/L). Initially, she received antibiotic treatment due to a suspected LNB but investigation showed no *Borrelia* specific antibodies in serum or CSF (negative AI). She recovered gradually but reported persistent headache at 6-month follow-up. The confirmatory TBEV neutralization test was negative and follow-up serology was unfortunately not available. Thus, a current TBE infection was highly suspected but not confirmed in this patient. Furthermore, 10 patients had elevated anti-TBEV IgG antibody titers, indicating a previous TBE infection or vaccination. No patient was anti-IgM antibody positive alone.

No positive PCR reactions for HSV 1 or 2, VZV or enterovirus were detected in CSF samples from any of the patients in the study (**Table 2**).

4. Discussion

In this retrospective study of children being evaluated for LNB, one patient with a highly suspected current TBE infection was identified whereas no indications of HSV 1 or 2, VZV or enteroviral infections were found by PCR analyses in CSF. These negative PCR findings may possibly raise questions about the reliability of the PCR methods. However, all CSF samples were run together with routine samples at the laboratory, including accurate positive and negative controls, assuring a high reliability. Handling and storage of samples has been correct and the amount of CSF in each patient sample has been sufficient (0.5 mL).

It is known that herpes viral RNA or DNA may be difficult to detect in CSF in the earliest phase of an acute facial nerve palsy as well as when viral re-activation occurs [34]. However, the duration of symptoms among patients in our study was not extremely short (median 1, 5 weeks) and negative results should probably not be explained by very short durations of symptoms. Re-activation of a viral infection is generally less likely in children than in adults. Furthermore, no patient in the study had vesicles or clinical manifestations of a current herpes viral infection. In this aspect, we are therefore prone to believe that our negative results on viral PCR are reliable. However, a number of patients had a rather long duration of symptoms and the virus might already have been cleared from CSF, which would explain the negative CSF findings. In such cases, neurological symptoms should be looked upon as sequelae after CNS infection.

Table 2. Viral investigations in serum and CSF.

	"Possible LNB" n = 44 n (%)	"Not determined" n = 55 n (%)
Anti-TBEV antibodies in serum[a]		
Anti-IgM	0 (0)	0 (0)
Anti-IgG	4 (9)	6 (11)
Anti-IgG and IgM	1 (2)	0 (0)
Herpes simplex virus 1 or 2[b]	0 (0)	0 (0)
Varicella zoster virus[b]	0 (0)	0 (0)
Enterovirus[b]	0 (0)	0 (0)

[a]Detected as elevated anti-TBEV antibodies in serum. [b]Detected by PCR in CSF. CSF = cerebrospinal fluid, TBEV = tick-borne encephalitis virus, Ig = Immunoglobulin, PCR = polymerase chain reaction.

Admittedly, a study designed for investigation of viral etiology should have included antibody evaluation of serum and CSF in parallel and as repeated samples over time, in addition to PCR detection in CSF. This could not be provided in our retrospective setting, nor were there fecal samples available for enteroviral isolation. In addition, due to ethical reasons, repeated lumbar punctures are rarely performed in young patients.

Furthermore, some of the patients might have been infected by neurotropic agents not tested for in our present study, *i.e.* Epstein-Barr virus (EBV), human herpes virus (HHV) 6 or 7, cytomegal virus (CMV), mycoplasma or influenza A or B [18]. Different refined method for detection of different causative agents in the central nervous system (CNS) has been in focus by Huttunen *et al.* [35]. A wide spectrum of analyses was used and a microbiological diagnosis was obtained in up to 85% of pediatric CNS infection. However, these patients all presented with distinct signs and symptoms of acute meningitis and/or encephalitis and are in this aspect not comparable to children in our study. In addition, the authors comment on the high costs of their extensive diagnostic approach and that hopefully future multi-array method may reduce the overall expense for such extensive evaluations.

Among children in our study with pleocytosis in CSF, all showed a clear dominance of mononuclear cells in CSF (>90% of the total white cell count). According to previous studies on LNB versus viral meningitis, mononuclear pleocytosis in CSF has been strongly predictive for LNB rather than viral meningitis and consequently, these patients are most probably early LNB patients [22] [36]. This is also in line with the negative PCR findings for enterovirus in CSF in our study. Furthermore, an interesting analysis of clinical features and etiology of CNS infections has been published by Waespe *et al.* [37]. They report that among children with acute facial nerve palsy and/or meningitis in combination with mononuclear pleocytosis in CSF, the most common etiology was LNB.

Some children had EM/lymfocytoma and/or *Borrelia* antibodies in serum in the group "Not determined" (**Table 1**), suggesting they may have a current LB (*i.e.* EM and headache), an early LNB (*i.e.* facial nerve palsy without pleocytosis) or a previous asymptomatic LB (anti-flagella IgG in serum). Some patients just had a low anti-flagella IgM response in serum indicating an unspecific cross reactivity. A few patients received antibiotic treatment on vague grounds (**Table 1**). There were also a few children with facial nerve palsy and/or headache who had no pleocytosis in CSF, no EM, no anti-*Borrelia* antibodies in serum and obviously, according to our results, no clinical or laboratory evidence of TBE-, herpes- or enteroviral infection. They should accordingly be classified as acute idiopathic Bell's palsy or unspecific headache.

The one patient with highly suspected current TBE infection (both anti-IgM and IgG antibodies in serum) showed unspecific symptoms and no neurological abnormalities, making her extra interesting as an example of how TBE infections may be deceptive for the clinician. However, one should keep in mind that anti-IgM antibodies in serum are essential for the TBE diagnosis whereas anti-IgG antibodies alone are a merely a measure of the seroprevalence or TBE vaccination coverage in the region.

5. Conclusion

In conclusion, our results suggest that undiagnosed herpes- or enteroviral infections are unlikely to explain CNS symptoms in children being evaluated for LNB, whereas missed TBE infections may occur. TBEV serology should be included when evaluating children for LNB in TBE endemic areas.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Abbreviations

CNS: Central Nervous System
CSF: Cerebrospinal Fluid
DNA: Deoxyribonucleic Acid
ELISA: Enzyme Linked Immunosorbant Assay
EM: Erythema Migrans
HSV: Herpes Simplex Virus
iv: Intravenous
Ig: Immunoglobulin
LNB: Lyme Neuroborreliosis
PCR: Polymerase Chain Reaction
po: Peroral
RNA: Ribonucleic Acid
TBEV: Tick-Borne Encephalitis Virus
VZV: Varicella Zoster Virus