CHARACTERISTICS OF PATIENTS WITH LYME NEUROBORRELIOSIS IN CENTRAL ROMANIA

BRÎNDUŞA ŢILEA[1], CRISTINA GÂRBOVAN[2], IOAN ŢILEA[3]

[1,2]Clinical County Hospital Tîrgu-Mureş, University of Medicine and Pharmacy of Tîrgu-Mureş, [3]Emergency Clinical County Hospital, University of Medicine and Pharmacy of Tîrgu-Mureş

Keywords: *Lyme neuroborreliosis, diagnosis, treatment, Romania*

Abstract: *Background: Lyme neuroborreliosis is a manifestation of Lyme borreliosis involving the central nervous system and/or peripheral nervous system following an infection with Borrelia burgdorferi spirochetes, with a reported incidence of 10–25%. The objective of our study was to evaluate the involvement of the central nervous system and peripheral nervous system and the associated lesions in the infection with Borrelia burgdorferi spirochetes, so as to better understand the disease transmission and incidence. Material and method: This retrospective study included 50 consecutive patients admitted between October 1, 2009, and October 1, 2012. Patients' inclusion criteria were based on the recommendations established by the US Centres for Disease Control (Atlanta, Georgia, USA) and the European Concerted Action on Lyme Borreliosis (EUCALB). Minor and adult patients were evaluated epidemiologically, clinically, and serologically. Computed tomography scans and magnetic resonance imaging were used to further evaluate the patients, and we monitored their treatment and evolution following infection. Results: Almost all (97%) of the included patients were diagnosed with early stage Lyme neuroborreliosis, and 3% with late Lyme neuroborreliosis. Acute lymphocytic meningitis was diagnosed in 86% of the patients and acute encephalitis in 72%; facial nerve lesions were identified in 38% of cases, peripheral nervous system lesions in 24%, and brainstem lesions in 12% of the patients. Serological examinations showed the presence of IgM and IgG antiBb antibodies in the serum and cerebrospinal fluid of the patients, confirming the diagnosis of Lyme neuroborreliosis. Conclusions: The presence of Lyme borreliosis in our geographic area is confirmed, with an increasing incidence in the recent years, due to the emergence of more vectorial agents, especially ticks from the Ixodes ricinus genus. The evolution of the disease was favourable for patients with early Lyme neuroborreliosis. Those patients with late Lyme neuroborreliosis experienced slow clinical evolution as well as relapses of the disease.*

Cuvinte cheie: *neuroborrelioză Lyme, diagnostic, tratament, România*

Rezumat: *Introducere: Neuroborrelioza Lyme este o manifestare clinică a borreliozei Lyme ce implică sistemul nervos central și/sau periferic, consecință a infecției cu spirocheta Borrelia burgdorferi, fiind raportată cu o incidență între 10-25%. Obiectivele studiului au constat în evaluarea afectării sistemului nervos central și periferic, precum și a leziunilor asociate infecției cu spirochete Borrelia burgdorferi, pentru o mai bună înțelegere a transmiterii și incidenței bolii. Material și metodă: Acest studiu retrospectiv a inclus 50 pacienți, internați în perioada 1 octombrie 2009 și 1 octombrie 2012. Criteriile de includere a pacienților în studiu s-au bazat pe recomandările Centrului de Control al Bolilor Infecțioase din Atlanta, Georgia, SUA (CDC) și Grupul de Acțiune European pentru Borrelioza Lyme (EUCALB). Pacienții, copii și adulți au fost evaluați epidemiologic, clinic, serologic, neuroimagistic prin efectuarea examenului computer tomografic și rezonanță magnetică cerebrală și de asemenea s-a monitorizat tratamentul și evoluția post-infecție. Rezultate: Aproape toți pacienții incluși în studiu (97%) au fost diagnosticați în stadiul precoce de boală Lyme cu manifestări neurologice și 3% în stadiul tardiv de neuroboreliază Lyme. Meningita acută limfocitară a fost diagnosticată la 86% din pacienți, encefalita acută la 72%, au fost identificate leziuni ale nervului facial la 38% din cazuri, leziuni de sistem nervos periferic la 24% și leziuni medulare la 12% din pacienți. Examinările serologice au evidențiat prezența anticorpilor anti-Bb, tip IgG și IgM din LCR și serul pacienților, confirmând diagnosticul de neuroboreliază Lyme. Concluzii: Prezența borreliozei Lyme în arealul nostru geografic s-a confirmat, cu o incidență în creștere în ultimii ani, din cauza numărului ridicat de agenți vectori, în special căpușe din genul Ixodes ricinus. Evoluția bolii a fost favorabilă la pacienții cu neuroboreliază Lyme precoce. Pacienții cu stadii tardive ale bolii au evoluat lent și au prezentat recăderi.*

INTRODUCTION

Transmission and manifestation of Lyme borreliosis

An outbreak of atypical pediatric arthritis presenting with a corresponding tick bite was first observed in the environs of the south-eastern Connecticut town of Lyme in the mid-1970s. This disorder, which came to be known as Lyme arthritis, was associated with cutaneous and neurological signs described in Europe by Garin and Bujadoux (1) and consisting of joint

[1]Corresponding author: Ioan Ţilea, Str. Revoluţiei, Nr. 35, Cod 540042, Tîrgu-Mureş, România, E-mail: i.tilea@clicknet.ro, Tel: +40265 211011
Article received on 03.06.2013 and accepted for publication on 12.08.2013
ACTA MEDICA TRANSILVANICA September 2013;2(3):263-269

pain, single limb paralysis, and cerebrospinal fluid alterations, secondary to erythema migrans (EM) that appeared subsequent to a tick bite. Lyme arthritis develops as a result of a tick-borne Lyme borreliosis (LB, also known as Lyme disease) infection.

LB is transmitted by arthropods of the *Ixodes* class and is produced by bacteria from the *Borrelia* genus; the disease is characterized by multisystemic clinical manifestations with a stadial evolution and polymorph clinical feature.(2) The main risk factor for LB is the permanent or temporary presence of man in areas with ticks with a high level of *Borrelia burgdorferi* spirochetal infection.

In this disease, the pathogen conveyed by the tick initially induces pathological manifestations on the skin in the form of EM and is frequently accompanied by general symptoms (e.g., fever, cephalalgia, myalgia, arthralgia, asthenia). EM is followed by hematogenous dissemination and the involvement of the central nervous system (CNS) and peripheral nervous system (PNS); to varying degrees, this systemic spread of the disease produces an immune response throughout the body, including meningeal, encephalitic, radicular, and brainstem lesions and inflammation of the joints (Lyme arthritis) and of the cardiovascular system (e.g., myocarditis, pericarditis, atrioventricular block). LB may appear at any age, but is more frequent in children under 14 years of age and in adults between 30 and 50 years of age, with males and females being equally likely to develop the disease.

Lyme neuroborreliosis (LNB), or the involvement of the CNS and/or PNS, accounts for 10–25% of LB cases.(3,4) The spirochete has been noted in the CNS in both the acute and late phases of the disease (4), pointing to the need to make a clear distinction between early LNB, which starts soon after t6he infection, and late (or "chronic") LNB, which starts no earlier than six months after initial LB infection. Accurate diagnosis of LNB has thus far proven difficult, with diagnoses of the condition generally being established based on the Centres for Disease Control (CDC)-established criteria for the condition; epidemiological data; clinical findings (e.g., acute lymphocytic meningitis, encephalomyelitis, radiculoneuritis); and paraclinical investigations.(5,6)

US and European LB incidence

LB is the most common vector-borne disease in the United States, with the majority of US cases being reported in the North-eastern and Midwestern areas of the country.(7) LB is a mandatory reportable disease in the United States, with an average of 15,000-20,000 new cases reported each year; 15 states are considered endemic areas.(8) In Europe, the incidence of LB is more difficult to track because of the disease not being mandatory reportable in all areas; most recently, the European incidence rate has registered approximately 85,000 cases per year.(9) Further hindering epidemiologists' tracking of European LB incidence are differences in testing patterns and persistent underdiagnosing of the disease.

Further hindering epidemiologists' tracking of European LB incidence are differences in testing patterns, including the use of varied serological diagnosis criteria for with early and late disease exposure. LB is thought to be continually underdiagnosed throughout Europe; however, of the reported cases, the highest incidence rate of LB is found in the northern, eastern, and central parts of the continent. The highest LB incidence was seen in the Baltic countries; Austria (300 cases/100,000 inhabitants); Bulgaria (55/100,000); Czech Republic (29/100,000); and Germany (25/100,000), with the reporting of LB cases and the distribution of LB-affected areas on the continent increasing significantly in recent years.(9) Historically, forest workers, farmers, veterinarians, hunters, and other individuals who spend significant amounts of time outdoors or in rural areas have been more likely to contract LB; however, in recent years, ticks carrying bacteria from the *Borrelia* genus have begun appearing in parks and other green areas frequented by children and young adults residing in urban areas, resulting in further challenges in diagnosis and geographic prediction of the disease.

LB has been shown to present a variety of clinical manifestations, likely because of the expansive geographic distribution of the disease. Specifically, the *Borrelia afzelli*, *Borrelia garinii*, *Borrelia sensu stricto*—and, less frequently, *Borrelia spielmanii*—are the most common LB genospecies in Europe, while *Borrelia burgdorferi sensu stricto* is the only pathogen species of the disease documented in the United States.(10)

Romanian LB incidence

In Romania, *Ixodes ricinus* (castor bean tick) is the vector agent of this increasingly common disease. The disease has come under surveillance by Romanian public health officials in recent years because of a continuing rise in vectors and reported cases, and in 2010 LB became a mandatory reportable disease. It is thought that global warming and the migration of vector agents from north-western Europe to Eastern Europe and Transylvania have contributed to the spread of LB into Romania and neighbouring countries in the past two decades.(11) The greatest number of LB cases has been reported in the central and northern parts of the country. *Borrelia burgdorferi* is the most common LB genospecies documented in Romania.(11)

The nation's estimated LB incidence rate in 2011 was 20 cases/100,000 inhabitants (12), but this incidence rate remains considerably lower than that of other European counties, most likely because of underdiagnosis of the disease in Romania. Mureş County, where this retrospective study took place, is located in central Romania and has an estimated population of approximately 588,000 inhabitants. While Mureş County's rates of 7.47 cases/100,000 inhabitants in 2010 and 12.91 cases/100,000 inhabitants were much lower than the national average (12), they represented a marked uptick in the county's reported LB cases in the past two years; it was this rapid increase in reported LB cases over such a short time period that led to expanded monitoring of the disease in this region.

As LB is becoming more and more prevalent in Romania, resulting in mandatory reporting of the disease, it is likewise becoming increasingly important to provide public health officials and epidemiologists with the knowledge and tools needed to accurately determine disease spread and incidence. Similarly, as LNB has the potential of resulting in long-term systemic problems, we considered it beneficial to more closely examine the incidence of this specific subdisorder.

PURPOSE

We sought to perform a retrospective study of LB patients' records in Mureş County to evaluate the involvement of the CNS and PNS and associated lesions in *Borrelia burgdorferi* spirochetes.

METHODS

Our teaching hospital, the Clinic of Infectious Diseases I in Tîrgu-Mureş, Romania, provides clinical services, including diagnosis, prophylaxis, ambulatory treatment, and/or hospital admission, related to tick bites and related vector-borne diseases to Mureş County residents. The retrospective study included 50 consecutive patients (mean age 29.12 ± 15.69 years) admitted to the Clinic of Infectious Diseases I between October 1, 2008, and October 1, 2011, for diagnosis and treatment of LB.

As noted previously, the objective of our study was to evaluate the involvement of the CNS and PNS and associated

lesions in patients infected with *Borrelia burgdorferi* (Bb) spirochetes. To avoid excessive diagnosis, patients' inclusion in the study was based on LNB criteria established by the CDC and the European Concerted Action on Lyme Borreliosis (EUCALB) (7,13,14), as described below:

- *Epidemiological*-presence of a tick bite, existence of EM
- *Clinical*-presence of neurological symptoms (e.g., headache, fever, intracranial hypertension syndrome, nausea, vomiting; nuchal rigidity, positive Kerning I and II signs, facial palsy and other pathologic neurological signs)
- *Paraclinical*-evaluation of pleocytosis (cell elements) and intrathecal-specific antibody synthesis

Methodology for assessing LNB

For the purposes of this research, we used ELISA and western blot analysis to demonstrate the presence of IgM and IgG anti-Bb (anti-*Borrelia burgdorferi*) antibodies in the cerebrospinal fluid (CSF), collected from all patients. ELISA was performed using Virion/Serion kits (Institute Virion/Serion GmbH, Würzburg, Germany). IgM and IgG antiBb antibodies were considered positive at values ≥9 U/l, negative at values <8 U/l, and equivocal at values in the range of 8–9 U/l. Western blotting was performed with a recomLine Borrelia IgG/ recomLine Borrelia IgM Mikrogen® kit (Mikrogen Diagnostik, Neuried, Germany) that uses a combination of antigens from 5 genospecies involved in the development of LB: *B. burgdorferi sensu stricto*, *B. garinii*, *B. afzelii*, *B spielmanii*, and *B bavariensis*. In the case of IgM antibodies, we obtained strips for the following antigens: p18, p41, OspC (*B. sensu stricto*), OspC (*B. afzelii*), OspC (*B. garinii*), OspC (*B. spielmanii*)-intensely positive-and OspC (surface proteins from *B. garinii*, *B. sensu stricto*, *B. afzelii*, *B. spilmanii*). In the case of IgG antibodies, we obtained strips for the following antigens: VisE (surface lipoprotein), p18, p39, p41, p58, p100, OspA, and OspC (*B. sensu stricto*). IgM and IgG antiBb antibodies were considered positive at values ≥ 7 points, negative at values ≤5, points and equivocal at 6 points. LNB diagnosis was based on the criteria described previously.

Patients who presented cerebral, meningeal, and brainstem manifestations were examined using computed tomography (CT), magnetic resonance imaging (MRI), and electroencephalography (EEG). To exclude false-positive reactions that could have occurred in the context of other diseases (e.g., syphilis, leptospirosis, tuberculosis, mononucleosis, autoimmune diseases, multiple sclerosis, tick-borne encephalitis), we tested patients' serum and CSF for these diseases. All results were negative. Multiple sclerosis was excluded by the hospital's neurologist, who used the 2010/2011 McDonald diagnostic criteria for the disease.(15) Patients who presented CNS manifestations were treated with a standard course of Ceftriaxone (50–100 mg/kg b.i.d., i.v.) for a period of 30 days, and those who presented PNS manifestations were treated with a standard course of Doxycycline (200–300 mg b.i.d., p.o.) for a period of 30 days. We followed the clinical and biological evolution of the patients, as well as the occurrence of relapses after stopping therapy.

Statistical analysis

Statistical analysis was performed using chi-square and T Student tests. A *p* value ≤0.5 was considered to be statistically significant.

Ethical considerations

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Tîrgu-Mureş, Romania and was performed in the accordance with standards of the Declaration of Helsinki. All study participants consented to use their clinical data for hospital research, and patients with a clear diagnosis of LNB upon treatment or hospital admission were provided additional details about this particular study. Written informed consent was obtained from each patient or legal guardian if the patient was a minor. No procedures beyond the standard of care were performed specifically for the purposes of this study.

RESULTS

We observed a higher incidence of LNB in children (<15 years - 19 patients, 38%), in the 16–35-years-old age group (14 patients, 28%) and adults below 36–45 years (12 patients, 24%), compared with adults over 45 years of age (5 patients, 10% of the cases). Distribution by gender shows a higher percentage of male patients (27 patients, 54%), compared with female patients (23 patients, 46%) without a statistically significant difference between the two genders.

A slight majority of patients resided in an urban environment (27 patients, 54%), compared with a slight minority of patients from a rural environment (23 patients, 46%); there was not a statistically significant difference between these two environs. Of these 50 patients, 37 (74%) came from Mureş County, and 13 (26%) came from neighboring counties (Harghita, Covasna, Cluj, Sibiu, and Braşov; figure no. 1).

Figure no. 1. Map of Romania, the central region with Mureş, Harghita, Covasna, Braşov, Sibiu and Cluj counties

A history of tick bite was present in 43 patients, or 86% of the cases, and EM was present in 39 patients, or 78% of cases; the remaining 11 patients (22%) did not notice the presence of EM. Almost all patients (47 patients, or 94%) were considered to be in the early stage of LNB based on CDC and EUCALB criteria, presenting neurological symptoms within 60 days following a tick bite; 3 patients, or 6% of the cases, were diagnosed with late LNB, presenting symptoms after a period of 6 months following the tick bite; for all of the LNB patients studied here, symptom onset did not start until 12 months after infection.

Acute lymphocytic meningitis was found with a high frequency (43 patients, 86% of cases), mostly in children ≤15 years old (17 patients, 34%), and in the 16–35-years-old age group (11 patients, 22%), compared with the age groups between 36–45 years (4 patients, 8% of cases) and over 45 years old (5 patients, 10% of the cases); p=0.54104 (table no. 1).

Table no. 1. Correlation between age groups and meningeal lesions

Age	Yes	No	Total
<15	17	2	**19**
16-25	11	3	**14**
26-35	6	2	**8**
36-45	4	0	**4**

over 45	5	0	**5**
Total	**43**	**7**	**50**

Pleocytosis in the CSF evidenced low levels of lymphocytes (20–99 cells/mm^3) in 28 patients, 56% of cases; medium levels (100–500 cells/mm^3) in 5 patients, 10% of cases; high levels (>500 cells/mm^3) in 5 patients, 10% of cases. The average value of pleocytosis in the CSF was 130.04 cells/mm^3 (table no. 2).

Table no. 2. CSF pleocytosis values (lymphocytes/mm^3)

Cells/mm^3	No. of patients	Percentage
<5	12	24%
20-99	11	22%
100-500	3	6%
>500	17	34%

Biochemistry of the CSF showed elevated protein levels in 33 patients, 66% of cases, with an average of 91.58 mg%, and high glucose levels in 17 patients, 34% of cases, with an average value of 49.08 mg%. Lymphocytic meningitis occurred in 40 patients (80% of the cases) in the early LNB stage, compared with the late stage of the disease, where lymphocytic meningitis was seen in only 3 patients (6% of the cases).

CNS involvement was observed in 36 patients, 72% of cases, 20 patients (40%) of whom presented severe forms (confusional state, drowsiness, hemi paresis, bladder retention, epileptic seizure); 14 patients, 28% presented medium forms (cerebellar ataxia, memory and concentration disorders); and 2 patients, 4%, had light forms (somnolence, behavioural disorders, vertigo), with a higher incidence among children (28 patients, 14% of cases), and among the 16–35-years-old age group (6 patients, 12% of cases), compared with patients over 45 years of age (p=0.95208). Associated meningoencephalic lesions were observed in 18 patients, 36% of cases, in the severe clinical forms, and in 10 patients, 20% of the cases, in the medium clinical forms, compared with the mild ones, 2 patients, 4% of the cases.

By analyzing the association between the approximate time span from the infecting moment to the onset of the neurological signs (<6 months), we have observed that 34 patients (68% of the cases) were categorized as suffering from early LNB and 2 patients (4%) from late LNB with neurological symptoms that started 12 months from the infecting moment.

Cranial nerve lesions were observed in 19 patients, 38% of cases, consisting of facial nerve lesions with unilateral or bilateral facial paralysis (18 patients, 36%; and 1 patient, 2% respectively). Their incidence was higher in children <15 years (11 patients, 22% of cases) and in the 16- to 35-year-old age group (4 patients, 8% of cases), compared with other age groups (1 patient, 2% of cases) (p=0.26083). The associated lesions were acute meningoradiculitis in 15 patients, 30% of cases (p=0.26052); encephaloradiculitis in 10 patients, 20% of cases (p=0.39047); acute polyradiculoneuritis in 6 patients, 12% of cases (p=0.32592); and acute myeloradiculoneuritis in 5 patients, 10% of cases (p=0.01474) also showing a statistically significant association. PNS involvement was recognized in 12 patients (24% of cases) who developed acute sensitive/motor isolated radiculoneuritis (3 patients, 6% of all cases). Several PNS-associated acute lesions were also observed, including: meningoradiculoneuritis (10 patients, 20% of cases, p=0.76008); encephalopolyradiculoneuritis (8 patients, 16% of cases, p=0.83658); and myelopolyradiculoneuritis in (3 patients, 6% of the cases, p=0.11192).

We observed PNS involvement in 11 patients, 22% of the cases in the early stage of the disease, compared with the late stage, where only 1 patient was included, 2% of the cases. Brainstem lesions were demonstrated in 6 patients, 12% of cases (3 patients, 6% of the cases in the <15 years old age group, and 3 patients, 6% of the cases in the 16- to 35-year-old age group), with associated lesions of acute meningomyelitis in 5 patients, 10% of cases (p=0.84095) and acute encephalomyelitis in 6 patients, 12% of cases (p=0.12741). Patients with brainstem involvement were included in the early LNB category because of the onset of symptoms in fewer than 6 months from the infecting moment (table no. 3).

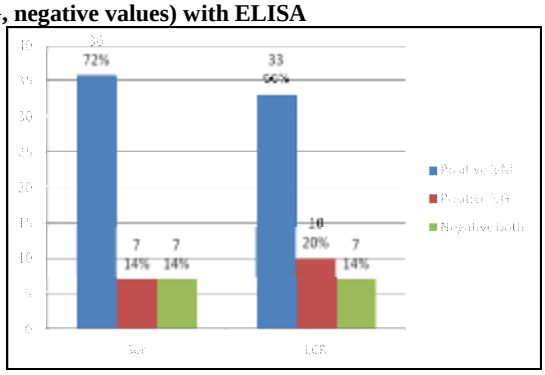
Serological determinations from the serum and CSF using ELISA demonstrated the presence of IgM antiBb antibodies in the serum of 36 patients, 72% of cases, IgG antiBb antibodies in 7 patients, 14% of cases, while the tests were negative for 7 patients, 14% of cases. In the CSF, IgM antiBb antibodies were present in 33 patients, 66% of cases, and IgG in 10 patients, 20% of cases; the tests were negative for 7 patients, 14% of cases (figure no. 2). Western blot analysis evidenced the presence of IgM antiBb antibodies in the serum of 33 patients, 66% of the patients, and IgG in 5 patients, 10% of cases; the test was negative for 12 patients, 24% of the cases, IgM antiBb antibodies were found in the CSF of 35 patients, 70% of cases, and IgG in 5 patients, 10% of cases; the test was negative for 10 patients, 20% of cases. By correlating the epidemiological data with the neurological symptoms and signs and with the IgM and IgG anti Bb antibodies readings from both serum and CSF, we observed that 35 patients (70% of the cases) were confirmed as definite or certain LNB; 5 patients (10% of the cases) with probable LNB; and 10 patients (20% of the cases) with possible LNB.

Table no. 3. Classification and clinical manifestation of Lyme neuroborreliosis

Early LNB		**Late LNB**	
No.	**Percentage**	**No.**	**Percentage**
47	94%	3	6%
Neurological clinical presentations			
Acute lymphocytic meningitis (43 patients, 86%)		**Acute lymphocytic meningitis** (3 patients, 6%)	
CNS involvement (36 patients, 72%)		**Brain involvement** (2 patients, 4%)	
• Acute meningoencephalitis (30 patients, 60%)		• Acute encephalitis (2 patients, 4%)	
Facial nerve involvement (VII) (19 patients, 38%)			
• Acute meningoradiculitis (15 patients, 30%)			
• Acute encephaloradiculitis (10 patients, 20%)			
• Polyradiculoneuritis (6 patients, 12%)			
• Acute myeloradiculitis (5 patients, 10%)			
PNS involvement (11 patients, 22%)		**PNS involvement** (1 patients, 2%)	
• Acute meningoradiculoneuritis (10 patients, 20%)		• Encephaloradiculoneuritis (1	

	patients, 2%)
• Acute encephalopolyradiculoneuritis acute (7 patients, 14%)	
• Sensitive and motor acute radiculoneuritis (3 patients, 6%)	
• Acute myelopolyradiculoneuritis (3 patients, 6%)	
Brainstem involvement (6 patients, 12%)	
• Acute meningomyelitis (5 patients, 10%)	
• Acute encephalomyelitis (6 patients, 12%)	

Figure no. 2. AntiBb antibodies in the serum and CSF (IgM, IgG, negative values) with ELISA



CT scans revealed modifications in 17 patients, 34% of cases, namely: localized or diffuse cerebral edema, hypodense in the white matter, periventricular calcifications, and/or hydrocephalus. MRI evidenced modifications in hyper- and hyposignal T1, T2 in the white matter, periventricularly and the border of white and gray matter, in 5 patients, 10% of the patients. EEG showed a localized irritative, diffuse line with spike-wave complexes in 5 patients, 10% of cases.

A total of 47 patients, 94% of cases, received antibiotic treatment with Ceftriaxone or Doxycycline. Dual therapy with Ceftriaxone plus Doxycycline was started in 10 patients, 20% of cases, with severe forms of acute meningoencephalitis. The evolution was favorable in 23 patients, 46% of cases, with remission of the clinical and biological status in fewer than 16 days from the beginning of treatment. Slightly more than half of the patients (27 patients, 54% of cases) had a slow evolution with prolonged symptoms, persisting pleocytosis in the CSF, and the presence of an acute inflammatory process at cerebral level, more than 16 days after the beginning of treatment. The monitoring of CSF constants (pleocytosis, glucose and protein concentration) was performed dynamically in the 1st, 10th, and 21st days after hospital admission.

Relapse was present in 14 patients, 28% of cases, with isolated and associated involvement of the CNS and PNS. We classified as relapse the recurrence of the initial symptoms (fatigue, drowsiness, headache, anxiety, and paresthesia) after a variable period, on average 60 days, following the end of therapy. No pathological changes were observed on the general clinical examination, and hence the relapses were interpreted as subjective. Nevertheless, in the 3 late LNB patients (6% of the cases), oral antibiotic treatment with Doxycycline 200 mg b.i.d. 14 days a month for 3 consecutive months was started.

DISCUSSIONS

Lyme borreliosis is a widespread disease, with an endemic evolution and small epidemic outbreaks in virtually all affected regions. Because of the predominance of asymptomatic forms of the disease and the impossibility and impracticality of performing all the laboratory tests needed for a positive diagnosis, LB almost certainly has a wider geographical spread than has been previously reported.(16)

In Mureş County, Romania, and its neighbouring areas, the number of vectorial agents (i.e., ticks from the *Ixodes ricinus* genus) has increased in numbers in recent years, leading to the initiation of a personal study on the subject.(2) We thus considered it likely that LB and the accompanying LNB disorder would have similarly increased in this region.

LNB did not present the characteristics of a professional disease in the studied group, and instead was observed most frequently in those individuals who spent time outdoors pursuing leisure activities such as jogging and hiking; interestingly, only 3 patients (a forest ranger and 2 animal caretakers), 6% of cases, came from a rural setting. In our study, LNB was observed most frequently in children and young adults, two groups likely to spend a significant amount of time engaging in leisure activities outdoors, where tick bites are more likely to occur. A history of tick bite was present in 43 patients, 86% of cases, and EM in 39 patients, 78% of cases.

The maximum incidence of the moment of infection was in the summer season, between April and September. Meningeal lesions and acute lymphocytic meningitis were present in a high percentage of cases (43 patients, 86% of cases). The high proportion of cases with meningeal inflammation in the second stage of the disease is in concordance with the results of Halperin et al. who found it to be the most frequent manifestation of early LNB.(17) A study carried out in Germany found that the second-most-common manifestation of pediatric LNB was aseptic meningitis (27.2%, n=46), and that LB was the third-most-common cause of aseptic meningitis in childhood (11.8%).(18) Cerebral lesions were found in 36 patients, 72% of cases in our study group, of whom 20 patients, 40% of cases, presented severe clinical forms.

The most frequently described clinical finding in the literature is Lyme encephalomyelitis, with variable incidence in the US and European literature.(19) In our study group, the frequency of acute meningoencephalitis was high (30 patients, 60% of cases) given the total number of patients, compared with other studies.(10,20) Facial paralysis was present in 19 patients, 38% of cases, being mostly unilateral (18 patients, 36%). We observed the association of cranial neuritis and acute lymphocytic meningitis (15 patients, 30% of cases), yielding acute meningoradiculitis or Bannwarth syndrome in 15 patients, 30% of cases. This syndrome is frequently reported, being interpreted to be the most typical manifestation of early LNB.(8) PNS lesions were present in 12 patients, 24% of cases, isolated or associated with radiculoneuritis with meningeal, cerebral, and/or brainstem lesions.

According to the literature, PNS lesions are present in approximately one-third of patients with LNB, being accompanied by pleocytosis in the CSF in 85% of cases and cranial neuropathy in 70% of cases;(21) these figures correspond relatively closely to our own findings in this study.

The patients in whom IgM and IgG antiBb antibodies could not be evidenced from the serum and CSF (ELISA 7 patients, 14% of cases; western blotting, 10 patients, 20% of cases) were classified as possibly having LNB, according to the classification of the CDC and EUCALB.(5,22) These patients presented an improvement of their general status, in concordance with the findings of Logigian et al.(20) In study

patients with LNB, the levels of IgM remained elevated even weeks or months after complete remission. Recent studies on the production of specific intrathecal antibodies have reported that a specific immune response may persist even 3 years after the completion of appropriate treatment. According to Halperin et al. (16), this fraction of intrathecal immunoglobulins is independent from the rate of IgM-IgG conversion in the blood, and a positive serology does not prove the presence of the disease, nor does a negative one does not confirm it. Diagnosis continues to be difficult, because of false positive reactions in the context of other diseases and because of the unexceptional sensitivity of diagnostic tests.

The absence of antiBb antibodies in the CSF does not, however, preclude the diagnosis of LNB, especially if there are neurological symptoms and epidemiological data supporting such a diagnosis.(8) In this study we have used two different serological methods: ELISA as a first reading because of its having a 70–90% sensitivity in early LNB and over 95% sensitivity in late LNB, and western blot analysis as a confirmation method.

Antibacterial therapy with i.v. Ceftriaxone in patients with CNS symptoms and p.o. Doxycycline in patients with PNS lesions for 30 days was tolerated well, without side effects. Approximately 25 patients, 50% of cases, presented a rapid and considerable clinical evolution following treatment with Ceftriaxone. Relapses were encountered in 14 patients, 28% of cases, with symptoms occurring after an average of 60 days following the end of therapy, in accordance with the studies of Fallon et al.(23) These patients received symptomatic therapy, vitamins, and antioxidants, with significant improvement of their clinical status. The exception were the late LNB cases in which antibiotic treatment with Doxycycline 200 mg b.i.d., 14 days a month, for 3 months was restarted, after which they did not show any symptoms.

CONCLUSIONS

In this study, we have observed that LB in Mureş County region of Romania-and, presumably, other areas of the country or the European continent-is becoming more prevalent in younger, urban populations engaging in outdoor leisure activities. This contradicts previous findings the highest incidence of LB in individuals contracting the disease through their living in rural regions and working outdoors in such areas.

Throughout this study we observed a predominance of early LNB cases compared with late LNB cases. Although the number of studied cases was relatively low, there was a high frequency of the severe forms of the illness. The evolution of the studied patients was favourable without the presence of irrecoverable motor deficits or neurological sequelae.

For more rigorous monitoring, we recommend increased education of the public to recognize the disease in time, as well as prophylactic measures. In addition, skin lesions should be properly treated to prevent spirochete dissemination and the disease becoming chronic. Finally, a national surveillance program should be instituted to monitor Lyme borreliosis in Romania.

Acknowledgement:

This paper is partially supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109.

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