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ORIGINAL RESEARCH ARTICLE

Tick bites and Erythema migrans in Transylvania

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Abstract. Erythema migrans (EM), the hallmark and the most frequent manifestation of Lyme Borreliosis (LB), has not been studied in Romanian patients until the present study. Forty patients with solitary or multiple EM that presented to the Teaching Hospital of Infectious Diseases, Cluj-Napoca, between June 2011 and August 2012, were prospectively studied. Clinical and serological outcome of the patients was investigated comparing two groups of patients: patients with a short observation period of 3 months and patients with a longer observation of 9 months-1 year. Only one patient was diagnosed at the follow-up with late LB with musculoskeletal manifestations, but a number of unspecific symptoms with no objective signs were described in other 16 patients. Our data prove that the serological profile is not correlated with the clinical course, and sustain the recommendation of clinical follow-up of EM patients with no repeated serological testing for assessing therapy efficacy. Epidemiological and clinical data are presented in comparison with the results of similar studies performed in Europe and USA.

Keywords: Erythema migrans; Lyme Borreliosis; Serology; Romania.

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Introduction

Erythema migrans (EM) is the most common and specific clinical manifestation of Lyme Borreliosis (LB). Several definitions have been proposed but best known among these are the definitions of EUCALB (Stanek et al., 1996) and the ESCMID study group (Broqui et al., 2004). EM is defined as an erythematous skin lesion that develops days to weeks after infection at the site where *B. burgdorferi* was inoculated into the skin by an *Ixodes* tick. Studies have shown epidemiological and clinical differences between EM lesions in Europe and USA (Strle et al., 1996; Strle et al., 2011). These differences are caused by different *B. burgdorferi* genospecies involved as etiological agents: in Europe the reports based on *B. burgdorferi*

cultivation showed mainly *B. afzelii*, less frequently *B. garinii* and *B. burgdorferi* sensu stricto (s.s.) and only exceptionally *B. bissettii* and *B. spielmanii*. In North America, EM is caused only by *B. burgdorferi* s.s. (Strle and Stanek, 2009). We have already showed that ticks collected from humans in our region are infected with the human pathogenic genospecies of *B. burgdorferi*, mainly *B. afzelii* and *B. garinii* (Briciu et al., in press). Long time prognosis of patients treated for EM is considered very good, though not many European studies have addressed this question (Lipsker et al., 2002; Cerar et al., 2010) and until now it has not been studied in Romania. Few data exists on LB in Romanian patients and these are mainly case reports (Cristea and Crișan, 2003; Rădulescu et al., 2009).

Patients and method

In our prospective observational study we have included adult and children patients who presented to the Teaching Hospital of Infectious Diseases, Cluj-Napoca, Romania, between June 2011 and August 2012, with the clinical diagnosis of EM, made in accordance with the definition criteria by a medical investigator. A typical EM lesion is shown in figure 1. Each patient was included in the study after an informed consent and received information on the aims and the protocol of the study. In case of patients under the legal age of consent, one of the parents signed the agreement. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Romania.

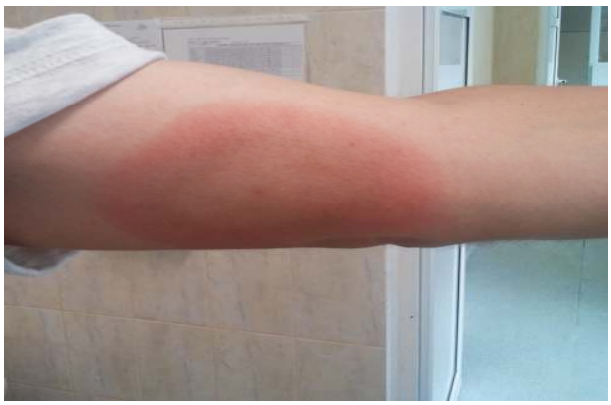

Figure 1. EM lesion with central clearing on the arm; the punctum (tick attachment site) may be noticed in the center of the lesion

A questionnaire was completed for each patient by the medical investigator regarding: occupational and recreational risk for tick bites, previous tick bites, date of tick bite, number of ticks detached, estimated time of tick attachment, the interval from the tick bite until the rash has appeared, the exact date when EM appeared, size and location of EM at presentation, chemoprophylaxis (if followed and the regimen), known allergies to antibiotics, pathological antecedents, pregnancy (if present). Nonspecific clinical symptoms or signs that accompany the cutaneous lesion were noted. A blood sample was provided for serological assays. The patients were treated as recommended by the guidelines (Wormser et al., 2006; Hansmann, 2009; Stanek et al., 2012) according to age and contra-indications.

Follow up. At the end of therapy patients were reevaluated using the same questionnaire and noting the day when EM disappeared, the clinical course of symptoms presented at the first visit, adverse reaction to medication and if other symptoms have appeared. Three months later patients were invited for a third visit. Those patients who did not return for the 3 months follow-up in 2011 were asked by telephone to return for a visit in May 2012.

The patients were divided in two groups: (group A) patients with a short time observation period and (group B) patients with a medium observation period (9 months-1 year). Complete response was defined as continued absence of objective manifestations of Lyme disease, with return to pre-Lyme disease health status. Partial response was defined as the presence of new or increased symptoms. A second blood sample was taken for serological assays.

Serum analyses. ELISA tests were performed for serological analysis. In 2011 we have used a Genzyme Virotech ELISA kit (Genzyme Virotech GmbH, Germany) and in 2012 we have used an Euroimmun ELISA kit (Euroimmun AG, Germany). Tests were performed and interpreted according to manufacturers' recommendations.

Data analyses. Fisher exact test with a level of significance $p < 0.05$ (two-tailed), was used for statistical comparisons.

Results

40 patients were included in the study group, 32 in 2011 and 8 in 2012. Most of the patients presented between June and August (31 cases; 77.5%), with no cases between November and April. Demographic and clinical patient data are summarized in table 1.

To increase the specificity of the diagnosis, a single primary lesion must reach 5 cm in size, in order to distinguish EM from inflammatory reaction to the bite of an arthropod that is not associated with infection and resolves spontaneously within a day or two. The size limitation should not be used alone to exclude the diagnosis of EM in individual patients who have otherwise suggestive clinical and epidemiologic features (Dandache et al., 2008). In the current study, 7 patients had the size of lesion at presentation between 2 and 5 cm, but were confirmed as EM due to the long interval between the tick bite and the rash appearance and/or persistence more than 4 days after presentation. Four patients (3 children and one adult) presented multiple EM.

Although the risk of infection transmission is considered minimum in the first 24 hours, 7 of our patients recalled a shorter tick attachment, suggesting the risk of early transmission. EM developed in 5 patients in spite of chemoprophylaxis administered in the first 72 hours. In 2 patients, 100 mg/day doxycycline were administered for 10 days after tick bite and EM developed after 75 days and 10 days respectively.

Recommended antibiotic treatment is presented in table 2. Doxycycline, the preferred oral regimen, was not indicated in: children younger than 8 years, known allergies, pregnancy, gastric or liver diseases and the impediment of avoiding sun exposure (photosensitivity). The dose and the treatment duration were according to the guidelines. Patients were treated as outpatient except multiple EM patients that were hospitalized for parenteral therapy.

In 2 cases treated with doxycycline therapy was prolonged from 14 days to 28 days due to the slow regression of EM that persisted until day 28. Ceftriaxone was administered in 3 cases of multiple EM, the 4th case, a 7 years old child, following treatment with oral amoxicillin (the mother refuses hospitalization and parenteral treatment). Two of our patients, pregnant at the moment of presentation were treated with amoxicillin 3 g/day, for 21 days, with no unfavorable effect on pregnancy or congenital malformations. In 2 patients, mild gastro-intestinal adverse events to antibiotics were described that ceased with symptomatic therapy. In other 2 patients antibiotics were stopped before the end of treatment due to adverse events (one patient stopped doxycycline treatment on the 12th day of therapy due to nausea and epigastric pain and for the second one, ceftiaxone treatment was stopped on the 16th day due to generalized pruritus).

Throughout the observation period, two patients were treated with antibiotics: (1) for cervical lymphadenopathy by family doctor; (2) prophylactic, after another tick bite. There were no other recognized tick bites recalled.

From the study group, 15 patients had a short observation period (group A), while 23 had a medium one (group B). Two patients did not return for the follow up.

Seventeen out of 38 followed-up patients had a partial response after therapy, presenting one or more symptoms at the final evaluation. No statistical significance was found regarding response to therapy between the 2 groups (p=1) (table 3). The main remaining or new symptoms presented alone or combined were: arthralgias (9 cases), paresthesia (7 cases), headache (6 cases), dizziness (4 cases), memory disturbances (3 cases), concentration deficits (2 cases), myalgias (2 cases). A single patient was diagnosed as LB with musculoskeletal manifestations, a patient already suffering of hands arthrosis when diagnosed with EM, who complained at the follow- up of augmented arthralgia, swelling and stiffness of the metacarpophalangeal and interphalangeal joins. The patient has stopped doxycycline treatment on the 12th day due to nausea and epigastric pain. For the musculoskeletal manifestations the patient was recommended 21 days of Amoxicillin 3 g/day. The rest of the cases with partial response did not fulfill the criteria of

post-Lyme disease syndrome (PLDS) defined by Wormser et al. (2006), as symptoms had low intensity and did not result in substantial reduction in previous levels of occupational, educational, social, or personal activities. None of these patients asked medical examination for their complains until the follow up and none did present any objective clinical finding. They were investigated for other rheumatological or neurological diseases and specific or symptomatic therapy was followed when indicated, but no antibiotic retreatment. They remained in clinical observation.

Table 1. Characteristics of patients with Erythema Migrans

Characteristic	Value
Adults (nr/%)	32 (80%)
Age (years): mean ± SD	50.8±15.5
Range	22-74
Children (nr/%)	8 (20%)
Age (years) mean ± SD	8.3±4.3
Range	3-17
Sex	
Female:Male (nr)	F=20; M=20
Residence	
Urban : Rural (nr/%)	U=29 (72.5%); R=11 (27.5%)
Cluj: Bistrita-Nasaud County	39:1
Tick bite at rash site recalled (nr./%)	34 (85%)
Recalled tick attachment < 24hours (nr. patients)	7
Chemoprophylaxis (nr./%)	5 (12.5%)
Solitary EM (nr./%)	36 (90%)
Location: Leg	24
Trunk and abdomen	9
Arm and shoulder	3
Multiple EM (legs, arms, trunk, abdomen, face)	4 (10%)
Time from bite to rash onset, days (median)	14
(range)	1-75
Largest diameter of solitary EM at presentation	
cm (median)	6
(range)	2-30
Nonspecific symptoms/signs (Nr. %)	
Fever	8 (20%)
Chills	7 (17.5%)
Fatigue	6 (15%)
Paresthesia	6 (15%)
Headache	5 (12.5%)
Arthralgia	6 (15%)
Myalgia	3 (7.5%)
Dizziness	3 (7.5%)
Local Pruritus	5 (7.5%)
Local necrosis	1 (2.5%)
*** local healing** (days) (median)	9.5
(range)	2-28

* time from the first day of antibiotic treatment until the rush disappeared.

The serological profile was investigated in 37 patients (3 patients did not present for serological analyses) and is shown in figure 2. We have studied distinct profiles of titer kinetics for each antibody (Atb) class: persistence of a positive antibody titer (PP), persistence of a negative antibody titer (PN), and decrease of a positive pretreatment antibody titer to a negative antibody titer throughout the observation period (P-N).

There is a predominance of the PN and P-N profile of the IgM antibodies with no statistical differences between group A and group B (P=0.46 and p=1, respectively). The third profile PP was present in both groups, no statistical difference (p=0.22). Less frequent were

described the N-P, N-B (negative-borderline), P-B (positive-borderline) or B-N (borderline-negative). Regarding the IgG antibodies, there is a predominance of the PN serological profile (figure 3).

Table 2. Antibiotherapy followed by the patients

Antibiotic/duration (days)	Solitary EM (N)	Multiple EM (N)
Doxycycline / 14-28	21	0
Amoxicillin / 21	12	1
Cefuroxim axetil / 14	2	0
Ceftriaxone / 14-21	0	3
Amoxicillin/clavulant (8 days)/ cefuroxim axetil (9 days)	1*	0
Total	**36**	**4**

N – patients nr.

* EM with central necrosis.

Table 3. Response to treatment in the two groups of patients

Patients	Partial response (nr.)	Complete response (nr.)	Total (nr.)
Group A (nr. /%)	7 (46.7)	8 (53.3)	15 (100)
Group B (nr. /%)	10 (43.5)	13 (56.5)	23 (100)
Total (nr. /%)	17 (44.8)	21 (55.2)	38 (100)

We have compared the response to treatment and the IgM PP or IgM P-N serological profile (figure 4) and no difference was found (p=0.7; p=0.29). Partial response to treatment was described even in the cases with PN IgM profile.

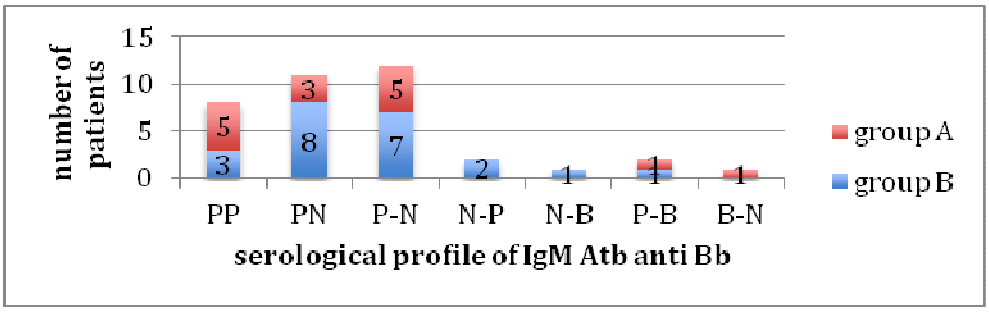

Figure 2. IgM serological profile in the 2 groups of patients

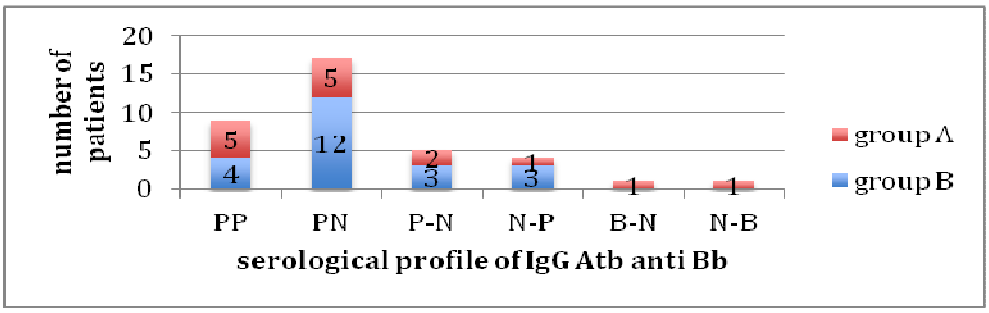

Figure 3. IgG serological profile in the 2 groups of patients

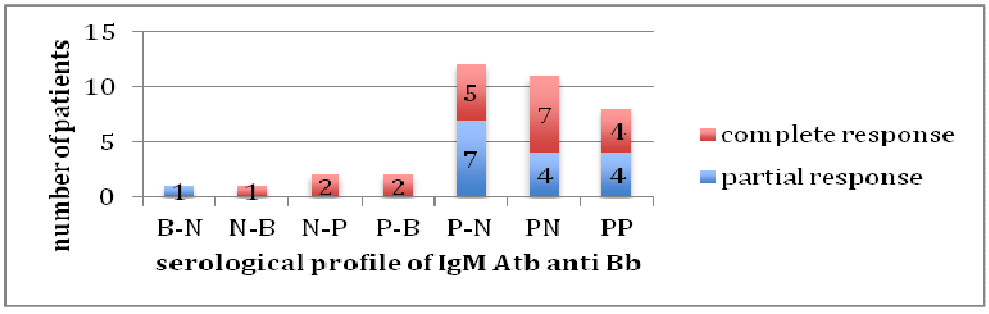

Figure 4. IgM serological profile and response to treatment

Discussion

EM, the hallmark of early LB is the most common manifestation of LB. In Slovenia, one of our neighbouring countries, where LB has been a notifiable disease for 20 years, EM represents 90% of all the cases (Strle and Stanek, 2009) while in USA it represents 70% of the cases (Dandache et al., 2008). The WHO report on LB in Europe (Lindgren and Jaenson, 2006) mentioned "no data" regarding Romania. As so far, no study has provided data regarding epidemiology, clinical characteristics and outcome of patients with EM in Romania, we have compared our results to the results of similar studies performed in Europe and North America.

In USA, only about 1 in 4 patients (14-32%) with EM recall a previous tick bite at the site of the skin lesion (Nadelman et al., 1996; Smith et al., 2002) while in the European studies, the proportion is substantially higher (73%) (Strle et al., 2002), close to the results in our study (85%). Patients with EM who do not recall a tick bite were most probably bitten, but were not aware of it. All of the patients presented a risk for tick bites due to professional or recreational activities in an endemic region, all the cases being diagnosed in warm months with tick activity. Tick bite is not painful and ticks (especially larvae or nymphs) may be unnoticed on the skin due to small dimensions. Although they do not have preferential site for attachment, the location was on the legs in 66% of our cases. This location was predominant in most of EM studies and might be explained by the questing activity of the ticks on the grass, which leads to attachment on humans' legs while walking.

The median diameter varies between 10 and 16 cm, but lesions may exceed 70 cm (Dandache et al., 2008). EM size is a function of its duration and lesions grow at a rate of 20 cm^2/day, presumably related to the migration of spirochetes in an outward direction from the inoculation site. The median diameter of the solitary EM in our study was 6 cm (smaller than in other European or American studies), maximum 30 cm. This might indicate a good medical education of the population in our region regarding LB and tick bites monitoring as patients present early in the course of the disease to the Department of Infectious Diseases.

Studies comparing clinical features in patients infected with *B. afzelii* and *B. garinii* in Slovenia versus patients infected with *B. burgdorferi* s.s. in USA revealed differences (Strle et al., 1999; 2011). The clinical presentation regarding nonspecific symptoms was quite similar in our patients with the Slovenian patients, explained by the predominance of *B. afzelii* and *B. garinii* isolated in ticks collected from humans in our region (Briciu et al., in press). The most common objective physical findings at the time of diagnosis in different studies is regional lymphadenopathy (3.4%-38.7%), more frequent in USA than Europe, but it was not described in our patients.

Multiple EM is defined as the presence of 2 or more skin lesions in an individual patient and is interpreted as a consequence of hematogeneous dissemination of *B. burgdorferi* from the primary EM skin lesion. The frequency of multiple EM is greater in USA (50% of the EM cases) (Steere et al., 1983) than in Europe (3-8% of EM in adults) (Asbrink et al., 1986; Strle et al., 2002) and in children than in adults. Four patients from our study presented multiple EM lesions, and 3 were children.

Five out of the 34 patients that recognized the tick bite at the lesion site developed EM in spite of chemoprophylaxis. The percentage (14.7%) is much higher than it was described in a study performed in Slovenia (Maraspin et al., 2002); 7 out of 5056 Slovenian patients (0.14%) developed EM after antibiotic prophylaxis. We have noticed in our patients a delay from the tick bite to rash onset, maximum 75 days in one of the cases that was administered 10 days of doxycycline. Our data did not enable us to assess the frequency of antimicrobial prophylaxis failure or the efficacy of individual antibiotics for the prevention of LB. However, the five patients presented demonstrate that antibiotic prophylaxis for LB after a tick bite is not entirely effective and it might postpone the onset of the disease.

The importance of serology in the diagnosis of EM is low and the probability of a positive

serology at presentation depends on the disease duration. There is substantial proof in the literature that antibody titer development after therapy is unpredictable and uncorrelated with the clinical course (Glatz et al., 2006). The PN serological profile of IgM antibodies was described in 11 cases and the PN profile of IgG antibodies in 17 of our patients, 6 of them having both results PN. In earlier studies, 40% to 100% of EM cases were found to remain seronegative during follow-up (Feder et al., 1992; Hulshof et al., 1997; Lomholt et al., 2000). Because of the absence of a specific immune response it could be questioned whether the diagnosis of EM was correct in these patients. However, the clinical diagnosis in EM is specific and the international guidelines do not recommend laboratory confirmation (Wormser et al., 2006; Stanek et al., 2012). Before therapy, IgG or IgM antibodies to *B burgdorferi* are lacking because seroconversion does not occur before 2 to 4 weeks of infection. After therapy, the lack of antibody development may be due to abrogation of infection by adequate antimicrobial treatment. A number of treated, initially nonreactive patients might, therefore, remain seronegative. The second profile, IgM PP was described in 8 patients and IgG PP was described in 9 of our patients. It was speculated that persistent IgM seropositivity indicates a continuing specific immune stimulation by noneradicated spirochetes with the potential consequence of ongoing symptoms or sequelae of Lyme disease. In our study the IgM PP profile was described in patient with both complete and partial response to therapy, and similar results were found by Glatz et al. (2006). Persistence of IgM and IgG antibodies was described even 10-20 years after infection in asymptomatic patients (Kalish et al., 2001).

So far, analyses of serum anti *B. burgdorferi* s.l. antibodies have been frequently used because of the misconception that serological follow-up examinations could support the assessment of the clinical course after treatment; in clinical practice physicians tend to retreat patients whose antibody titers do not decline soon after therapy. Our data brings additional evidence that the serological profile is unpredictable and uncorrelated with the clinical course, and sustain the recent recommendation of avoiding repeated serologic testing for assessing therapy efficacy (Mullegger and Glatz, 2009). The assessment of patients with EM in the follow-up rests primarily on the clinical picture.

The long time prognosis is considered good based on the studies from USA (Plörer et al., 1993; Breier et al.,1996; Kowlaski et al., 2010), while there are only a few studies performed in Europe (Lipsker et al., 2002; Cerar et al., 2010). We have to be aware of the fact that if different genospecies on the 2 continents are associated with different clinical presentations, long time clinical outcome might also be different. The French study (Lipsker et al., 2002) evaluated in 2000, by telephone interview, 37 patients treated for EM between 1995-1999, with no record of late LB. Though we have described nonspecific symptoms in 10 of the patients followed for 9 months to 1 year, only one patient suffering for degenerative arthrosis was diagnosed as LB with musculoskeletal manifestations (sustained clinically and serologically by high IgG anti *B. burgdorferi* antibody titers as recommended by the guidelines) and retreated. Though a prospective and a large retrospective clinical trial have shown that for doxycycline 10 days of treatment is as effective as 20 days (Wormser, 2003; Kowalski, 2010) and the 10-21 days regimen has been included in the treatment guideline for LB of the Infectious Diseases Society of America, in Europe most of the recommendations include 14-21 days for doxycycline similar to amoxicillin or cefuroxim axetil. The patient diagnosed as late LB in our study group was the only one who has stopped the treatment before the 14th day, due to side effects. When analyzing clinical outcome in LB patients we have to consider the possible confusion factor due to stress and publicity regarding LB. Another explanation for the unspecific symptomatology could be found in the study performed by Cerar et al. (2010); the clinical outcome was compared in EM and a control group of healthy persons and the authors reported similar symptomatology in both groups, though providing evidence for unexplained symptoms in the general population that could be misinterpreted as “chronic LB”.

The present study represents the first epidemiological, serological and clinical study on EM in Romania. The small number of the

patients included and the short time follow up represent limits in interpreting the clinical outcome and prognosis. Continuing the study in the following years could bring new data regarding early LB in our region.

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