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Serological and Clinical one year follow-up of Patients with Erythema migrans treated in a Romanian Infectious Disease Hospital

Monitorizarea serologică și clinică pe durata unui an a pacienților cu eritem migrator într-un spital de boli infecțioase românesc

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Abstract

*Erythema migrans (EM) is the most common and recognized clinical manifestation of early Lyme Borreliosis (LB) in Europe. **Purpose:** to evaluate clinically and serologically all the patients with EM and to correlate the serology with clinical approach. **Material and method:** A prospective analyses of all patients that came with EM in our University Hospital of Infectious Diseases from Cluj-Napoca Romania between 1st of April to 31st of August 2011. We registered data regarding: age, gender, interval between tick bite and EM occurrence, interval between EM appearance and hospital presentation, serology (IgM and IgG for *Borrelia burgdorferi* sl) at presentation and one year after; antibiotic treatment, duration of antibiotic treatment and clinical outcome. **Results:** 44 patients with EM, 2 had Multiple EM, 23 male (52 %), average age 41.3 years (min 3 years, max 84 years, median 43.5 years). Serology at presentation was positive in 15 patients for IgM and in 9 patients for IgG with ELISA and for 13 for IgM and 16 for IgG with WB. At the one year follow up positive results for IgM were found in 14 patients with ELISA and in 11 patients with WB. IgG was positive in 7 patients with ELISA and in 5 with WB. The average time interval between the tick bite and EM was not significant different for patients with negative or positive IgM or IgG. The interval between EM occurrence and treatment initiation had a strong significance for IgG. After one year no statistical significance was found for positive IgM for any of the analyzed factors while for IgG positivity we found a significant importance for age and duration of incubation. No influence of antibiotic or duration of treatment was found on serology of our patients. **Conclusions:** Our data brings additional evidence that the serological profile is unpredictable.*

Keywords: Lyme disease, Erythema migrans, serology, follow up, Borreliosis

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Rezumat

*Eritemul migrator (EM) este cea mai comună și recunoscută manifestare clinică precoce a Borreliozei Lyme (LB) în Europa. Scop: de a evalua clinic și serologic toți pacienții cu EM și de a corela serologia cu abordarea clinică. Material și metodă: Un studiu prospectiv incluzând toți pacienții care au venit cu EM la Spitalul Clinic de Boli Infecțioase din Cluj - Napoca România între 1 aprilie și 31 august 2011. Am înregistrat date referitoare la: vârstă, sex, intervalul între înțepătura de căpușă și apariția EM, intervalul între apariția EM și prezentarea la spital, serologia (IgM și IgG pentru *Borrelia burgdorferi* sl) la prezentare și după un an, tratamentul cu antibiotice, durata tratamentului cu antibiotice și evoluția clinică. Rezultate: 44 pacienți cu EM, 2 au avut EM multiplu, 23 bărbați (52%), cu vârsta medie de 41.3 ani (min 3 ani, maxim 84 ani, în medie 43.5 ani). Serologia la prezentare a fost pozitivă la 15 pacienți pentru IgM și la 9 pacienți pentru IgG cu ELISA și la 13 pentru IgM și la 16 pentru IgG cu WB. După un an de urmărire rezultate pozitive pentru IgM au fost găsite la 14 pacienți cu ELISA și la 11 de pacienți cu WB. IgG a fost pozitivă la 7 pacienți cu ELISA și la 5 cu WB. Intervalul mediu de timp între mușcătura de căpușă și EM nu a fost semnificativ diferit pentru pacienții cu IgM sau IgG negativ sau pozitiv. Intervalul dintre apariția EM și inițierea tratamentului a avut semnificație statistică pentru IgG pozitiv. După un an nu a fost găsită nici o semnificație statistică pentru IgM pozitiv pentru niciunul dintre factorii analizați, în timp ce pentru IgG pozitivitate a fost semnificativ asociată cu vârsta și durata perioadei de incubație. Tratamentul antibiotic și durata acestuia nu au influențat serologia pacienților noștri. Concluzii: Datele noastre aduc dovezi suplimentare că profilul serologic este imprevizibil la pacienții cu Borrelioză Lyme.*

Cuvinte cheie: Boala Lyme, Eritem migrator, serologie, monitorizare, Borrelioza

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Lyme borreliosis (LB) has been reported throughout Europe as the most common tick-borne infection and it became an important pathology in the Eastern part of Europe in the last 10 years. The sharp increase in the number of ticks and the extension of the tick's geographic range have increased the risk that tick-borne infections will be transmitted more frequently to humans.[1] Erythema migrans (EM) is the most common and recognized clinical manifestation of early LB in Europe.[2-5] In Romania preliminary studies have proved that 11-18% of *Ixodes ricinus* ticks are infected with *Borrelia burgdorferi* sensu lato (sl) strains mainly *B afzelii*. [6,7]

The seroprevalence of *Borrelia burgdorferi* sl antibodies in humans differs between general population (4.3%) and at risk population (9.3%). [8]

Public perception of the disease in Romania has been distorted by the media and the degree of awareness increased, so the rule is that patients with tick bite and EM are examined by

infectious diseases specialists.

The aim of our study was to evaluate clinically and serologically all the patients presented with EM during a tick season (from 1st of April 2011 to 31 of August 2011). For one year they were followed up and serology was correlated with clinical approach.

Material and methods

This study involved a prospective analysis of all patients that presented with EM in our University Hospital of Infectious Diseases in Cluj-Napoca Romania between 1st of April to 31st of August 2011.

The clinical recognition of skin lesions was performed by an infectious diseases specialist in accordance with the definition of EUCALB. [9] In all patients we recorded data regarding: age, gender, date of tick bite, interval between tick bite and EM occurrence, interval between EM appearance and hospital presentation, diameter and localization of EM, serology (IgM and

IgG for *Borrelia burgdorferi* sl) at presentation and one year after antibiotic treatment (between April and June 2012), antibiotic treatment, duration of antibiotic treatment and clinical outcome.

All patients were treated in accordance with the EUCALB and IDSA recommendation for 10 to 28 days.[10] The antibiotics used were: doxycycline (Doxiciclina, Antibiotice SA), ceftriaxone (Medaxone, Medochemie LTD), amoxicillin (Amoxicilina, Antibiotice SA), clarithromycin (Klabax, Terapia SA), azithromycin (Sumamed, Teva PH), cefuroxime (Zinnat, GlaxoSK). Doxycycline, the preferred oral regimen, was not prescribed for children younger than 8 or pregnant women, those with known allergic, gastric or liver diseases and those at risk from exposure to the sun (photosensitivity).

Serology was performed from the same blood sample with Anti-Borrelia EUROLINE-RN-AT plus VlsE (IgG) ELISA and EUROLINE Borrelia-RN-AT (p18, p19, p20, p21, p58, OspC, p39, p83, LBb, LBa, VlsE Bg, VlsE Bb, VlsE Ba) WB. The antigens used in the Anti-Borrelia plus VlsE ELISA are whole extracts of the strains *B. burgdorferi*, *B. afzelii* and *B. garinii*, as well as recombinant VlsE. The IgM kit uses recombinant, covalently bound dimeric OspC antigens from *B. burgdorferi*, *B. afzelii*, *B. garinii* and *B. spielmannii*. We used the interpretation criteria recommended by the EUROIMMUN provider: for IgM/ IgG ELISA < 16 RU/ml nonreactive, $\geq 16 - <22$ RU/ml borderline, and ≥ 22 RU/ml reactive. We confirmed with WB according with: IgM positive if one of OspCBa, OspCBb, OspCBg, OspC Bs is intense positive or if is weak positive p39 or VlsEBb should be positive; borderline means that OspCBa or OspCBg are weak positive; and for positive IgG should have at least one VlsEBb intense positive or weak positive in addition with one or more other bands (p18, p19, p20, p21, p58, OspC (p25), p39, p83, Lipid Bb, Lipid Ba, VlsEBa, VlsEBg) and borderline when alone VlsE Bb is weak positive or two other bands are positive.[11] We have inter-

preted WB borderline results as positive when ELISA was positive and negative when ELISA was borderline or negative.

The clinical evolution was assessed for each patient by the same physician at the beginning of treatment and at the one-year follow up, by completing a questionnaire and medical examination.

The study was approved by the Hospital Ethic Committee (No 2/2011) and all patients signed an informed consent.

Statistical comparison of percentages was performed by Chi squared test and comparison of means by Student's test [12]. The time intervals between tick bite and EM and the start of treatment were analyzed with Kaplan-Meier curves and differences between curves were evaluated by log-rank test. ROC (receiver operator characteristics curve) curves were used in the classic way to highlight influences of numerical factors in the status of patients at one year follow-up (positivity of IgM and IgG). The cut-off on the ROC curves was evaluated by identifying the closest point of the curve to the point (0,1) of absolute classification. Confidence interval (CI) where needed was evaluated at 95% level.

Results

44 patients (8 children and 36 adults) were diagnosed with EM between Apr – Aug 2011, 2 had Multiple EM. The demographic data of our patients are shown in *Table I*.

Table I. Demographic characteristics of patients with EM

Patients		No
sex	female	21
	male	23
Age	Min	3
	Max	84
	Average	41.3
	Median	43.5

Because of the close temporal proximity of tick bites and onset of EM, this manifestation of LB has a pronounced seasonal occurrence. More than 60% of our patients were diagnosed in June and July. (Figure 1) All patients, except 3 of them, noticed recent tick bites (93%).

The time interval between tick bite and EM occurrence varied between 1 day and 60 days with an average of 15 days (95% CI: 10.4-18.8) and a median of 11 days. (Figure 2)

The time interval between EM occurrence and hospital presentation varied between 1 day

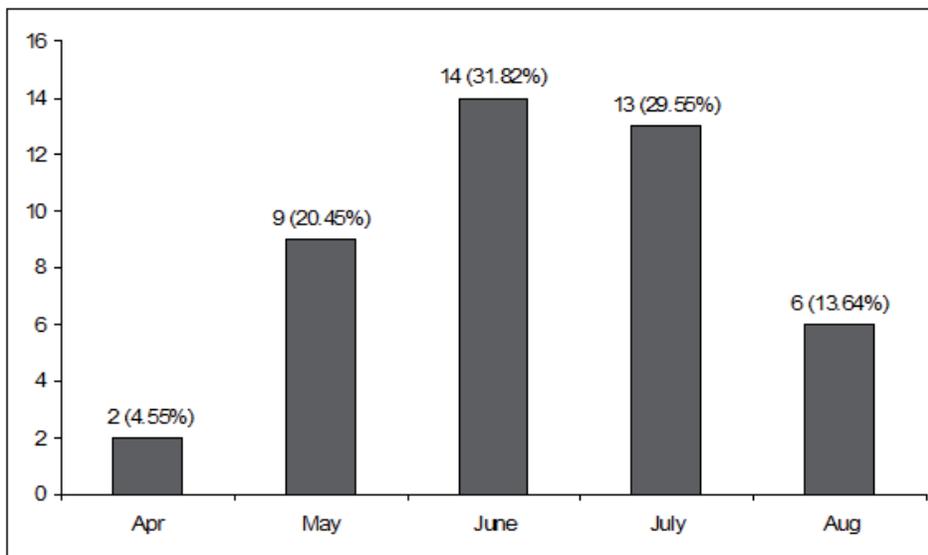


Figure 1. Seasonality of Erythema migrans.

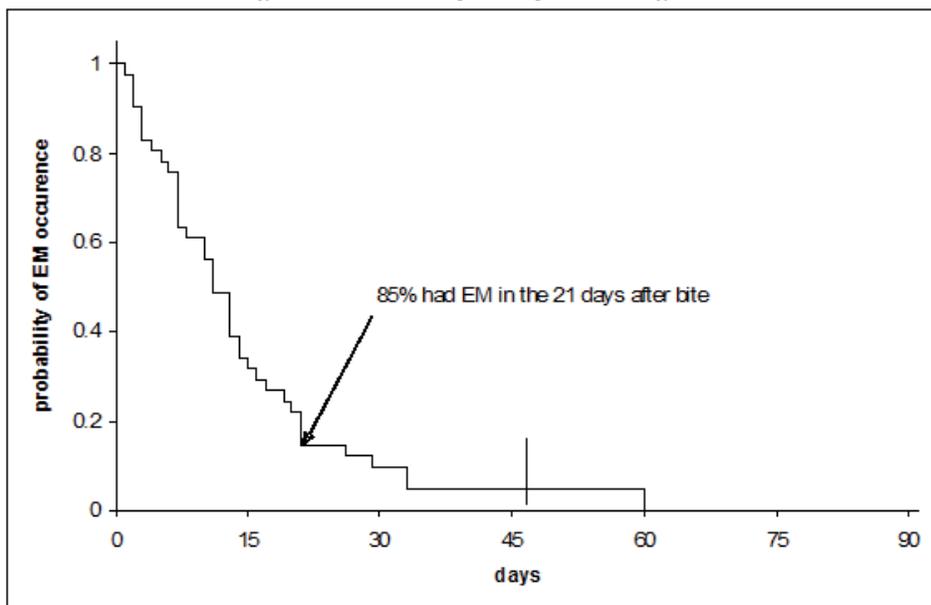


Figure 2. Kaplan-Meier curve for the interval between tick bite and Erythema migrans.

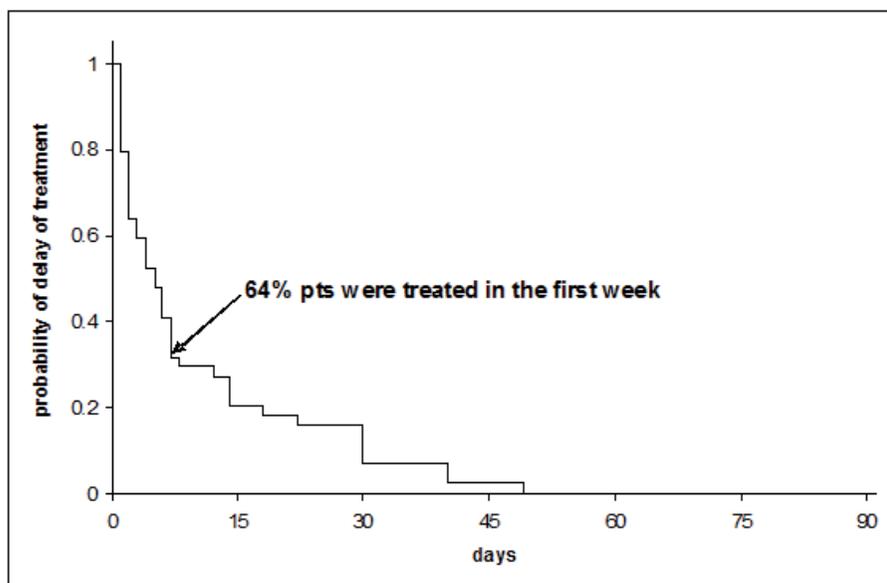


Figure 3. Kaplan-Meier curve for the interval between Erythema migrans occurrence and start of treatment.

Table II. Serological (IgM and IgG) evaluation at presentation for patients with Erythema migrans

		WB*** 1 IgM				
		negative	borderline	positive	?	Total
ELISA*1	negative	21 (72%)	3(10%)	4 (14%)	1 (4%)	29
IgM**	positive	5 (33%)	1 (7%)	9 (60%)		15
	Total	26 (59%)	4 (9%)	13 (30%)	1 (2%)	44

		WB 1 IgG				
		negative	borderline	positive	?	Total
ELISA1	negative	11 (31%)	12 (35%)	11 (31%)	1 (3%)	35
IgG****	positive	1 (11%)	1 (11%)	7 (78%)		9
	Total	12 (27%)	13 (30%)	18 (41%)	1 (2%)	44

* ELISA = enzyme-linked immunosorbent assay

***WB =Western blot

**IgM = Immunoglobulin M

****IgG = Immunoglobulin G

and 49 days with an average of 10.2 days (95% CI: 6.4-14.0) and a median of 5 days.(Figure 3)

The localization of EM was mainly on the leg 28/44 (64%) and the median diameter was 6 cm (range 2-30 cm).

Serology at presentation was positive in 15 patients for IgM (34%) and in 9 patients for IgG (20%) with ELISA and for 13 out of 43 for IgM

(30%) and 16 out of 43 for IgG (37%) with WB (1 patient was not tested with WB). (Table II)

31 patients were treated with doxycycline 100 mg bid, 14 patients for 10 - 14 days and 17 for 21-28 days. Amoxicillin was used in 9 patients at a dose of 500 - 1000 mg tid for 21 days, 4 patients were treated with clarithromycin 500 mg bid for 14 days, azithromycin 500 mg qd for

Table III. Antibiotic treatment of patients with Erythema migrans

Treatment	Duration of treatment (days)	No of patients	Remarks
amoxicilline	21	9	6 children < 8years, one pregnant woman, 2 allergic adults to doxycycline
azithromycin	8	1	1 allergic adult
ceftriaxone	21	1	1 child with MEM
clarithromycin	14	1	1 allergic adult
doxycycline	10	3	All adults except one child >8 years old
doxycycline	14	11	
doxycycline	21	16	
doxycycline	28	1	
cefuroxime	14	1	
Total		44	

8 days, cefuroxime 500 mg bid for 14 days or ceftriaxone 2g qd for 21 days due to Multiple Erythema Migrans (MEM) and contraindication of doxycycline treatment. (Table III)

Time for local response (clearing of EM lesion) under treatment was 8.5 days (3-14 days). 8 out of 44 patients presented different complains during the one year follow up period. Fever without another cause appeared in one patient, arthralgia in 4 patients, arthritis in one patient, headache and memory disorder in 2 patients. 5 of these patients were treated with doxycycline, 4 patients for 21 days and one for

14 days, 2 were treated with amoxicillin and one with azytromycin. Retreatment recommended during the one year follow-up was performed for 21 days with: doxycycline in 2 patients, amoxicillin in 3 patients and ceftriaxone in 3 patients. After the second treatment the clinical evolution was good.

At the one year follow up serology was performed in 40 patients, and 4 patients could not be followed up. Positive results for IgM were found in 14 patients (35%) with ELISA and in 11 patients (27%) with WB. IgG was positive in 7 patients (17%) with ELISA and in 5 (12%) with WB. (Table IV)

Table IV. Serological (IgM and IgG) evaluation at one year follow up for patients with Erythema migrans

		WB 2 IgM			
		negative	borderline	positive	Total
ELISA 2 IgM	negative	23 (88%)		3 (12%)	26
	positive	3 (21.5%)	3 (21.5%)	8 (57%)	14
	Total	26 (65%)	3 (7.5%)	11 (27.5%)	40
		WB 2 IgG			
		negative	borderline	positive	Total
ELISA 2 IgG	negative	26 (79%)	5 (15%)	2 (6%)	33
	positive	4 (57%)		3 (43%)	7
	Total	30 (75%)	5 (12.5%)	5 (12.5%)	40

Table V. The evolution of serology during one year in patients with treated Erythema migrans

Evolution of serology	No of patients				Total
	P-P	P-N	N-N	N-P	
IgM	10	4	22	4	40
IgG	4	13	22	1	40

(P-P= positive-positive; P-N= positive-negative; N-N=negative-negative; N-P=negative-positive before treatment and one year after treatment)

Taking into account both ELISA and WB results, IgM and IgG stayed negative in 22/40 patients (55%). Ten out of 14 patients with positive IgM (71%) and 4 out of 17 patients with positive IgG (23%) in the moment of diagnosis remained positive one year after treatment. One year after treatment 4 patients were found to have negative IgM and 13 patients negative IgG after being positive at the beginning of the study. From 26 IgM negative patients 4 became positive and from 23 IgG negative patients one became positive.(Table V)

The average time interval between the tick bite and EM occurrence was not significantly different for patients with negative or positive IgM or IgG (11.9 days for negative IgM com-

pared to 19.9 days for positive IgM, $p = 0.067$ respectively 11.4 days versus 15.2 days with $p = 0.497$ for IgG). When we considered the interval between EM occurrence and treatment initiation we had a strong significance for IgG (7.9 days for negative compared to 19.2 for positive, with $p = 0.013$).

When we analyzed the results using ROC curves for one year positivity of IgM we did not find statistical significance for any of the analyzed factors: EM - treatment interval, tick bite - EM interval, age, antibiotic treatment and duration of antibiotic treatment.(Figure 4)

Looking also at one year IgG positivity, ROC curves show a significant importance for age ($p = 0.03$, cut off = 57 years) and duration of

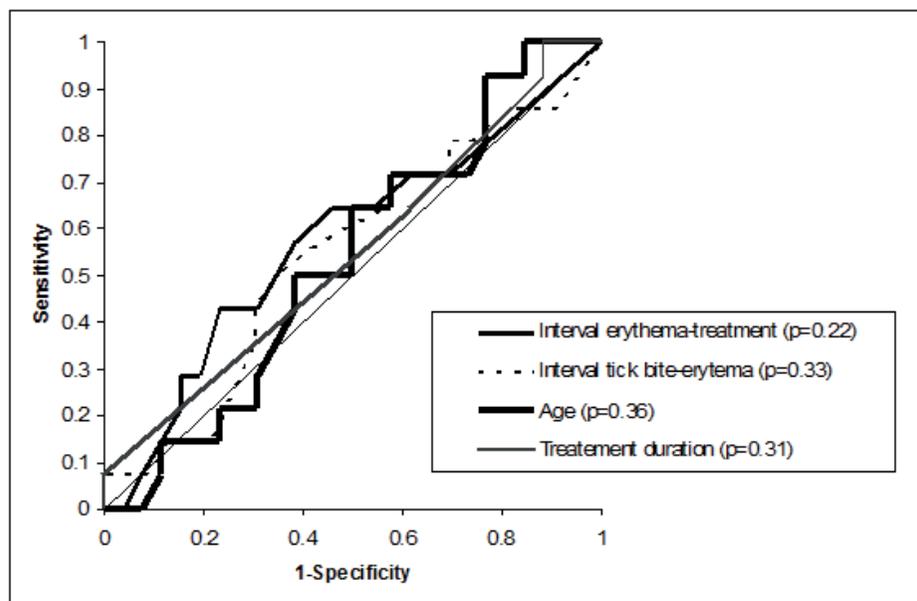


Figure 4. IgM positive at one year follow-up in patients with treated Erythema migrans

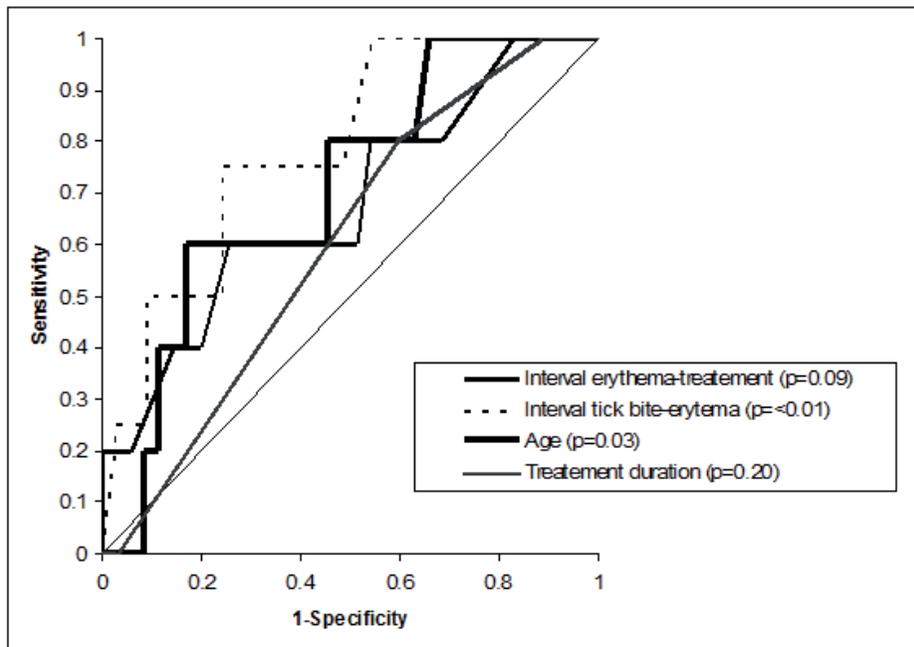


Figure 5. IgG positive at one year follow-up in patients with treated Erythema migrans

incubation ($p = 0.01$, cut off = 16 days) but for delayed treatment only a trend was observed ($p = 0.09$, cut off = 16 days). (Figure 5).

When we tried to assess the influence of antibiotics on serology of our patients one year after treatment we found no differences: 3/9=33% positive with amoxicillin versus 10/31=32% positive with doxycycline for IgM ($p=0.73$) and 2/9=22% with amoxicillin versus 3/27=16% positive with doxycycline for IgG ($p=0.78$). ROC curve for the duration of antibiotic treatment with doxycycline (10-14 days compared with 21-28 days) also revealed no influence for both IgG and IgM ($p=0.20$ and 0.31).

Discussions

Lyme borreliosis is a tick borne infection transmitted by *Ixodes ricinus* ticks in Europe.[13] It is caused by different *Borrelia* species belonging to the *B. burgdorferi* sl complex. EM is more associated with infection due to *B afzelii*. [14]

The seasonal pattern of *Ixodes ricinus* ticks activity is observed also in EM, the most frequent clinical manifestation of Early Lyme borreliosis in our country as in all European endemic countries.[15,16] According to the literature all *Borrelia* species can cause EM although in middle Europe it is most commonly related to *B afzelii*. [17]

EM usually appears at the site of a tick bite after a median time of 17 days in Europe and 11 days in the USA.[18] In our study the incubation period was similar to that found in USA patients from the previous study. One explanation could be the fact that the incubation period for *B afzelii* infection is shorter than for other genospecies and our studies of infection in ticks from human showed that *B afzelii* was the most prevalent genospecies (data not published). [19]

The median duration of rash at presentation in our study was shorter than in the Slovenian patients from the study performed in 1999 (14

days), but similar to that reported in a recent study from Slovenia.[18,20] This might indicate a good medical education of the population in the recent years regarding Lyme borreliosis and tick bites monitoring, as patients come in the early stage of the disease to the Department of Infectious Diseases.

To increase the specificity of the diagnosis, a single primary lesion must reach 5 cm in size, in order to distinguish EM from an 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.[21] 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. In USA the median diameter of EM varies between 10 and 16 cm, but lesions may exceed 70 cm.[21] EM size is a function of its duration and lesions grow at a rate of 20 cm²/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 be explained by the early presentation of the patients in our department after the clinical onset, due to accessible medical information.

The clinical diagnosis in EM is specific and the international guidelines do not recommend laboratory confirmation.[10,22] 71.6% of patients with culture confirmed EM had positive IgM and/or IgG anti *B. burgdorferi* s.l. antibodies at presentation.[23] The probability of a positive serology at presentation depends on the disease duration. In our study we found a strong correlation of positive IgG at presentation with a delay in starting treatment (longer than 19.2 days).

There is substantial proof in the literature that antibody titer development after therapy is unpredictable and uncorrelated with the clinical course.[24] 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 may therefore remain seronegative. More than half of the patients included in our study remained negative both for IgM and/or IgG specific antibodies. However, we cannot exclude the possibility that some of these patients had seroconverted after the start of treatment, and had become seronegative again before the 1-year follow-up visit. The short interval between clinical onset and treatment initiation was proved to be associated with persistent IgG negative antibodies.

It was speculated that persistent IgM seropositivity indicates a continuing specific immune stimulation by non-eradicated spirochetes with the potential consequence of ongoing symptoms or sequelae of Lyme borreliosis. In our study the IgM persistent positive profile was described in 10 patients, all with good response to antibiotic therapy and similar results were found by Glatz et al. [23]. In the Slovenian study of Stupica et al., [24], 44.14% of patients presented were seropositive at the one year follow up, while 96.1% of the patients had a complete clinical response to therapy. The former study showed that the proportion of seropositive patients significantly decreased between the 2nd and the 6th month follow-ups. Seropositivity to *Borrelia burgdorferi* s.l, either at presentation or follow-up, was found in independent studies not to be a risk factor for treatment failure.[23,25-29] Persistence of IgM and IgG antibodies was described even 10-20 years after infection in asymptomatic patients.[30] It might be assumed that sustained positive titers indicate a long-term serologic memory that results from an antigen-independent polyclonal activation and differentiation of memory B cells.[31]

The age of the patients, the interval between tick bite and EM occurrence, the interval between EM occurrence and treatment initiation or duration of antibiotic treatment were not associated in our study with positive IgM antibodies at the one year follow up. Glatz et al [24], and Agüero Rosenfeld et al [32] found that the IgG antibody response was significantly correlated with the duration of EM before therapy and the large size of the EM, which was not concordant with our results even if there was a tendency for longer interval for positive IgG serology at one year.[24,32] In our study the IgG positive antibodies were significantly higher at the follow up in patients older than 57, and with longer incubation periods. The type or duration of antibiotic treatment did not influence serological profile at one year in our study or in the larger study on 113 patients of Glatz et al.[24].

Our data brings additional evidence that the serological profile is unpredictable and not influenced by the treatment recommended or duration of antibiotics and support the recent recommendation of avoiding repeated serologic testing for assessing treatment efficacy.[33] The assessment of patients with EM in the follow-up rests primarily on the clinical picture.

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