CLINICAL STUDY

Antibodies to *Borrelia burgdorferi* in erythema migrans patients

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Abstract

Determination of antibodies against *Borrelia burgdorferi* has supporting value in the diagnose of Lyme disease. The purpose of this study was to determine the production of antibodies in a defined group of patients.
Material and methods: **The study analysed antibodies in the group of 25 patients with erythema migrans. For the detection of antibodies Immunofluorescence methods, ELISA and Western blot were used.**
Results: **The detection of antibodies by Imunoflorescence methods proved positivity of the titre 1:256 in 14 patients and 9 patients were borderline, with the titre 1:128. Majority of the antibodies detected were of IgM class. The ELISA IgM test found positive reaction in 12 patients and 2 borderline results. IgG antibodies were found significantly more often by ELISA test than by Immunofluorescence. The Western blotting results were IgM positive in 9 patients, 6 were borderline. Only 5 patients have positive IgM results in all tests (Immunofluorescence, ELISA and at least one positive result out of three IgM Western blots).**
Discussion and conclusion: **The tests which detect *Borrelia burgdorferi* antibodies are not standardized. They have variable sensitivity and specificity and their standardization is complicated with respect to great heterogenicity of *Borrelia burgdorferi* strains circulating in individual regions of Europe. The high specificity of antibodies to individual borrelia antigens are presently pointing towards the need to use, when in diagnostic confusion, more tests, which could detect antibodies also to other borrelia antigens *(Tab. 3, Ref. 14)*. Full Text (Free, PDF) *www.bmj.sk*.**
Key words: *Borrelia burgdorferi*, erythema migrans, ELISA, Western blot, immunofluorescence.

Lyme borreliosis is a natural focality disease, which occurs in moderate climates of the north globe. It is the most common tick borne disease in Europe. Its incidence peaks towards the end of spring and in summer months. In 70 % of patients it manifests as erythema migrans. In the year 2005, the annual prevalence was reported to be 15.65 per 100 000, which represented 843 cases (12). It is assumed that a share of cases is unreported, which correlates with a high prevalence of *Borrelia burgdorferi* antibodies in healthy population (10.4 %), although a part of cases has probably clinically unapparent course (2). High prevalence of *borrelia* in ticks reaches in some regions of Slovakia 33 %, which suggests frequent contact of human population with infected ticks and the possibility of acquiring this ailment (4, 7). Defects in reporting systems can be related to problems in diagnosing the disease, especially in its advanced stages. Diagnosis of erythema migrans is based on clinical presentation and epidemiologic history. Specific antibodies are present in acute phase approximately in 30–40 % of patients.

The goal of this study was to compare results of antibody testing in patients with diagnosed erythema migrans while using various laboratory methods.

Material and methods

The study was conducted in cooperation with Department of Dermatovenerology at hospital in Bratislava. Within the group of patients diagnosed with erythema migrans during 2001–2005, patient age and gender, area of residence, data about tick bite

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Tab. 1. Borrelia burgdorferi antibody test results in erythema migrans patients.

Immunofluorescence				ELISA		Western blot		
Patient	**general**	**IgM**	**IgG**	**IgM**	**IgG**	**B.a.**	**B.b.s.s.**	**B.g**
1	neg	1:128	neg.	pos	pos	pos	neg.	pos
2	1:512	1:128	1:256	pos	neg	bd.poz.	neg	neg
3	1:128	1:512	neg	pos	neg	pos	pos	pos
4	1:256	1:128	1:128	pos	neg	pos	pos	pos
5	1:256	1:256	1:128	pos	pos	pos	pos	pos
6	1:1024	1:256	1:512	bd.pos	neg	pos	neg	bd.poz.
7	1:512	1:512	neg	pos	pos	pos	pos	pos
8	1:2048	1:2048	1:128	pos	neg	pos	pos	pos
9	1:512	1:512	1:128	neg	pos	bd.pos	bd.pos	bd.pos
10	1:1024	1:1024	neg	bd.pos	pos	neg	neg	bd.pos
11	1:512	1:512	1:128	neg	neg	neg	bd.pos	bd.pos
12	1:256	1:128	1:128	neg	neg	neg	neg	neg
13	1:128	1:256	neg.	neg	neg	neg	neg	neg
14	1:128	1:128	1:128	neg	pos	neg	neg	neg
15	1:512	1:256	1:128	pos	pos	neg	neg	bd.pos
16	1:128	1:256	neg.	pos	neg	neg	bd.pos	neg
17	1:128	1:256	neg.	pos	pos	neg	pos	pos
18	1:256	1:256	neg.	pos	neg	neg	neg	neg
19	1:128	1:256	neg	neg	pos	neg	neg	neg
20	1:128	1:512	neg.	neg	pos	neg	neg	neg
21	1:256	1:1024	neg	neg	neg	neg	neg	neg
22	1:128	1:256	1:128	neg	neg	neg	neg	neg
23	1:128	1:256	neg	neg	neg	neg	neg	neg
24	1:256	1:128	1:128	pos	neg	neg	pos	pos
25	neg	1:128	neg.	neg	neg	neg	neg	neg

Abbreviations: B.a. - *Borrelia afzelii*, B.g. - *Borrelia garinii*, B.b. – *Borrelia burgdorferi s.s.*
pos – positive result, neg – negative, bd.pos – borderline positive

and the topography of erythema migrans focus were analyzed. The study included 25 patients, 15 of those were women with average age of 58 (ranging from 33 to 81 years) and 10 were men with average age of 30 (23 to 68 years). Samples of serum were obtained prior to antibiotic therapy initiation.

Detection of antibodies

Via immunofluorescence, IgG+IgM+IgA were tested as a group, and IgG and IgM also individually. Whole cell cultures of *Borrelia burgdorferi sensu lato* (*Borrelia burgdorferi s.s*. American strain B31, isolated from *Ixodes scapularis* and *Borrelia garinii* K48, an isolate from *Ixodes ricinus* from western Slovakia), were used as antigens (9). Titres of 1:256 and higher were read as positive.

IgM and IgM were also tested via ELISA, using commercially available (EUROIMMUN), which contained extracts of *Borrelia burgdorferi s.s. Borrelia afzelii* and *Borrelia garinii*. The test for IgG detection also contained recombinant antigen VlsE from *Borrelia burgdorferi.* Results were determined based on manufacturer's guidelines.

Also the qualitative IgM via Western blot (WB) was detected. Individual commercial kits contained electrophoretically separated antigens from the manufacturer (EUROIMMUN):

1. WB *Borrelia garinii*, 2.WB *Borrelia afzelii*, 3. WB *Borrelia burgdorferi s.s.*

Results

Patients originated from western Slovakia, especially from Bratislava County. Erythema migrans was in majority of cases (68 %) present on lower extremity.

While testing general antibodies via indirect IF, we found their presence in titres of 1:256 and higher in 14 from 25 patients (56 %) (Tabs 1 and 2); 9 of the patients had a borderline titre of 1:128. Majority of the cases were represented by IgM. IgG antibodies were present only in two patients.

Tab. 2. Immunofluorescence, ELISA and WB tests results in erythema migrans patients.

n=25		**pos.**	**bd.pos.**	**neg.**
Immunofluorescence	**IgM**	18 (72%)	7 (28%)	0
	IgG	2 (8%)	10 (40%)	13 (52%)
	general	14 (56%)	9 (36%)	2 (8%)
ELISA	**IgM**	12 (48%)	2 (8%)	11 (44%)
	IgG	10 (40%)	0	15 (60%)
WB	**B. afzelii**	7(28%)	2(8%)	16(64%)
	B. burg. s.s.	7(28%)	3(12%)	15(60%)
	B. garinii	8(32%)	5(20%)	12(48%)

Abbreviations: n – number of patients, general – IgA+ IgM+IgG, WB – western blot

Tab. 3. Antibodies to individual *B. burgdorferi* antigens via Western blot in erythema migrans patients.

	western blot			incubation period
	B. afzelii	**B. burgdorferi s.s.**	**B. garinii**	
1	**Osp C**, **p30**,35,41,62		**OspC, p30,** 41	1,5m
2	p41,60,62			3w
3	**Osp C**	**OspC**	**OspC**	2w
4	**Osp C**, p41, 60	**OspC, OspA,** p41, 36, 62**, 83**	**OspC**, p41, 57, **83**	3m
5	**Osp C,** p35,41,60,62,75	**OspC,** p41, **83**	**OspC,** p41, **83**	1m
6	**Osp C**, p41		**OspC***	unknown
7	**Osp C**, p41,62,	**OspC**	**OspC,** p41	1m
8	**Osp C,** p41,35	**OspC**, p41, 43, 57	**OspC**, p41	1m
9	p41,43,60,62,75**,83***	**OspA*,** p41*	p41	1m
10	p41*	p41*	p41	3w
11	p41*,50,60,62	**p83***	**p83***	1m
12	p41*			1m
13	p 41*	p62	p3	unknown
14	p41*, 60,62			2w
15	p41*	p41*	**OspC*,** p41	2m
16	p41*,60,62	p41, **83**	p37, 41*, 50, 57, 62	1m
17	p41*	**OspC**, p41	**OspC**, p41	3w
18	p41*			3w
19	p41*,60,62			2m
20	p41*			1,5m
21	p41*		p62, p67	2m
22	p41*,60,62	p41*	p41*	2w
23	p41*	p62, 57,	p62	2m
24		**OspC**, p41	**OspC**, p41	3w
25				1m

Abbreviations: Incubation period-period from tick bite to erythema migrans formation, w – week, m – month, * weak line

ELISA IgM was positive in 12 (48 %) patients and 2 patients were borderline positive (Tab. 2). Presence of IgG antibodies was more frequently detected via ELISA when compared to IFT. (Tab. 1). In five patients a concurrent presence of IgM and IgG was detected.

Western blot confirmed the presence of specific antibodies detected via general IFT in 6 patients; 5 had borderline positive results and in 3 cases the results were negative.

In all ELISA IgM positive results, western blot confirmed specificity in 8 cases, 3 results were borderline positive and 1 was negative. Western blot was borderline positive in 2 cases of negative IgG ELISA. When comparing results of 3 Western blots, 5 patients had specific antibodies in all tests (Tab. 1), 3 patients tested positive in 2 tests only and 1 patient had only 1 test positive with antigens of *Borrelia afzelii*.

The most common antibodies were against flagellar antigen p41, which were present in 23 patients (Tab. 3). In 3 patients these were the only antibodies present, although the tests were performed 3 weeks, 1 month and 1.5 month after the initial disease presentation. Antibodies to OspC antigen, which is highly specific, were present in 10 (Tab. 3) patients, in 1 of them these were the only antibodies, performed 2 weeks after the disease onset (Tab. 3). OspA antibodies were present in 2 patients, 1 month and 3 months following the tick bite, respectively. Among other highly specific antibodies, 4 patients tested positive for p83 (Tab. 3). Among antibodies to nonspecific antigens, the most prevalent ones were to p60, p62, in lesser extent to p35, p57 and occasionally to p30, 43, 50 and 75 (Tab. 3).

Discussion

In this study we compared the immune response of erythema migrans patients, with the use of various laboratory methods. Routine diagnostics uses IFT and ELISA, but in early stages of Lyme disease the humoral response is delayed and limited to certain antigens, which may cross-react with another microorganisms. These are responsible for low specificity of the tests and for more detailed analysis of antibody response, immunoblot is used for the detection of specific *Borrelia burgdorferi* antigens. In our study, we focused on IgM antibodies. As various antigens were used in individual tests, there may be discrepancies in the results.

The antibodies to flagellar antigen p41, which are the first to form in *Borrelia burgdorferi* infection, are probably best detected via IgM IFT, for which endemic antigen was used. 72 % of samples were positive, which is, when compared to results of other authors, very high (2,4). The tests which detect *Borrelia burgdorferi* antibodies are not standardized. They have variable sensitivity and specificity and their standardization is complicated with respect to great heterogenicity of *Borrelia burgdorferi* strains circulating in individual regions of Europe. (7, 9). IgM western

blot confirmed the presence of p41 antibodies in 23 of 25 patients and further mainly antibodies to OspC, which is highly variable and antibodies to it are formed in early stages of the disease. In patients positive via IFT general, western blot confirmed the positivity in only 43 %. Positivity was related mainly to p41 antibodies. On the other hand, the production of OspC at the time of testing might not have been initiated yet, which would have been critical for western blot positivity. Presence of antibodies to all 3 types of OspC in some patients suggests the possibility of mixed infection. The surveillance of prevalence of *Borrelia burgdorferi* in ticks, which repeatedly proved mixed infection (4, 7, 8), also points to this fact. Presence of p83 antibodies in some patients could be reflecting more remote infection.

According to the literature, all types of borrelia can cause erythema migrans, although in middle Europe it is most commonly *Borrelia afzelii* (1, 14). Data about strains circulating in Slovakia point towards the dominance of *Borrelia garinii*, then *Borrelia afzelii*, then *Borrelia burgdorferi*, and relatively common is *also Borrelia valaisiana* (5). In our western blot testing of 25 patients with erythema migrans, we confirmed the presence of specific antibodies in 8 patients (36 %), 5 of them tested positive in all 3 immunoblots, but immunoblot testing overall did not suggest the dominance of some of the *Borrelia burgdorferi* genospecies in our patients.

IgG testing suggested its presence mainly with the use of ELISA. 40 % of this could be a result of test enrichment with lipoprotein V1sE, which is highly immunogenic and specific. Antibodies against VlsE form in relatively early stages of infection. In some patients it could have reflected more remote infection, which ran unapparent (2). Prevalence of antibodies in healthy population also suggests this fact (2). Serum of our erythema migrans patients was drawn in various time frames after the tick bite, which could suggest longer persistence of the infection. Even after two months, some patients were positive only to p41. On the other hand, some patients were 2–3 weeks after the tick bite already positive for anti-OspC. This could reflect a variable immune response of the patients, or various characteristics of borrelias which caused the infection.

The great variability of borrelias circulating in our region, problems with standardization of laboratory diagnostics and high specificity of antibodies to individual borrelia antigens are suggesting the need to use, when in diagnostic confusion, more tests, which could detect antibodies also to other borrelia antigens.

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