ORIGINAL PAPERS

Adv Clin Exp Med 2010, **19**, 4, 489–496
ISSN 1230-025X

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Antibodies Against *in vivo B. burgdorferi* Antigens Evaluated in Patients with Lyme Arthritis with Reference to Treatment

Przeciwciała przeciwko antygenom *in vivo B. burgdorferi* u pacjentów ze stawowymi objawami boreliozy w odniesieniu do leczenia

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Abstract

Background. Highly immunogenic proteins produced *in vivo* after spirochete transmission into the human body are significant antigens for the diagnostics of *B. burgdorferi* s.1. infections. Antigens VlsE, BBA36, BBO323, Crasp 3 and pG demonstrate *in vivo* expression and comprise highly immunogenic epitopes, common for *B. burgdorferi* s.l., which are important IgG serological markers of advanced stages of borreliosis.
Objectives. The purpose of the investigation was to determine the frequency of the development of erythema migrans following tick bites and to evaluate the pattern of IgM and IgG antibodies against *B. burgdorferi* antigens in patients who developed symptoms of Lyme arthritis with reference to treatment.
Material and Methods. The study was conducted in the group of 200 patients: 180 patients, 100 men (aged 21–65) and 80 women (aged 24–60) suspected of borreliosis hospitalized in 2007–2008; 20 patients, 10 men (aged 25–66) and 10 women (aged 24–70) with the second stage of Lime disease hospitalized in 2007–2008. All patients were asked to respond to a questionnaire to gather information about the dates and frequency of tick bites, incidents of EM, symptoms that occurred over first 30 days from tick bite, symptoms developed from 30th day of hospitalization, diagnostic tests preformed and antibiotics taken. The presence of anti-B. burgdorferi IgM and IgG antibodies was determined in the patients' serum using the diagnostic tests ELISA and Wb. The presence of antibodies against *in vivo B. burgdorferi* antigens were evaluated in 20 hospitalized patients with Lyme arthritis.
Results. Single tick bites were reported by 145 patients (73%). There were 104 cases of migratory erythema (52%), among them 88 patients sustained single tick bites and 16 were tick bitten several times. In case of ELISA test, positive results were obtained in 105 (52%) patients – all the results were confirmed by the WB test; 63 (31%) patients were negative for the presence of anti-Borrelia antibodies in the ELISA assay. Serological tests performed in the patients with symptoms of Lyme arthritis before Biotraxon (Ceftriaxonum) therapy detected IgG anti-VlsE antibodies in 75% cases. Antibodies against other antigens occurred with varied frequency: anti-BBO323 in 45%, anti-Crasp3 in 35%, anti-p39 in 30%, anti-p83 in 30%, anti-BBA36 in 20% and anti-pG in 15%. The dynamics of IgG antibodies against antigens exprimated *in vivo* observed after 14 days of Biotraxon (Ceftriaxonum) administration and after 6 weeks from the completion of treatment seems an interesting issue. Altered production of IgG antibodies against *in vivo* antigens, like BBA36, BBO323, Crasp3 and pG were determined in 6 patients (30%) after 14 days of Biotraxon (Ceftriaxonum) administration and in 4 patients (20%) after 6 weeks from the completion of treatment.
Conclusions. Among patients enrolled in the study the EM developed in every second patient including persons who were bitten by ticks only once and those who declared repeated tick bites. The results of the present study indicate that the WB test is a valuable, confirmatory test in the diagnosis of *B. burgdorferi* infection. The constant presence of antibodies against antigenic proteins OspC, VlsE, p39 or p83 was detected in the study group, including the period before, during, and after 6-week etitropic therapy. The development of or, on the contrary, elimination of IgG antibodies from serum directed against the following *B. burgdorferi* antigens – *in vivo* BBA36, BBO323,

Crasp3, and pG observed directly after antimicrobial therapy or 6 weeks after finishing the therapy confirms appropriateness of studies on complex mechanisms of immune response to *B. burgdorferi* infection (**Adv Clin Exp Med 2010, 19, 4, 489–496**).

Key words: Lyme arthritis, *B. burgdorferi*, *in vivo* antigens.

Streszczenie

Wprowadzenie. Antygenami istotnymi z punktu widzenia diagnostyki zakażeń wywołanych przez *B. burgdorferi* są wysoko immunogenne białka pojawiające się *in vivo* po transmisji krętka do organizmu człowieka. Antygeny VlsE, BBA36, BBO323, Crasp3, pG wykazują ekspresję *in vivo* i mają wysoko immunogenne epitopy wspólne dla *B. burgdorferi sensu lato*, będące ważnym oznacznikiem dla zaawansowanych stadiów boreliozy w serologii IgG.
Cel pracy. Określenie częstości występowania *erythema migrans* (EM) w aspekcie narażenia na pokłucie przez kleszcze oraz ocena występowania przeciwciał IgM i IgG dla antygenów *in vivo B. burgdorferi* u pacjentów z objawami boreliozy stawowej w aspekcie leczenia biotraksonem (Ceftriakson).
Materiał i metody. Badanie przeprowadzono u 200 pacjentów: 180 pacjentów: 100 mężczyzn (21–65 lat) i 80 kobiet (24–60 lat) z podejrzeniem boreliozy hospitalizowanych w latach 2007–2008; 20 pacjentów: 10 mężczyzn (25–66 lat) i 10 kobiet (24–70 lat) w drugiej fazie boreliozy hospitalizowanych w latach 2007–2008. Wszyscy pacjenci wypełniali ankietę, w której informowali o dacie i częstości pokłucia przez kleszcze, zaobserwowanych objawach klinicznych występujących po pokłuciu, wykonanych testach diagnostycznych i stosowanym leczeniu. Oznaczenia przeciwciał anty-B. burgdorferi w surowicy pacjentów wykonano testami ELISA i Western blot zgodnie z zasadami dwustopniowej diagnostyki boreliozy. Obecność przeciwciał przeciwko antygenom z grupy *in vivo* określono u 20 hospitalizowanych pacjentów z objawami boreliozy stawowej.
Wyniki. U 104 osób wystąpił EM, przy czym 88 pacjentów informowało o jednokrotnym pokłuciu przez kleszcze, a 16 o pokłuciu wielokrotnym. W teście ELISA wyniki dodatnie uzyskano u 105 osób (52%), wszystkie potwierdzono testem Wb. U 63 osób (31%) w teście ELISA otrzymano wyniki ujemne. U pacjentów z objawami boreliozy stawowej przed zastosowaniem leczenia biotraksonem (Ceftriakson) stwierdzono obecność przeciwciał IgG anty-VlsE u 75% badanych. Przeciwciała IgG skierowane przeciwko innym antygenom występowały z różną częstością: anty-BBO323 u 45%, anty-Crasp3 u 35%, anty-p39 u 30%, anty-p83 u 30%, anty-BBA36 u 20% i anty-pG u 15%. Pewne zmiany w wytwarzaniu przeciwciał IgG przeciwko antygenom z grupy *in vivo*, jak BBA36, BBO323, Crasp3 i pG stwierdzono u 30% badanych po 14 dniach leczenia i u 20% 6 tygodni po zakończeniu leczenia.
Wnioski. Na podstawie przeprowadzonych badań stwierdzono, że EM wystąpił u co drugiego pacjenta i to zarówno u osób jednokrotnie kłutych przez kleszcze, jak i u osób deklarujących pokłucia wielokrotne. Przeprowadzone badania potwierdzają, że w praktyce klinicznej test Western blot jest wartościowym, potwierdzającym zakażenie *B. burgdorferi* testem diagnostycznym. W badanej grupie wykazano stałą obecność przeciwciał dla białek antygenowych OspC, VlsE, p39 lub p83 przed, podczas oraz po 6 tygodniach etiotropowej terapii. Pojawianie lub zanikanie przeciwciał IgG przeciwko antygenom *vivo* BBA36, BBO323, Crasp3 and pG *B. burgdorferi* bezpośrednio po antybiotykoterapii lub 6 tygodni po jej zakończeniu potwierdza celowość badań nad złożonymi mechanizmami odpowiedzi immunologicznej w zakażeniu *B. burgdorferi* (**Adv Clin Exp Med 2010, 19, 4, 489–496**).

Słowa kluczowe: borelioza z Lyme, *B. burgdorferi*, antygeny *in vivo*.

Clinically varied course of borreliosis is associated with the properties of *B. burgdorferi sensu lato* bacterial genospecies as well as the effectiveness of the immune system of an infected person. The symptoms observed in the course of chronic infection produce different clinical and serological picture. Main signs and symptoms are located in the locomotor system, affecting big joints of the limbs, in the central and peripheral nervous system and the heart. The disease can have a long term history and in some patients the symptoms can persist despite repeated antibiotic treatment. Other symptoms include persistent inflammatory infiltrations containing various lymphocyte subpopulations [1]. The diagram of serological response to infection varies with account for the level of IgM and IgG antibodies and their distribution with reference to a wide variety of antigens tested. The presence of specific antibodies with no clinical manifestations does not prove the disease has been present [2, 3].

B. burgdorferi does not produce toxins and numerous destructive processes in the course of borreliosis are not connected with direct activity of spirochete but with enhanced inflammatory response to their presence in the tissues. In late borreliosis autoimmune processes may sustain excessive and inadequate inflammatory response may be responsible for persistent pathological manifestations. For therapeutic reasons it is essential to differentiate between active *B. burgdorferi* infection and autoimmune process [4, 5].

Despite large knowledge on the course of infections triggered by *B. burgdorferi* it is impossible to define explicitly the correlation between host immunocompetence and bacterial antigen proteins, those expressed *in vivo* especially. Dependences between them determine either the elimination of pathogen or developing infection. Learning and understanding the mechanisms responsible for the disease development can improve methods of prevention and treatment of *B. burgdorferi* infections.

The purpose of own investigation was to determine the frequency of the development of erythema migrans following tick bites and to evaluate the pattern of IgM and IgG antibodies against *B. burgdorferi* antigens in patients who developed symptoms of Lyme arthritis with reference to treatment.

Material and Methods

The study was conducted in the group of 200 patients:

– 180 patients, 100 men (aged 21–65) and 80 women (aged 24–60) suspected of borreliosis hospitalized in Clinic of Gastrology and Infectious Diseases, I Military Hospital in Lublin, Clinic Infectious Diseases, Hospital in Łuków in 2007–2008;

– 20 patients, 10 men (aged 25–66) and 10 women (aged 24–70) with the second stage of Lime disease hospitalized in the Department of Infectious Diseases, Medical University of Lublin in 2007–2008.

The study group involved patients demonstrating manifestations of joint involvement including arthralgia and/or arthritis treated symptomatically and receiving parenteral etiotropic antimicrobial therapy. The diagnosis of borreliosis was established on the basis of the patient's medical history, physical examination, clinical picture, and serologic investigation (ELISA and Western Blot tests were performed to detect the presence of specific anti-Borrelia burgdorferi antibodies).

All patients were asked to complete a questionnaire to gather information about the dates and frequency of tick bites, incidents of migratory erythema (EM), symptoms that occurred over first 30 days from tick bite, symptoms developed from 30th day of hospitalization, diagnostic tests preformed and antibiotics taken.

The presence of anti-Borrelia burgdorferi IgM and IgG antibodies was determined in the 200 patients' serum using the following diagnostic tests:

1. ELISA (Euroimmun):

– for IgM and IgG: a mixture of antigenic lysate originating from *Borrelia burgdorferi sensu lato*.

2. The doubtful results and positive results were subsequently verified using Western blot assay with the use of the EUROLine Scan (Euroimmun) reading programme for IgM and IgG: VlsE, p83, p41, p39 (BmpA), p31 (OspA), p30, p25 (OspC), p21, p19, and p17.

Among patients hospitalized at the Department of Infectious Diseases, Medical University of Lublin an additional Immunoblot (Genzyme Virotech GmbH) test was performed for IgG and IgM antibodies to *Borrelia burgdorferi sensu lato* which included the following antigens:

– for IgM: OspC, p39 – standard antigens used for diagnostic tests, EBV-VCA-gp125 (Epstein Barr Virus antigen-gp125) – highly specific antigen for the serology of IgM in primary EBV infection, used in so called excluding diagnostics;

– for IgG: VlsE, p39, p83 – standard antigens for diagnostic tests; BBA36 (iv1), BBO323 (iv2), Crasp3 (iv3), pG (iv4) – antigens from *in vivo* group.

The serologic investigation was conducted according to the following scheme:

– before antibiotic treatment with Biotraxon (Ceftriaxonum) was started, 2 × 1 g over 14 days,

– after 14 days of Biotraxon (Ceftriaxonum) administration,

– 6 weeks from the day the treatment with Biortaxon (Ceftriaxonum) was completed.

Results

Questionnaire information obtained from 200 patients with symptoms of Lyme arthritis was analyzed.

Single tick bites were reported by 145 patients (73%). None of them connected the incident with their professional work in the forest. Other 55 patients (27%) reported having been tick bitten in the years 1980–2007, however they were unable to precisely define the time of tick bites. In that group 19 persons (34%) associated tick bites with work done (farmers, forest workers, people cutting down trees, nature photographer).

There were 104 cases of migratory erythema (52%), among them 88 patients sustained single tick bites and 16 were tick bitten several times. All of them reported taking antibiotics to treat the infection (single or multiple therapies): Doxyciline (doxycycline hydrochloricum), Sumamed (azitromycin), and other.

Over the first 30 days from tick bites 19 patients (10%) observed other symptoms but EM, (fever, headache, flu-like symptoms) which could have been referred to tick bites. In the period from 30th day until hospitalization the patients reported headaches, pains in the bones and joints (knee, shoulder), muscular and spinal pains, all occurring with various frequency. There were also single cases of tingling in the ears, chronic sinusitis, swollen lower limbs, weakened concentration, cardiac arrhythmia, heart valve failure and skin changes.

60 patients (36 absence of EM and 24 presence of EM, (30%), reported taking antibiotics to treat pains in the bones and joints, 60 patients (30%) remained untreated.

Table 1. Patients' history findings of tick bites, EM and antibiotic treatment of EM or/and bony and articular manifestations

Tabela 1. Informacje uzyskane od pacjentów w wywiadzie chorobowym dotyczące pokłucia przez kleszcze, wystąpienia EM i kuracji antybiotykowych przyjętych w związku z objawami EM lub/i dolegliwościami ze strony układu kostno-stawowego

Study group (Grupa badana) N = 200	Tick bite (Pokłucia przez kleszcze)	Erythema migrans (Rumień wędrujący) (EM)	Treatment (Antybiotykoterapia)
9	¤¤	+	O O O
2	¤¤	+	O O O
4	¤¤	+	O
1	¤¤	+	O
2	¤¤	–	O O O
1	¤¤	–	O O O
14	¤¤	–	O
3	¤¤	–	O
16	¤¤	–	Ø
3	¤¤	–	Ø
81	¤	+	O
7	¤	+	O
14	¤	–	O
2	¤	–	O
40	¤	–	Ø
1	¤	–	Ø

☐ Patients with the second stage of borreliosis.
Pacjenci w drugim okresie boreliozy.

☐ Patients suspected of borreliosis.
Pacjenci z podejrzeniem boreliozy.

¤¤ Double or more tick bite.
Pokłucia dwukrotne lub wielokrotne.

¤ Tick bite.
Pokłucia jednokrotne.

– Absence of EM.
Brak rumienia wędrującego.

+ Presence of EM.
Obecny rumień wędrujący.

Ø No treatment.
Brak leczenia.

O Treatment.
Antybiotykoterapia.

O O O Multiple therapy.
Wielokrotna antybiotykoterapia.

Table 1 lists the history of tick bites, EM and antibiotic treatment of EM or/and bony and articular complaints.

ELISA and Western blot tests were done in 200 patients with Lyme arthritis symptoms. The presence of antibodies directed against *B. burgdorferi* was detected in patient's sera using ELISA tests (Euroimmun). The doubtful results and positive results were subsequently verified using Western blot (Wb) assay with the use of the EUROLine Scan (Euroimmun) reading programme.

In case of ELISA test, positive results were obtained in 105 (52%) patients including:

– 28 (14%) patients positive for IgM and IgG antibodies – all the results were confirmed as positive by the WB test;

– 35 (17%) patients positive for IgM; 30 of them were confirmed as positive by the WB test;

– 42 (21%) patients positive for IgG among which all the results were confirmed as positive by the WB test.

63 (31%) patients were negative for the presence of anti-Borrelia antibodies in the ELISA assay.

The obtained results are included in the table 2.

Table 3 shows IgM and IgG antibodies anti specific *B. burgdorferi* antigens prior to treatment.

Western blot test was performed in 20 patients who developed symptoms of Lyme arthritis before Biotraxon (Ceftriaxonum) was started. IgM and IgG antibodies against specific *Borrelia burgdorferi* antigenes were detected in 12 patients. IgM antibodies alone were found in 4 patients and other 4 patients had only IgG antibodies.

IgM anti-OspC were present in 16 patients (80%) and IgM anti other antigens (VlsE, p39) were detected with various frequency in 6 patients (37%).

IgG antibodies anti-VlsE, BmpA (p39), p83, BBA36, BBO323, Crasp3 and pG were observed in 16 patients (80%) with various frequency. The most frequently detected were IgG anti *in vivo* antigens, like VlsE – in 15 patients (75%) BBO323 – in 9 patients (45%) and Crasp3 – in 7 patients (35%). Less frequently detected were IgG anti-p39 (30%), anti-p83 (30%), anti-BBA36 (20%) and anti-pG (15%).

Table 4 shows IgM and IgG antibodies against *B. burgdorferi* specific antigens after 14 days of Biotraxon (Ceftriaxonum) administration and after 6 weeks from the completion of therapy.

Serological testing for IgM performed after 14 days of Biotraxon (Ceftriaxonum) treatment and after 6 weeks from the completion of therapy did not differ from the results obtained before treatment.

Altered production of IgG antibodies against *in vivo* antigens, like BBA36, BBO323, Crasp3 and pG were determined in 6 patients (30%) after 14 days of Biotraxon (Ceftriaxonum) administration and in 4 patients (20%) after 6 weeks from the

Table 2. ELISA and Western blot test results in 200 patients

Tabela 2. Wyniki testu ELISA I Western blot u 200 pacjentów z podejrzeniem boreliozy

Study group (Grupa badana) N = 200	Anti-B.burgdorferi antibodies (Przeciwciała anty-B. burgdorferi)				
	ELISA		N=200	Western blot	
	IgM	IgG		IgM	IgG
28	positive	positive	28	positive	positive
35	positive	negative	30	positive	not done
			5	negative	
42	negative	positive	42	not done	positive
4	doubtful	doubtful	1	negative	doubtful
			3	negative	negative
18	doubtful	negative	18	negative	not done
10	negative	doubtful	6	not done	doubtful
			4		negative
63	negative	negative	63	not done	not done

Positive (wynik pozytywny).
Negative (wynik ujemny).

Doubtful (wynik graniczny).
Not done (badanie niewykonane).

completion of treatment. After 14 days of antibiotic treatment IgG antibodies anti-pG disappeared in 2 patients (10%) and IgG anti-BBO323 in 1 person (5%). However BBA36 and BBO323 antibodies were determined in 10% cases and Crasp3 and pG were noted in 5% cases.

Serological determinations done after 6 weeks from the completion of treatment revealed IgG anti-pG present in 1 person (5%) and the absence of IgG anti-BBO323 in 2 persons (10%), anti-BBA36 in 1 person (5%), anti-Crasp3 in 1 person (5%) and anti-pG in 1 person (5%).

Antibodies against other *B. burgdorferi* specific antigens followed the same pattern as before treatment.

Discussion

Typically Lyme arthritis leads to asymmetrical intermittent migratory synovitis of the large joint, the change often preceded by migratory erythema. The analysis of clinical data by Dinser R. et al. found that 60% patients with EM untreated in the past developed intermittent or chronic migratory arthritis. Another study carried out in Germany revealed that 30% patients with Lyme arthritis reported having had migratory erythema [6]. Other authors reported late form of borreliosis in ca. 60% cases left untreated. Patients complained of intermittent pains and oedema in one or more joints, in the knee and hip mainly [7]. According to other data, knees and ankles were the joints most often affected, however other joints were mentioned in some cases. Some patients developed symmetrical arthritis and synovitis in five or more joints. Some patients with chronic arthritis and intermittent arthritis also experienced single episodes of synovitis [6].

Own results revealed 52% patients having symptoms of borreliosis reported migratory erythema in their history and being treated with antibiotics. Despite treatment the patients developed late pains in the bones and joints, arthritis and muscular pains. Out of the total 30% patients reported having been treated with antibiotics in the past for other complaints than migratory erythema, like the pains in the bones and joints, which were often associated with tick bites. Other 20% patients were untreated although their complaints could have been related to *B. burgdorferi* infection. In the clinical practice the evaluation of the active stage of infection (including both the first and second phase of illness) is primarily based on the clinical symptomatology, routine enzyme im-

Table 3. IgM and IgG antibodies anti specific *B. burgdorferi* antigens prior to treatment in 20 patients

Tabela 3. Przeciwciała IgM i IgG skierowane przeciwko specyficznym antygenom *B. burgdorferi* przed leczeniem u 20 pacjentów

Study group (Grupa badana) N = 20	Presence of IgM anti-*B. burgdorferi* antigens Przeciwciała IgM anti-*B. burgdorferi*)				Presence of IgG anti-*B. burgdorferi* antigens Przeciwciała IgG anti-*B. burgdorferi*						
	OspC	p39	VlsE	EBV	VlsE	p39	p83	BBA36	BBO323	Crasp3	pG
3	+	+	–	–	no band						
1	+	–	–	–	no band						
1	+	–	–	–	+	+	+	+	+	+	–
1	+	–	–	–	+	+	–	–	+	+	–
1	+	–	–	–	+	–	–	–	+	–	+
1	+	–	–	–	+	–	+	–	+	–	–
1	+	–	+	–	+	–	–	–	–	+	–
1	+	–	+	–	+	–	–	–	+	–	–
1	+	–	–	–	+	–	–	–	+	–	–
3	+	–	–	–	+	–	–	–	–	–	–
1	+	+	+	–	+	–	–	–	–	–	–
1	+	–	–	–	–	+	–	–	–	–	–
2	no band				+	+	+	+	+	+	+
1	no band				+	+	+	–	+	+	–
1	no band				+	–	+	+	–	+	–

– Absence of antibodies.
Brak przeciwciał.
+ Presence of antibodies.
Obecne przeciwciała.

munoassays, and confirmatory tests such as Western Blot.

As the disease progresses the production of antibodies against various antigens evolves. At the early stage of *B. burgdorferi* infection, i.e. within 2–4 weeks from tick bite the immune system recognizes some antigens only, e.g. flagelin p41protein and Osp proteins; OspC is considered immunodominant antigen in IgM response. The highest level of IgM is observed after 6–8 weeks and the lowest after 4–6 months.

IgG antibodies appear last, after 2 months from tick bites and their maximum level is observed after 4–6 months. The level of IgG anti *B. burgdorferi* may increase and persist even when the symptoms have already subsided. As the infection develops immune responses extend onto bigger number of antigen proteins, like p39, p58, p83, p53, p43, p31, p30, p21, p19, p17 and p14 [8].

The diagnostic and therapeutic problems associated with *Borrelia burgdorferi* infections necessitate the search for additional immunologic parameters which would support the diagnosis of active phase of the disease.

Highly immunogenic proteins produced *in vivo* after spirochete transmission into the human body are significant antigens for the diagnostics of *B. burgdorferi* s. l. infections. Antigens VlsE, BBA36 (22kDa), BBO323 (42 kDa), Crasp3 (21 kDa) and pG (22 kDa) demonstrate *in vivo* expression and comprise highly immunogenic epitopes, common for *B. burgdorferi sensu lato*, which are important IgG serological markers of advanced stages of borreliosis [4, 8–10].

Hoffmann et al. found OspC and VlsE are main antigens produced in early stages of infection and antigens BBA36, BBO323, Crasp3 and pG are typical of late stage of Borrelia infection. In all patients with Lyme arthritis they found IgG antibodies against VlsE and p39. Relatively less frequent were antibodies against BBO323 (90%), BBA36 (67%), p83 (71%), antibodies against Crasp3 (38%) and

Table 4. IgM and IgG antibodies against *B. burgdorferi* specific antigens after 14 days of Biotraxon (Ceftriaxonum) administration and after 6 weeks from the completion of therapy in 20 patients

Tabela 4. Przeciwciała IgM i IgG skierowane przeciwko specyficznym antygenom *B. burgdorferi* po 14 dniach leczenia Biotraxonem (Ceftriakson) i po 6 tygodniach od zakończenia leczenia u 20 pacjentów

Study group (Grupa badana) N = 20	Presence of IgM anti-*B. burgdorferi* antigens (Przeciwciała IgM anti-*B. burgdorferi*)				Presence of IgG anti-*B. burgdorferi* antigens (Przeciwciała IgG anti-*B. burgdorferi*)						
	OspC	p39	VlsE	EBV	VlsE	p39	p83	BBA36	BBO323	Crasp3	pG
3	+	+	–	–	no band						
1	+	–	–	–	no band						
1	+	–	–	–	+	+	+	+	+	+	▪
1	+	–	–	–	+	+	–	–	Ø	#	–
1	+	–	–	–	+	–	–	–	#	–	#
1	+	–	–	–	+	–	+	–	+	–	–
1	+	–	+	–	+	–	–	•	•	+	–
1	+	–	+	–	+	–	–	•	+	•	–
1	+	–	–	–	+	–	–	–	+	–	–
3	+	–	–	–	+	–	–	–	–	–	–
1	+	+	+	–	+	–	–	–	–	–	–
1	+	–	–	–	–	+	–	–	•	–	•
2	no band				+	+	+	#	#	+	Ø
1	no band				+	+	+	–	+	+	–
1	no band				+	–	+	+	–	+	–

– Absence of antibodies.
Brak przeciwciał.

\+ presence of antibodies.
Obecne przeciwciała.

Ø no antibodies present after 14 days of Biotraxon (Ceftriaxonum) administration.
zanik przeciwciał po 14 dniach leczenia Biotraxonem (Ceftriakson).

\# no antibodies present after 6 weeks from treatment completion.
zanik przeciwciał po 6 tygodniach od zakończenia leczenia.

• antibodies present after 14 days of Biotraxon (Ceftriaxonum) administration.
pojawienie przeciwciał po 14 dniach leczenia Biotraxonem (Ceftriakson).

▪ antibodies present after 6 weeks from treatment completion.
pojawienie przeciwciał po 6 tygodniach od zakończenia leczenia.

pG (33%) were the least frequent. IgM antibodies against OspC and VlsE were determined despite later stage of the disease [9].

Own results revealed concomitant IgM and IgG against specific *B. burgdorferi* antigens in 60% patients, in 20% cases only IgM were detected and in another 20% cases only IgG were present. The most common were IgM anti-OspC (80%), less frequent anti-p39 (20% patients) and anti-VlsE (15% patients). IgM were determined both before antibiotic treatment and after 14 days of Biotraxon (Ceftriaxonum) administration and 6 weeks after the treatment was completed.

IgG anti specific *B. burgdorferi* antigens (VlsE, p83, BBA36, BBO323, Crasp3 and pG) occurred with varied frequency in 80% patients with the symptoms of Lyme arthritis.

The researchers believe VlsE protein is the most sensitive recombinant *B. burgdorferi* s. l. antigen used in the diagnostics. It is possible to detect *B. burgdorferi* s. l. in all pathogenic *Borrelia burgdorferi* sensu lato genospecies and the risk of

false positive results is ten times lower in comparison to other Borrelia antigens [11]. Serological tests performed in the patients with symptoms of Lyme arthritis before Biotraxon (Ceftriaxonum) therapy detected IgG anti-VlsE antibodies in 75% cases. Antibodies against other antigens occurred with varied frequency: anti-BBO323 in 45%, anti-Crasp3 in 35%, anti-p39 in 30%, anti-p83 in 30%, anti-BBA36 in 20% and anti-pG in 15%. The dynamics of IgG antibodies against antigens exprimated *in vivo* (VlsE, BBA36, BBO323, Crasp3 and pG) observed after 14 days of Biotraxon (Ceftriaxonum) administration and after 6 weeks from the completion of treatment seems an interesting issue.

Based on the preliminary results obtained in the present study the authors suggest that the search for additional/new immunologic parameters of active stage of *B. burgdorferi* infection is justified and desirable the more so because the available literature data and the results of recent clinical studies indicate the need for the introduction of extended diagnostic panels. It is conceivable that the modified approach to the diagnosis would enable to treat this illness more effectively; it would also constitute a valuable parameter of the evaluation of the course of the disease.

The authors concluded that among patients enrolled in the study the EM developed in every second patient including persons who were bitten by ticks only once and those who declared repeated tick bites. The results of the present study indicated that the WB test is a valuable, confirmatory test in the diagnosis of *B. burgdorferi* infection. The constant presence of antibodies against antigenic proteins OspC, VlsE, p39 or p83 was detected in the study group, including the period before, during, and after 6-week etitropic therapy. The development of or, on the contrary, elimination of IgG antibodies from serum directed against the following *B. burgdorferi* antigens – *in vivo* BBA36, BBO323, Crasp3, and pG observed directly after antimicrobial therapy or 6 weeks after finishing the therapy confirms appropriateness of studies on complex mechanisms of immune response to *B. burgdoferi* infection.

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Conflict of interest: None declared

Received: 01.03.2010
Revised: 26.04.2010
Accepted: 09.08.2010