ORIGINAL PAPERS

Adv Clin Exp Med 2008, **17**, 6, 635–641
ISSN 1230-025X

ANNA DUDA, URSZULA KASPRZYKOWSKA, BEATA SOBIESZCZAŃSKA

Non-Specific Arthralgia as the Main Manifestation of *Borrelia burgdorferi* Sensu Lato Infection*

Nieswoiste bóle stawowe jako główny objaw kliniczny zakażenia krętkami *Borrelia burgdorferi* sensu lato

Department of Microbiology, Silesian Piasts University of Medicine in Wrocław, Poland

Abstract

Background. In European countries, muscoskeletal symptoms associated with Lyme arthritis appear in 7–20% of disseminated cases of borreliosis. Lyme arthritis is a late manifestation of the disease; however, like erythema migrans, arthritis may occur as the only or the initial manifestation of infection with *Borrelia burgdorferi* sensu lato.
Objectives. The present study was carried out to determine seroreactivity to *B. burgdorferi* sensu lato antigens in inhabitants of the Lower Silesian region of Poland suffering from undiagnosed arthralgia.
Material and Methods. To detect IgM and IgG antibodies specific to *B. burgdorferi* sensu lato in sera, an immunoenzyme assay with recombinant antigens was performed. All immunoenzyme assay-positive samples were confirmed by Western blot.
Results. Immunoenzyme assay followed by Western blot analysis revealed that of 64 serum samples examined, 23 (35.9%) demonstrated the presence of IgM and/or IgG antibodies confirmed by reactivity with antigens specific to *B. burgdorferi* sensu lato. The results allow assuming that at least 9 (14.1%) subjects with IgG or IgG and IgM immune responses present active Lyme arthritis.
Conclusions. The results strongly suggest that in the wooded region of Lower Silesia, arthralgia should be diagnosed paying special attention to Lyme disease at least in subjects with a history of tick bite (**Adv Clin Exp Med 2008, 17, 6, 635–641**).

Key words: arthralgia, Lyme arthritis, *Borrelia burgdorferi* sensu lato, serological tests, recombinant antigens.

Streszczenie

Wprowadzenie. W krajach europejskich objawy kostno-stawowe związane z chorobą z Lyme dotyczą 7–20% przypadków rozsianej boreliozy. Boreliozowe zapalenie stawów dotyczy przewlekłej boreliozy, ale podobnie jak rumień migrujący, może być wczesnym, a czasem jedynym objawem zakażenia krętkami *Borrelia burgdorferi* sensu lato.
Cel pracy. Zbadanie obecności przeciwciał dla antygenów *Borrelia burgdorferi* sensu lato w surowicach mieszkańców Dolnego Śląska cierpiących z powodu nieokreślonych bólów stawowych, którzy w przeszłości mieli kontakt z kleszczami.
Materiał i metody. Przeciwciała swoiste dla *Borrelia burgdorferi* sensu lato oznaczano testem immunoenzymatycznym z rekombinowanymi antygenami, a następnie ich obecność potwierdzano testem Western blot.
Wyniki. Obecność przeciwciał klas IgM i/lub IgG swoistych dla *Borrelia burgdorferi* sensu lato wykryto w 23 (35,9%) spośród 64 badanych surowic. Uzyskane wyniki badań pozwalają przypuszczać, że co najmniej 9 (14,1%) badanych seropozytywnych osób prezentowało aktywną postać boreliozowego zapalenia stawów.
Wniosek. Nierzadkie na zalesionym terenie Dolnego Śląska zakażenie krętkami z gatunku *Borrelia burgdorferi* sensu lato powinno być brane pod uwagę w różnicowej diagnostyce bólów stawowych (**Adv Clin Exp Med 2008, 17, 6, 635–641**).

Słowa kluczowe: ból stawu, boreliozowe zapalenie stawów, *Borrelia burgdorferi* sensu lato, testy serologiczne, antygeny rekombinowane.

* This work was financed by Silesian Piasts University of Medicine Specialistic Microbiology Laboratory MicroFAM.

Lyme borreliosis caused by spirochetes of the species *Borrelia burgdorferi* sensu lato, consisting of *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* genospecies, is a multisystem disorder that can afflict many organs, but it is most commonly associated with the skin, nervous system, heart, and joints [20, 23]. Lyme arthritis is most often associated with *B. burgdorferi* sensu stricto infection, which is common in North America but less frequent in Europe. In European countries, muscoskeletal symptoms appear in 7–20% of disseminated cases of borreliosis; however, apart from *B. burgdorferi* sensu stricto, *B. garinii* can also cause arthritis [2, 12, 26].

Lyme arthritis may occur early in disseminated disease or at late stages of borreliosis and usually presents an intermittent course as the majority of patients have only a few attacks. The disease most commonly affects asymmetric peripheral large joints, especially the knees, ankles, and elbows [3, 6]. As in other joint inflammatory diseases, Lyme arthritis presents with warmth, erythema, swelling, and also pain in joints during motion [21, 22].

Although Lyme arthritis is clinically distinct from rheumatoid arthritis (RA), the disease resembles the oligoartricular form of juvenile or adult RA and can cause joint destruction due to chronic proliferative synovitis with inflammatory exudates rich in protein and infiltration of granulocytes [1, 6]. Moreover, muscoskeletal symptoms may persist for a long time, mimicking RA, in untreated patients. Rheumatoid factor and anti-nuclear antibodies characteristic of RA are usually negative in Lyme arthritis, but may be detected in some patients [25]. In contrast to RA, most patients with Lyme arthritis can be treated successfully with appropriate antibiotic therapy, even in long-standing disease [8, 18]. However, despite adequate antibiotic therapy, approximately 10% patients with Lyme arthritis, mainly those with HLA DR2 and HLA DR4 haplotypes, develop chronic arthritis with histology of the synovial lesions typical of that found in all forms of chronic inflammatory arthritis [10, 14, 28]. Unfortunately, clinical and laboratory signs of joint inflammation may be minimal in the course of Lyme arthritis, making the diagnosis and differentiation from other forms of inflammatory joint involvement difficult [6].

The present study was carried out to determine seroreactivity to *B. burgdorferi* sensu lato antigens in inhabitants of the region of Lower Silesia in Poland suffering from undiagnosed arthralgia. Since individuals with nonspecific symptoms such as arthralgia are frequently misdiagnosed as having borreliosis, the aim of the present study was to find out how often joint complaints might be associated with Lyme arthritis.

Material and Methods

Serum Samples

Serum samples were obtained from 64 randomly selected adults (age average: 50.5 years), inhabitants of the region of Lower Silesia in Poland who had joint complaints and who had never been treated for Lyme borreliosis. All persons answered a questionnaire regarding exposure to arthropod bites before the onset of arthralgia and antibiotic therapy before serum sample testing.

Serological Testing

Serological testing was performed according to the guidelines of the German Society of Hygiene and Microbiology (GSHM) in a two-step procedure: a sensitive immunoenzyme test differentiating IgM and IgG followed by immunoblot (IgM and IgG) of all seropositive samples.

ELISA

To detect IgM and IgG antibodies specific to *B. burgdorferi* sensu lato in the sera, an immunoenzyme assay with recombinant antigens (Borrelia IgM, IgG Recombinant Antigen, Bellco Biomedica Medizinprodukte GmbH & Co. KG, Austria) was performed. The levels of antibodies detected in the serum samples were calculated according to the manufacturer's instructions and expressed as predefined borrelial units (BBU/ml). Serum samples with ≤ 11 BBU/ml were considered negative, 11–20 BBU/ml weakly positive, and 20–30 BBU/ml highly positive.

Immunoblot

All serum samples positive in the immunoenzyme assay were confirmed by the Western blot assay (*recom*Blot Borrelia$_{NB}$ IgM and IgG, Mikrogen GmbH, Germany). Nine recombinant proteins combined from *B. burgdorferi* sensu lato genospecies are employed in the test. These proteins are p100, p41, p39, OspA, OspC, p41/i *B. garinii* (p41/iG), p41/i *B. afzelii* (p41/iA), p18 (DbpA), and VlsE, an antigen conserved among *B. burgdorferi* sensu lato strains with broad reactivity to IgM- and IgG-specific antibodies. The results of Western blot were interpreted according to the manufacturer's instructions by point evaluation of the borrelial antigens reacting with the serum samples and then adding the point values. A total score of ≤ 4 points indicates a negative result, 5–6 points a borderline result, and ≥ 7 points a positive result. Moreover, the Western blot

results were also interpreted according to GSHM recommendations. The blot was consider IgG positive if two of the following bands were present: p100, p58, p43, p39, p30, OspC, p21, DbpA, and p14, and IgM-positive if at least one band of the following was present: p41 (strong reactivity), p39, OspC, and DbpA [7, 27].

Results

Of the 64 serum samples examined by the immunoenzyme assay, 23 (35.9%) showed the presence of antibodies specific to *B. burgdorferi* sensu lato. Specific IgM and IgG antibodies were present in 17 (26.6%) and 9 (14.1%) serum samples, respectively, and both classes of antibodies were detectable in 3 (4.7%) samples (Table 1). Western blot confirmed the presence of antibodies specific to *B. burgdorferi* sensu lato antigens in all the serum samples in spite of borderline scores obtained in two samples (Table 2, serum samples 25 with IgM and 56 with IgG).

A questionnaire revealed that of the 23 individuals positive in the serological tests, as many as 17 (73.9%) remembered a tick bite. The period between the tick bite and serological testing varied from several weeks to five years and all these persons complained of transitory painful joint swelling and/or migratory arthralgia as the main symptoms.

Analysis of the specific antigens reacting with IgM antibodies present in the 17 IgM-positive serum samples revealed that OspC protein (88.2% reactivity) and p41 *B. burgdorferi* flagellum antigen (82.3% reactivity) followed by p41/i *B. afzelii* (70.6%) and and p41/i *B. garinii* flagellar antigens (64.7%) were the most frequently recognized antigens (Table 3). As many as 14 (60.9%) of the 23 seropositive samples demonstrated the presence of IgM antibodies alone and all these samples fulfilled the requirements for a positive result in the immunoblot assay. IgM reactivity with only one (OspC) antigen was demonstrated in one serum sample and reactivity with two antigens in 2 (14.3%) of the 14 IgM-positive samples. Interestingly, none of these serum samples revealed reactivity with flagellar antigens, but the antigens recognized were VlsE and OspA in one serum sample and p39 and OspC in the second one. The remaining serum samples were positive for three or more of the nine antigens examined by Western blot (Table 4). As many as 12 (85.7%) of the 14 IgM-positive serum samples reacted with OspC protein, besides with flagellar antigens (p41, p41/i B. garinii, p41/i B. afzelii).

Of all the serum samples examined, only 9 (14.1%) showed the presence of IgG or IgM and IgG antibodies. The most common antigens recognized by the specific IgG antibodies were VlsE and p41 (100% reactivity), followed by p41/i *B. garinii* and p41/i *B. afzelii* (77.8% reactivity) and p39 (66.7% reactivity) (Tables 2 and 3). In all except one sample (Table 2, sample 56) specific IgG reactivity with VlsE and flagellar antigens was accompanied by reactivity with p18, and p39 or OspC, and/or p100. Only 2 (22.2%) of the 9 serum samples demonstrated IgG response to three of the nine antigens in Western blot and the remaining 7 (77.8%) samples revealed IgG reactivity with four to eight of the nine antigens (Table 4).

Table 1. Immunoenzyme and Western blot assay evaluation

Tabela 1. Test immunoenzymatyczny i Western blot

Serum sample no. (Numer próbki surowicy	EIA (BBU/ml)		WB (score)	
	IgM	IgG	IgM	IgG
4	12.3	≤ 11	7	≤ 4
17	10.5	≤ 11	13	≤ 4
18	12.5	≤ 11	13	≤ 4
22	19	≤ 11	9	≤ 4
25	20.6	≤ 11	5*	≤ 4
41	17.8	≤ 11	11	≤ 4
45	10.6	≤ 11	20	≤ 4
51	> 30	≤ 11	13	≤ 4
54	> 30	≤ 11	16	≤ 4
62	13.5	≤ 11	13	≤ 4
64	18.1	≤ 11	9	≤ 4
79	13.8	≤ 11	30	≤ 4
80	18.4	≤ 11	13	≤ 4
84	17.3	≤ 11	13	≤ 4
56	12.8	15.9	12	6*
74	17.2	> 30	8	21
87	12.3	20.7	12	11
11	≤ 11	> 30	≤ 4	37
14	≤ 11	> 30	≤ 4	31
38	≤ 11	24	≤ 4	21
42	≤ 11	> 30	≤ 4	31
63	≤ 11	20.5	≤ 4	14
81	≤ 11	> 30	≤ 4	31

BBU/ml – borrelial units.
EIA – immunoenzyme assay.
WB – Western blot.
* Borderline results.

BBU/ml – umowne jednostki boreliozowe.
EIA – test immunoenzymatyczny.
WB – western blot.
* Wyniki graniczne/wątpliwe.

Table 2. Reactivity of specific IgM and IgG antibodies in serum samples examined with specific recombinant antigens in Western blot assay

Tabela 2. Reaktywność przeciwciał IgM and IgG w próbkach surowicy z rekombinowanymi antygenami badana testem Western blot

Serum sample no. (Numer próbki surowicy)	Reaction of antibodies with specific antigens (Reakcja przeciwciał ze swoistymi antygenami)	
	IgM	IgG
4	VlsE, OspA	–
17	p41, OspC, p41/iG[a], p41/iA[b]	–
18	p41, OspC, p41/iG, p41/iA	–
22	p41, OspC	–
25	p41, p41/iG, p41/iA	–
41	p39, OspC	–
45	p100, VlsE, p41, OspC, p41/iG, p41/iA	–
51	p41, OspC, p41/iG, p41/iA	–
54	p41, p39, OspC, p41/iG, p41/iA	–
62	p41, OspC, p41/iG, p41/iA	–
64	p41, OspC, p41/iA	–
79	p41, p39, OspA, OspC, p41/iG, p41/iA	–
80	p41, OspC, p41/iG, p41/iA	–
84	p41, OspC, p41/iG, p41/iA	–
56	p41, OspC, p41/iG	VlsE, p41, p41/iG
74	OspC	VlsE, p41, p39, OspC, p41/iG, p41/iA
87	p41, OspC, p41/iG	VlsE, p41, OspC
11	–	p100, VlsE, p41, p39, OspC, p41/iG, p41/iA, p18
14	–	p100, VlsE, p41, p39, p41/iG, p41/iA, p18
38	–	p100, VlsE, p41, p39, p41/iG, p41/iA, p18
42	–	p100, VlsE, p41, p39, p41/iG, p41/iA, p18
63	–	VlsE, p41, p39, p41/iA
81	–	p100, VlsE, p41, p39, p41/iG, p41/iA, p18

[a] Flagellar antigen specific to *B. garinii.*
[b] Flagellar antigen specific to *B. afzelii*.

[a] Antygen rzęskowy swoisty dla *B. garinii.*
[b] Antygen rzęskowy swoisty dla *B. afzelii*.

Discussion

The diagnosis of Lyme disease is based on serological tests; however, in Europe borreliosis is caused by at least three genospecies of *B. burgdorferi* sensu lato and serological assays must consider the heterogeneity of borrelial antigens [24]. The antigenic diversity among the genospecies associated with Lyme disease, the wide variety of clinical symptoms of the disease, and a demonstrated seropositivity of the normal healthy population varying from 5–20% depending on age and outdoor activities make an interpretation of serological test results difficult, especially when clinical manifestations of the disease are nonspecific [4, 27].

In their laboratory, the present authors have noticed that first-contact doctors quite often refer individuals experiencing muscoskeletal symptoms for serological examination for Lyme disease. Nonspecific symptoms such as arthralgia may be associated with many other clinical disorders besides borreliosis, such as reactive or rheumatoid arthritis, systemic lupus erythematosus (SLE), or simply the wearing down of joint cartilage, common in the elderly.

Lyme arthritis is a late manifestation of the disease and most patients present elevated levels of IgG antibodies to a wide variety of borrelial antigens. However, similarly to erythema migrans, arthritis may occur as the only or the initial mani-

Table 3. Specific *B. burgdorferi* sensu lato antigens recognized by IgM and IgG antibodies present in the serum samples

Tabela 3. Antygeny swoiste dla *B. burgdorferi* sensu lato wykryte przez przeciwciała IgM and IgG obecne w próbkach surowicy

Antigen (Antygen)	IgM n = 17	IgG n = 9
p100	1 (5.9%)	5 (55.5%)
VlsE	2 (11.8%)	9 (100%)
p41	14 (82.3%)	9 (100%)
p39	3 (17.6%)	6 (66.7%)
OspA	2 (11.8%)	0
OspC	15 (88.2%)	2 (22.2%)
p41/iG	12 (70.6%)	7 (77.8%)
p41/iA	11 (64.7%)	7 (77.8%)
p18 (DbpA)	0	5 (55.5%)

festation of infection with *B. burgdorferi*. In these cases, both IgM- and IgG-specific antibodies are often detectable [9, 11, 21, 22].

In this study more than one third of the individuals with arthralgia revealed the presence of antibodies specific to *B. burgdorferi* sensu lato, but about 60% of the immunoenzyme assay-positive serum samples demonstrated IgM without accompanying IgG antibodies, indicating an early immune response. Early in the disease, some patients may experience migratory intermittent muscoskeletal pain in joints which without antibiotic treatment may evolve into more severe musculoskeletal disorders, especially among individuals with an IgM response to OspC outer membrane protein. OspC (21–25 kDa) is the most immunodominant antigen early after infection and seems to be an important virulence factor responsible for the infectivity and invasiveness of *B. burgdorferi* sensu lato species [1]. IgM response to OspC involves about 35–53% of European patients with early manifestations of borreliosis, especially those with neuroborreliosis. At a late stage of the disease, IgG response to OspC is associated with 3–60% of subjects [1]. On the other hand, immunological response to OspC might be predictive of the development Lyme arthritis since, according to Fung et al. [5], individuals with prominent IgM response to OspC early in the illness developed IgG reactivity with this protein, often apparent in arthritis cases, months to years later. Panelius et al. [16] also demonstrated IgM antibodies against OspC in 53% patients with Lyme arthritis. In the present study, a large proportion of the tested sera showed strong IgM reactivity with the antigen and, taking into consideration that most of the individuals reported in the questionnaire that they had symptoms for at least several weeks, it seems possible that although they did not reveal an IgG response, they might develop IgG reactivity with the antigen and their symptoms will evolve into arthritis [29].

Table 4. Number of antigens recognized by specific IgM and IgG antibodies present in the serum samples

Tabela 4. Liczba antigenów wykrytych za pomocą przeciwciał dla IgM and IgG obecne w próbkach surowicy

No. of antigens (Liczba antygenów)	IgM n = 17	IgG n = 9
1	1 (5.9%)	0
2	3 (17.6%)	0
3	4 (23.5%)	2 (22.2%)
4	6 (35.3%)	2 (22.2%)
5	1 (5.9%)	0
6	2 (11.8%)	0
7	0	4 (44.4%)
8	0	1 (11.1%)

In about 80% of the serum samples with IgM against OspC there were also IgM antibodies to flagellar antigens of *B. burgdorferi* sensu lato genospecies. Flagellar protein p41 (41 kDa) is also an immunodominant antigen inducing strong IgM and IgG response early after infection. Unfortunately, antibody reactivity only with flagellar antigen may be associated with a cross-reaction of antibodies induced by infection with other spirochetes, for example *Treponema*, *Leptospira*, or relapsing fever of borrelial species, and these results should be interpreted cautiously [1]. However, in the study there was only one serum sample with IgM antibodies reacting only with flagellar antigens, as confirmed by Western blot analysis. Although the level of the IgM antibodies in the sample was quite high (20.6 BBU/ml), Western blot evaluation indicated a borderline score. In this case, other spirochetal or viral diseases, such as mononucleosis, should be excluded.

Interestingly, two of the tested samples indicated IgM reactivity with OspA antigen. Borreliacidal antibodies to OspA (31 kDa) antigen are detected very rarely among European patients with Lyme disease and are characteristic of late infection [1]. In this study, in one sample IgM reactivity to OspA was accompanied by VlsE antigen and in the second by OspC, p39, and flagellar antigens, which are highly specific to borrelial infection [19]. In some patients with Lyme disease, IgG response is delayed, and even during late stages, 5–10% of patients do not have elevated antibody levels, which might also be true of these

two cases of the study [23]. Moreover, it cannot be also excluded that these two subjects, although they had claimed not to have been treated with antibiotics, might have been treated for another infection. Patients who received inadequate antibiotic therapy early in the disease may have an abrogated IgM to IgG switch [27].

Although in rare cases the presence of IgM antibodies against specific borrelial antigens together with clinical symptoms may indicate Lyme arthritis, it is mostly the IgG class of specific antibodies with or without accompanying IgM antibodies that indicates joint involvement in the course of Lyme disease. Patients with Lyme arthritis usually have IgG antibodies to a broad spectrum of antigens, whereas a negative IgG result argues against late Lyme disease [27]. In the present study about 14% of the samples showed IgG response against at least three borrelial antigens, i.e. p18, p39, and p100, that are particularly reliable markers for late manifestations of Lyme disease. Moreover, all IgG-positive sera presented reactivity with VlsE antigen, which is the most sensitive protein for IgG antibody detection in all stages of Lyme disease, reacting with more than 90% of patients' samples [6]. VlsE, a 35-kDa surface-exposed lipoprotein, is highly immunogenic and conserved among *B. burgdorferi* sensu lato species and causes a broad reactivity during infection [1, 13, 15]. About 87% of patients with early infection show IgG against this antigen, but the antigen also often accompanies the immune response in late manifestations of Lyme disease [1, 13]. In the present study the IgG immune response against VlsE was predominantly accompanied by flagellar proteins, which is in accordance with the study by Panelius et al. [17] in which 86% of serum samples from Lyme arthritis patients reacted with one to three recombinant flagellar antigens of *B. burgdorferi* sensu lato species. Moreover, according to Lawrence et al. [13], as many as about 90% of patients with Lyme arthritis showed antibodies against VlsE, in contrast to no patient with non-borrelial arthritis. Additionally, antibodies to VlsE antigen wane rapidly after antibiotic treatment, making them also good indicators of successful treatment [20]. Besides that, p39, p100, and p18 (DbpA) antigens were the most frequently recognized by IgG antibodies in the present study. Decorin-binding protein A (p18, DbpA, 17 kDa) is responsible for binding the spirochetes to the host collagen-associated proteoglycan decorin and seems to be a sensitive and specific antigen for the serodiagnosis of disseminated Lyme disease, as seropositivity to the protein is as high as 85% in patients with late borreliosis [8]. The second antigen, p39 (BmpA), is less useful in the serodiagnosis of Lyme disease because of genetic and antigenic differences among *B. burgdorferi* sensu lato species [1, 6]. However, Goettner et al. [6] showed IgG reactivity against recombinant DbpA, p100, and VlsE antigens in 100%, p41 in 80%, and OspC and p39 in 60% of serum samples of European patients with Lyme arthritis.

Considering all the data, the fact that arthralgia was the predominant disorder experienced by the examined individuals, as well as the fact that they had never been treated for Lyme disease, the results of the study allow the assumption that all the nine subjects with IgG or IgG and IgM immune response presented active Lyme arthritis. The study demonstrated that arthralgia should be diagnosed paying special attention to Lyme disease and that Western blot analysis of specific to *B. burgdorferi* sensu lato antigens seems to be a useful diagnostic tool for evaluating arthritis caused by *Borreliae*. Serodiagnosis of Lyme disease should be considered a supplemental assay in individuals with arthralgia, at least among subjects with a history of tick bite.

References

[1] Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP: Diagnosis of Lyme borreliosis. Clin Microbiol Rev 2005, 18, 484–509.

[2] Balmelli T, Piffareti JC: Association between different clinical manifestations of Lyme disease and different species of *Borrelia burgdorferi* sensu lato. Res Microbiol 1995, 146, 329–340.

[3] Bingnan MA, Christen B, Leung D, Vigo-Pelfrey C: Serodiagnosis of Lyme boreliosis by western immunoblot: reactivity of various antibodies against *Borrelia burgdorferi*. J Clin Microbiol 1992, 30, 370–376.

[4] Blaauw AM, van Loon AM, Schellekens JF, Bijlsma JW: Clinical evaluation of guidelines and two-test approach for Lyme disease. Rheumatol 1999, 38, 1121–1126.

[5] Fung BP, McHugh GL, Leong JM, Steere AC: Humoral immune response to outer surface protein C of *Borrelia burgdorferi* in Lyme disease: role of the immunoglobulin M response in the serodiagnosis of early infection. Infect Immun 1994, 62, 3213–3221.

[6] Goettner G, Schulte-Spechtel U, Hillermann R, Liegl G, Wilske B, Fingerle V: Improvement of Lyme borreliosis serodiagnosis by a new developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and addition of VlsE and DbpA homologes. J Clin Microbiol 2005, 43, 3602–3609.

[7] Hauser U, Lehnert G, Wilske B: Validity of interpretation criteria for standardization western blots (immunoblots) for serodiagnosis of Lyme borreliosis based on sera collected throughout Europe. J Clin Microbiol 1999, 37, 2241–2247.
[8] Heikkila T, Seppala I, Saxen H, Panelius J, Yranainen H, Lahdenne P: Species-specific serodiagnosis of Lyme-arthritis and neuroborreliosis due to *Borrelia burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* by using decorin binding protein A. J Clin Microbiol 2002, 40, 453–460.
[9] Huppertz HI, Karch H, Suschke HJ, Doring E, Ganser G, Thon A, Benats W: Lyme arthritis in European children and adolescents. The Pediatric Rheumatology Collaborative Group. Arthritis Rheum 1995, 38, 361–368.
[10] Kalish RA, Leong JM, Steere AC: Association of treatment-resistant chronic Lyme arthritis with HLA-DR4 and antibody reactivity to OspA and OspB of *Borrelia burgdorferi*. Infect Immun 1993, 61, 2774–2779.
[11] Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC: Persistence of immunoglobulin M or immunoglobulin G antibody responses to *Borrelia burgdorferi* 10–20 years after active Lyme disease. Clin Infect Dis 2001, 33, 780–785.
[12] Kamradt T: Lyme disease and current aspects of immunization. Arthritis Res 2002, 4, 20–29.
[13] Lawrenz MB, Hardham JM, Owens RT, Nowakowski J, Steere AC, Wormster GP, Norris SJ: Human antibody responses to VlsE antigenic variation protein of *Borrelia burgdorferi*. J Clin Microbiol 1999, 37, 3997–4004.
[14] Limbach FX, Jaulhac B, Puechal X, Monteil H, Kuntz JL, Piemont Y, Sibilia J: Treatment resistant Lyme arthritis caused by *Borrelia garinii*. Ann Rheum Dis 2001, 60, 284–286.
[15] Norris SJ: Antigenic variation with twist – the *Borrelia* story. Mol Microbiol 2006, 60, 1319–1322.
[16] Panelius J, Lahdenne P, Heikkila T, Peltomaa M, Oksi J, Seppala I: Recombinant OspC from *Borrelia burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* in the serodiagnosis of Lyme boreliosis. J Med Microbiol 2002, 51, 731–739.
[17] Panelius J, Lahdenne P, Saxen H, Heikkila T, Seppala I: Recombinant flagellin A proteins from *Borrelia burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* in serodiagnosis of Lyme borreliosis. J Clin Microbiol 2001, 39, 4013–4019.
[18] Renaud I, Cachin C, Gerster JC: Good outcomes of Lyme arthritis in 24 patients in an endemic area of Switzerland. Joint Bone Spine 2004, 71, 39–43.
[19] Ryffel K, Peter O, Rutti B, Suard A, Dayer E: Scored antibody reactivity determined by immunoblotting shows an association between clinical manifestations and presence of *Borrelia burgdorferi sensu stricto*, *B. garinii*, *B. afzelii*, and *B. valaisiana* in humans. J Clin Microbiol 1999, 37, 4086–4092.
[20] Schnarr S, Franz JK, Krause A, Zeidler H: Lyme borreliosis. Clin Rheumatol 2006, 20, 1099–1118.
[21] Steere AC: Lyme disease. N Engl J Med 2001, 345, 115–125.
[22] Steere AC: Musculoskeletal manifestations of Lyme disease. Am J Med 1995, 98, 44S–51S.
[23] Steere AC, Gross D, Meyer AL, Hubber BT: Autoimmune mechanisms in antibiotic treatment-resistant Lyme arthritis. J Autoimmun 2001, 16, 263–268.
[24] Tilton RC: Laboratory aids for the diagnosis of *Borrelia burgdorferi* infection. J Spirochetal and Tick-Borne Dis 1994, 1, 18–23.
[25] Valckenare C, Guillemin F, Pourel J. Schiele F, Heller R, Jaulhac B: Seroreactivity to *Borrelia burgdorferi* antigens in early rheumatoid arthritis: a case – control study. Br J Rheumatol 1997, 36, 945–949.
[26] Wang G, van Dam AP, Schwartz I, Danbert J: Molecular typing of *Borrelia burgdorferi* sensu lato: taxonomic, epidemiological, and clinical implications. Clin Microbiol Rev 1999, 12, 633–365.
[27] Wilske B: Diagnosis of Lyme borreliosis in Europe. Vector-Borne Zoonotic Dis 2003, 3, 215–227.
[28] Wormser GP, Nadelman RB, Nowakowski J, Shwartz I: Asymptomatic *Borrelia burgdorferi* infection. Med Hypotheses 2001, 57, 435–438.
[29] Wooten RM, Weis JJ: Host-pathogen interactions promoting inflammatory Lyme arthritis: use of mouse models for dissection of disease processes. Curr Opin Microbiol 2001, 4, 274–279.

Address for correspondence:

Beata Sobieszczańska
Department of Microbiology
Silesian Piasts University of Medicine
Chałubińskiego 4
50-368 Wrocław
Poland
Tel.: +48 71 784 12 78
E-mail: mapasobie@provider.pl

Conflict of interest: None declared

Received: 3.09.2008
Revised: 14.11.2008
Accepted: 21.11.2008