Arch. Immunol. Ther. Exp. (2011) 59:69–77
DOI 10.1007/s00005-010-0111-0

ORIGINAL ARTICLE

Serodiagnosis of Borreliosis: Indirect Immunofluorescence Assay, Enzyme-Linked Immunosorbent Assay and Immunoblotting

Iwona Wojciechowska-Koszko · Iwona Mączyńska · Zbigniew Szych · Stefania Giedrys-Kalemba

Received: 19 March 2010 / Accepted: 20 August 2010 / Published online: 22 January 2011

Abstract Lyme disease is an infectious, multi-system, tick-borne disease caused by genospecies of *Borrelia burgdorferi* bacteria sensu lato, characterized by remarkable heterogeneity. In this situation choosing an optimal antigen array for diagnostic tests seems problematic. The serological tests for borrelia routinely done in laboratories often produce ambiguous results, which makes a proper diagnosis rather complicated and thus delays the implementation of an appropriate treatment regimen. Thirty-seven outpatients and eight inpatients with suspected borreliosis diagnosis hospitalized at the Clinics of the Pomeranian Medical University (Szczecin, Poland), participated in the study. In order to detect the antibodies against *Borrelia* sensu lato three kinds of serological tests were used: indirect immunofluorescence assay (IIFA), enzyme-linked immunosorbent assay (ELISA), and immunoblot. The IIFA and immunoblot tests conducted on 45 patients (100%) produced positive results for both the IgM and IgG antibody types. In the case of ELISA, positive or borderline results were observed in only 24 patients (53.3%). The immunoblot test for IgM most frequently detected antibodies against the outer surface protein C (OspC) antigen (p25), and, in the case of IgG, against the recombinant variable surface antigen (VlsE). The IIFA screening test used for diagnosing Lyme borreliosis produced the highest percentage of positive results, which were then confirmed by immunoblot, but not by ELISA. Therefore using only ELISA as a screening test or for diagnosing Lyme borreliosis seems debatable.

Keywords Lyme borreliosis · ELISA · IIFA · Immunoblot · VlsE protein · OspC protein

Abbreviations

ELISA	Enzyme-linked immunosorbent assay
IIFA	Indirect immunofluorescence assay
VlsE	Variable surface antigen
OspC	Outer surface protein C
EM	Erythema chronicum migrans
ACA	Acrodermatitis chronica atrophicans

Introduction

Borreliosis is a multi-organ, infectious disease transmitted by ticks of several different species of the genus *Ixodes*. Following the bite of an infected tick, the spirochetes undergo a rapid hematogenous dissemination and can be found in many of the major organs. In the early phase (the first stage), within a few days or weeks of the time of the original infection, erythema chronicum migrans (EM), flu-like symptoms, and sometimes lymphadenosis benigna cutis may appear. Unfortunately, EM is present, remembered or observed by only 50–70% of patients and the other signs of an early infection vary and are non-specific (Sigal 1992; Steere 1989). Without the correct diagnosis and medical treatment the disease progresses towards the secondary and late stages. The symptoms of the second stage may include cardiovascular system complaints, nervous system complaints and musculoskeletal system conditions.

I. Wojciechowska-Koszko (✉) · I. Mączyńska · S. Giedrys-Kalemba
Department of Microbiology and Immunology, Pomeranian Medical University, Powstańców Wielkopolskich 72, 70-111 Szczecin, Poland
e-mail: IwonaKoszko@interia.pl

Z. Szych
Department of Informatics and Biocybernetics, Pomeranian Medical University, Szczecin, Poland

Late borreliosis (the third, chronic, persistent stage) is characterized by acrodermatitis chronica atrophicans (ACA), joint inflammation, neuroborreliosis manifesting as encephalitis, encephalomyelitis and peripheral neuropathy, as well as chronic encephalopathy. In Europe neurological symptoms appear in 30%, and musculoskeletal symptoms in 20%, of untreated patients. The polymorphic nature of the disease makes a proper diagnosis difficult (Aberer 2007; Altpeter and Meier 1992; Figlerowicz 2006; Steere 1989).

In Europe three pathogenic genospecies are prevalent: *Borrelia garinii*, *B. afzelii and B. burgdorferi* sensu stricto. The remarkable heterogeneity of these genospecies along with the many different types of antigens of specific character used in diagnostic tests pose additional difficulties in recognizing borreliosis and interpreting the results of the tests (false positive and false negative test results). In accordance with European directives, a two-stage diagnostic process is recommended. The first stage consists of highly sensitive serological tests [enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence assay (IIFA)], which determine the IgM and IgG antibody levels. In the second stage, in order to verify a positive or ambiguous result, the high quality immunoblot method is used. It contains antigens against the three pathogenic genospecies or common antigens (Niścigorska et al. 2003; Tylewska-Wierzbanowska and Chmielewski 2005; Wilske 2003; Zajkowska et al. 2007).

The aim of this study is to compare test results using IIFA, ELISA and immunoblot conducted in parallel in patients with a suspected borreliosis diagnosis.

Materials and Methods

The Study Group

The tests were performed on 45 patients: 37 outpatients and 8 inpatients hospitalized in the clinical wards of Szczecin hospitals [the Clinic of Skin and Venereal Diseases (6 patients), the Clinic of Vascular Surgery (1 patient) and the Clinic of Rheumatology (1 patient)]. In every patient the interview and clinical symptoms indicated a possible borreliosis diagnosis: possible ($n = 14$) or definite ($n = 31$) exposure to a tick, the presence of erythema migrans ($n = 34$), lymphocytoma ($n = 1$), joint inflammation ($n = 5$), radiculoneuropathy ($n = 2$), polyneuropathy ($n = 3$). In patients with definite recent exposure to a tick, the tests were done at least 4 weeks after the time of the exposure.

Serodiagnosis

Serum obtained from centrifuged peripheral blood was used for the serological tests. The blood taken from the patients was put directly into a test tube (Sarstedt Monovette) without any anticoagulant on the day they came for the examination.

IIFA

The tests were done with fresh serum using 1st-generation anti-*Borrelia* IgM and IgG indirect immunofluorescence from Euroimmun (Germany) containing whole cell antigens against *B. burgdorferi* sensu stricto, in accordance with the procedure recommended by the manufacturer. The test for IgM antibodies was assumed to be positive when a fluorescence pattern was present in the 1:10 initial titer, and for the IgG class antibodies in the 1:100 titer. In order to determine the quantity of IgG antibodies, the serum was diluted in accordance with the recommended schedule: 1:320, 1:1,000, 1:3,200, 1:10,000, etc. When assaying the IgM antibodies, IgG/RF (rheumatoid factor) absorbent was used (goat's antibodies against human IgG immunoglobulin), which eliminates the possibility of a potential cross-reaction between the RF and the IgM antibodies, thus excluding a false positive result. The sensitivity of the test used to detect the antibodies against *Borrelia* is 80% for IgM and 95% for IgG.

ELISA

In the immunoenzymatic ELISA method, a third-generation test was used: recombinant *Borrelia* IgM and IgG manufactured by Biomedica (Austria), containing recombinant proteins characteristic of the three species of *Borrelia*: *B. garinii, B. afzelii* and *B. burgdorferi* sensu stricto. It detects IgM antibodies for the antigens outer surface protein C (OspC; p25) and p41int (an internal part of the flagellin protein); and for IgG it detects OspC (p25), p18, p100 and the variable surface (VlsE) recombinant antigen. The tests were done in accordance with the procedure recommended by the manufacturer. The results of the quantitative assay of both immunoglobulin types were expressed in Biomedica Borrelia units (BBU)/ml. Results with the value $\geq$11 BBU/ml were assumed to be positive, those in the range 9–11 BBU/ml were assumed to be ambiguous, and those $\leq$9 BBU/ml were assumed to be negative.

Immunoblot

The sera from which the positive or ambiguous results were obtained in the first diagnostic stage, regardless of which test was used (IIFA or ELISA) and which antibody class was tested for, were then verified by the immunoblot anti-*Borrelia* test EUROLINE IgM and IgG (Euroimmun). This test contains the antigen p83/100 against the whole *B. afzelii* cell, p41(flagellin), basic membrane protein A

(BmpA; p39), OspA (p31), p30, OspC (p25), p21, p19, p17 and the VlsE recombinant antigen. The immunoblots were assayed automatically using EuroLine Scan, the analysis software from Euroimmun.

To be positive for the IgM antibody type the result had to contain:

- a distinct OspC (p25) band;
- a distinct or weak OspC (p25) band and one band containing any of the antigens listed above with the exception of the p41 antigen (according to the manufacturer's evaluation protocol, p41 antigen is not genus specific and may give cross-reactivity to other Spirochaetaceae and bacteria having flagella);
- a distinct band of all the antigens listed above with the exception of p41 or OspC (p25). The limen result for IgM antibodies was determined in the case of a weakly marked OspC (p25) band.

To be positive for the IgG antibody class the result had to contain:

- a distinct VlsE band;
- a distinct or weak VlsE band and at least one distinct band of one of the antigens listed above with the exception of the p41 antigen;
- at least two distinct bands of the antigens listed above with the exception of the p41 or VlsE antigens. To be ambiguous for the IgG class, such a borderline result had to include a weakly marked VlsE band or one distinct band of any of the antigens listed above with the exception of the p41 and VlsE antigens.

Statistical Analysis

The statistical analysis of the results was done using a test for independence of χ^2 or a test for independence of χ^2 with Yates' correction on Statistica 6.0 Pl software. Acceptable probability (p) of a type 1 error (the level of test significance) was assumed to be equal to 0.05.

Results

The detailed compilation of the results of assaying anti-*Borrelia* IgM and IgG type antibodies, using serological methods applied to all the patients with a suspected borreliosis diagnosis, is shown in Table 1.

During the first stage of laboratory diagnostics, using the IIFA method, specific antibodies against borreliosis were detected in all the 45 tested patients (100%): for both IgM and IgG in 24 patients (53.3%), for only IgM in 1 patient (2.2%), and for only IgG in 20 patients (44.4%).

Using the ELISA method, positive or borderline results in the same group of patients were obtained in 24 patients (53.3%): for both IgM and IgG in 11 patients (24.4%), for only IgM in 5 patients (11.1%), and for only IgG in 8 patients (17.8%).

In the immunoblot confirmation test positive results were obtained in all the patients (100%): in 16 patients (35.6%) for both IgM and IgG, in 2 patients (4.4%) for only IgM, and in 27 patients (60%) for only IgG. The compilation of the positive results obtained from the serological methods applied is shown in Table 2.

Comparing $\sum$ for positive results obtained for IgM and IgG shown in Table 2, statistically significant differences were observed in the case of:

- both antibody types obtained from ELISA and IIFA (Fig. 1a);
- IgM antibody obtained from IIFA and immunoblot (Fig. 1b);
- IgG antibody obtained from ELISA and immunoblot (Fig. 1c).

While in the case of positive results for IgG obtained by IIFA and immunoblot, as well as in reference to the positive results for IgM obtained from ELISA and immunoblot, no statistically significant differences were observed (Fig. 1b, c).

In the immunoblot confirmation test for IgM, the most frequently detected antibodies were against the p25 antigen, OspC ($n = 17$; 37.7%), in addition to which antibodies against other antigens were occasionally detected—OspA ($n = 2$) and p83 ($n = 1$). The most frequently detected antibodies against IgG were antibodies against the VlsE recombinant antigen ($n = 29$; 64.4%), followed by antibodies against the following antigens: p83 ($n = 15$), p31–OspA ($n = 14$), p17 ($n = 12$), p30 ($n = 11$), p25–OspC ($n = 9$), p39–BmpA ($n = 7$), p19 ($n = 6$), p21 ($n = 3$)—Fig. 2.

Examples of immunoblot results for both anti-*Borrelia* antibody types from patients are shown in Fig. 3.

As shown in the analysis in Table 2, comparing the results of different tests carried out on the same person reveals the high conformity of the immunoblot and IIFA methods (97.8%) as far as detecting specific IgG antibodies is concerned (only in patient number 17 in Table 1, who returned a positive IIFA, were no IgG antibodies detected by immunoblot). The conformity of the detection rates for IgM antibodies was lower (84.5% in 7 IIFA-positive patients, no IgM antibodies detected by immunoblot).

The conformity of ELISA and immunoblot in detecting IgM antibodies was comparable (82.2% in 3 ELISA-positive patients, no antibodies detected by immunoblot). However, five patients who were ELISA-negative were immunoblot positive. Four patients in this group had a positive OspC (p25) antigen band (proteins included in both tests), and one patient was positive for the p31

Table 1 Detailed compilation of results of assaying anti-*Borrelia* IgM and IgG antibodies from tested patients

Patient number	IIFA		ELISA		EUROLINE—IgM										EUROLINE—IgG									
	IgM	IgG	IgM	IgG	VlsE	p83	p41	BmpA (p39)	OspA (p31)	p30	OspC (p25)	p21	p19	p17	VlsE	p83	p41	BmpA (p39)	OspA (p31)	p30	OspC (p25)	p21	p19	p17
1	+	**1:1000**	**15.883**	**13.661**	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	+	–	+
2	–	**1:1000**	7.548	6.736	–	–	–	–	–	–	–	–	–	–	+	+	(+)	–	–	–	–	–	–	–
3	+	**1:100**	**19.766**	6.145	–	–	+	–	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–
4	–	**1:100**	8.378	5.281	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
5	+	**1:100**	**35.094**	3.452	–	–	+	–	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–
6	+	**1:100**	**11.184**	**11.129**	–	–	–	–	–	–	–	–	–	–	+	–	(+)	–	–	–	–	–	–	+
7	+	**1:100**	8.396	**10.416**[a]	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
8	+	**1:320**	8.454	4.265	–	–	–	–	–	–	–	–	–	–	–	–	(+)	+	+	+	+	–	–	–
9	+	**1:100**	**11.531**	4.738	–	–	(+)	–	–	–	+	–	–	–	–	(+)	–	–	–	–	–	–	–	–
10	+	**1:320**	**10.984**[a]	**41.965**	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+	+	+	–	+	–
11	+	**1:100**	**13.773**	**39.644**	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–
12	–	**1:100**	6.930	5.267	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–
13	+	**1:1000**	**10.984**[a]	**>150.000**	–	–	–	–	–	–	(+)[a]	–	–	–	+	+	+	+	–	+	(+)	(+)	–	+
14	+	**1:100**	7.864	**14.921**	–	–	+	–	+	–	–	–	–	–	+	–	+	–	+	–	–	–	+	+
15	+	**1:1000**	**14.370**	**>150.000**	–	–	(+)	–	–	–	(+)[a]	–	–	–	+	+	–	–	(+)	+	–	–	(+)	–
16	+	**1:320**	7.164	**9.468**	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
17	+	**1:100**	**16.317**	2.548	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
18	–	**1:1000**	2.574	6.762	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	–	–	–	–	–
19	+	**1:100**	**13.248**	**18.732**	–	–	–	–	–	–	+	–	–	–	–	–	(+)	–	–	–	(+)[a]	–	–	–
20	+	**1:320**	**13.934**	**16.969**	–	–	–	–	–	–	+	–	–	–	(+)	+	(+)	–	+	–	–	–	–	–
21	+	**1:320**	6.706	4.288	–	–	+	–	–	–	+	–	–	–	–	–	+	–	–	–	+	–	–	–
22	–	**1:320**	8.721	4.857	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–
23	+	–	**29.240**	4.149	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
24	+	**1:100**	6.168	4.271	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	+
25	–	**1:1000**	4.424	3.538	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
26	+	**1:1000**	**36.866**	**>150.000**	–	–	+	–	–	–	+	–	–	–	+	–	+	–	–	–	+	–	–	–
27	–	**1:1000**	7.663	4.031	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–
28	–	**1:1000**	4.438	3.455	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–	–	–
29	–	**1:1000**	5.219	**58.773**	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	+	–	+	+
30	+	**1:320**	6.356	5.976	–	–	+	–	–	–	+	–	–	–	+	–	+	–	+	–	–	–	–	+
31	–	**1:100**	1.020	7.902	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
32	+	**1:10000**	**34.554**	**>150.000**	–	+	+	–	–	–	+	–	–	–	+	+	+	+	–	–	+	+	–	+
33	+	**1:1000**	3.639	8.193	–	–	–	–	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	+
34	–	**1:320**	3.810	**17.681**	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–

Table 1 continued

Patient number	IIFA		ELISA		EUROLINE—IgM				EUROLINE—IgG									
	IgM	IgG	IgM	IgG	VlsE	p83	p41	BmpA (p39)	OspA (p31)	p30	OspC (p25)	p21	p19	p17	VlsE	p83	p41	BmpA (p39)	OspA (p31)	p30	OspC (p25)	p21	p19	p17
35	–	**1:320**	2.710	3.274	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
36	–	**1:100**	2.340	4.421	–	–	–	–	–	–	–	–	–	–	+	–	(+)	–	–	–	–	–	–	–
37	–	**1:100**	8.854	3.737	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
38	–	**1:100**	2.531	6.287	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–
39	–	**1:100**	2.963	2.930	–	–	–	–	–	–	–	–	–	–	+	–	+	–	+	–	–	–	–	–
40	–	**1:320**	4.630	3.255	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
41	+	**1:320**	8.587	**>150.000**	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	+	+
42	–	**1:100**	4.533	5.951	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	–	–	–	–
43	–	**1:100**	2.688	**15.112**	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–
44	+	**1:1000**	**11.092**	**102.614**	–	–	(+)	–	–	–	+	–	–	–	+	+	+	–	–	+	–	–	–	+
45	–	**1:10000**	3.502	**>150.000**	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	+

Borderline and positive results were marked by using thick heavy lines

(+), weakly marked band in the EUROLINE test

[a] Borderline result

antigen, which is not included in the immunoenzymatic test (Table 3).

Conformity between ELISA and immunoblot in detecting IgG antibodies was observed in only 19 patients (42.2%). The remaining 24 patients, who were ELISA negative, were found to be immunoblot positive for antibodies against different antigens. Within this group 14 patients had a positive band of the VlsE recombinant antigen, included in both tests used; in 8 patients the presence of p83 or p25 (OspC) antigens (included in both tests) was detected combined with antigens p31, p30, p39 (included only in immunoblot), while in 2 patients p31 and p30 antigens (not included in ELISA) were detected (Table 3).

Discussion

Lyme borreliosis is usually diagnosed at the early stage of the infection on the basis of clinical symptoms such as erythema migrans. However, since erythema migrans only appears in some cases, while in others it may not appear, it cannot be the sole criterion for diagnosis. Some patients may also have atypical cutaneous alterations, or no skin symptoms. Thus one of the criteria for making a Lyme borreliosis diagnosis is a positive result of a laboratory test. The tests are usually aimed at detecting specific IgM and/or IgG anti-*Borrelia* antibodies. However, about 40% of erythema migrans patients are seronegative. Seropositivity persists for years after infection and is also seen in the healthy population (Cetin et al. 2006; Stanek and Strle 2009). Less frequently spirochete cultures are grown on growth medium or borrelial DNA is detected by PCR, mostly used in seronegative patients (Tylewska-Wierzbanowska and Chmielewski 2005).

In accordance with European standards, in the two-stage serological diagnosis of borreliosis, highly sensitive tests such as ELISA or IIFA are used during the first stage, and only then are positive and ambiguous results verified by the Western blot. In Europe the method requires taking into account antigens of three different pathogenic genospecies or including common antigens (Tylewska-Wierzbanowska and Chmielewski 2005; Wilske et al. 1993, 2007; Wilske 2003). The accurate choice of suitable antigens in constructing a serological test is difficult. Many authors believe that the most reliable serological indicators of borrelia infection are antibodies against the antigens OspC and VlsE, both during the early phase of the infection (the OspC antigen in particular) and in the later phase (mostly the VlsE recombinant antigen) (Chmielewska-Badora et al. 2006; Zajkowska et al. 2006). However, other antigens, such as p83 (wall protein), p39 (BmpA), p58, p14, p30, p43 and Osp 17 (DbpA), are also important markers,

Table 2 Number (*n*) and percentage (%) of positive and ambiguous results obtained from patients using the IIFA, ELISA and immunoblot methods

Serological test	Positive patients for only IgM		Positive patients for only IgG		Positive patients for both IgM and IgG		∑ for positive patients for IgM		∑ for positive patients for IgG		∑ for all positive patients only for IgM and only for IgG and for both IgM and IgG	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
IIFA	1	2.2	20	44.4	24	53.3	25	55.5	44	97.8	45	100
ELISA	5	11.1	8	17.8	11	24.4	16	35.5	19	42.2	24	53.3
Immunoblot	2	4.4	27	60	16	35.6	18	40	43	95.5	45	100

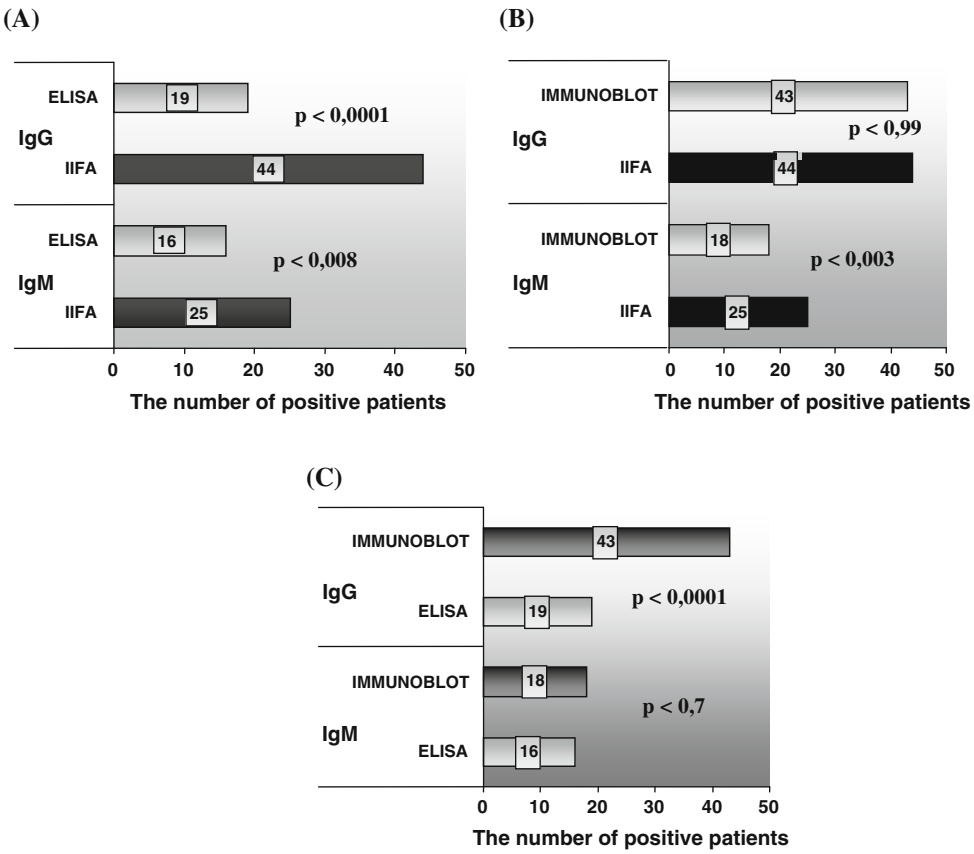

Fig. 1 The number of positive patients for anti-*Borrelia* IgM and IgG types antibodies obtained by using: **a** IIFA and ELISA test, **b** IIFA and immunoblot and **c** ELISA and immunoblot

mostly for IgG antibodies, particularly in the later stage of infection (Aguero-Rosenfeld et al. 2005; Hauser et al. 1997; Tylewska-Wierzbanowska and Chmielewski 2005).

The cross-reactions between some species of bacteria and viruses which set off polyclonal antibody production should also be taken into account in interpreting the results. False positive serological results for *Borrelia* obtained from screening tests may be positive in cases of syphilis, *Salmonella typhimurium* infection, Epstein-Barr virus and exposure to *Bacillus subtilis*, but also in collagenosis and when RF is present (Marangoni et al. 2005). Therefore serological test for borreliosis routinely done in laboratories may produce questionable results. Moreover, such results may differ from the standard sequence in which antibodies usually appear (IgM antibodies are characteristic for acute phase of infection or remission, and then IgG antibodies are characteristic for acute or former phase of infection) in the case of other infectious diseases (Zajkowska et al. 2006).

The tests commonly used during the first diagnostic stage, which are based on immunoenzymatic or immunofluorescence methods, may produce both false positive and false negative results, which may delay the correct diagnosis and therefore the necessary treatment. Such an

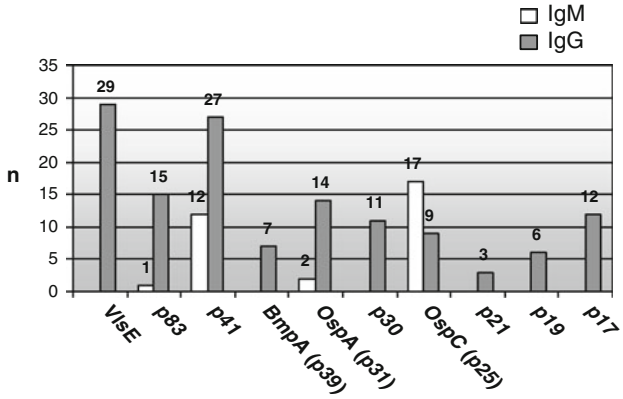

Fig. 2 The number of positive results (***n***) obtained from the researched group of patients for the IgM and IgG antibody types for particular antigens included in the Immunoblot test

opinion was confirmed by our own research, in particular by the ELISA results, where positive IgM and/or IgG anti-*Borrelia* antibody results were obtained only in 53.3% of patients when compared to the immunoblot confirmation test; for IgG these differences were statistically significant. Comparing the positive and negative results obtained from ELISA and immunoblot in the same patient showed a higher conformity for IgM (82.2%) than for IgG (44.4%). Using ELISA, only three patients returned a false positive for IgM compared to immunoblot, while the remaining IgM results and all the IgG results were false negatives. The number of positive results obtained from IIFA was higher for both IgM and IgG than the number obtained from ELISA, and the difference was statistically significant. All the positive IIFA results were confirmed by

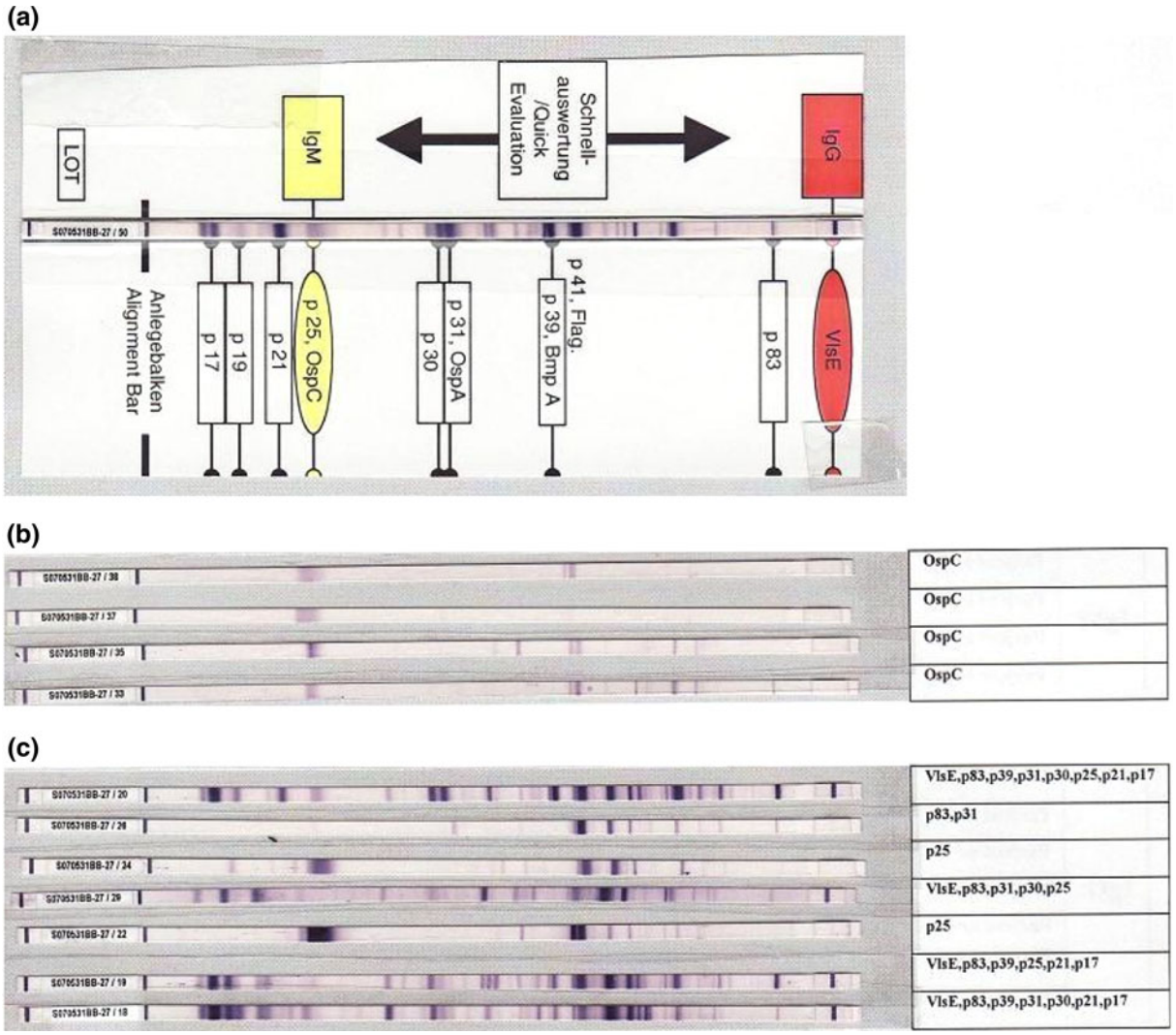

Fig. 3 Examples of immunoblots of patients with suspected borreliosis: **a** anti-*Borrelia*—Euroline-WB evaluation matrix **b** examples of immunoblots of patients for IgM type and **c** examples of immunoblots of patients for IgG

Table 3 Number of patients positive for anti-*Borrelia* IgM and IgG antibodies when tested by immunoblot, but negative when tested by ELISA

Antibody type/n (number of positive patients)	Obtained antigens present in both the ELISA and immunoblot tests	Obtained antigens present only in the immunoblot test	Number of patients positive in immunoblot, but negative in ELISA	
IgM	Osp C (p25)	–	4	∑=5
(n=5)	–	OspA (p31)	1	
IgG	VlsE	–	13	∑=14
(n=24)	VlsE, p83	–	1	
	OspC (p25)	p30, OspA (p31), p39	1	∑=8
	OspC (p25)	OspA (p31)	1	
	OspC (p25)	–	1	
	p83	OspA (p31)	1	
	p83	p39	1	
	p83	OspA (p31), p30	1	
	p83	p30	1	
	p83	–	1	
	–	p30, p31 (OspA)	2	∑=2

immunoblot, while the conformity of the two methods in detecting specific IgG antibodies was 97.8% (1 false positive IIFA result) and for IgM it was 84.5% (7 false positive results using IIFA); no false negatives were observed. On the one hand, the results obtained indicate that the IIFA test might be hypersensitive, as it produces more false positive results, particularly for IgM antibodies. On the other hand, using it lowers the risk of not detecting actual *Borrelia* sensu lato infections in comparison to the ELISA method, which gives a high percentage of false negatives when compared to immunoblot, even though they both include similar antigens (immunoblot: VlsE, $p83^{B.afzelii}$ – disintegration protein of p100 antigen, p25 – $OspC^{B.afzelii}$, ELISA: VlsE, $p100^{B.afzelii}$, p25 – $OspC^{B.burgdorferi}$ sensu stricto, *B.garinii*).

The difference in the number of positive anti-*Borrelia* results obtained by ELISA and immunoblot has also been pointed out by other authors. Marangoni et al. (2005) found that IgG positive results were 6.6% higher in immunoblot than in ELISA. Similarly, Chmielewska-Badora et al. (2006) observed a lower percentage of positive results for both antibody types (3.3% for IgM and 4.4% for IgG) in ELISA tests compared with immunoblot tests.

Our own research confirms the observations of other authors regarding the high specificity of immunoblot in diagnosing borreliosis. In the case of detecting IgM antibodies, the specificity of the test increases with the presence of antigen OspC (p25; in our research positive results were observed in 37.7% of patients), while for the IgG type, when the VlsE recombinant antigen is present, positive results were observed in 64.4% of patients. Similar research results were obtained by Zajkowska et al. 2006, 2007 and Glatz et al. 2008.

In conclusion, it appears that ELISA, which is recommended as the only screening test for diagnosing borreliosis in Poland, carries a high risk of error, particularly as far as negative results are concerned. Relying on ELISA leads to omitting further confirmation tests, which therefore often makes a correct diagnosis impossible. As a consequence the time of starting treatment is delayed, allowing the infection to develop further in the patient, often leading to serious complications. The results of our research indicate that use of the IIFA test, which is mentioned in European directives, during the first stage of the diagnostic process should be recommended. The IIFA test gives a higher percentage of positive results compared to ELISA. In our research all the positive IIFA results were confirmed by the immunoblot reference test. Admittedly, the IIFA test gave some false positive results for IgM antibodies compared with the immunoblot; but no patients were left undiagnosed, as was the case with the negative results from ELISA, in which some patients turned out to be positive for one or both antibody types when ELISA was verified by immunoblot. The same patients also tested positive for both antibody types in the IIFA test.

References

Aberer E (2007) Lyme borreliosis—an update. J Dtsch Dermatol Ges 5:406–413

Aguero-Rosenfeld ME, Wang G, Schwartz I et al (2005) Diagnosis of lyme borreliosis. Clin Microbiol Rev 18:484–509

Altpeter ES, Meier C (1992) Epidemiologische Aspekte der Neurologischen Komplikationen der Lyme-*Borreliose* in der Schweiz. Schweiz Med Wochenscher 122:22–26

Cetin E, Sotoudeh M, Auer H, Stanek G (2006) Paradigm Burgenland: risk of *Borrelia burgdoferi* sensu lato infection indicated by variable seroprevalence rates in hunters. Wien Klin Wochenschr 118:677–681

Chmielewska-Badora J, Cisak E, Wójcik-Fatla A et al (2006) Correlation of tests for detection of *Borrelia burgdorferi* sensu lato infection in patients with diagnosed borreliosis. Ann Agric Environ Med 13:307–311

Figlerowicz M (2006) Borelioza–pamiątka z wakacji. Przew Lek 8:56–59

Glatz M, Fingerle V, Wilske B et al (2008) Immunoblot analysis of seroreactivity to recombinant *Borrelia burgdorferi* sensu lato antigens, including VlsE, in the long-term course of treated patients with erythema migrans. Dermatology 216:93–103

Hauser U, Lehnert G, Lobentanzer R, Wilske B (1997) Interpretation criteria for standardized Western Blots for three European species of *Borrelia burgdorferi* sensu lato. J Clin Microbiol 35:1433–1444

Marangoni A, Sparacino M, Mondardini V et al (2005) Comparative evaluation of two enzyme linked immunosorbent assay methods and three Western Blot methods for the diagnosis of culture-confirmed early Lyme *Borreliosis* in Italy. New Microbiol 28:37–43

Niścigorska J, Skotarczak B, Wodecka B (2003) *Borrelia burgdorferi* infection among forestry workers—assessed with an immunoenzymatic method (ELISA), PCR, and correlated with the clinical state of the patients. Ann Agric Environ Med 10:15–19

Sigal LH (1992) Current recommendations for the treatment of Lyme disease. Drugs 43:683–699

Stanek G, Strle F (2009) Lyme borreliosis: a European perspective on diagnosis and clinical management. Curr Opin Infect Dis 22:450–454

Steere AC (1989) Lyme disease. N Engl J Med 321:586–596

Tylewska-Wierzbanowska S, Chmielewski T (2005) Serological diagnosis of Lyme disease—European guidelines. Postepy Mikrobiol 44:289–293

Wilske B (2003) Diagnosis of Lyme borreliosis in Europe. Vector Borne Zoonotic Dis 3:215–227

Wilske B, Fingerle V, Herzer P et al (1993) Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Comparison with indirect immunofluorescence and enzyme-linked immunosorbent assay. Med Microbiol Immunol 182:255–270

Wilske B, Fingerle V, Schulte-Spechtel U (2007) Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 49:13–21

Zajkowska J, Kondrusik M, Pancewicz S et al (2006) [Western-blot with VLSE protein and "in vivo" antigens in Lyme borreliosis diagnosis]. In Polish. Przegl Epidemiol 60(suppl 1):177–185

Zajkowska JM, Kondrusik M, Pancewicz SA et al (2007) Comparison of test with antigen VlsE (C6) with tests with recombinant antigens in patients with Lyme borreliosis. Pol Merkur Lekarski 23:95–99 in Polish