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CORRELATION OF TESTS FOR DETECTION OF *BORRELIA BURGDORFERI* SENSU LATO INFECTION IN PATIENTS WITH DIAGNOSED BORRELIOSIS

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Abstract: A group of 180 patients with diagnosed Lyme borreliosis were examined for the presence of infection with *Borrelia burgdorferi* sensu lato (*B. burgdorferi* s.l.) by serologic tests with *B. burgdorferi* s.l. antigens (IgM-ELISA, IgG-ELISA, IgM-immunoblot, IgG-immunoblot) and by polymerase chain reaction (PCR, nested-PCR) for detection of *B. burgdorferi* s.l. DNA in peripheral blood. A total of 61.7%, 53.9%, 62.2%, and 59.4% of the examined patients' sera showed positive or borderline results in the serologic tests IgM-ELISA, IgG-ELISA, IgM-immunoblot, and IgG immunoblot, respectively. The results of the tests IgM-ELISA and IgM-immunoblot were significantly correlated ($p<0.001$). A higher degree of the correlation ($p<0.000001$) was found at the comparison of results obtained with IgG-ELISA and IgG-immunoblot. The correlation between the positive findings in the IgM-ELISA and detection with IgM-immunoblot the diagnostically important *B. burgdorferi* s.l. OspC surface protein was relatively low but statistically significant ($0.01<p<0.05$). Much higher correlation was found between the positive findings in the IgG-ELISA and detection with IgG-immunoblot other diagnostically important *B. burgdorferi* s.l. antigen, the VlsE protein ($p<0.000001$). The presence of *B. burgdorferi* s.l. DNA was found by PCR in 20 out 180 examined blood samples (11.1%). No correlation was found to exist between the PCR results and the results of any of the serologic tests for detection of anti *B. burgdorferi* s.l. antibodies of IgM class. PCR results correlated significantly at a relatively low level ($0.01<p<0.05$) with the results of IgG-ELISA, but not with the results of IgG-immunoblot with regard to total reactions ($0.2<p<0.1$). By contrast, a distinctly significant correlation was found between the PCR results and detection of the VlsE protein with IgG-immunoblot ($0.001<p<0.01$). In conclusion, the results of the present study suggest that antibodies of IgG class are the most reliable marker in laboratory diagnostics of Lyme borreliosis, in particular those directed against VlsE surface protein of *Borrelia burgdorferi* sensu lato.

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INTRODUCTION

Lyme borreliosis, caused by the spirochete *Borrelia burgdorferi* sensu lato (*B. burgdorferi* s.l.) transmitted by ixodid ticks, is a multiphasic disease manifested by dermal, arthral, cardiac and neural symptoms. Their diversity and similarity to many other diseases often hinder the proper diagnosis [1, 4]. In Poland, borreliosis is

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diagnosed mainly on the basis of clinical symptoms, epidemiological interview and serologic tests [3, 20], and less frequently with the use of polymerase chain reaction (PCR) method for detection of spirochetes in body fluids and tissues, which is considered as a specific and sensitive diagnostic test [20, 21, 26]. The serological diagnosis of Lyme borreliosis is based on detection in patient's serum of the presence of specific antibodies anti *B. burgdorferi* s.l., directed against its proteins (VlsE, p100, p39, p18, OspA, OspB, OspC), usually by the ELISA and immunoblot (Western blot) tests [5, 8, 16, 18, 27].

The aim of the present work was to study a group of patients with clinically diagnosed borreliosis and assess the degree of correlation between: • the ELISA test for detection of specific IgG and IgM antibodies anti *B. burgdorferi* s.l. antigens, • the immunoblot test for detection of specific IgG and IgM antibodies anti *B. burgdorferi* s.l. antigens, • and PCR test for detection of the *B. burgdorferi* s.l. antigen in peripheral blood.

MATERIALS AND METHODS

Examined patients. A total group of 180 persons, 56 women and 124 men, mean age 44.0 ± 12.2 years, were examined. The group consisted of 116 forestry workers occupationally exposed to tick bite and 64 patients treated in neurological departments of various hospitals and clinics in the city of Lublin, and in the Outpatient Unit of Occupational Diseases at the Institute of Agricultural Medicine in Lublin. Lyme borreliosis was diagnosed in all members of the group on the basis of clinical symptoms, history of tick bite and laboratory tests. The disease was either acute or chronic and showed different clinical manifestations and degree of severity.

All patients were subjected to epidemiological interview, including circumstances of exposure to tick bite and connections between the exposure and appearance of symptoms, in particular those affecting the skin, joints, and nervous system. The samples of peripheral blood were drawn by puncture of elbow vein. The sample was divided into 2 parts: one of 4 ml for separation of serum by centrifugation and the other of 1 ml for performing PCR test. All subjects gave formal consent to participate in the study. The Ethics Commission of the Institute of Agricultural Medicine approved human subjects protocols.

Serological tests. The presence of specific IgM and IgG antibodies against *B. burgdorferi* s.l. was determined with the use of ELISA and immunoblot tests using commercial kits Borrelia IgM Rekombinant and Borrelia IgG Rekombinant (Bellco Biomedica Medizinprodukte GmbH & Co. KG, Vienna, Austria), and *recom*Blot Borrelia$_{NB}$ IgM and *recom*Blot Borrelia$_{NB}$ IgG (Mikrogen, Neuried, Germany), respectively. In all kits recombinant proteins specific for *B. burgdorferi* s.l. were used as diagnostic antigens.

Detection of *B. burgdorferi* s.l. DNA by PCR. Total DNA was extracted from samples of peripheral blood taken from the patients by using a Dneasy Tissue Kit (Qiagen AG, Basel, Switzerland) according to the manufacturer's instructions. Polymerase chain reaction (PCR) and nested-PCR methods were used for identification of the *B. burgdorferi* s.l. DNA in patients' blood. Two pairs of oligonucleotide primers, amplifying fragments of the highly conservative *fla* gene encoding flagellin, a protein building flagellum of *B. burgdorferi* s.l. [14] were applied: • BF1 (5`- CAC ACC AGC ATC ACT TTC AGG GTC -3`) and BF2 (5`- CAA CCT CAT CTG TCA TTG TAG CAT CTT TT -3`) for PCR (first amplification), • and BF3 (5`- GGA GCA ACC CAA GAT GA -3`) and BF4 (5`- AGG TGC TGG CTG TTG AG -3`) for nested-PCR (second amplification). Primers were synthesized by Oligo JBB PAN, Warsaw, Poland. DNA of the Bo-148c/2 strain of *B. burgdorferi* s.l. (obtained by courtesy of Dr. B. Wodecka, University of Szczecin) was used as a positive control.

Amplifications were performed in a reaction volume of 50 µl containing 1 µl (100 pM) of each 5` and 3` primer (BF1-BF2 for PCR and BF3-BF4 for nested-PCR), 1 µl (200 µM) of deoxynucleoside triphosphate (dNTP) mixture (DNA Gdańsk, Poland), 0.5 µl (1 U) of DNA polymerase (Finnzymes Oy, Espoo, Finland) and sterile redistilled water to total volume of 50 µl. 10 µl of isolated DNA in PCR and 5 µl of the PCR mixture in nested-PCR were used as template DNA. Amplifications were carried out in 30 cycles, with the following cycle parameters: preliminary denaturation at 94ºC for 5 min, denaturation at 94ºC for 1 min, annealing at 58ºC for 1 min, elongation at 72ºC for 1 min. In nested-PCR annealing was performed at 54ºC. After the last cycle, samples were incubated at 72ºC for 10 min. All amplifications were performed in a PTC-150 thermal cycler (MJ Research Inc., Waltham, MA, USA).

Amplification products were identified in 1.5% agarose gel, after electrophoresis in standard conditions and staining with ethidium bromide solution (2 µg/ml). Amplified fragments were visualised in transilluminator under UV light (UV-953, JW Electronic, Warsaw, Poland). The size of the amplification products was 437 base pairs (bp) for PCR and 144 bp for nested-PCR.

Statistical analysis. The data were analysed by Spearman's correlation test and χ^2 test with the use of STATISTICA for Windows v. 5.0 package (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS

A total of 61.7%, 53.9%, 62.2%, and 59.4% of the examined patients' sera showed positive or borderline results in the serologic tests IgM-ELISA, IgG-ELISA, IgM-immunoblot, and IgG immunoblot, respectively (Tab. 1-2). The results of the tests IgM-ELISA and IgM-immunoblot with *B. burgdorferi* s.l. antigens were

Table 1. Correlation of serological detection of IgM antibodies against *Borrelia burgdorferi* s.l. with tests IgM-ELISA and IgM-immunoblot in the group of 180 patients with diagnosed borreliosis.

		IgM-immunoblot			
		Positive	Borderline	Negative	Total
IgM-ELISA	Positive	66 (36.7%)	2 (1.1%)	24 (13.8%)	92 (51.1%)
	Borderline	11 (6.1%)	2 (1.1%)	6 (3.3%)	19 (10.6%)
	Negative	30 (16.7%)	1 (0.6%)	38 (21.1%)	69 (38.3%)
	Total	107 (59.4%)	5 (2.8%)	68 (37.8%)	180 (100%)
Conformance = 58.9%; r = 0.263; p = 0.000369; correlation significant					

Table 2. Correlation of serological detection of IgG antibodies against *Borrelia burgdorferi* s.l. with tests IgG-ELISA and IgG-immunoblot in the group of 180 patients with diagnosed borreliosis.

		IgG-immunoblot			
		Positive	Borderline	Negative	Total
IgG-ELISA	Positive	84 (46.7%)	3 (1.7%)	5 (2.8%)	92 (51.1%)
	Borderline	4 (2.2%)	0	1 (0.6%)	5 (2.8%)
	Negative	13 (7.2%)	3 (1.7%)	67 (37.2%)	83 (46.1%)
	Total	101 (56.1%)	6 (3.3%)	73 (40.6%)	180 (100%)
Conformance = 83.9%; r = 0.692; p<0.000001; correlation highly significant					

Table 3. Correlation of serological detection of IgM antibodies against *Borrelia burgdorferi* s.l. with test IgM-ELISA and serological detection of antibodies against *B. burgdorferi* s.l. OspC protein with IgM-immunoblot in the group of 180 patients with diagnosed borreliosis.

		IgM-immunoblot: presence of OspC protein		
		Positive	Negative	Total
IgM-ELISA	Positive	56 (31.1%)	36 (20.0%)	92 (51.1%)
	Borderline	10 (5.6%)	9 (5.0%)	19 (10.6%)
	Negative	29 (16.1%)	40 (22.2%)	69 (38.3%)
	Total	95 (52.8%)	85 (42.2%)	180 (100%)
Conformance = 58.9%*; r = 0.179; p = 0.016418; correlation significant				

*The borderline IgM-ELISA results were considered positive when comparing with the results of IgM-immunoblot for the presence of OspC protein.

Table 4. Correlation of serological detection of IgG antibodies against *Borrelia burgdorferi* s.l. with test IgG-ELISA and serological detection of antibodies against *B. burgdorferi* s.l. VlsE protein with IgG-immunoblot in the group of 180 patients with diagnosed borreliosis.

		IgG-immunoblot: presence of VlsE protein		
		Positive	Negative	Total
IgG-ELISA	Positive	73 (40.5%)	19 (10.6%)	92 (51.1%)
	Borderline	3 (1.7%)	2 (1.1%)	5 (2.8%)
	Negative	5 (2.8%)	78 (43.3%)	83 (46.1%)
	Total	95 (52.8%)	85 (42.2%)	180 (100%)
Conformance = 85.5%*; r = 0.724; p<0.000001; correlation highly significant				

*The borderline IgG-ELISA results were considered positive when comparing with the results of IgG-immunoblot for the presence of VlsE protein.

significantly correlated ($p<0.001$), and the conformance of the results was 58.9% (Tab. 1). A much higher degree of the correlation ($p<0.000001$) and of the conformance (83.9%) was found at the comparison of results obtained with IgG-ELISA and IgG-immunoblot with *B. burgdorferi* s.l. antigens (Tab. 2).

Out of 111 sera showing positive and borderline results in the test IgM-ELISA with *B. burgdorferi* s.l. antigen, 66 showed in IgM-immunoblot the presence of antibodies against OspC antigen which is the surface protein of *B. burgdorferi* s.l., considered as an important marker in early detection of borreliosis. The correlation between the positive findings in the IgM-ELISA and detection of the OspC protein with IgM-immunoblot was relatively low but statistically significant ($0.01<p<0.05$), and the conformance was 58.9% (Tab. 3).

Out of 97 sera showing positive and borderline results in the test IgG-ELISA with *B. burgdorferi* s.l. antigen, 76 showed in IgG-immunoblot the presence of antibodies against VlsE surface protein antigen of *B. burgdorferi* s.l., which is regarded as an important indicator of *Borrelia* infection. The correlation between the positive findings in the IgG-ELISA and detection of the VlsE protein with IgG-immunoblot was highly significant ($p<0.000001$) and the conformance was 85.5% (Tab. 4).

The presence of *B. burgdorferi* s.l. DNA was found by PCR in 20 out of 180 examined blood samples (11.1%). No correlation was found to exist between the PCR results and the results of any of the serologic tests for detection of anti *B. burgdorferi* s.l. antibodies of IgM class ($p>0.5$), including IgM-ELISA, IgM-immunoblot with regard to total reactions, and IgM-immunoblot with regard to reactions with OspC protein (Tab. 5-6).

PCR results correlated significantly at a relatively low level ($0.01<p<0.05$) with the results of IgG-ELISA (Tab. 5) but not with the results of IgG-immunoblot with regard to total reactions ($0.2<p<0.1$) (Tab. 7). By contrast, a distinctly significant correlation was found between the PCR results and detection of the VlsE protein with IgG-immunoblot ($0.001<p<0.01$) (Tab. 7).

It is noteworthy that the positive PCR reactions occurred significantly more frequently in the patients who showed positive reactions in all 6 serological tests under consideration (IgM-ELISA, IgG-ELISA, IgM-immunoblot, IgG-immunoblot, presence of OspC protein, presence of VlsE protein) ($\chi^2=7.06$, $p=0.0079$).

DISCUSSION

Heterogeneity of the pathogenic genospecies forming the *B. burgdorferi* s.l. complex and diversity of the clinical manifestations caused by these species create a need for the application of reliable laboratory methods for making proper diagnosis of borreliosis [22, 23, 28]. In Europe a 2-step procedure for serological diagnosis of borreliosis is recommended, using a high sensitive but less specific ELISA test as a first step, and a more specific immunoblot as a confirmatory, second step assay [2, 8, 26, 27].

Table 5. Correlation of serological detection of antibodies against *Borrelia burgdorferi* s.l. with IgM-ELISA and IgG-ELISA tests and detection of *Borrelia burgdorferi* s.l. DNA in peripheral blood with PCR test in the group of 180 patients with diagnosed borreliosis.

		IgM-ELISA				IgG-ELISA			
		Positive	Borderline	Negative	Total	Positive	Borderline	Negative	Total
PCR	Positive	9 (5.0%)	2 (1.1%)	9 (5.0%)	20 (11.1%)	16 (8.9%)	0	4 (2.2%)	20 (11.1%)
	Negative	83 (46.1%)	17 (9.4%)	60 (33.3%)	160 (88.9%)	76 (42.2%)	5 (2.8%)	79 (43.9%)	160 (88.9%)
	Total	92 (51.1%)	19 (10.6%)	69 (83.3%)	180 (100%)	92 (51.1%)	5 (2.8%)	83 (46.1%)	180 (100%)
		Conformance = 39.45%*; r = - 0.049; p=0.518112; correlation not significant				Conformance = 52.8%*; r = 0.185; p = 0.012814; correlation significant			

*The borderline IgM-ELISA and IgG-ELISA results were considered positive when comparing with the results of PCR test.

Table 6. Correlation of serological detection of antibodies against *Borrelia burgdorferi* s.l. with IgM-immunoblot test (with regard to total positive and positive against OspC protein) and detection of *Borrelia burgdorferi* s.l. DNA in peripheral blood with PCR test in the group of 180 patients with diagnosed borreliosis.

		IgM-immunoblot: total reactions				IgM-immunoblot: presence of OspC protein		
		Positive	Borderline	Negative	Total	Positive	Negative	Total
PCR	Positive	13 (7.2%)	0	7 (3.9%)	20 (11.1%)	12 (6.7%)	8 (4.4%)	20 (11.1%)
	Negative	94 (52.2%)	5 (2.8%)	61 (33.9%)	160 (88.9%)	84 (46.6%)	76 (42.2%)	160 (88.9%)
	Total	107 (59.4%)	5 (2.8%)	68 (37.8%)	180 (100%)	96 (53.3%)	84 (46.6%)	180 (100%)
		Conformance = 41.1%*; r = 0.020; p=0.787236; correlation not significant				Conformance = 48.9%; r = 0.047; p = 0.528824; correlation not significant		

*The borderline IgM-immunoblot results were considered positive when comparing with the results of PCR test.

Table 7. Correlation of serological detection of antibodies against *Borrelia burgdorferi* s.l. with IgG-immunoblot test (with regard to total positive and positive against VlsE protein) and detection of *Borrelia burgdorferi* s.l. DNA in peripheral blood with PCR test in the group of 180 patients with diagnosed borreliosis.

		IgG-immunoblot: total reactions				IgG-immunoblot: presence of VlsE protein		
		Positive	Borderline	Negative	Total	Positive	Negative	Total
PCR	Positive	15 (8.3%)	0	5 (2.8%)	20 (11.1%)	15 (8.3%)	5 (2.8%)	20 (11.1%)
	Negative	86 (47.8%)	6 (3.3%)	68 (37.8%)	160 (88.9%)	66 (36.7%)	94 (52.2%)	160 (88.9%)
	Total	101 (56.1%)	6 (3.3%)	73 (40.6%)	180 (100%)	81 (45.0%)	99 (55.0%)	180 (100%)
		Conformance = 41.1%*; r = 0.112; p=0.134386; correlation not significant				Conformance = 60.5%; r = 0.213; p = 0.004059; correlation significant		

*The borderline IgG-immunoblot results were considered positive when comparing with the results of PCR test.

According to Schulte-Spechtel *et al.* [17], VlsE surface protein is the most sensitive of all recombinant antigens of *B. burgdorferi* s.l. in the laboratory detection of *Borrelia* infections. This opinion is shared by other authors who report that the detection of antibodies against the immunodominant VlsE antigen is of the greatest value in serological diagnostics of Lyme borreliosis [6, 7, 12, 15]. Over 85% of IgG-positive sera could be quickly diagnosed by the detection of VlsE band in IgG-immunoblot [17]. The VlsE antigen enables the detection of antibodies against all pathogenic genospecies of *B. burgdorferi* s.l. and the risk of false-negative reactions is 10-fold lower compared to other *Borrelia* antigens [25]. Such reactions could occur in the first stage of borreliosis and in the late dermal borreliosis [28].

A high diagnostic value of the VlsE antigen was also confirmed in the present study. Detection of the VlsE protein in IgG-immunoblot showed a highly significant correlation with IgG-ELISA, much higher compared to that found between the detection in IgM-immunoblot of OspC, another indicator surface protein of *B. burgdorferi* s.l., and IgM-ELISA. The presence of the VlsE protein in IgG-immunoblot also showed the distinctly significant correlation with the presence of the *B. burgdorferi* s.l. DNA found by the PCR, while in the other serological tests compared the correlation was either low or none.

PCR is recommended in laboratory diagnostics of Lyme borreliosis as a sensitive method for detection of the presence of *Borrelia burgdorferi* s.l. in body fluids and tissues, in particular in those cases where serological tests fail to confirm the clinical diagnosis of the disease [1, 7, 9, 10, 11, 13, 19, 24]. The method is mostly effective in the case of bacteremia but may give false-negative results in chronic borreliosis, when spirochetes are encysted within tissues. Hence, the results of the PCR test for the presence of borreliae performed by different

authors could be variable. Skotarczak *et al.* [20] found by PCR the presence of *B. burgdorferi* s.l. DNA in 23 out 30 examined blood samples of patients with the diagnosis of borreliosis. By contrast, examination of 52 blood samples of forestry workers exposed to borreliae carried out by PCR by Niścigorska *et al.* [15] gave negative results. The low proportion of positive PCR results found in the present work could be due to the fact that most of the examined patients had chronic borreliosis. The percentage of positive PCR results and degree of correlation with serologic tests could probably be higher if instead of flagellar the VlsE primers were used and if, apart from blood, samples of cerebrospinal fluid, synovial fluid and skin biopsy specimens were examined [26].

CONCLUSION

Results of the present study suggest that antibodies of IgG class are the most reliable marker in laboratory diagnostics of Lyme borreliosis, in particular those directed against VlsE surface protein antigen of *Borrelia burgdorferi* sensu lato.

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