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Analysis of selected serological parameters in patients with diagnosed Lyme borreliosis and in seropositive patients with no clinical symptoms

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Abstract

Objectives. The aim of the study was to analyze some metalloproteinases, cytokines, and chemokines in LB patients and healthy seropositive subjects. The presence of IgM/IgG antibodies against specific *Borreliella* antigens was analyzed in the presence or absence of clinical manifestations of LB.

Materials and method. The study involved 38 patients diagnosed with LB and arthralgia and/or arthritis symptoms, and 57 foresters presenting no clinical symptoms of LB. The ELISA test was applied for general screening of anti-*Borreliella* IgM/IgG. Western blot was used for confirmatory diagnosis of LB for the positive and borderline results. Serum IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ, TNF, IL-8, CCL5, CXCL9/MIG, CCL2/MCP-1, CXCL10/IP-10 concentrations were measured with the use of the Human Cytometric CBA test. The concentration of MMP-2 and MMP-9 in the serum was determined with the use of ELISA tests.

Results. Analysis of the cytokines and chemokines revealed that only the concentration of IL-2 was significantly higher (2.4 pg/m; p=0.00641) in patients with LB symptoms than in the seropositive individuals (0.4 pg/ml). The MMP2 concentration was significantly higher (233.3 ng/ml; p=0.00294) in patients with clinical manifestations of LB than in those occupationally exposed to tick bites, but did not have anti-*Borreliella* antibodies (192.0 ng/ml).

Conclusions. The presence of IgG antibodies against a number of *Borreliella* antigens and the differences in the IL-2 and MMP2 levels in seropositive or seronegative individuals and symptomatic LB patients, may indicate differences in the intensity of the immune response to the infection and, consequently, may induce development of clinical manifestations of the disease in seropositive and seronegative individuals.

Key words

cytokines, MMP-9, MMP-2, Lyme borreliosis, *Borreliella*

INTRODUCTION

Lyme borreliosis (LB) is caused by spirochetes of the Lyme Disease (LD) group of the *Borreliaceae* species which are transmitted by ticks [1]. LB is a disease with diverse clinical presentations the most common of which is the manifestation of *erythema migrans* (EM). However, the infecting pathogen can spread to other tissues and organs, causing more severe manifestations involving the patient's skin, nervous system, joints, or heart [2, 3, 4].

More than 10 genospecies are included to LD group of *Borreliaceae* species: *Borreliella afzelii, Borreliella garinii, Borreliella bavariensis, Borreliella burgdorferi*, and occasionally *Borreliella spielmanii* and *Borreliella lusitaniae,* are pathogenic to humans in Europe, whereas *Borreliella burgdorferi* and, in certain areas, *Borreliella mayonii*, are human pathogens in North America [1, 5]. The spirochetes *B. afzelii* is mostly associated with skin manifestations and *B. garinii* and *B. burgdorferi* seem to be the most neurotropic and the most arthritogenic species, respectively [3].

In accordance with current recommendations, the presence of at least one of the clinical symptoms: *erythema migrans*, *borrelial lymphoma*, and *acrodermatitis chronica athrophicans,* or a set of symptoms comprising *Lyme carditis*, *Lyme arthritis*, and *neuroborreliosis,* is the basis for the diagnosis of Lyme borreliosis. Another indispensable element of diagnostics (besides EM skin lesions localized early) is the detection of specific anti-*Borreliella* antibodies. Laboratory diagnostics consists of two stages: detection of specific antibodies with the enzyme immunoassay and confirmation with the Western blot test [6, 7, 8].

An interesting phenomenon is the presence of specific anti-*Borreliella* antibodies without clinical signs of infection [9–19]. Detection of antibodies alone does not evidence the disease [5, 7]. The reasons why some patients present clinical signs of Lyme borreliosis while others only undergo seroconversion are not fully known. It seems that the type of generated immune reactions may have key importance for the development of Lyme borreliosis. Neutrophils, acidophils, mast cells, and macrophages are involved in the immune response against

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of *Borreliella* species. The inflammatory response mediators produced by these cells, as well as the synergistic interactions of macrophages and T cells, influence the intensity of immune response to infection [20]. *B. burgdorferi* spirochetes do not secrete enzymes degrading the extracellular matrix that would facilitate entrance into the host organism and migration in tissues [21]. However, they are able to activate proteolytic enzymes, e.g. matrix metallproteinases (MMPs), and thus penetrate human tissues [22]. Metalloproteinases are involved in degradation of protein components of the extracellular matrix and hydrolysis of molecules released from the cell surface. Thus, they can activate or inactivate many cytokines, chemokines, and growth factors [23, 24]. Cytokines secreted by activated T cells have a considerable impact on the regulation and effectiveness of immune response. Th1 cells secrete IFN-γ and TNF, thus promoting cytotoxic phagocyte-dependent immune response. In turn, Th2 cells secrete IL-4, IL-5, and IL-9 inducing humoral phagocyte-independent immune response [10].

OBJECTIVES

The aim of the study is to analyze some serological parameters (metalloproteinases, cytokines, and chemokines) in Lyme borreliosis patients and healthy seropositive subjects. The presence of IgM/IgG antibodies against specific *Borreliella* antigens was analyzed in the presence or absence of clinical manifestations of Lyme borreliosis.

MATERIALS AND METHODS

Study group. The examinations involved 95 subjects, including: 38 patients diagnosed with Lyme borreliosis and arthralgia and/or arthritis symptoms, who were patients in the Clinic for Infectious Diseases at the Medical University, in Lublin, eastern Poland. The diagnosis was based on medical history, physical examination, clinical picture, and serological tests in accordance with the recommended procedure for Lyme borreliosis diagnosis [7]. The patient group included 22 females and 16 males between the ages of 21 – 80, average age – 57 (SD 13.3), and 57 foresters occupationally exposed to tick bites, presenting no clinical symptoms of Lyme borreliosis. The 3 females and 54 males in this group were between the ages of 26 – 70, average age 49 (SD 9.8). They worked in the forest districts of Lublin Province of eastern Poland.

Information on tick bites was collected from all 95 subjects. Serological tests were carried out to detect the presence of anti-*Borreliella* IgM/IgG antibodies, and selected serological parameters (metalloproteinases and cytokines) were analyzed. In the patient group, blood was collected for testing before application of the treatment (2017 – 2018). Blood was collected from the foresters in 2014–2015.

Serological tests. The presence of anti-*Borreliella* IgM/IgG antibodies was assessed in accordance with the recommendations in a two-stage diagnostic scheme: the first stage – ELISA tests and the second stage – confirmation Western blot tests in the case of a positive or borderline result obtained in the first diagnostic stage [6, 7, 8].

Anti-*Borrelia* ELISA IgM and anti-*Borrelia* plus VlsE ELISA IgG (Euroimmun, Germany) were used in the study. The reaction wells were coated with a mixture of antigens derived from *B. burgdorferi*, *B. afzelii*, and *B. garinii,* as well as the recombinant *B. burgdorferi* VlsE antigen. Results below 16 relative units/ml (RU/ml), between 16 – 22 RU/ml, and above 22 RU/ml were regarded as negative, borderline, and positive, respectively.

The Western blot Anti-*Borrelia* EUROLINE-WB IgM test (Euroimmun, Germany) was used for confirmation of the positive and borderline IgM results. The test strips contained a complete *B. afzelii* antigen extract and a membrane chip with recombinant VlsE antigen. In the IgG class, an Anti-*Borrelia* EUROLINE-RN-AT-IgG kit (Euroimmun, Germany) was employed as a confirmation test. The test strips contained highly specific recombinant *B. burgdorferi* s.s. antigens (p83, p58, p21, p20, p19, p18), highly specific recombinant dimeric OspC (advance, p25) from *B. burgdorferi*, *B. garinii*, and *B. afzelii*, purified recombinant flagellin (p41) and BmpA (p39) from *B. afzelii*, *Borrelia afzelii* lipid (LBa), *B. burgdorferi.* lipid, and highly purified recombinant VlsE antigens.

Serological parameters: cytokines and metalloproteinases. Serum IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ, and TNF concentrations were measured with the use of the Human Cytometric Bead Array Kit Th1/Th2/Th17 test (Becton Dickinson; BD). The level of IL-8 (CXCL8), CCL5 (RANTES, Regulated on Activation, Normal T-cell Expressed and Secreted), monokine induced by interferon gamma (CXCL9/MIG), monocyte chemoattractant protein-1 (CCL2/MCP-1), and interferon gamma-induced protein-10 (CXCL10/IP-10) in serum samples were determined using the Human Chemokine I Cytometric Bead Array Kit (Becton Dickinson; BD). Equipment: FACSCanto™II cytometer and FCAP Array™ Software Version 3.0. (Becton Dickinson; BD).

The concentration of MMP-2 in the serum was determined (ELISA, R&D): a monoclonal antibody specific for MMP-2 has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any MMP-2 present was bound by the immobilized antibody. Minimum detectable dose (MDD) of MMP-2 ranged from 0.014 – 0.082 ng/mL. The concentration of MMP-9 in the serum was determined (EISA, R&D): a monoclonal antibody specific for human MMP-9 has been pre-coated onto a microplate. Standards and samples were pipetted into the wells, and MMP-9 was bound by the immobilized antibody. The minimum detectable dose (MDD) of human MMP-9 is typically less than 0.156 ng/mL.

Equipment: ELISA plate reader (Thermo Scientific). All serological assays were carried out and results were interpreted according to the manufacturers' instructions. The data were analyzed statistically using the Statistica v.10 programme. The Mann-Whitney U test and the Kruskal-Wallis test were employed to determine the statistical significance of the results. Values of $p < 0.05$ were considered statistically significant.

The Bioethical Committee of the Medical University in Lublin authorized the project (Permission No. KE-0254/177/2014; KE-0254/27/2016).

RESULTS

Tick bite episodes. Tick bites were reported by 30 of the 38 patients with clinical manifestations of Lyme borreliosis

(79%). A single bite was declared by 6 patients (16%), whereas 24 subjects (63%) reported multiple events. Eight Lyme borreliosis patients (21%) had not noticed any tick bites.

In the group of the 57 examined foresters, 43 (75%) workers reported tick bites, with 11 individuals who had been bitten once, and 32 (56%) subjects declaring multiple attacks. No tick infestations were declared by 14 (25%) foresters.

Anti-B. burgdorferi antibodies. In all patients with clinical manifestations of Lyme borreliosis (38 persons), ELISA results were confirmed by Western blot tests. The presence of anti-*Borreliella* antibodies by Western blot was confirmed in 33 foresters (57.9%).

Antibodies against specific *Borreliella* antigenic proteins were detected in all patients: 2 of them (5.3%) had IgM class antibodies, 9 patients (23.7%) had IgM and IgG class antibodies, and 27 subjects (71%) had IgG class antibodies. IgM antibodies for the p25 antigen were detected most frequently (28.9% of the patients), whereas IgG for the VlsE and p41 antigens were usually found (94.7% of the patients). Detailed data on the presence of anti-*Borreliella* IgM/IgG are shown in Table 1.

Table 1. Anti-*Borreliella* IgM/IgG antibodies in patients with clinical manifestations of Lyme borreliosis

Anti-*Borreliella* IgM/IgG antibodies		Patients with Lyme borreliosis N(%)			
		Patients with IgM antibodies present	Patients with IgM and IgG antibodies present	Patients with IgG antibodies present	Total
IgM	p17	-	1(2.6)	-	1(2.6)
	p25	2(5.3)	9(23.7)	-	11(28.9)
	p31	-	2(5.3)	-	2(5.3)
	p39	-	1(2.6)	-	1(2.6)
	83	-	1(2.6)	-	1(2.6)
	VlsE	-	1(2.6)	-	1(2.6)
IgG	p18	-	-	5(13.1)	5(13.1)
	p19	-	2(5.3)	5(13.1)	7(18.4)
	p20	-	1(2.6)	1(2.6)	2(5.3)
	p21	-	2(5.3)	4(10.5)	6(15.8)
	p25	-	7(18.4)	8(21.0)	15(39.5)
	p39	-	1(2.6)	8(21.0)	9(23.7)
	p41	-	9(23.7)	27(71.0)	36(94.7)
	p58	-	1(2.6)	5(13.1)	6(15.8)
	p83	-	3(7.9)	8(21.0)	11(28.9)
	VlsE	-	9(23.7)	27(71.0)	36(94.7)
	Lba	-	1(2.6)	2(5.3)	3(7.9)
	Lbb	-	1(2.6)	1(2.6)	2(5.3)
Total		2(5.3)	9(23.7)	27(71.0)	38(100.0)

N – Number of people in the test group

No anti-*Borreliella* IgM antibodies were detected in any of the examined foresters. In this group, anti-*Borreliella* IgG antibodies were found in the case of 33 (57.9%) but not in the other 24 forest workers (42.1%). No person from this group declared any clinical symptoms of Lyme borreliosis.

The prevalence of the specific *Borreliella* IgG antibodies was compared between the groups of the seropositive foresters and patients with advanced (IgM and IgG) and late (IgG only) anti-*Borreliella* response (Tab. 2). Patients with Lyme borreliosis symptoms exhibited a significantly higher level of anti-p25 IgG (p=0.00009), anti-VlsE IgG (p=0.000001), and anti-p41 IgG (p=0.000001) antibodies than that determined in the group of the seropositive foresters. In turn, anti-p18 IgG antibodies were detected in the sera of the seropositive foresters, with a significantly higher frequency than in the Lyme borreliosis patients (p=0.00003).

Serological parameters: cytokines and metalloproteinases. The concentration of the selected serological parameters was analyzed in 3 groups:
- patients diagnosed with Lyme borreliosis before the application of antibiotic therapy (38 subjects);
- foresters with anti-*Borreliella* IgG but with no clinical Lyme borreliosis symptoms (33 subjects, positive control);
- foresters with no anti-*Borreliella* antibodies (24 subjects, negative control) (Tab. 3 and 4).

Analysis of the cytokines revealed that only the concentration of IL-2 was significantly higher (2.4 pg/ml; p=0.00641) in the patients with Lyme borreliosis symptoms than in the seropositive individuals (positive control, 0.4 pg/ml). The levels of IL-8, TNF, IL-6, and IL-4 in the serum samples collected from the patients with Lyme borreliosis symptoms, were higher than in the control groups, but these differences were not significant. There were no significant differences in the concentrations of the IP-10, MCP-1, MIG-1, and RANTES chemokines in the serum of the examined subjects.

The MMP2 concentration was significantly higher (233.3 ng/ml; p=0.00294) in the patients with clinical manifestations of Lyme borreliosis than in the subject that were occupationally exposed to tick bites but did not have anti-*Borreliella* antibodies (192.0 ng/ml). In turn, no significant differences were found in the concentration of this metalloproteinase between the patients and the seropositive individuals (with anti-*Borreliella* IgG) presenting no clinical symptoms of the disease (MMP2–213.1 ng/ml). A similar trend was observed in the case of metalloproteinase MMP9; however, the differences in the values of this parameter between the analyzed groups did not have statistical significance.

DISCUSSION

According to the recommendations, specific clinical symptoms are a basis for the diagnosis of Lyme borreliosis, which must be confirmed by detection of specific anti-*Borreliella* antibodies. The presence of anti-*Borreliella* antibodies without clinical signs of infection is not an indication for medical treatment [25], since some *Borreliella* antigen-seropositive subjects do not present clinical signs of disease [9–19].

Anti-*Borreliella* antibodies were detected in the sera of 57.9% of foresters occupationally exposed to tick bites, but did not report symptoms of Lyme borreliosis. The moment of tick attachment to the skin is not always noticed and therefore cannot be a decisive criterion for assessment of the risk of the disease. Studies conducted by Shkilna et al. [26] have shown that 70% of patients with clinical symptoms of Lyme borreliosis and current anti-*Borreliella* antibodies declared the occurrence of tick bites (single bites – 35%, double bites

Table 2. Anti-*Borreliella* IgG antibodies in patients with clinical manifestations of Lyme borreliosis (only those with advanced (IgM and IgG) and late anti-*Borreliella* response (only IgG)) and seropositive foresters

	Anti-*Borreliella* IgG antibodies											
	p18*	p19	p20	p21	p25**	p39	p41***	p58	p83	VlsE****	Lba	Lbb
Patients with Lyme borreliosis N=36 (100%)	5(13.9)	7(19.4)	2(5.6)	6(16.7)	15(41.7)	9(25.0)	36(100.0)	6(16.7)	11(30.6)	36(100.0)	3(8.3)	2(5.6)
Seropositive foresters N=33 (100%)	20(60.6)	16(48.5)	-	2(6.0)	3(9.1)	9(27.3)	9(27.3)	-	13(39.4)	20(60.6)	-	-

* – p=0.00003; ** – p=0.00009; *** – p=0.000001; **** p=0.000001
N – Number of people in the test group

Table 3. Concentrations of selected cytokines in Lyme borreliosis patients prior to treatment and in subjects occupationally exposed to tick bites

	Parameter [pg/ml]															
	IL-2	SD	IL-4	SD	IL-6	SD	IL-10	SD	IL-17A	SD	INF-g	SD	TNF	SD	IL-8	SD
Patients with symptoms of Lyme borreliosis before deployment of treatment, N=38	2.4*	8.8	1.0	2.9	2.2	7.4	0.3	2.4	0.06	0.4	0.06	0.4	2.7	13.2	19.3	14.0
People professionally exposed to tick bites – IgG *B. burgdorferi* antibodies present (positive control) N=33	0.4*	0.7	0.4	0.8	1.0	1.5	0.2	0.4	1.14	3.6	0.04	0.2	0.01	0.05	17.5	14.4
People professionally exposed to tick bites – no IgG *B. burgdorferi* antibodies present (negative control) N=24	0.5	0.9	0.2	0.5	5.5	15.7	0.1	0.3	0.89	2.7	0.05	0.2	0.09	0.3	16.5	6.6

* p=0.00641
N – The number of people in the test group

Table 4. Selected serological parameters in patients with Lyme borreliosis before implementation of treatment, and in people professionally exposed to tick bites

	Serological parameters [pg/ml]											
	IP-10	SD	MCP-1	SD	MIG-1	SD	RANTES	SD	MMP2	SD	MMP9	SD
Patients with symptoms of Lyme borreliosis before deployment of treatment N=38	188.4	130.7	132.8	73.4	82.4	82.7	11438.2	3149.2	233.3*	54.6	598.5	228.3
People professionally exposed to tick bites – IgG *B. burgdorferi* antibodies present (positive control) N=33	136.3	51.4	120.3	84.6	54.5	46.0	13146.7	2404.2	213.1	43.6	572.9	209.8
People professionally exposed to tick bites – no IgG *B. burgdorferi* antibodies present (negative control) N=24	170.4	130.3	138.0	51.2	53.7	71.4	13654.9	2176.1	192.0*	46.9	490.8	213.0

* p=0.00294
N – Number of people in the test group

– 10%, multiple bites – 25%). Other studies indicate that only 12.5% of rural residents bitten by ticks performed diagnostic tests to detect anti-*Borreliella* antibodies [27]. In the group of patients with Lyme borreliosis symptoms qualified for antibiotic therapy, 20% did not notice a tick attached to their skin. These observations confirm that clinical symptoms combined with the presence of antibodies to specific antigenic proteins of LD group of *Borreliaceae* species are crucial in the diagnosis of Lyme borreliosis. Simultaneously, the absence of contact with a tick reported in a patient's medical history cannot be a decisive criterion. Great importance for the diagnosis of Lyme disease is attached to the quality of *Borreliella* antigens. The use of recombinant antigens (p100, p58, p41, VlsE, OspC, DbpA), especially in the Western blot test, instead of antigens from cell lysates, is diagnostically preferable [7, 25, 28].

One of the most immunogenic *Borreliella* proteins is flagellin, which elicits a strong early humoral response. This protein shows a high degree of homology with the flagellin of *Bacillus subtilis, Salmonella Typhimurium, T. pallium*, *Borrelia recurrentis, Borrelia duttoni, Borrelia hermsii,* and *Leptospira sp.*, hence the possibility of cross-reactions. Epitopes characteristic of *Borreliella* p41 are present only between amino acids 129 and 251, and only such a protein can be regarded as a specific antigen of the spirochete with diagnostic importance [29]. The interpretation of the Western blot results took into account the presence of antibodies against diagnostically important *Borreliella* antigens: early phase markers (OspC, VlsE), highly specific p39 antigen (BmpA), late phase markers (p83, lipid markers), and recombinant antigens (p58, p21, p20, p19, and p18). Analysis of IgG antibodies in patients with advanced (IgM and IgG) or late (only IgG) anti-*Borreliella* and in the seropositive foresters showed significantly greater frequency of anti-VlsE and p25 IgG antibodies in the patients. In turn, anti-p18 IgG were significantly more prevalent in the sera from the

seropositive foresters than in the patients. Therefore, it seems that the type of immunological reactions has key importance in the development of the symptomatic form of infection, especially in chronic cases.

Clinical manifestations of Lyme borreliosis may be associated with the ineffectiveness of host immune mechanisms to eliminate the pathogen, development of autoimmune reactions, or immunopathological mechanisms related to cytokine production [10]. The LD group of *Borreliaceae* species are able to stimulate immunocompetent cells for production of both pro-inflammatory and anti-inflammatory cytokines [30, 31] and activation of CD14 mononuclear cells. In turn, this leads to the production of TNF-α, INF-α, IL-1, IL-6, IL-8, IL-12, chemokines, and reactive oxygen species and, consequently, has an impact on the further production of cytokines, chemokines, and molecules with adhesive and signaling functions [21]. Chemokines play an important role in the generation of inflammatory response in tissues during *Borreliella* infection. Early induction of macrophages and dendritic cells to secrete CCL3 (a chemoattractant for monocytes, natural killer cells, and T cells) and CCL4 (a chemoattractant for monocytes and some T cells) is indispensable for initiation of the migration of inflammatory cells. Species causing LD directly influences CD14+ monocytes/macrophages via induction of secretion of CCL3, CCL4, and CXCL8 (a neutrophil chemoattractant) and stimulation thereof to secrete CCL2 with the help of IFN-γ. Additionally, IFN-γ induces secretion of CXCL9 and CXCL10 by these cells [32]. Chemokine IP-10 (CXCL10) is secreted by leukocytes, neutrophils, eosinophils, monocytes, endothelial cells, and keratinocytes in response to IFN-γ. CXCL10 exerts a strong effect on biological functions, chemotactic activity, apoptosis, and regulation of cell growth and proliferation. It also plays an important role in infectious and cancer diseases; however, the mechanism of CXCL10 interactions in the pathogenesis of infectious, viral, and bacterial diseases has not been fully elucidated. It is known that impaired production of CXCL10 may lead to, e.g., increased susceptibility to *Legionella pneumophila* and *Candida albicans* infection. IP-10 has been shown to play a role in *Helicobacter pylori, Mycoplasma,* and *Chlamydia* infections [33]. The role of CXCL10 in Lyme borreliosis has not been demonstrated to-date.

Skogman et al. [14] found no differences in the number of *Borreliella* -specific IFN-γ-, IL-4-, and IL-17-secreting cells when comparing *Borreliella*-exposed asymptomatic children, patients with clinical Lyme borreliosis and a control group. Neither were significant differences found in the *Borreliella*-induced cytokine secretion (IL-1β, IL-6, IL-10, TNF) between the groups in this study. As shown by Ekerfelt et al. [10], seropositive subjects without clinical Lyme borreliosis symptoms had a similar number of *Borreliella*-specific IFN-producing cells as patients with symptomatic *Borreliella* infection. The authors suggest that IFN-γ acting as part of a set of cytokines produced in response to infection rather than alone, may play a role in elimination of *Borreliella* spirochetes. As demonstrated in other studies, IL-6 is able to induce IL-17 secretion from naive T cells and, therefore, together with IL-17 may be involved in the inflammatory mechanisms and pathogenesis of Lyme borreliosis [34, 35].

Research carried out by Zhi et al. [36] in an animal model have shown that C1qα$^{-/-}$ mice with impaired activation of the classical complement pathway exhibit altered Th1/Th2 response balance after infection with *B. burgdorferi* B31. Elevated levels of cytokines produced by Th1 (MIP-1α, IL-2, IL-12, and TNFα), Th2 (IL-4, IL-10 and MCP -1), and Th17 (IL-17), were detected in the infected C1qα$^{-/-}$ mice. C1q has been shown to affect cytokine production associated with both T cell and B cell responses in *B. burgdorferi* infection [36]. It cannot be excluded that these relationships can affect the quality and intensity of anti-*Borreliella* responses.

In the presented study, there were no statistically significant correlations between the concentrations of IL-6 and IL-17A, IFN-γ, IL-4, IL-8, and IL-10 in the Lyme borreliosis patients, compared with the *Borreliella*-seropositive subjects (positive control) and those with no such antibodies (negative control). However, a significantly higher concentration of IL-2 and a higher, although statistically insignificant, level of TNF were noted in the patients with Lyme borreliosis symptoms.

In their studies, Sjöwall et al. [37] and Jarefors et al. [38] did not show significant differences in the production of IL-1β, IL-6, IL-10, TNF, and IFN-γ by PBMC between groups of patients with neuroborreliosis symptoms, asymptomatic *Borreliella*-seropositive subjects, and seronegative individuals. As shown by Cerar et al. [39], the serum levels of cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α, and IFN-γ) and chemokine IL-8 did not differ between patients with clinical neuroborreliosis symptoms and those with suspected neuroborreliosis. In contrast, serum CXCL13 chemokine levels were higher in the patients with neuroborreliosis. The elevated levels of chemokine CXCL13 in the cerebrospinal fluid were shown to be significantly associated with intrathecal synthesis of *Borreliella* antibodies. Burgasova et al. [40] reported higher levels of IL-2, IL-4, and IL-8 in patients with Lyme borreliosis during the acute phase; however, the IL-4 and IL-2 levels in patients with arthritis remained high during the recovery phase.

Analysis of the serum samples collected from the symptomatic Lyme borreliosis patients, as well as the seropositive and control subject, did not reveal significant differences in the levels of chemokines IP-10, MCP-1, MIG-1, and RANTES. However, there was a significant difference in the serum MMP2 metalloproteinase levels in the untreated patients with Lyme borreliosis symptoms (233.3 ng/ml), compared to those determined in the seronegative individuals (192.0 ng/ml). Similarly, the MMP9 levels in the patients with Lyme borreliosis symptoms were higher but not statistically significant in comparison with the results in the seronegative individuals (negative control). It is possible that the immune response to *B. burgdorferi* infection increases serum MMP2 levels. Metalloproteinases, including MMP2 and MMP9, may be important for the degenerative processes developing in Lyme borreliosis. MMP-2 is active against collagen type I, II, and MCP-3 chemokine, whereas MMP-9 has affinity for plasminogen, myelin, and cartilage proteoglycans [23, 24, 41]. As reported by Zhao et al. [21], the serum MMP9 concentration in patients with acute Lyme borreliosis is significantly higher than in healthy controls. Determination of MMP2 and MMP9 in a larger group of patients and control groups, including subjects occupationally exposed to tick bites, could confirm or exclude the observed relationship and show the potential importance of this parameter for monitoring the development and suppression of the infection.

CONCLUSIONS

1. The failure to notice a tick bite by a patient suspected of Lyme borreliosis cannot be a decisive criterion for exclusion from the need to carry out serological tests.
2. The presence of IgG antibodies against a number of *Borreliella* antigens and the differences in the IL-2 and MMP2 levels in seropositive or seronegative individuals and symptomatic Lyme borreliosis patients, may indicate differences in the intensity of the immune response to the infection and, consequently, may induce development of clinical manifestations of the disease in seropositive and seronegative individuals.
3. Studies on a larger group of patients with clinical symptoms of Lyme borreliosis and seropositive subjects focused on innate defense mechanisms, e.g. the complement system, will prove or exclude their significance.

Competing interests
The authors declare that they have no competing interests.

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