

Levels of sVCAM-1 and sICAM-1 in patients with Lyme disease

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KEY WORDS

endothelial activation
markers,
Lyme disease,
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ABSTRACT

INTRODUCTION Lyme disease is a multi-organ animal-borne disease caused by the spirochete *Borrelia burgdorferi* (Bb).

OBJECTIVES As the pathogenesis of Lyme borreliosis is not fully understood, the study has been designed to examine levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1) in serum and the cerebrospinal fluid (CSF) of patients with Lyme borreliosis and their associations with clinical signs and symptoms and anti-*Borrelia burgdorferi* (anti-Bb) antibody titers.

PATIENTS AND METHODS Sixty-four patients were enrolled in the study, including 39 patients treated for Lyme borreliosis and 25 without the disease (control group). In both groups sVCAM-1 and sICAM-1 levels were determined in serum and the CSF.

RESULTS Mean serum sICAM-1 and sVCAM-1 levels were higher in patients with Lyme borreliosis than in the control group. Serum sICAM-1 levels were significantly lower among patients with results positive for immunoglobulin M seroreactivity with Bb than among those with negative antibody responses. In patients with Bb-specific serum immunoglobulin G (IgG) antibodies, significantly higher serum sICAM-1 levels were found. Higher sVCAM-1 and sICAM-1 levels in the CSF were observed in patients positive for anti-Bb IgG antibody titers in the CSF.

CONCLUSIONS In patients with Lyme borreliosis, endothelial cell activation results in elevated levels of sICAM-1 and sVCAM-1 in serum and the CSF.

INTRODUCTION Lyme borreliosis (Lyme disease) is a tick-borne disease presenting as a wide spectrum of clinical manifestations affecting the skin, joints, nervous system, and heart. The characteristic skin manifestation of the disease is erythema migrans (EM).¹⁻³ Lyme borreliosis is caused by the spirochete *Borrelia burgdorferi* (Bb), which in Poland are transmitted to humans and animals by tick species *Ixodes ricinus* and *Ixodes persulcatus*.¹⁻⁵

Clinical presentations of borreliosis are diverse and depend on the stage of the disease and affected organs. In the majority of cases, the disease presents as EM within 3–30 days, up to 3 months from the tick bite. In some patients signs and symptoms may first appear after many months, or even years since the infection.^{2,3} The universally

accepted Asbrink-Hovmark classification involves early (localized and disseminated) and late (chronic) disease.^{6,7} Apart from EM, patients with early disseminated disease may also develop borreliolymphocytoma of the skin. Manifestations of the early disseminated disease include multiple EM, early Lyme neuroborreliosis, Lyme arthritis, and sometimes Lyme carditis. Manifestations of late Lyme disease are acrodermatitis chronica atrophicans and neurological and rheumatic disorders of >12 months' duration.^{2,3,8}

The diagnosis of Lyme borreliosis is based on clinical presentation and results of laboratory tests. A two-step diagnostic approach is mandatory, with the initial immunoglobulin M (IgM) and immunoglobulin G (IgG) enzyme-linked immunosorbent assays (ELISA) and if their results

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TABLE 1 Serum soluble intercellular adhesion molecule-1 levels in patients with Lyme borreliosis and in the control group

	n	Mean (ng/ml)	SD	Min (ng/ml)	Max (ng/ml)	P
patients with Lyme borreliosis	39	233.5	86.2	130.1	634	0.73
control group	25	203.1	43.1	144.0	323.9	

TABLE 2 Serum soluble vascular cell adhesion molecule-1 levels in patients with Lyme borreliosis and in the control group

	n	Mean (ng/ml)	SD	Min (ng/ml)	Max (ng/ml)	P
patients with Lyme borreliosis	39	723	299.1	366.9	1945	0.31
control group	25	707.3	204.3	470.2	1301.2	

are positive, they are confirmed by the Western blotting.¹

Inflammatory state resulting from Bb dissemination in the human body is accompanied by endothelial activation and leukocyte migration. Central nervous system involvement is associated with cerebral vasculitis caused by active invasion of the vascular wall by spirochetes.⁸⁻¹⁰

Adhesive molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), mediate leukocyte adhesion to the endothelium. The intercellular adhesion molecule is a transmembrane glycoprotein present in multiple cell types, including monocytes, epithelial cells and endothelial cells. A soluble form of ICAM-1 (soluble ICAM-1 – sICAM-1) occurs in body fluids. The level of sICAM-1 is increased during inflammation in proportion to the level of transmembrane ICAM-1. A physiological role of sICAM-1 is not fully understood, however it has been suggested to take part in the inhibition of inflammation by affecting cell-cell interactions.^{2,11} The vascular cell adhesion molecule is absent on the surface of unstimulated endothelial cells, and its expression is increased by lipopolysaccharide, tumor necrosis factor- α , interleukin-1 and interleukin-4. Expression of VCAM-1 and ICAM-1 is stimulated by proinflammatory cytokines during endothelial activation, which enables mutual leukocyte interactions and their migration outside the vessels.¹²

The study has been designed to investigate:

- 1 Serum soluble VCAM-1 (sVCAM-1) and sICAM-1 levels in patients with Lyme borreliosis;
- 2 Correlation of sVCAM-1 and sICAM-1 levels with clinical signs and symptoms and anti-Bb antibody titers in serum and the cerebrospinal fluid (CSF).

PATIENTS AND METHODS Sixty-four patients were enrolled in the study, including 39 patients treated for Lyme borreliosis and 25 without the disease (control group; age range 22–50). The Lyme borreliosis patients were 17 men (43.5%) aged 24–64 and 22 women (56.4%) aged 20–60. Patients from the control group were healthy and had no signs or symptoms of any diseases. The diagnosis of Lyme borreliosis was based on the clinical and laboratory criteria, including a history,

clinical signs and symptoms, serological tests (IgG and IgM ELISA assay, Biomedica; positive results were confirmed by the Western blotting; Recomblot Borrelia IgG and IgM, Mikrogen) – according to the recommendations issued by the Polish Society of Epidemiologists and Infectious Diseases Physicians.¹³

In patients with suspected neuroborreliosis lumbar puncture was performed. Pleocytosis, protein, glucose and chloride levels and serology for Lyme borreliosis using the ELISA assay were determined in the CSF. In both groups sVCAM-1 and sICAM-1 levels were measured in serum and the CSF using the commercially available ELISA assays (kits, Human sVCAM-1 Quantikine ELISA Kit and Human sICAM-1/CD54 ELISA Kit, Biokom).

Antibody synthesis in the CSF was assessed using a formula based on serum and CSF albumin levels. Total IgG and specific IgG titers were determined in serum and the CSF.^{8,14}

Laboratory tests were performed at the Department of Laboratory Diagnostics, University Hospital, Jagiellonian University, Kraków, Poland.

Statistical analysis included a nonparametric Mann-Whitney test performed using the Statistica 8.0 software. A $p < 0.05$ was considered statistically significant.

RESULTS In the Lyme borreliosis group 21 patients (53.84%) had a history of a tick bite, including 12 patients (30.8%) with multiple bites. Twenty-three patients (58.97%) had a history of EM, and 10 patients (43.5%) had been treated with antibiotics for this reason. Twenty-three patients (58.97%) reported headaches, 8 patients (20.5%) had paresis, 17 patients (43.6%) muscle pain, 16 patients (41%) sensory abnormalities and 22 patients (56.4%) complained of joint pain. Lumbar puncture was performed in 33 patients. The CSF inflammatory response was found in 8 patients (25%). Twelve patients (30.8%) had positive IgG reactivity against Bb antigens in the CSF and 13 patients (33.33%) had elevated IgM anti-Bb antibody titers.

Serum sICAM-1 and sVCAM-1 levels in patients with Lyme borreliosis and controls are presented in **TABLES 1** and **2**. Mean serum sICAM-1 and sVCAM-1 levels were higher in patients with Lyme borreliosis than in the control group, but the difference was not statistically significant. There

FIGURE 1 Serum sICAM-1 levels in patients with positive serum anti-Bb IgM antibodies titers comparing with patients from the control group.

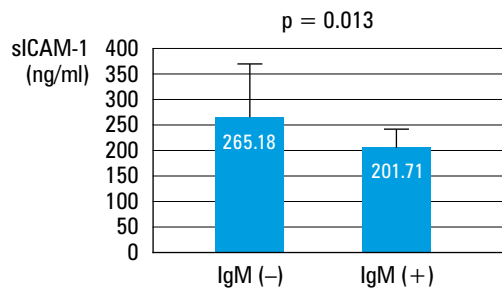


FIGURE 2 Serum sICAM-1 levels in patients with positive serum anti-Bb IgG antibodies titers comparing with patients from the control group.

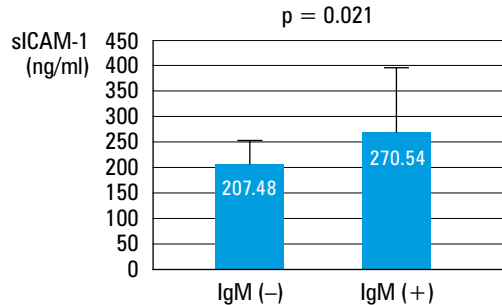


FIGURE 3 CSF sICAM-1 levels in patients with positive CSF anti-Bb IgG antibodies titers comparing with patients from the control group.

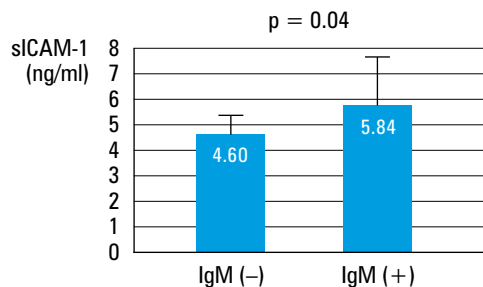
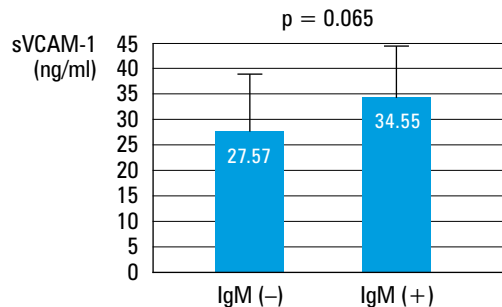


FIGURE 4 CSF sVCAM-1 levels in patients with positive CSF anti-Bb IgG antibodies titers comparing with patients from the control group.



Abbreviations: anti-Bb – anti-*Borrelia burgdorferi*, CSF – cerebrospinal fluid, IgG – immunoglobulin G, IgM – immunoglobulin M

were no statistically significant correlations between sVCAM-1 and sICAM-1 levels and the presence or absence of typical Lyme borreliosis signs and symptoms. However, a statistically significant association was observed between seropositivity for anti-Bb IgM antibodies and serum sICAM-1 levels, which were significantly lower ($p = 0.013$) in positive patients than in those with low serum titers of these antibodies (FIGURE 1). Significantly higher serum sICAM-1 levels were demonstrated ($p = 0.021$) in patients with positive titers of serum anti-Bb IgG antibodies (FIGURE 2).

In patients with positive anti-Bb IgG antibody titers in the CSF, slightly higher sVCAM-1

levels were observed in the CSF ($p = 0.065$). This group had also higher sICAM-1 levels in the CSF ($p = 0.04$), particularly in patients with positive IgG antibody titers and active intrathecal antibody synthesis. There was a statistically significant correlation of these antibodies and sICAM-1 ($p = 0.045$), and it was close to the level of statistical significance for sVCAM-1 (FIGURES 3 and 4).

Patients with positive IgG reactivity against Bb antigens in the CSF and active intrathecal antibody synthesis had no history of EM, an early sign of Lyme borreliosis. Results positive for IgG seroreactivity with Bb in the CSF were significantly more prevalent ($p = 0.007$) in patients with inflammatory responses in the CSF (increased pleocytosis and protein concentrations). Moreover, a correlation was observed between elevated anti-Bb IgG titers determined in serum and the CSF and the results of Western immunoblotting, which was significantly higher in such cases ($p = 0.002$).

DISCUSSION Adhesive molecules in blood and the CSF have previously studied by other investigators. Zajkowska et al. demonstrated increased levels of sICAM-1, sICAM-2, and sICAM-3 in circulating blood and the CSF obtained from patients with neuroborreliosis; higher blood sVCAM levels were also noted in patients with EM and late Lyme borreliosis.^{15,16} Pietruczuk et al. observed elevated sICAM-1, sICAM-2, and sICAM-3 levels in patients with neuroborreliosis compared to the control group. The authors found higher levels of adhesive molecules in patients with neuroborreliosis prior to antibiotic therapy compared to the posttreatment levels.¹⁷ Lewczuk et al. reported six-fold higher central nervous system (CNS) sICAM levels in patients with neuroborreliosis compared to the control group, while serum sICAM levels were similar to those detected in the controls.²

The findings of the present study are consistent with the results published by other authors. Slightly higher serum sVCAM and sICAM levels were observed in patients with neuroborreliosis compared to the control group, although the differences were not statistically significant.

Of note is a correlation between blood sICAM-1 levels and positive serum anti-Bb antibody titers. Higher sICAM-1 levels in patients with elevated anti-Bb IgG antibody titers, but not in those with IgM antibodies, may indicate that sICAM becomes more important with a longer duration of infection. The adhesive molecules investigated as markers of endothelial activation seem to play even a greater role in neuroborreliosis. The patients with results positive for IgG seroreactivity with Bb in the CSF had significantly higher both sVCAM-1 and sICAM-1 levels in the CSF and the correlation was stronger in patients with positive IgG antibody titers and intrathecal antibody synthesis. The current study corroborates the findings of other authors on sICAM-1 and sVCAM-1 levels in Lyme borreliosis. However,

their role in the pathomechanism of this disease still remains to be elucidated.

Patients with suspected neuroborreliosis pose a major diagnostic challenge. During dissemination of the Bb infection it may involve the nervous system causing radicular pains, lymphocytic meningitis, cerebral nerve palsy, peripheral neuropathy and encephalomyelitis. These conditions may be isolated or coexistent. The intensity of clinical signs and symptoms varies from mild headaches and radicular pains to altered consciousness and focal neurological symptoms. Late Lyme borreliosis affecting the nervous system usually has a form of encephalomyelitis, chronic peripheral neuropathy, cognitive disorders and memory impairment.^{8,10,18-20}

The observation regarding intrathecal anti-Bb IgG antibody synthesis found in patients without a history of EM indicates effectiveness of antibiotic treatment for early Lyme borreliosis. Difficulties in Lyme borreliosis diagnostic evaluation arise from the absence of tests that allow physicians to assess the stage of Bb infection, and, particularly, confirm an active infection when serological tests are positive. Clinical signs and symptoms are not pathognomonic, with the exception of EM, and may be present in other conditions (multiple sclerosis, cerebrovascular ischemia, rheumatoid arthritis). Antibodies against Bb may be found positive in serological tests for a long time even after eradication of the infection and are not unequivocal indicators of an ongoing chronic infection.

We hope that elucidation of a role that is played by the sICAM-1 and sVCAM-1 in the pathomechanism of Lyme borreliosis may help use these molecules in the diagnosis of patients with active forms of the disease. In patients with Lyme borreliosis, endothelial cell activation results in elevated sICAM-1 and sVCAM-1 levels in serum and the CSF.

Proper antibiotic therapy initiated in the phase of EM is highly effective and prevents spirochete CNS invasion. Levels of endothelial activation markers in patients with clinical signs and symptoms of Lyme borreliosis mandate further studies. In our opinion they are an important element of pathogenesis of this disease.

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Stężenie sVCAM-1 i sICAM-1 u pacjentów z chorobą z Lyme

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SŁOWA KLUCZOWE

choroba z Lyme,
markery aktywacji
śródbłonna, sICAM,
sVCAM

STRESZCZENIE

WPROWADZENIE Borelioza jest wieloukładową chorobą odzwierzęcą, wywoływaną przez bakterie należące do krętków z rodzaju *Borrelia*, zaliczanych do gatunku *Borrelia burgdorferi* (Bb).

CELE Patogeneza boreliozy nie została w pełni poznana. Podjęto badania mające na celu ocenę stężenia rozpuszczalnych cząsteczek adhezyjnych (*soluble vascular cell adhesion molecule-1* – sVCAM-1, *soluble intercellular adhesion molecule-1* – sICAM-1) w surowicy i płynie mózgowo-rdzeniowym chorych na boreliozę oraz korelacji sVCAM-1 i sICAM-1 z objawami choroby i mianem przeciwciał przeciw Bb.

PACJENCI I METODY Badanie przeprowadzono u 64 chorych, w tym 39 leczonych z powodu boreliozy i 25 bez tej choroby (grupa kontrolna). W obu grupach badanych oznaczono stężenie sVCAM-1 i sICAM-1 w surowicy i płynie mózgowo-rdzeniowym.

WYNIKI Średnie wartości sICAM-1 i sVCAM-1 w surowicy były większe w grupie pacjentów z boreliozą niż w grupie kontrolnej. Stężenie sICAM-1 w surowicy było zmiennie mniejsze u pacjentów z przeciwciałami przeciwko Bb w klasie immunoglobuliny M w surowicy niż u pacjentów, u których nie stwierdzono tych przeciwciał. W grupie chorych, u których wykryto przeciwciała przeciw Bb w klasie immunoglobuliny G (IgG) w płynie mózgowo-rdzeniowym, stwierdzono również większe stężenie sICAM-1 i sVCAM-1 w płynie mózgowo-rdzeniowym.

WNIOSKI U chorych na boreliozę dochodzi do aktywacji śródbłonna naczyńowego ze zwiększeniem stężeń cząsteczek adhezyjnych sICAM-1 i sVCAM-1 w surowicy i płynie mózgowo-rdzeniowym.

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