Health Problems of Civilization 2017, Volume 11, Issue 2
ISSN 2353-6942

ROLE OF VlsE/C6 ANTIGEN AS A MARKER FOR EARLY LYME BORRELIOSIS DIAGNOSIS AND MONITORING THE EFFECTIVENESS OF ITS TREATMENT

ZNACZENIE ANTYGENU VlsE/C6 JAKO MARKERA W DIAGNOSTYCE WCZESNEJ BORELIOZY Z LYME I BADANIU SKUTECZNOŚCI JEJ LECZENIA

Paweł Jan Krzemień[1(E,F)]

[1]Euroimmun Polska Sp. z o.o., Poland

Authors' contribution
Wkład autorów:
A. Study design/planning
zaplanowanie badań
B. Data collection/entry
zebranie danych
C. Data analysis/statistics
dane – analiza i statystyki
D. Data interpretation
interpretacja danych
E. Preparation of manuscript
przygotowanie artykułu
F. Literature analysis/search
wyszukiwanie i analiza literatury
G. Funds collection
zebranie funduszy

Summary

Diagnosing Lyme borreliosis, despite years of standardization, continues to encounter difficulties. They result primarily from the lack of a good marker of active infection and one helpful in assessing the effectiveness of the treatment. So far, a certain diagnosis of Lyme borreliosis can be made only in a patient with erythema migrans (EM). Unfortunately, this symptom occurs only in some patients. According to the recommendations of the Polish Society of Epidemiologists and Doctors of Infectious Diseases, the effectiveness of treatment is determined by the disappearance of clinical symptoms. For this reason, for years, we have been looking for highly sensitive and diagnostically specific laboratory markers. These would allow for rapid identification of fresh infections with *Borrelia* spirochetes as well as simple monitoring of treatment efficacy. According to many of the recently published publications, the solution to the second of the presented problems may be the measurement of IgG antibodies to the surface antigen of *Borrelia burgdorferi* s.l. VlsE / C6.

Keywords: VlsE, C6, Lyme borreliosis, *Borrelia burgdorferi*, serology

Streszczenie

Diagnostyka boreliozy z Lyme, pomimo iż od lat jest wystandaryzowana, nadal spotyka się z kilkoma problemami. Wynikają one przede wszystkim z braku dobrego markera aktywnej infekcji, oraz markera pomocnego w ocenie skuteczności leczenia. Jak dotąd, pewne rozpoznanie boreliozy z Lyme można postawić jedynie w przypadku wystąpienia u pacjenta rumienia wędrującego (EM - Erythema migrans). Niestety objaw ten występuje tylko u części pacjentów. Według rekomendacji Polskiego Towarzystwa Epidemiologów i Lekarzy Chorób Zakaźnych za podstawę oceny skuteczności leczenia uważa się zanik objawów klinicznych. Z tego powodu od lat poszukuje się markerów laboratoryjnych, które z wysoką czułością i specyficznością diagnostyczną pozwalałyby z jednej strony na szybkie rozpoznanie świeżych infekcji krętkami *Borrelia*, a z drugiej na proste monitorowanie skuteczności leczenia. Według wielu spośród pojawiających się w ostatnich latach publikacji rozwiązaniem drugiego z przedstawionych problemów może być pomiar przeciwciał IgG przeciwko antygenowi powierzchniowemu *Borrelia burgdorferi* s.l. VlsE/C6.

Słowa kluczowe: VlsE, C6, borelioza z Lyme, *Borrelia burgdorferi*, serologia

Tables: 0
Figures: 0
References: 27
Submitted: 2017 Mar 30
Accepted: 2017 Apr 24

Introduction

Lyme borreliosis is the most common infectious disease in Northern Europe carried by ticks in the genus *Ixodes*. The disease is caused by spirochetes of the genus *Borrelia burgdorferi* sensu lato, whereas in Europe they are usually strains of *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. The strains of *B. spielmanii* and *B. bavariensis* also play their role in the disease spread [1]. Ticks which primarily feed on wild animals' blood become vectors of *Borrelia* spirochetes. When these ticks bite people, they transfer spirochetes to their bodies. According to the data published by the National Institute of Public Health - National Institute of Hygiene (Laboratory for Monitoring and Analyzing Epidemiological Situations), the incidence of Lyme borreliosis in Poland is constantly increasing. In 2016, 21 220 new cases were reported (a growth by 55.7% compared to the year 2015, when 13 624 cases were reported) [2]. The clinical manifestations of Lyme borreliosis may include skin, joint, neurological or cardiological changes. According to the recommendations by the Polish Society of Epidemiologists and Doctors of Infectious Diseases [1], the diagnosis of Lyme borreliosis is based on clinical symptoms, out of which the most characteristic one is migratory erythema (*Erythema migrans*).

Krzemien PJ. Role of VlsE/C6 antigen as a marker for early Lyme borreliosis diagnosis and monitoring the effectiveness of its treatment. Health Problems of Civilization. 2017; 11(2): 87-92. doi: 10.5114/hpc.2017.69023.

Address for correspondence / Adres korespondencyjny: Paweł Jan Krzemień, Euroimmun Polska Sp. z o.o., Widna 2a, 50-543 Wrocław, Poland, e-mail: p.krzemien@euroimmun.pl, phone: +48 509 657 480

When the migratory erythema appears after a tick bite or following exposure period in endemic areas, it is a sufficient condition to make a diagnosis and introduce an antibiotic therapy. Then, additional serological diagnosis is not recommended. However, if erythema does not develop, but symptoms of disseminated Lyme borreliosis do (*Borrelial lymphoma*, Neuroboreliosis, *Lyme arthritis*, *Acrodermatitis chronica athrophicans* have been recognised in patients with erythema multiforme, then serological tests for IgG and IgM *anti-Borrelia* antibodies are needed. According to the two-stage diagnostic protocol in Poland [1, 3], the first test is a highly sensitive ELISA immunoassay. The test should be performed not earlier than 4 weeks after the tick bite. Positive or limit score ELISA results require confirmation by an independent, highly specific Western blot. In the classic case of *B. burgdorferi* infection, the IgM antibodies appear early to target specific spirochyma proteins. After about 2 weeks after the appearance of IgM antibodies, seroconversion occurs and antibodies of the IgG class appear. In the case of active Lyme borreliosis, an increase in IgG antibody titer is visible with time.

IgM antibodies, which are most often considered to be early-onset markers, can in many cases be permanent and persist for several years after successful treatment. They are most often seen in patients who have been rapidly treated (before seroconversion). Long-term persistence of IgM antibodies does not indicate a need for continued treatment in asymptomatic patients. Antigens in the IgG class can persist in the patient's blood even for the rest of their lives, with a slight downward trend. The best and currently the only proof of the effectiveness of the antibiotic therapy is the remission of clinical symptoms. Unfortunately, ELISA and Western blot tests are often repeated by the patient who has finished therapy, which is a mistake. It should be remembered that antibodies can be present in human serum for many years, so their persistence or even a slight growth does not indicate failure of treatment [1].

Aim of the study

One of the problems encountered in the Lyme borreliosis diagnosis is the lack of a sensitive and a specific marker of the disease activity to assess the effectiveness of treatment. The ideal marker should react flexibly to infection, i.e. it should appear rapidly after infection in the patient's serum and rapidly disappear after successful treatment. The following paper presents an analysis of available literature describing the possibility of a quantitative measurement of levels of antibody against *B. burgdorferi* VlsE / C6 protein in monitoring the activity and efficacy of Lyme borreliosis treatment.

Role of VlsE in the pathogenesis of Lyme borreliosis

VlsE (a variable major protein (VMP)-*like sequence, Expressed*) is a lipoprotein of mass 36 kDa occurring on the surface of the outer cell wall of human pathogenic *Borrelia* spirochetes. The VlsE lipoprotein expression cassette is located in locus vls and consists of 6 variable regions (VRs) alternating with 7 regions of low variability (IR) [4]. It is believed that a continuous recombination in highly variable regions significantly impedes an effective response from the host immune system, which leads to a development of chronic infection and the survival of spirochetes in mammals [5]. Also, crystallographic analysis of recombinant VlsE showed that the variable domains (VRs) partially override the conservative ones (IR), which further hinders the achievement of effective host immune responses [6].

As it was demonstrated by mouse models, VlsE variable domain (VR) recombination occurs only *in vivo* [7]. At the same time, no changes have been observed in the amino acid sequence of variable regions in *in vitro* spirochaetes and those isolated from the infected ticks. Moreover, in the spirochetes kept in such conditions, the level of expression of the VlsE protein decreases in time. A transfer of bacteria from the tick to a warm-blooded host causes significant changes in the expression of bacterial genes. So far, it has not been possible to identify the factor that causes an increased expression of VlsE protein in mammals [4]. It seems that the key role in spirochete survival in the host, which was also shown in mouse models, is the loss of plasmid lp28-1 encoding the sequence of the expressed VlsE protein, which results in a significant decrease in infectivity [8, 9]. As Rogovskyy et al. indicate, the variability of VlsE protein is an essential factor for *Borrelia burgdorferi* reinfection [10]. The results obtained by the researchers confirm the key role of VlsE in avoiding the host's immune response.

Role of VlsE protein in diagnostics of Lyme borreliosis

Despite the presence of variable regions (VR) and tertiary protein conformation, which partially overrides the invariant regions [IR], the VlsE protein exhibits high immunogenicity and is therefore commonly used in serological diagnostics of Lyme borreliosis. As mentioned above, this protein is strongly expressed only in *in vivo* conditions [7], thus, the only way to obtain the right antigen for the construction of diagnostic tests is to

rely on genetic engineering. As demonstrated in several independent studies [11, 12], the enrichment of classical diagnostic *Borrelia burgdorferi* s.l. tests with recombinant VlsE protein significantly increases the sensitivity of the test. In Schulte-Spechtel et al. study, sensitivity increases from 63.8% to 86.1% without loss of specificity [12]. The VlsE protein itself as an antigen also displays the highest diagnostic sensitivity among other *Borrelia burgdorferi* s.l. antigens used in diagnostic tests [11, 13].

VlsE has been proved to be the most sensitive marker for the IgG antibody class. Its prevalence in patients with early Lyme borreliosis symptoms ranges from 20% to 50%, increasing to 70% - 90% in second-stage patients and reaching 100% in patients with chronic Lyme borreliosis (the third phase) [13]. Further, studies have shown that these invariant (IR) VlsE regions induce a strong immune response in mammals [14]. Then, particular importance is attributed to the invariant region of IR6, often referred to in literature as C6.

C6 in diagnostics of Lyme borreliosis

Out of all 6 invariant regions [IR] of VlsE (IR1-IR6), the most prominent, despite a fairly tight curtain by variable regions (VR), is the IR6, also known as region C6 [6]. Therefore, it is precisely C6 that appears to be the most commonly recognized antibody, the invariant (IR) epitope of the VlsE protein [15, 16]. As Embers *et al.* showed in a study on 37 patients with a clinically confirmed Lyme borreliosis, the most commonly recognized VlsE epitope is IR6 (C6) (78% of the patients with LB). The N- and C-terminal domains of VlsE were recognized in fewer than 40% of the samples and IR2 or IR4 fragments in fewer than 12% of the samples. What was also surprising was that only 15 patients observed reactions with C6, but not with full recombinant VlsE. According to the authors, in the full VlsE molecule used in the diagnostic tests, the C6 epitope is well covered by variable regions, and it is the variable regions of the protein surface that are responsible for the positive results [17]. Attention has also been paid to the possibility of using C6 in diagnostics of early Lyme borreliosis. In studies by Zajkowska *et al*. [18], in the group of patients with migratory erythema, positive or limit score results in C6 protein test were found in 61% of the patients, whereas in the *Borrelia* IgM recombinant (OspC, ip41 - *B. afzelii*, *B. garinii*) test, positive results were obtained in 33% of those tested, and in *Borrelia* IgG recombinant (OspC, ip41 - *B. afzelii*, *B. garinii*, p18, p100), only in 16% of the persons. Among the patients with chronic Lyme borreliosis, C6 IgG was detected in 84% of the patients with 100% of positive recombinant results for *Borrelia* IgG. An analogous study of patients in Northern Italy has shown a C6 sensitivity in 63% of the patients with early Lime borreliosis and 100% with late Lyme borreliosis and specificity of 100% [19].

In the Liang *et a.l* study, the C6-based test gave a positive result for 74% of the patients with early Lyme borreliosis, 80% - 95% of the patients after treatment, and 100% of the patients with Lyme borreliosis. In the control group of patients with other spirochetes, the specificity of C6 antigen was 99% [14]. According to Johnsson, in early (acute) Lyme borreliosis, the C6 peptide test increases the diagnostic sensitivity to 60% at this stage of the disease [20]. C6 antigen has also been evaluated as an effective marker for the diagnosis of neuroboreliosis. Assuming that highly pathogenic strains that cause neuroborreliosis in Europe are highly heterogeneous, Fingerle *et al*., have used a conservative and immunogenic C6 peptide epitope to detect IgG antibodies in early neuroborreliosis. Out of the 36 persons, 34 were positive and 2 were questionable [21]. Similarly, in the studies by Stanek *et al*. covering 25 patients with neuroborreliosis, the highest sensitivity and specificity was obtained in studies using the peptide IR6 VlsE as an antigen [22]. Also, van Burgel *et al*. assess C6-based tests as suitable for the routine diagnosis of patients with suspected neuroboreliosis [23].

The analysis of the above work indicates that the use of C6 antigen in examining patients with early Lyme borreliosis enables getting a positive result faster in those infected with *Borrelia burgdorferi* s.l., especially in patients with atypical symptoms and without erythema.

C6 as a marker of disease activity and treatment efficacy

In recent years, there have appeared numerous publications suggesting that quantitative IgG class antibody marking can become a helpful tool in identifying VlsE / C6 antigen and monitoring the disease activity. Unlike the other IgG antibodies, anti-VlsE / C6 disappear relatively quickly after the antibiotic therapy is introduced, which prompted researchers to think of monitoring them in order to assess the effectiveness of the treatment. In one of the first publications on this subject, Philipp *et al*. examined antibody titers against C6 in rhesus monkeys and dogs infected with *Borrelia* [24]. The study included 7 adult rhesus, in whom there was observed a rise of anti-C6 IgG antibody titer for 12 weeks after the infection to the antibiotic therapy. In 6 out of 7 of the rhesus, a decrease in anti-C6 IgG antibody levels was observed during 9-weeks antibiotic therapy until their total disappearance 13 weeks after the end (the 34 week after infection).

In the same study, anti-C6 IgG antibody titers were observed in 8 dogs infected with *Borrelia* spirochaete, 4 of which were treated with antibiotics, while the treatment of the others was postponed over time. In the group of the treated dogs, the level of test antibodies was reduced until they disappeared completely several weeks after treatment. At the same time, in the group of untreated dogs, anti-C6 IgG antibodies reached a high level that persisted throughout the study period. In another study, Levy *et al*. identified 68 infected dogs, 53 of which received treatment, whereas the treatment of further 15 was postponed over time. Then, the level of anti-C6 antibody was measured at the time of therapy initiation as well as after 3, 6, and 12 months after its beginning. The dogs are divided into 2 groups. The first group included individuals with a pre-high level of anti-C6 IgG (30 individuals) and the other one with a relatively low level (23 individuals).

In the first group, a significant decrease in anti-C6 IgG antibodies was observed in relation to the baseline level. In the other, a decrease was noticeable, but it was not so spectacular. No significant change in anti-C6 IgG titre was observed in the untreated dogs [25]. In 2005, Philipp *et al*. examined the dynamics of anti-VlsE / C6 IgG antibodies in 120 patients with clinically confirmed Lyme borreliosis in whom antibiotic therapy was implemented. Between 4 and 15 months after the treatment initiation, 59.0% of the treated patients showed a total decrease in anti-C6 IgG antibodies and 32.4% of the patients showed at least a 4-fold drop in their level. In another study, Marangoni *et al*. observed anti-VlsE / C6 IgG antibodies in 15 patients with clinical symptoms of Lyme borreliosis who underwent treatment. Each patient was tested every 30 days from the onset of antibiotic therapy.

After 1-6 months, all tested patients were seronegative for anti-VlsE / C6 IgG antibodies, including symptom remission [26]. Another study [27] assessed the possibility of using anti-C6 IgG antibodies to evaluate patients with the so called post Lyme syndrome. Patients selected for the study had a well documented Lyme borreliosis, including chronic muscular and neurological symptoms. They were then enrolled in a double-blind trials. Samples were collected twice, at the start of the treatment and 6 months later. In this study, there was no correlation between the decrease in anti-C6 IgG antibody levels and the treatment or disappearance of clinical symptoms. Simultaneously, anti-C6 IgG antibodies were noted in lower titres and less frequently in patients with myelodysplastic syndrome, than in patients with active Lyme borreliosis. Despite this, the authors of the publication evaluate the diagnostic value of anti-C6 IgG antibodies in patients with a syndrome as low.

Conclusions

Rich scientific literature on lipoprotein VlsE allows to treat this antigen as a well-studied marker of confirmed significance in the diagnosis of Lyme borreliosis. Particular attention has been paid in recent years to examining the significance of antibody levels against the unchanged IR6 fragment of the VlsE protein. VlsE / C6 antigen has proven to be a significant marker in the diagnosis of early Lyme borreliosis, and appears to meet the conditions of an effective marker for monitoring efficacy. It is also interesting to use the VlsE / C6 marker in optimizing the current strategy for diagnosing Lyme borreliosis, but this matter requires more research.

Basing on available publications, it would seem reasonable to suggest a strategy of a quantitative assessment of anti-VlsE / C6 anti-VlsE / C6 antibody levels in monitoring the efficacy of treating patients with early Lyme borreliosis, through examination performed at three stages - first before or at the time of the introduction of antibiotic therapy, and then another two - 6 and 12 months after the treatment. Disappearance of clinical symptoms combined with a decrease in anti-VlsE / C6 IgG antibody titer will testify about the success of antibiotic therapy. For now, the anti-VlsE / C6 IgG level test to assess the activity of the disease is devoid of a scientific basis. As of today, the VlsE / C6 test is not included in any recognition criteria in diagnosis of Lyme borreliosis. It remains just an additional tool and requires further research.

References:

1. Pancewicz SA, Garlicki AM, Moniuszko-Malinowska A, Zajkowska J, Kondrusik M, Grygorczuk S, et al. Polish Society of Epidemiology and Infectious Diseases. Diagnosis and treatment of tick-borne diseases recommendations of the Polish Society of Epidemiology and Infectious Diseases. Przegl Epidemiol. 2015; 69(2): 309-16, 421-8.
2. Zachorowania na wybrane choroby zakaźne w Polsce od 1 stycznia do 31 grudnia 2016 r. oraz w porównywalnym okresie 2015 r, Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny, Zakład Epidemiologii, Departament Zapobiegania oraz Zwalczania Zakażeń i Chorób Zakaźnych u Ludzi GIS [Internet] 2017 Jan [cited 2017 mar 24] Available from: http://wwwold.pzh.gov.pl/oldpage/epimeld/2016/INF_16_12B.pdf (in Polish)
3. Chmielewski T, Dunaj J, Gołąb E, Gut W, Horban A, Pancewicz S, et al. „Diagnostyka laboratoryjna chorób odkleszczowych" Rekomendacje Grupy Roboczej: Krajowa Izba Diagnostów Laboratoryjnych, Narodowy

Instytut Zdrowia Publicznego - Państwowy Zakład Higieny, Konsultant Krajowy w dziedzinie chorób zakaźnych, Klinika Chorób Zakaźnych i Neuroinfekcji Uniwersytet Medyczny w Białymstoku, Polskie Towarzystwo Wirusologiczne, Krajowa Izba Diagnostów Laboratoryjnych, Warszawa 2014, p. 20 [Internet] 2014 [cited 2017 mar 24] Available from: http://kidl.org.pl/uploads/rekomendacje/05_kleszcze%20z%20okladka.pdf (in Polish)

4. Norris SJ. vls Antigenic Variation Systems of Lyme Disease Borrelia: Eluding Host Immunity through both Random, Segmental Gene Conversion and Framework Heterogeneity. Microbiol Spectr. 2014 Dec;2(6), doi: 10.1128/microbiolspec.MDNA3-0038-2014.
5. Bubeck-Martinez S. Immune evasion of the Lyme disease spirochetes. Front Biosci. 2005 Jan 1;10:873-8.
6. Eicken C, Sharma V, Klabunde T, Lawrenz MB, Hardham JM, Norris SJ, et al. Crystal structure of Lyme disease variable surface antigen VlsE of Borrelia burgdorferi. J Biol Chem. 2002 Jun 14; 277(24): 21691-6.
7. Sung SY, McDowell JV, Marconi RT. Evidence for the contribution of point mutations to VlsE variation and for apparent constraints on the net accumulation of sequence changes in VlsE during infection with Lyme disease spirochetes. J Bacteriol. 2001 Oct; 183(20): 5855-61.
8. Labandeira-Rey M, Baker E, Skare JT. Decreased infectivity in Borrelia burgdorferi strain B31 is associated with loss of linear plasmid 25 or 28-1. J. Infect. Immun. 2001; 69: 446–455.
9. Purser JE, Norris SJ. Correlation between plasmid content and infectivity in Borrelia burgdorferi. Proc. Natl. Acad. Sci. U. S. A. 2000; 97(25): 13865–13870.
10. Rogovskyy AS, Bankhead T. Variable VlsE is critical for host reinfection by the Lyme disease spirochete. PLoS One. 2013 Apr 8; 8(4): e61226
11. Goettner G, Schulte-Spechtel U, Hillermann R, Liegl G, Wilske B, Fingerle V. Improvement of Lyme borreliosis serodiagnosis by a newly developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and addition of VlsE and DbpA homologues. J Clin Microbiol. 2005 Aug; 43(8): 3602-9.
12. Schulte-Spechtel U, Lehnert G, Liegl G, Fingerle V, Heimerl C, Johnson BJ, et al. Significant improvement of the recombinant Borrelia-specific immunoglobulin G immunoblot test by addition of VlsE and a DbpA homologue derived from Borrelia garinii for diagnosis of early neuroborreliosis. J Clin Microbiol. 2003 Mar; 41(3): 1299-303.
13. Wilske B. Epidemiology and diagnosis of Lyme borreliosis. Ann Med. 2005; 37(8): 568-79.
14. Liang FT, Steere AC, Marques AR, Johnson BJ, Miller JN, Philipp MT. Sensitive and specific serodiagnosis of Lyme disease by enzyme-linked immunosorbent assaywith a peptide based on an immunodominant conserved region of Borrelia burgdorferi VlsE. J Clin Microbiol. 1999 Dec; 37(12): 3990-6.
15. Liang FT, Philipp MT. Analysis of antibody response to invariable regions of VlsE, the variable surface antigen of Borrelia burgdorferi. Infect Immun. 1999; 67: 6702–6706.
16. Liang FT, Philipp MT. Epitope mapping of the immunodominant invariable region of Borrelia burgdorferi VlsE in three host species. Infect Immun. 2000; 68: 2349–2352.
17. Embers ME, Jacobs MB, Johnson BJ, Philipp MT. Dominant epitopes of the C6 diagnostic peptide of Borrelia burgdorferi are largely inaccessible to antibody on the parent VlsE molecule. Clin Vaccine Immunol. 2007 Aug; 14(8): 931-6.
18. Zajkowska JM, Kondrusik M, Pancewicz SA, Grygorczuk S, Jamiołkowski J, Stalewska J. Comparison of test with antigen VlsE (C6) with tests with recombinant antigens in patients with Lyme borreliosis. Pol Merkur Lekarski. 2007 Aug; 23(134): 95-9 (in Polish).
19. Cinco M, Murgia R. Evaluation of the C6 enzyme-linked immunoadsorbent assay for the serodiagnosis of Lyme borreliosis in north-eastern Italy. New Microbiol. 2006 Apr; 29(2): 139-41.
20. Johnson B. Advantages and limitations of testing for antibodies to B.burgdorferi sensu stricto. IX International Conference on Lyme Borreliosis and Other Tick Borne Diseases. August 18-22, 2002, NY. Abstracts
21. Fingerle V, Schulte-Spechtel U, Levin A. i wsp.: Evaluation of an ELISA based on the C6 peptide of VlsE for diagnosis of early neuroborreliosis. IX International Conference on Lyme Borreliosis and Other Tick Borne Diseases. August 18-22, 2002, NY. Abstracts
22. Stanek G, Lusa L, Ogrinc K, Markowicz M, Strle F. Intrathecally produced IgGand IgM antibodies to recombinant VlsE, VlsE peptide, recombinant OspC and whole cell extracts in the diagnosis of Lyme neuroborreliosis. Med Microbiol Immunol. 2014 Apr; 203(2): 125-32.
23. van Burgel ND, Brandenburg A, Gerritsen HJ, Kroes AC, van Dam AP. High sensitivity and specificity of the C6-peptide ELISA on cerebrospinal fluid in Lyme neuroborreliosis patients. Clin Microbiol Infect. 2011 Oct; 17(10): 1495-500.
24. Philipp MT, Bowers LC, Fawcett PT, Jacobs MB, Liang FT, Marques AR, et al. Antibody response to IR6, a conserved immunodominant region of the VlsE lipoprotein, wanes rapidly after antibiotic treatment of Borrelia burgdorferi infection in experimental animals and in humans. J Infect Dis. 2001 Oct 1; 184(7): 870-8.

25. Levy SA, O'Connor TP, Hanscom JL, Shields P, Lorentzen L, Dimarco AA. Quantitative measurement of C6 antibody following antibiotic treatment of Borrelia burgdorferi antibody-positive nonclinical dogs. Clin Vaccine Immunol. 2008 Jan; 15(1): 115-9.
26. Marangoni A, Sambri V, Accardo S, Cavrini F, Mondardini V, Moroni A, et al. A decrease in the immunoglobulin G antibody response against the VlsE protein of Borrelia burgdorferi sensu lato correlates with the resolution of clinical signs in antibiotic-treated patients with early Lyme disease. Clin Vaccine Immunol. 2006 Apr; 13(4): 525-9.
27. Fleming RV, Marques AR, Klempner MS, Schmid CH, Dally LG, Martin DS, el al. Pre-treatment and post treatment assesment of the c(6) test in patients with persistent symptoms and history of Lyme borreliosis. Eur. J. Clin. Microbiol. Infect. Dis. 2004; 23(8): 615-618.