

THE USE OF ANTI-C6VlsE IgG IN THE ASSESSMENT OF THE EFFECTIVENESS OF LYME DISEASE TREATMENT – A PRELIMINARY REPORT

ZASTOSOWANIE PRZECIWCIAŁ ANTY-C6VlsE W OCENIE SKUTECZNOŚCI LECZENIA BORELIOZY Z LYME – DONIESIENIE WSTĘPNE

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Summary

Background. The aim of this study was to evaluate the dynamics of changes in IgG anti-C6VlsE concentration in patients treated for Lyme borreliosis in relation to clinical manifestations of the disease.

Material and methods. The study group consisted of 20 patients from Ternopil and surrounding areas (Western Ukraine) with clinical symptoms of Lyme borreliosis. IgG anti-C6VlsE was measured three times: before starting the patients on antibiotic therapy, immediately after its completion and 3-4 months after the end of antibiotic therapy.

Results. In 40% of the patients the IgG anti-C6VlsE concentration decreased 3-4 months after the treatment, but only in half of them it was associated with elimination of symptoms. 60% of patients did not show any tendency to decrease in IgG anti-C6VlsE concentrations after 3-4 months after the treatment, and 25% of patients in this group had an improvement in health condition.

Conclusions. It cannot be excluded that IgG anti-C6VlsE may be important in the serological evaluation of the efficacy of Lyme borreliosis treatment, especially in early stage patients. However, this requires further research, which should be extended to a larger group of patients. It is also important that the assessment of anti-C6VlsE antibody concentration should be performed additionally for a period longer than 4 months from the end of antibiotic therapy.

Keywords: VlsE, Borrelia burgdorferi, Lyme borreliosis, Ukraine

Streszczenie

Wprowadzenie. Celem badań była ocena dynamiki zmian stężenia IgG anty-C6VlsE u pacjentów leczonych w związku z boreliozą z Lyme w stosunku do manifestacji klinicznych choroby.

Materiał i metody. Grupę badaną stanowiło 20 pacjentów z klinicznymi objawami boreliozy z Lyme z Tarnopola i okolic (Ukraina Zachodnia). IgG anty-C6VlsE oznaczano trzykrotnie: przed wdrożeniem antybiotykoterapii, bezpośrednio po jej zakończeniu i 3-4 miesiące od zakończenia antybiotykoterapii.

Wyniki. U 40% badanych stwierdzono obniżenie stężenia IgG anty-C6VlsE po 3-4 miesiącach po leczeniu, przy czym tylko u połowy z nich wiązało się to z eliminacją dolegliwości. U 60% pacjentów nie stwierdzono tendencji obniżania stężeń IgG anty-C6VlsE po 3-4 miesiącach po leczeniu, a u 25% tej grupy nastąpiła poprawa stanu zdrowia.

Wnioski. Nie wykluczone, że IgG anty-C6VlsE może mieć znaczenie w serologicznej ocenie skuteczności leczenia boreliozy z Lyme, zwłaszcza u osób we wczesnym okresie choroby. Wymaga to jednak dalszych badań, którymi należałoby objąć liczniejszą grupę pacjentów. Istotne jest także, by ocena stężenia przeciwciał anty-C6VlsE dokonywana była dodatkowo w okresie dłuższym niż 4 miesiące od zakończenia antybiotykoterapii.

Słowa kluczowe: VlsE, Borrelia burgdorferi, borelioza z Lyme, Ukraina

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Introduction

Borrelia burgdorferi sensu lato (*B. burgdorferi* s. l.) including *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii* are responsible for generating symptoms of Lyme borreliosis in European countries. Clinical manifestations of the disease include early skin lesions such as erythema migrans (EM), Lyme arthritis, neuroborreliosis and skin lesions such as acrodermatitis chronica atrophicans (ACA) [1, 2]. The laboratory diagnosis of Lyme borreliosis is based on the results of serological tests and clinical manifestations observed in the patient [2, 3]. According to the recommendations valid in Poland and many other European countries, serological diagnosis of Lyme borreliosis should be carried out according to a two-stage protocol (ELISA test, Western blot test) [4]. There are no uniform diagnostic recommendations for Lyme borreliosis in Ukraine, but according to the Order of the Minister of Health of Ukraine N133 from 19th July 1995, the disease was classified as a particularly dangerous infection.

Serological tests used in the diagnosis of Lyme borreliosis cannot be used to assess the efficacy of treatment. The effectiveness of antibiotic therapy should be assessed on the basis of the disappearance of clinical manifestations of the disease [5]. As a result, laboratory markers with high sensitivity and diagnostic specificity are still being sought to monitor the effectiveness of the treatment of this disease. High hopes in this respect were associated with anti-C6VlsE *B. burgdorferi* antibodies. In some patients there was a decrease in their titre after effective antibiotic therapy. Therefore, the possibility of their use in the evaluation of the efficacy of Lyme borreliosis treatment is still considered [6]. VlsE (variable major protein-like sequence, expressed), is an immunogenic molecule responsible for the antigenic variability of *B. burgdorferi*. Its construction distinguishes six variable regions and six unchanging regions. Unchanging regions (IR1-IR6) are conservative for the genotypes of the *B. burgdorferi* s. l. complex. [7]. It is suggested that supplementing the diagnostic panel with recombinant *B. burgdorferi* s. l. proteins, including C6 peptide from VlsE, may significantly increase the sensitivity of serological tests [3]. The aim of this study was to evaluate the dynamics of changes in IgG anti-C6VlsE concentration in patients treated for Lyme borreliosis in relation to clinical manifestations of the disease.

Material and methods

Study group

The study group consisted of 20 patients with clinical symptoms of Lyme borreliosis and antibodies for specific *B. burgdorferi* antigen proteins: 18 adults aged 25 to 62 years (mean 50.83, SD 9.54) and 2 children aged 4 and 9 years. The subjects came from Ternopil and surrounding areas (Western Ukraine). The process of diagnosis and treatment for Lyme borreliosis was carried out in the Department of Infectious Diseases and Epidemiology, Dermatology and Venereology, I. Ya. Horbachevsky Ternopil State Medical University.

Blood was collected three times to determine IgG anti-C6VlsE antibodies:

- before starting antibiotic therapy (sample 1),
- after the end of antibiotic therapy (sample 2),
- 3-4 months after the end of antibiotic therapy (sample 3).

The study was approved by the Bioethics Committee of I. Ya. Horbachevsky Ternopil State Medical University, No 42, 04/09/2017.

Questionnaire

All patients answered questions concerning: tick bites (the place where the tick was inserted into the body, the number of bites, time elapsed from the moment of inserting the tick until its removal from the body, the method of removing the tick), occurrence of erythema migrans (EM) and other symptoms that were related to the fact of tick bites and the coexistence of chronic diseases.

Serological tests

Serum IgG anti-C6VlsE antibodies were assayed using the Lyme Trace ELISA (IgG) test (Euroimmun, Germany). This test is based on recombinant VlsE from *B. burgdorferi* sensu stricto and *B. afzelii*, which are the etiological factors of Lyme borreliosis in Europe. IgG anti-C6VlsE concentration is expressed in RU/ml. The test was performed according to the manufacturer's instructions.

Statistical analyses

Statistical analyses were performed on the basis of Statistica v. 10. The ANOVA Friedman test was used to analyse the variance of repeated measurements. This test is used when measurements of the test variable are made several times ($k \geq 2$). Kendall's coefficient of concordance: values close to 0 are the mismatch between cases, values close to 1 are the correspondence between repeated measurements. Assumed significance level $p < 0.05$.

Results

Analysis of answers given by respondents in the questionnaire

Detailed data on the history of tick bites and symptoms associated with this event as well as the general health status of the respondents are presented in Table 1.

Seventy percent (70%) of people from the study group declare the occurrence of tick bites: single bites (35%), double bites (10%), multiple bites (25%). The most frequent bites occurred in the forest areas (40%). Ticks inserted into the skin were found in the lower limbs (50%), back (20%), upper limbs and abdomen (15% each), and the in the neck area (5%). Respondents often used the help of medical personnel (doctor, nurse) to remove ticks (25%). Some people used incorrect actions, such as pulling the tick with their fingers (25%), lubricating the tick with fat so the insect gets out by himself (15%) or scratching the tick with their fingernails (20%). Only 5% of the respondents removed the tick within 12 hours of its insertion into the skin. 40% of patients reported EM after a tick bite - 25% of patients up to 30 days after the tick bite, while 15% of respondents did not remember the amount of time that passed from the tick bite. Other symptoms reported by subjects due to tick bite are presented in Table 1. Fifty-five percent (55%) of the study group remains under the care of a cardiologist, neurologist, dermatologist or other specialist in chronic diseases. Data concerning drugs taken by patients are presented in Table 1.

Table 1. Exposure to ticks and general health characteristics

Answers provided		N(%)
Tick bites		
The area	agricultural land	2(10%)
	forest	8(40%)
	meadow/grassland	1(5%)
	fruit orchard	1(5%)
	green areas	2(10%)
The number of tick bites	bitten once	7(35%)
	bitten twice	2(10%)
	multiple bites	5(25%)
Tick bites which were not noted		6(30%)
Place of tick bite	upper limb	3(15%)
	lower limb	10(50%)
	neck	1(5%)
	back	4(20%)
	stomach	3(15%)
Removal of the tick	up to 12 hours	1(5%)
	up to 24 hours	6(30%)
	up to 48 hours	3(15%)
	3 days	1(5%)
	1 month	1(5%)
	I do not remember	2(10%)

Method of removal	by a physician/nurse	5(25%)
	pulled out with fingers	5(25%)
	scratching with a nail	4(20%)
	spreading a tick with oily substance to make it go out on its own	3(15%)
	disinfecting a place after removing a tick	4(20%)
General health		
Patients who are under the care of a cardiologist, neurologist, dermatologist or other specialist in the field of chronic diseases		11(55%)
Used medicinal preparations	in cardiovascular diseases	2(10%)
	in digestive system diseases	1(5%)
	antihistamines	1(5%)
	hormones	4(20%)
	antibiotics	4(20%)
	other	4(20%)

N (%) - the number of people (percentage)

Serological tests - IgG-antigen concentration C6VlsE

IgG anti-C6VlsE concentrations were measured three times in serum samples: before antibiotic therapy (sample 1), after antibiotic therapy (sample 2), 3-4 months after antibiotic therapy (sample 3).

Depending on the obtained results of IgG-antigen C6VlsE concentration, subsequent serum samples were divided into groups:

- group A - patients with a decrease in IgG anti-C6VlsE concentration in three consecutive serum samples;
- group B - patients with an increase in IgG anti-C6VlsE concentration after antibiotic therapy (sample 2) compared to the pre-treatment status (sample 1), followed by a decrease in IgG anti-C6VlsE concentration 3-4 months after treatment completion (sample 3);
- group C - patients with an increase in IgG anti-C6VlsE concentration in three consecutive serum samples;
- group D - patients in whom IgG anti-C6VlsE concentration decreased after antibiotic therapy (sample 2) compared to the pre-treatment status (sample 1), followed by an increase in IgG anti-C6VlsE concentration 3-4 months after treatment completion (sample 3);
- group E - patients with high concentrations (<250 RU/ml) of IgG anti-C6VlsE in subsequent serum samples;
- group F - patients with low concentrations (3.57 - 8.94 RU/ml) of IgG anti-C6VlsE in subsequent serum samples.

A detailed summary of the results together with information on antibiotic therapy and symptoms of Lyme borreliosis before and after treatment is presented in Table 2 .

Table 2. IgG anti-C6VlsE concentrations and observed clinical signs before and after the treatment for Lyme borreliosis

N(%)	Group	IgG anty-C6VlsE			Symptoms before treatment	Treatment	Result of treatment
		Sample 1	Sample 2	Sample 3			
3(15)	A	211	115	101	EM simultaneously diagnosed with anaplasmosis	3	CR
		178	85.1	32.4	EM	1	CR
		98.6	14.1	5.59	EM, concentration disorders	3	CR

5(25)	B	112	153	55.5	EM	1	LTC – neurological complications
		5.88	13.7	4.12	muscle pain, bones and joints pain	4,5	LTC – musculoskeletal system
		77.4	177	70.4	bones and joints pain	1	LTC – musculoskeletal system
		36.4	44.4	25.5	EM	1	LTC – musculoskeletal system, neurological complications
		11.1	14.3	5.9	EM, muscles pains, bones and joints pain	1	CR
1(5)	C	3.53	111	139	bones and joints pain	3	LTC – musculoskeletal system, uveitis
2(10)	D	46.5	34.6	54.4	bones and joints pain	1	CR
		14.1	7.16	15.9	fever, bones and joints pain, concentration disorders	1	CR
5(25)	E	>250	>250	>250	bones and joints pain	3	LTC – musculoskeletal system
		>250	>250	>250	EM	6	LTC – problems with the pulmonary system
		>250	>250	>250	EM, bones and joints pain arthritis, concentration disorders	7	LTC – musculoskeletal system, uveitis
		>250	>250	>250	meningitis	8	CR
		>250	>250	>250	bones and joints pain, concentration disorders	1	LTC – musculoskeletal system
4(20)	F	4.15	3.57	5.62	bones and joints pain	1,5	LTC – musculoskeletal system
		7.16	7.81	8.94	erythema nodosum, bones and joints pain	1,5	LTC – musculoskeletal system
		7.57	6.12	5.68	muscle pain, bones and joints pains	1,5	LTC – musculoskeletal system
		3.77	4.24	4,8	bones and joints pains	1,5	LTC – musculoskeletal system

Markings in the table:

N (%) - the number of people (percentage)

Groups of patients: A, B, C, D, E, F - explanation of the abbreviations is included in the text

Treatment:

1. Doxycycline, 100mg twice daily for 21 days
2. Azithromycin, 500mg twice daily for 10 days
3. Doxycycline, 100mg twice daily for 14 days + Azithromycin, 500mg twice daily for 10 days
4. Doxycycline, 100mg twice daily for 14 days + Bicillin 5 for 10 days
5. Treatment with hormones in anamnesis
6. Doxycycline, 100mg twice daily for 14 days + treatment of ascaris and lamblia
7. Doxycycline, 100mg twice daily for 21 days +Levofloxacin, 500mg twice daily for 10 days
8. Ceftriaxone 1000mg twice daily for 12 days + Azithromycin, 500mg twice daily for 12 days+ hormones

Result of treatment:

- CR- Complete recovery
- LTC - Long-term consequences

Group A patients

In 15% of patients a decrease in IgG anti-C6VlsE concentration was observed in three consecutive serum samples (Figure 1). The mean pre-treatment IgG anti-C6VlsE (sample 1) was 162. 53 RU/ml (SD 57.77); after antibiotic therapy (sample 2) 71.40 RU/ml (SD 51.82); 3-4 months after treatment (sample 3) 46.33 RU/ml (SD 49.20). In the analysed cases significant differences were found between measurements ($p=0.04979$) and strong correlation (1.00). In all patients in this group the symptoms of Lyme borreliosis were eliminated (Table. 2).

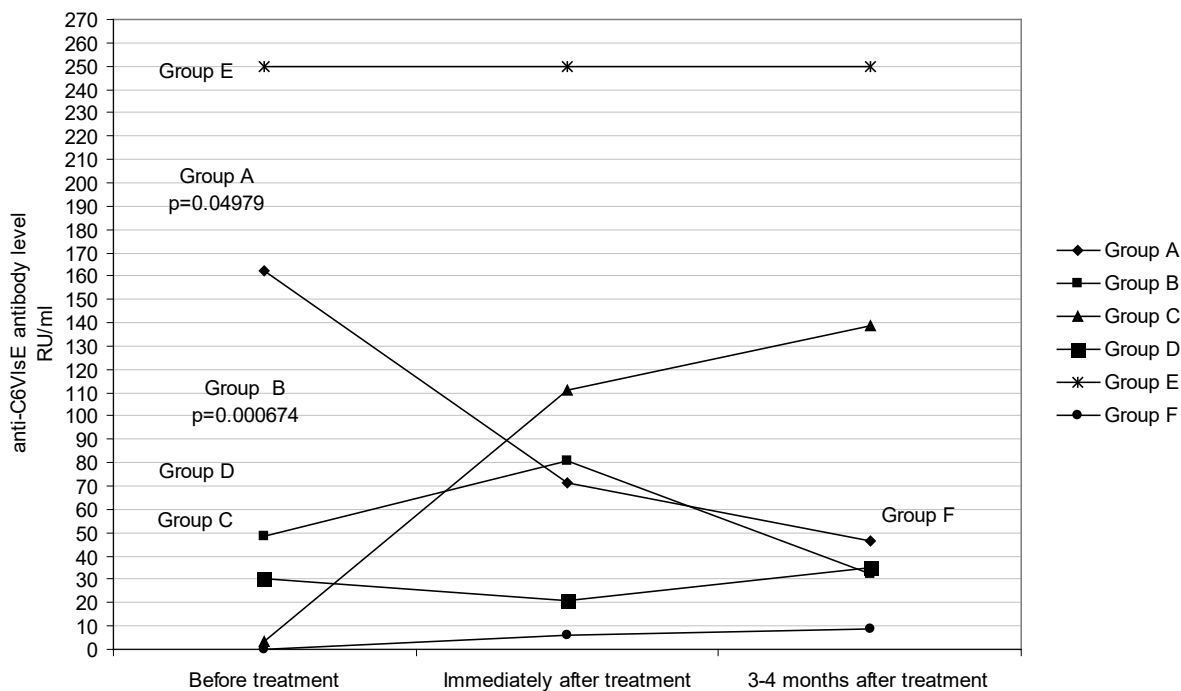


Figure 1. Dynamics of IgG anti-C6VlsE changes in patients treated for Lyme borreliosis

Group B patients

In 25% of patients, including 1 child, IgG anti-C6VlsE concentration increased after antibiotic therapy (sample 2) and then IgG anti-C6VlsE concentration decreased 3-4 months after treatment completion (sample 3) (Figure 1). Mean IgG anti-C6VlsE values were: sample 1 - 48.55 RU/ml (SD 45.35); sample 2 - 80.48 RU/ml (SD 78.60); sample 3 - 32.28 RU/ml (SD 29.69). In the analysed cases significant differences were found between measurements ($p=0.00674$) and strong correlation (1.00). In 80% of patients in this group the symptoms of Lyme borreliosis were not eliminated (Table 2).

Group C patients

In 5% of the subjects an increase in IgG anti-C6VlsE concentration was observed in subsequent samples (1 - 3.53 RU/ml; 2 - 111 RU/ml; 3 - 139 RU/ml). The symptoms of Lyme borreliosis were not eliminated (Figure 1, Table 2).

Group D patients

In 10% of patients, IgG anti-C6VlsE concentration decreased after antibiotic therapy (sample 2) compared to sample 1, followed by an increase in IgG anti-C6VlsE concentration 3-4 months after treatment completion (sample 3) (Figure 1). Average IgG anti-C6VlsE values were: sample 1 - 30.30 RU/ml (SD 22.91); sample 2 - 20.88 (SD 19.40); sample 3 - 35.15 (SD 27.22). The differences were not significant ($p=0.13534$). The symptoms of Lyme borreliosis were eliminated (Table 2).

Group E patients

In 25% of patients, including 1 child, high concentrations (>250 RU/ml) of IgG anti-C6VlsE were found in three consecutive serum samples (Figure 1, Table 2). In one adult in this group of patients the symptoms of Lyme borreliosis were eliminated.

Group F patients

In 20% of three consecutive serum samples low IgG anti-C6VlsE concentrations were found, ranging from 3.57 to 8.94 RU/ml. (Figure 1, Table 2). There was no elimination of Lyme borreliosis symptoms in the subjects.

Discussion

In the absence of a Lyme borreliosis vaccine, the only way to avoid infection with *B. burgdorferi* s. l. spirochetes is to consciously apply the principles of prevention. Among other things, it is important to remove the tick embedded in the skin as quickly and correctly as possible. Unfortunately, many people still use inappropriate practices such as lubricating the tick with fat so it detaches from the skin on its own (15%) or scratching the tick off (20%) and only 5% of respondents removed the tick within 12 hours of its insertion into the skin. In other studies (not published) carried out among Ukrainian foresters, 18.8% of them declared tick lubrication with greasy substance. It is therefore important to strive for continuous education for people at risk of tick bites in connection with their work or staying in areas where these arachnids are found.

The diagnosis of Lyme borreliosis is based on characteristic skin lesions such as EM and borreliolymphoma. Other early symptoms include Lyme carditis, Lyme arthritis and early neuroborreliosis. Late changes include neuroborreliosis, Lyme arthritis, acrodermatitis chronica atrophicans (ACA). In about 80% of patients EM is diagnosed, which appears between 3 to 30 days after infection with *B. burgdorferi* s. l. and spontaneously disappears within about 4 weeks. The diagnosis is based on the clinical picture and does not require confirmation by serological tests [8]. This early symptom of Lyme borreliosis was reported by 40% of patients in the study group. Bone and joint pain occurred in 65% of subjected individuals, concentration disorders in 20% and muscle pain in 15%.

Antibiotic therapy implemented in patients with early symptoms of the disease is usually effective. However, in individuals with advanced clinical manifestations such as Lyme arthritis, it may take a few weeks or months for the patient to notice improvement in their health from the time of starting them on antibiotics. Treatment may also not bring the improvement in health expected by the patient. It is estimated that about 10% of individuals experience chronic fatigue, muscle pain and other non-specific problems of a continuous or recurrent nature, despite starting them on antibiotic therapy for Lyme borreliosis. These symptoms may be subjective and the mechanism of their generation has not been explained [9]. In some cases, the group of signs and symptoms are referred as the post-treatment Lyme disease syndrome (PTLDS) [10].

From the group of 20 patients treated for Lyme borreliosis, 35% had an improvement in health after antibiotic therapy. In 65% of individuals the symptoms of the disease persisted or exacerbated 3-4 months after the end of the treatment. These were most often bone and joint ailments and muscle pains (55%), while neurological disorders occurred in 10% of the patients.

It cannot be excluded that in some patients accompanying chronic diseases and the treatment used in this connection had an impact on the ailments experienced by the examined patients. It is also difficult to assess

whether some of these ailments could have been subjective feelings. In diagnostic practice, the possibility of using anti-C6VlsE antibodies to evaluate the efficacy of Lyme borreliosis treatment is taken into account, as some patients showed a decrease in their titre after effective antibiotic therapy [6]. IR6 (C6) specific antibodies were detected in 78% of serum samples from patients with symptoms of Lyme borreliosis, while less than 12% of samples responded to IR2 or IR4 [7]. Studies conducted by Philipp have shown that in patients with symptoms of Lyme borreliosis (early localized, early disseminated symptoms) who received treatment, the anti-C6VlsE *B. burgdorferi* antibody titre decreased fourfold or even to undetectable levels within 6 to 12 months after treatment. This decrease was observed in 89% of patients with EM and in 100% of patients with multiple-EM [9]. Withdrawal of IgG antibodies to VlsE antigen within 2 to 6 months after treatment was also found in other studies in patients with early localized Lyme borreliosis [2]. According to Philipp and his team, the decrease in anti-C6VlsE antibody titre after antibiotic treatment may result due to the properties of VlsE protein [11]. The analysis of the results showed that the dynamics of IgG anti-C6VlsE concentration changes in patients treated for Lyme borreliosis may vary. In 40% of the patients IgG anti-C6VlsE concentration decreased 3-4 months after the treatment, but only in half of them it was associated with the elimination of the ailments. 60% of patients did not show any tendency of IgG anti-C6VlsE concentrations decrease after 3-4 months after treatment, and 25% of this group had an improvement in health condition. The dynamics of IgG anti-C6VlsE changes may depend on the severity and duration of clinical signs of Lyme borreliosis. In patients with PTLDS there was no correlation between the decrease in C6 antibody titre and the outcome of treatment and it was therefore concluded that they could not be used to assess the outcome of treatment in this population [12].

Conclusions

It is not excluded that IgG anti-C6VlsE may be important in the serological evaluation of the efficacy of Lyme borreliosis treatment, especially in patients in the early stage of the disease. However, this requires further research, which should be extended to a larger group of patients. It is also important that the assessment of anti-C6VlsE antibody concentration is performed additionally for a period longer than 4 months from the end of antibiotic therapy.

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