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Lyme disease – treatment assessment based on anti-VIsE antibodies: a pilot study

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ABSTRACT

Introduction: Although the causative agent of Lyme disease (LD) has been known for a long time, and so far it has been possible to develop patterns useful in diagnosis and treatment, several factors continue to complicate the management of infection with *Borrelia burgdorferi* sensu lato. These include high species diversity of the spirochete causing LD and the lack of a treatment that could guarantee a complete and sustained eradication of infection in all patients. Therefore, the current study aimed to evaluate the effectiveness of treatment of patients with LD based on the measurement of LD-related antibodies generated in response to the highly immunogenic VlsE antigen evaluated using enzyme-linked immunosorbent assay (Lyme Trace ELISA).

Materials and methods: The study group consisted of 10 healthy volunteers (control group) and 21 outpatients (experimental group) with LD, living in the West Pomeranian Province of Poland. The serum samples of the experimental and control groups were tested with the anti-*Borrelia* IgG plus VlsE ELISA, anti-*Borrelia* IgG immunoblot, and anti-VlsE IgG Lyme Trace ELISA.

Results: Research showed that the mean value of anti-VIsE IgG antibody concentration decreased after treatment by 41.6%, 35.9%, and 31.7% in the serum dilutions 1:101, 1:1010, and 1:10100, respectively. A statistically significant difference was obtained in antibody concentration in the serum dilution 1:101 before (R) and after (R*) treatment. The R/R* ratio presented at least a 4-fold decrease in antibody concentration in 2 patients (9.5%), thereby suggesting the effectiveness of the therapy. In serum samples diluted at 1:101 and 1:1010, antibody levels showed an increase after treatment in 7 patients (33.3%), and at a dilution of 1:10100, this increase was found in 6 patients (28.6%). The R/R* ratio differed significantly between the subgroups, where the antibody concentration increased after treatment and then decreased.

Conclusion: Summing up, it can be concluded that the Lyme Trace ELISA assay used in the study to assess the level of anti-VlsE IgG antibodies showed insufficient satisfactory results in the assessment of monitoring the effectiveness of treatment in patients with LD before and after the treatment. The assessment of the effectiveness of treatment should, as such, still be based on the evaluation of the clinical symptoms of the disease, treating the quantification of IgG antibodies with the use of recombinant VlsE antigens as an additional tool.

Keywords: Lyme disease; treatment; healthcare; VIsE; antibodies.

INTRODUCTION

Lyme disease (LD) is a multi-systemic infectious disease caused by Gram-negative bacteria from the *Borrelia burgdorferi* sensu lato complex. Even though the etiological factor of LD has been known for 40 years and several standards of effective diagnosis of the disease have been developed, many cases are still recorded in endemic regions [1, 2, 3, 4]. Studies show that if LD therapy is started too late, it can't guarantee complete and permanent eradication of the infection for patients [5, 6]. Currently, in Europe, treatment of LD depends on clinical and laboratory diagnosis, except in the case of typical erythema migrans (EM) [5, 7, 8], per the European Union Concerted Action on Lyme Borreliosis (EUCALB). Thus, in Europe, several treatments regimes for LD have been recommended [9, 10]. In some patients then, chronic LD symptoms persist despite the antibiotic therapy [6, 11, 12, 13, 14, 15]. The current EUCALB recommendations do not provide any guidelines regarding the use of assays in determining the level of eradication of the infection [10]. Most antibodies, even those with high specificity to *Borrelia* spp., can be detected even after eliminating the pathogens, so cannot be evidence of an active infection, and thus cannot be an indication for restarting antibiotic therapy [16, 17].

As no current method could provide reliable information on the eradication of the infectious agent from the body, evaluation of the effectiveness of treatment is based therefore on the assessment of clinical symptoms. There is therefore a necessity to develop a serological method that uses a specific and sensitive marker to reflect the ongoing activity of the LD pathogens after treatment with antibiotics. According to literature [6, 18, 19, 20, 21, 22], the quantification of antibodies generated in response to the *B. burgdorferi* surface lipoprotein (namely the VlsE antigen or more precisely its most immunogenic epitope component C6) may be valuable in the diagnosis of LD. In reaction to this protein,

a rapid and specific humoral response is triggered, which has been studied to be detectable in the active phase of the disease.

Therefore, this pilot study aimed to examine VIsE as a potential biomarker for assessing treatment effectiveness by quantitative measurement of anti-VlsE IgG antibodies using enzyme-linked immunosorbent assays (ELISA) before and after treatment.

MATERIALS AND METHODS

Patients

The study included a group of 21 outpatients (13 females and 8 males) with LD aged 11–74 years (average age – 53 years) living in the West Pomeranian Province of Poland. The study participants were included based on the following criteria: a confirmed contact with the Ixodes tick, confirmed in the interview; symptoms characteristic of stage 1 (EM, n = 13) and 2 (arthritis without EM in the initial stage of the disease, n = 8) of *Borrelia* spp. infection; positive results in commercial assays: screening (anti-*Borrelia* IgG plus VlsE ELISA by Euroimmun, Lübeck, Germany) and confirmation (anti-*Borrelia* EUROLINE-RN-AT IgG by Euroimmun, Lübeck, Germany); the presence of anti-VlsE IgG antibodies in the serum (anti-VlsE *B. afzelii* and/or anti-VlsE *B. burgdorferi* and/or anti-VlsE *B. garinii*); and treated with antibiotic therapy as recommended by the treating physician (doxycycline, n = 12; amoxicillin, n = 9) between the 1st and 2nd blood sampling (Note: the 1st sample was collected on the day of reporting, the 2nd sample was taken 6 months after the date of the 1st assay).

Additionally, a randomly selected control group of healthy volunteers were tested. This group consisted of 10 people (i.e., 6 women and 4 men) aged 27–46 years (average age – 35 years). These study participants were included based on the following criteria: in the collected interview they confirmed a lack of contact with the tick; they did not have symptoms characteristic of LD; they were found negative by the screening assay and the confirmation assay, and during the last 3 months they had not received any antibiotic therapy. The confirmation assay was performed in this group of people, regardless of the negative result of the screening assay, to eliminate any false-negative results that may have appeared in the 1st assay [23]. Detailed data on the characteristics of the assay and control groups is presented in Tables 1 and 2 respectively.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Pomeranian Medical University of Szczecin (KB-0012/147/18).

ELISA

Serum samples of the tested and control groups were tested using the anti-*Borrelia* IgG and VlsE ELISA screening assay by Euroimmun (Lübeck, Germany), according to the manufacturer's guidelines. *Borrelia burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* cell lysate, as well as recombinant VIsE protein, were used to detect anti-*Borrelia* IgG antibodies. The measured immunoglobulins are expressed in relative units/mL (RU/mL). According to the European principle of interpretation of results used in the diagnosis of LD, as well as the information from the assay manufacturer, sera with an Ig level ≥22 RU/mL are assessed as positive, the range from 16–22 RU/mL is borderline, and <16 RU/mL is considered negative [10, 24]. Results were read at 450 nm using a Biochrom Asys Expert 96 Microplate Reader (Biochrom Ltd., Cambridge, UK) with MicroWin 2000 S.C. Reader software (Biogenet, Józefów, Poland).

Immunoblot

Serum samples of the test and control groups were verified by anti-*Borrelia* IgG EUROLINE-RN-AT Immunoblot (IB) assay (Euroimmun, Lübeck, Germany). All determinations were performed according to the manufacturer's instructions. Antigens were used to detect IgG antibodies, as described in detail in a previous work [25]. The IB results were analyzed using EURO-LineScan software (Euroimmun, Lübeck, Germany).

Determination of anti-VIsE immunoglobulin G antibodies by Lyme Trace ELISA

The quantitative determination of anti-VlsE IgG antibodies by Euroimmun Lyme Trace ELISA IgG (Lübeck, Germany) was performed for the serum samples of the test group. Following the manufacturer's recommendations, blood serum samples were diluted: before treatment (R1 = 1:101, R2 = 1:1010, R3 = 1:10100), and after treatment (R1* = 1:101, R2* = 1:1010, R3* = 1:10100). Multipliers were used to calculate the final results of anti-VlsE IgG antibody levels from which the antibody concentration values were obtained. For this purpose, the following calculations were made (equation):

C anti-VIsE = antibody concentration at specified serum dilution × multiplier

where: for dilution 1:101 – multiplier 1, for dilution 1:1010 – multiplier 10, for dilution 1:10100 – multiplier 100.

The spectrophotometric evaluation of the optical density (OD) of the analyzed samples was performed at 450 nm using a Biochrom Asys Expert 96 Microplate Reader (Biochrom Ltd., Cambridge, UK) with MicroWin 2000 S.C. Reader software (Biogenet, Józefów, Poland). Based on the obtained OD results, the final anti-VlsE IgG antibody concentration (RU/mL) was calculated according to the manufacturer's instructions.

The effectiveness of the treatment was assessed according to the manufacturer's criteria, in which at least a 4-fold decrease in the level of anti-VlsE IgG antibodies in the serum indicated the success of the therapy. For this purpose, the concentration relation (R/R*) was calculated, by dividing the antibody concentration in a given dilution R before the treatment with the antibody concentration in the same dilution R* after the treatment. The resulting ratio showed the effectiveness of the antibiotic therapy, where R/R* ≤1.0 meant no success of the therapy, R/R* from 1.0–4.0 indicated a mediocre success (a slight decrease in the level of antibiotics after treatment), and R/R* ≥4.0 showed the success of the antibiotic therapy.

TABLE 1. Characteristics of the group of outpatients with Lyme disease

TABLE 1. Characteristics of the group of outpatients with Lyme disease

No.	Age	Sex	Days from contact with the tick	EM	LA	Antibiotic administered during therapy	Date of serum collection before treatment	Date of serum collection after treatment	ELISA (RU/mL)	IB – IgG														Result
										VlsE B.a	VlsE B.b	VlsE B.g	Lipid B.a	Lipid B.b	p83 B.b	Flagellin B.a	BmpA B.a	OspC B.a/B.b/B.g	BB_A 34	BB_K 53	BB_Q 03	BB_N 38	BB_P 38	
1.	38	F	21	+	–	AMX	3 Jun 2019	10 Jan 2020	**176.807**	+	+	+	–	–	+	+	+	+	+	–	–	–	–	POS
2.	48	M	140	–	+	DOX	31 May 2019	3 Feb 2020	**33.867**	+	+	+	–	–	+	+	+	+	+	–	–	–	–	POS
3.	43	M	25	+	–	AMX	2 Sep 2019	6 Mar 2020	**41.513**	–	+	(+)	–	–	(+)	+	–	(+)	(+)	–	–	–	–	POS
4.	64	M	19	+	–	DOX	19 Jul 2019	6 May 2020	**32.537**	+	+	+	–	–	+	+	+	+	+	–	–	–	–	POS
5.	61	F	20	+	–	DOX	17 Jun 2019	10 Jan 2020	**102.267**	+	–	+	–	(+)	–	–	+	–	–	–	–	–	–	POS
6.	67	M	18	+	–	AMX	20 Jun 2019	6 Jan 2020	**44.112**	–	–	+	–	–	+	+	–	+	–	–	–	–	–	POS
7.	74	M	26	+	–	AMX	8 Aug 2019	3 Feb 2020	**32.537**	+	+	+	–	–	–	–	–	+	–	–	–	–	–	POS
8.	48	F	60	–	+	DOX	10 Apr 2019	25 Apr 2020	**>200.000**	+	+	+	–	–	–	(+)	–	–	+	–	–	–	–	POS
9.	11	F	27	+	–	AMX	14 Oct 2019	6 May 2020	**164.502**	(+)	+	+	–	–	–	+	–	+	–	(+)	–	–	–	POS
10.	74	F	45	-	+	DOX	22 Nov 2019	25 May 2020	**77.237**	+	–	–	–	–	–	(+)	–	+	–	–	–	–	–	POS
11.	20	F	17	+	–	AMX	2 Dec 2019	29 May 2020	**91.750**	+	+	+	–	–	–	+	(+)	–	–	–	–	–	–	POS
12.	53	F	23	+	–	AMX	3 Jun 2019	2 Mar 2020	**28.134**	+	+	(+)	–	–	(+)	+	–	(+)	(+)	–	–	–	–	POS
13.	71	F	155	–	+	DOX	5 Jun 2019	21 Jan 2020	**30.248**	–	+	–	–	–	+	+	–	+	–	–	–	–	–	POS
14.	50	F	26	+	–	AMX	2 Aug 2019	3 Feb 2020	**30.248**	+	+	+	–	–	(+)	+	(+)	–	–	–	–	–	–	POS
15.	48	F	75	–	+	DOX	13 Jun 2019	9 Jan 2020	**(19.764)**	+	–	+	–	(+)	–	+	–	+	–	–	–	–	–	POS
16.	66	F	29	+	–	DOX	3 Sep 2019	28 Feb 2020	**82.707**	+	+	+	–	–	–	+	–	–	–	–	–	–	–	POS
17.	66	M	120	–	+	DOX	17 Jun 2019	6 Feb 2020	**163.413**	+	+	+	–	(+)	+	+	+	+	–	–	–	–	–	POS
18.	61	M	27	+	–	DOX	23 Sep 2019	2 Apr 2020	**96.061**	+	–	+	–	(+)	–	+	–	–	–	–	–	–	–	POS
19.	64	F	105	–	+	AMX	20 Jun 2019	17 Jan 2020	**90.463**	+	+	+	–	–	–	+	–	–	(+)	–	–	–	–	POS
20.	35	M	90	–	+	DOX	30 Aug 2019	20 Mar 2020	**116.515**	–	+	–	–	–	–	+	+	+	–	–	–	–	–	POS
21.	50	F	18	+	–	DOX	30 Sep 2019	23 Apr 2020	**25.044**	+	+	+	–	+	–	+	–	–	–	–	–	–	–	POS

B.a – *Borrelia afzelii*; B.b – *Borrelia burgdorferi;* B.g – *Borrelia garinii*; F – female; M – male; EM – erythema migrans; LA – Lyme arthritis; AMX – amoxicillin; DOX – doxycycline; RU/mL – relative units/mL; IB – immunoblot, POS – positive; ELISA – enzyme-linked immunosorbent assay.
ELISA ratios: ≥22 RU/mL – positive result; 16–22 RU/mL – borderline result; <16 RU/mL – negative result. IB: "+" – positive result; "–" – negative result; "(+)" – borderline result. ELISA/IB assays: positive/borderline results are marked in bold

Additionally, to check the seroreactivity of the assay and to exclude the possibility of false positive results, a single quantitative determination of anti-VlsE IgG antibodies was performed for the serum samples from the control group with the same assay in 3 dilutions (R1–R3) according to the procedure described by the manufacturer.

Statistical analysis

The description of calculations includes the number of cases and the percentage. In order to compare the values of anti-VlsE IgG antibodies in the serum of patients before treatment with the values of antibody levels after treatment, non-parametric Wilcoxon pairwise tests and Mann–Whitney U tests were used, where $p \leq 0.05$ was

TABLE 2. Characteristics of healthy individuals (control group)

No.	Age	Sex	Days from contact with the tick	EM	LA	Date of serum collection	ELISA (RU/mL)	IB – IgG														Result
								VlsE B.a	VlsE B.b	VlsE B.g	Lipid B.a	Lipid B.b	p83 B.b	Flagellin B.a	BmpA B.a	OspC B.a/B.b/B.g	BB_A 34	BB_K 53	BB_Q 03	BB_N 38	BB_P 38	
1.	27	M	–	–	–	28 Aug 2019	5.600	–	–	–	–	(+)	–	–	–	–	–	–	–	–	–	NEG
2.	33	M	–	–	–	12 Sep 2019	2.729	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NEG
3.	45	M	–	–	–	11 Sep 2019	4.172	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NEG
4.	44	F	–	–	–	27 Sep 2019	11.454	–	–	–	–	–	–	+	–	–	–	–	–	–	–	NEG
5.	31	M	–	–	–	13 Aug 2019	4.112	–	–	–	–	–	–	+	–	–	–	–	–	–	–	NEG
6.	29	F	–	–	–	16 Sep 2019	3.876	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NEG
7.	46	F	–	–	–	26 Aug 2019	1.628	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NEG
8.	33	F	–	–	–	13 Aug 2019	8.950	–	–	–	–	–	–	+	(+)	–	–	–	–	–	–	NEG
9.	28	F	–	–	–	23 Sep 2019	2.764	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NEG
10.	34	F	–	–	–	15 Sep 2019	5.197	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NEG

B.a – *Borrelia afzelii*; B.b – *Borrelia burgdorferi*; B.g – *Borrelia garinii*; F – female; M – male; EM – erythema migrans; LA – Lyme arthritis; AMX – amoxicillin; DOX – doxycycline; RU/mL – relative units/mL; IB – immunoblot; NEG - negative; ELISA – enzyme-linked immunosorbent assay.
ELISA ratios: ≥22 RU/mL – positive result; 16 - 22 RU/mL – borderline result; <16 RU/mL – negative result. IB: "+" – positive result; "–" – negative result; "(+)" – borderline result. ELISA/IB assays: positive/borderline results are marked in bold

considered statistically significant. Statistical analysis was performed using Statistica v.13.0 software (StatSoft, Inc, Palo Alto, USA).

RESULTS

Thirteen patients possessed anti-VIsE IgG antibodies concurrently against 3 genospecies – *B. afzelii*, *B. burgdorferi*, and *B. garinii*. The lowest number (i.e., single cases) of patients were related to the individual genospecies (namely, *B. afzelii*, n = 1; *B. burgdorferi*, n = 1; *B. garinii*, n = 1). Detailed data on the number and frequency of patients with LD and anti-VlsE IgG antibodies against *B. afzelii*, *B. burgdorferi*, and *B. garinii* is presented in Table 3.

TABLE 3. Number and frequency of patients with Lyme disease and anti-VlsE IgG antibodies against *B. afzelii*, *B. burgdorferi*, and *B. garinii*

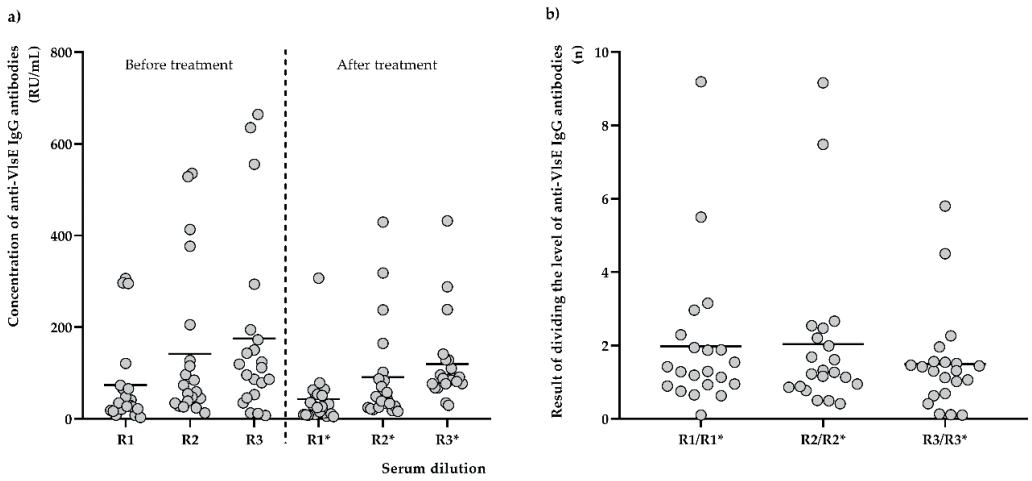
Patients with anti-VIsE IgG antibodies n = 21 (100%)	*B. afzelii*	*B. burgdorferi*	*B. garinii*
11 (52.4)	+	+	+
3 (14.3)	+	–	+
2 (9.5)	–	+	–
1 (4.8)	+	+	(+)
1 (4.8)	(+)	+	+
1 (4.8)	–	+	(+)
1 (4.8)	+	–	–
1 (4.8)	–	–	+

"+" – positive result; "–" – negative result; "(+)" – borderline result

Figure 1 presents the anti-VlsE IgG antibody levels in individual dilutions before treatment (R1–R3) and after the end of therapy (R1*–R3*) – Figure 1a, along with the relation (R/R*) – Figure 1b. The mean concentrations of anti-VlsE IgG antibodies after treatment decreased by 41.6%, 35.9% and 31.7% in serum dilutions 1:101, 1:1010, and 1:10100, respectively, compared to the value before treatment.

In order to assess the effectiveness of each LD treatment in the study participants, the ratios of the concentration of anti--VlsE antibodies in samples R1, R2, R3 were compared to the concentration of antibodies in samples R1*, R2*, R3*. In this way, the results of 3 relations were obtained for each patient, which allowed us to classify the study participants into 3 groups according to the effect of the applied therapy (Tab. 4), namely: R/R* ≥4.0 – success of the applied antibiotic therapy; R/R* = 1.0–4.0 – a mediocre therapy success (i.e., a slight decrease in the level of antibodies after treatment); and R/R* ≤1.0 – the lack of success of the therapy undertaken. In 2 of the patients, the R/R* value was ≥4.0, which indicated that a positive effect from the applied therapy. In most of the other patients tested, a slight decrease in anti-VIsE IgG antibodies was observed in each dilutions. The obtained results were in the range R/R* between 1.0–4.0, indicating a mediocre therapy success, or the R/R* values were below 1.0, which showed a failure of the antibiotic. Detailed information on the levels of anti-VIsE IgG antibodies after treatment is presented in Table 4.

In order to compare the levels of anti-VlsE IgG antibodies in the serum of patients before and after receiving the particular antibiotic therapies, Wilcoxon pair-order tests for dependent variables were used, and showed that only the lowest dilution of serum tested with the ELISA method (R1 = 1:101) presented a statistically significant reduction in the level of anti-VlsE IgG antibodies between the samples taken before and after the treatment (p = 0.011738). In the samples with higher dilutions there were no statistically significant difference in antibody concentrations before and after receiving the treatment



R1 – serum dilution 1:101 before treatment; R1* – serum dilution 1:101 after treatment; R2 – serum dilution 1:1010 before treatment; R2* – serum dilution 1:1010 after treatment; R3 – serum dilution 1:10100 before treatment; R3* – serum dilution 1:10100 after treatment; R/R* – the result of dividing the level of anti-VlsE IgG antibodies in the sample in a given dilution before treatment with the concentration of antibodies in a serum sample of the same dilution after treatment. The lower positive detection limit of the assay according to the manufacturer is 0.6 RU/mL

FIGURE 1. Determination of the anti-VlsE IgG antibody level in the serum of outpatients with Lyme disease before treatment (R1–R3) compared to the concentration of antibodies after the end of therapy (R1*–R3*) – a), along with the relation (R/R*) – b)

TABLE 4. Ratios (R/R*) of the concentrations of anti-VlsE IgG antibodies

Interpretation of post-treatment anti-VlsE IgG antibody levels	R/R* (RU/mL)	R1/R1* n = 21 (%)	R2/R2* n = 21 (%)	R3/R3* n = 21 (%)
Decrease	≥4.0	2 (9.5)	2 (9.5)	2 (9.5)
	1.0–4.0	12 (57.1)	12 (57.1)	13 (61.9)
Increase	≤1.0	7 (33.3)	7 (33.3)	6 (28.6)

R/R* – the result of dividing the level of anti-VlsE IgG antibodies in the sample at a given dilution before treatment with the concentration of antibodies in a serum sample of the same dilution after treatment; RU/mL – relative units/mL; R1 – serum dilution 1:101 before treatment; R1* – serum dilution 1:101 after treatment; R2 – serum dilution 1:1010 before treatment; R2* – serum dilution 1:1010 after treatment; R3 – serum dilution 1:10100 before treatment, R3* – serum dilution 1:10100 after treatment

(p = 0.204565 for the dilution R2 = 1:1010 and p = 0.217242 for the dilution R3 = 1:10100).

Using Mann–Whitney U tests, the ratios of antibody concentrations before and after treatment were compared in 2 groups: the group with no treatment effect (R/R* ≤1.0) and the group in which the antibody level showed a decrease after treatment (R/R* >1.0). In the 2nd group, there were 2 cases in which the effectiveness of the therapy was noted (R/R* ≥4.0). The results revealed a statistically significant difference in the R/R* value between the group in which the antibody level decreased after treatment (p = 0.000297) and the group in which the antibody concentration increased (p = 0.000532).

Concerning the control group, the obtained negative results for the presence of anti-VIsE IgG antibodies confirmed the lack of seroreactivity, regardless of serum dilution.

DISCUSSION

Taking into account the constantly increasing incidence of LD, one fundamental limitation in this field of medicine is the lack of a sensitive and specific marker of active infection to assess the success of the applied therapy. The features which such a marker should characterize include high specificity for the pathogen causing LD, the ability to detect infections of even low intensity, and a kind of flexibility to infection. An important feature relates to the reflection of the infection dynamics, that is, the parameter used should appear soon enough after the infection occurs and decrease along with an effective treatment. According to several researchers, these conditions can be met by quantitative measuring specific anti-VlsE antibodies, which are produced in response to the highly immunogenic VlsE antigen (in particular, its most immunogenic component – the C6 peptide) [26, 27]. The immunogenic VlsE antigen is a *B. burgdorferi* surface lipoprotein subject to antigenic variability during infection [21]. In reaction to this protein, a rapid humoral response is triggered which can be detected during the disease. Studies have shown that the titer of anti-VlsE antibodies is higher in people with advanced disease than in people without residual LD symptoms after effective antibiotic therapy [18, 19, 20, 21, 28]. A decrease in the titer of these antibodies correlates perfectly with the disappearance of clinical symptoms in patients.

The current study found that anti-VlsE IgG antibodies were most often detected simultaneously in the serum of people with LD against 3 genotypes: *B. afzelii*, *B. burgdorferi*, and *B. garinii* (61.9% of patients). The study results confirm opinions about the high proportion of the indicated species in the pathogenesis of LD in Europe, as presented in literature [1, 25, 29] and in the meta-analysis by Estrada-Peña et al. on the most common species of ticks of the *B. burgdorferi* group sensu lato [30].

In this study, it was also noted that the overall effects of antibiotic therapy used during the treatment of patients manifested primarily as a decrease in the average level of antibodies after treatment by 41.6%, 35.9%, and 31.7% in the 1:101, 1:1010 and 1:10100 dilutions of tested sera, respectively. This, in fact, may prove the positive effect of antibiotic therapy on the eradication

of infection in the group of people with LD. A discrepancy in non-outliers was found to decrease in all dilutions of the test serum after treatment. A detailed comparison of the antibody concentration in each patient only showed a statistically significant difference in the level of antibodies before and after treatment in the lowest serum dilution (1:101). These results are also confirmed and corroborated by the data available in the literature. One of the first studies on changes in the level of anti--VlsE/C6 antibodies was presented in 2001 by Philipp et al. [31]. In animal model studies on dogs and rhesus monkeys infected with *Borrelia* spirochetes, a significant increase in the level of IgG anti-VlsE/C6 antibodies was found 12 weeks after infection, until the introduction of antibiotic therapy with gentamicin and kanamycin. During the 9-week treatment, a decrease in antibodies was noted in 6 out of 7 of the monkeys, and by the 13th week after treatment, the level of antibodies was noted to have completely disappeared. In a group of 8 dogs with LD, divided into equal groups due to undergoing or delaying antibiotic therapy, the level of antibodies in the group treated with antibiotics gradually decreased during therapy until they disappeared several weeks after treatment. In the group of dogs not treated with antibiotics, the levels of IgG anti-VlsE/C6 antibodies increased significantly throughout the study. In another study, Marangoni et al. observed the dynamics of anti-VlsE/C6 antibodies in 15 patients with clinically proven LD at monthly intervals from the start of treatment (no data on treatment). The researchers noticed that after 1–6 months, all test subjects were found to be seronegative for the antibodies they measured [27]. On the other hand, in another study conducted by the team of Phillipp et al., the dynamics of antibodies against the conserved C6 region in VlsE protein was assessed in 120 patients with LD, who were in stage 1 or 2 at the time of treatment initiation (no data on treatment) [32]. Based on the conducted studies, the total disappearance of anti-VlsE/C6 IgG antibodies was noted between 4–15 months after the introduction of antibiotic therapy in as many as 59% of patients, while in almost 1/3 (32.4%) of the patients the concentration decreased at least 4-fold. This study provided significant evidence for the correlation of the decline in anti-VlsE antibodies with a positive response to treatment in humans in the early phase of LD. Also, Peltomaa et al. conducted a study on 77 patients with LD, a min. 4-fold decrease in IgG anti-VlsE antibodies was found in 33% of patients with the early manifestation of LD and 86% of patients with late symptoms. Importantly, in a group of 32 additional patients, 50% of people in the early stage of LD and as many as 83% in the late stage were still positive for anti-VlsE antibodies even 8–15 years after successful antibiotic therapy with doxycycline, amoxicillin, penicillin, or ceftriaxone [26]. Therefore, studies show that persistent anti-VlsE antibody responses within months (or years) after treatment may not be synonymous with the presence of live *Borrelia* spp. in the body.

However, potential reasons for the persistence of the antibodies are still unknown. Hence, it becomes prudent to consider the individual differences in patient immune response resulting from the different clinical ages of the disease. According to scientific reports, the level of antibodies in the subjects' serum may differ regardless of such factors as age, sex, environmental exposures, or the presence of metabolic abnormalities [33]. When analyzing the results of serological assays monitoring the assessment of treatment effectiveness, it is worth remembering that a slight decrease in the level of antibodies may be related to the low sensitivity of spirochetes to some antibiotics and the emergence of persistent 'blebs' during infection [34]. In the treatment of LD, amoxicillin and doxycycline are among the most frequently used first-line drugs, but they show little activity against *in vitro* stationary cultures enriched with persistent forms, including round bodies and microcolonies [12, 35, 36, 37]. It has been shown experimentally that doxycycline and penicillin, the commonly used antibiotics in patients suffering from LD, induce forms in *B. burgdorferi* culture from spirochetes to persistent round forms [35]. Feng et al. used this observation in their studies and compared the sensitivity of persistent forms to amoxicillin with other drugs. They tested whether other drugs not used in routine clinical practice demonstrated better activity against the amoxicillin-induced round body forms. They found that other drugs, such as artemisinin, ciprofloxacin, nifuroxime, phosphomycin, chlortetracycline, sulfacetamide, sulfamethoxypyridazine, and sulfathiazole, had higher activity than amoxicillin against stationary round body forms of *B. burgdorferi*. These studies confirm the thesis that further laboratory and clinical assays concerning accurate diagnosis and effective treatment of LD are necessary [37].

CONCLUSIONS

The Lyme Trace ELISA assay used in the study to assess the level of specific anti-VlsE IgG antibodies showed insufficient satisfactory results in the assessment of monitoring the effectiveness of treatment in patients with LD 6 months after the onset of symptoms. The concentration of IgG anti-VlsE antibodies was never found to completely disappear after the treatment, but did decrease with the use of the antibiotics. According to the manufacturer's recommendation, the level of antibodies should also be assessed 12 months after treatment. At the moment, assessment of the effectiveness of treatment should still be based on the assessment of the clinical symptoms of the disease, treating the quantification of IgG antibodies with the use of recombinant VlsE antigens as an additional tool.

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