Original Article
J Immunol Clin Microbiol. 2020;5(3):85-93

J Immunol Clin Microbiol

Testing for Lyme borreliosis: could serology tell more?

András Zóka*[1], Márton Gönczi[1], Veronika Barbai[1], Radka Nikolova[1], Eszter Ujhelyi[1], Zsuzsa Kienle[2], Gabriella Bekő[1]

1 South-Pest Central Hospital, National Institute for Infectology and Haematology, Budapest, Hungary. 2 National Public Health Institute, Budapest, Hungary.

Abstract

Background: Remnant antibodies might be prevalent in the general population, thus recognizing relevant seropositivity might be challenging. The sequential nature of the anti-Borrelia antibody response might be diagnostically utilized. We aimed to specify details which might potentially be useful in orientating the diagnostic schedule.

Materials and Methods: We processed the sera of 1304 patients using a recombinant antigen-based ELISA between Aprils of 2017 and 2019. Seroreactivity (when coherent with the anamnestic data) was confirmed with a line immunoassay (LIA). ELISA testing (IgG and/or IgM) was reactive in 539 cases. 107 patients with persistent symptoms tested positive or borderline with IgG ELISA.

Results: A significant difference was observed (Mann-Whitney U-test p=0.003) between the LIA scores of patients with characteristic (arthritis, acrodermatitis, neuropathy, other objective neurologic disorder; n=83; median LIA score: 16) and non-specific symptoms (entirely subjective complaints, other known disease, lone subfebrility, uveitis; n=24; median LIA score: 6). 101 of the 107 patients tested positive for IgG against any specific protein by LIA. Those with a LIA score reaching the group median of 15 (n=51) displayed strong anti-VlsE IgG positivity or a typical late IgG antibody (against p100, p18 or p39) more often than those below (88,2% vs. 30% and 100% vs. 38% respectively, X2 p<0.0001).

Conclusions: Weak anti-VlsE IgG positivity in combination with the lack of late antibodies might suggest the presence of remnant antibodies and prompt scrupulous differential diagnosis in patients with persistent symptoms.

Key words: *Serology, Lyme borreliosis, differential diagnosis*

***Corresponding Author:** Zóka András M.D., Ph.D. Institution: South-Pest Central Hospital, National Institute for Infectology and Haematology, Budapest, Hungary. **Phone:** +36-70-948-10 **E-mail:** zoka.andras@dpckorhaz.hu **Received:** Apr, 2020. **Accepted:** Sep, 2020.

Introduction

Whereas erythema migrans (EM) remained a clinical diagnosis, the gold standard method for diagnosing postprimary Lyme borreliosis remained two-tier serology (screening with a sensitive enyme-immunoassay, confirmation of reactivity with an immunoblot or line immunoassay -LIA- according to the anamnestic data, as summarized in Figure 1. (1-3). Nucleic acid amplification methods are highly specific, but their sensitivity is inferior to serology and depends on the clinical presentation and the sample. Borreliae can be cultured in vitro, however this method is extremely time-consuming and needs special expertise. Although further methods have been introduced, their true efficiacy and benefit in diagnostic practice is not yet clear (1). In late stage Lyme borreliosis the sensitivity of serology is excellent, however, it has limited capability to differentiate between remnant antibodies and ongoing infection. A background seropositivity of 10% or above in asymptomatic individuals has been reported in multiple areas of Central Europe (4-6). The antibody response against Borrelia antigens is sequential, which might be diagnostically utilized. This forms the basis of the measurement of paired sera in order to detect seroprogression, however, this might also consume time and delay the definitive diagnosis. Positive LIAs often display antibody patterns inconsistent with ongoing late-stage infection (eg. weak positivity against few early antigens). Markers capable of giving an instant clue, whether Lyme borreliosis is a likely or unlikely etiology for the current symptoms, might save time and help the clinician in chosing the preferences of the diagnostic shedule. The VlsE antigen is known to evoke early, robust IgG class antibody response, which might or might not persist after successful treatment (7–9). The p17/p18, p39, p83/p100 antigens typically evoke a late, IgG class antibody response (3). Characteristics of certain antigens are summarized in Table 1. We aimed to specify certain details of the antibody response which might be useful in orientating the further diagnostic schedule.

Materials and Methods

Samples and study setup

Between Aprils 2017 and 2019 we received 1408 test requests for Lyme borreliosis from the samples of 1304 patients in the Central Laboratory of the National Institute of Hematology and Infectology, Central Hospital of Southern Pest. Our study population consisted of the 539 patients (female/male: 312/227; mean age: 48,1±18,1 years) in whom the enzyme-linked immunosorbant assay (ELISA, IgG and/or IgM) was reactive. We summarized the results of two-tier serology, as well as the essential clinical data (symptoms, their duration, tick exposure, and previous antibiotic treatment if known). In order to avoid terminological confusion one data point always refers to one patient in our analysis. In cases of repeated sampling, only one result was included as follows: when no therapy was applied, the latest confirmed result; in cases of repeated sampling after antibiotic treatment the confirmed result before the treatment.

Two-tier serology and data analysis

The ELISA (until September 2017 NovaTec NovaLisa recombinant IgM and IgG, NovaTec GmbH, Dietzenbach, Germany; thence Biomedica Borrelia Recombinant Antigen IgM and IgG tests, Biomedica Ltd, Budapest, Hungary) results, which were possibly coherent with ongoing infection according to the anamnestic data (Figure 1.), were confirmed with a LIA (Mikrogen RecomLine IgM and/or IgG, Mikrogen GmbH, Neuried, Germany). When EM

was recognized according to the accessible documantation, or a lone IgM was detected in cases with persistent symptoms (over three months) confirmation was not done. LIA results were visually evaluated according to the manufacturers recommendations. The sum of the scores for certain antibodies determined the final result as follows: negative below and borderline at 6 points, positive for 7 points and above. In our analysis the density of certain stripes were were documented semiquantitatively (weak: barely equivalent with the cutoff; strong: clearly stronger than cutoff; very strong: near maximum). Kyplot 5.0 software (KyensLab Inc., Tokyo, Japan) was used for statistical analysis.

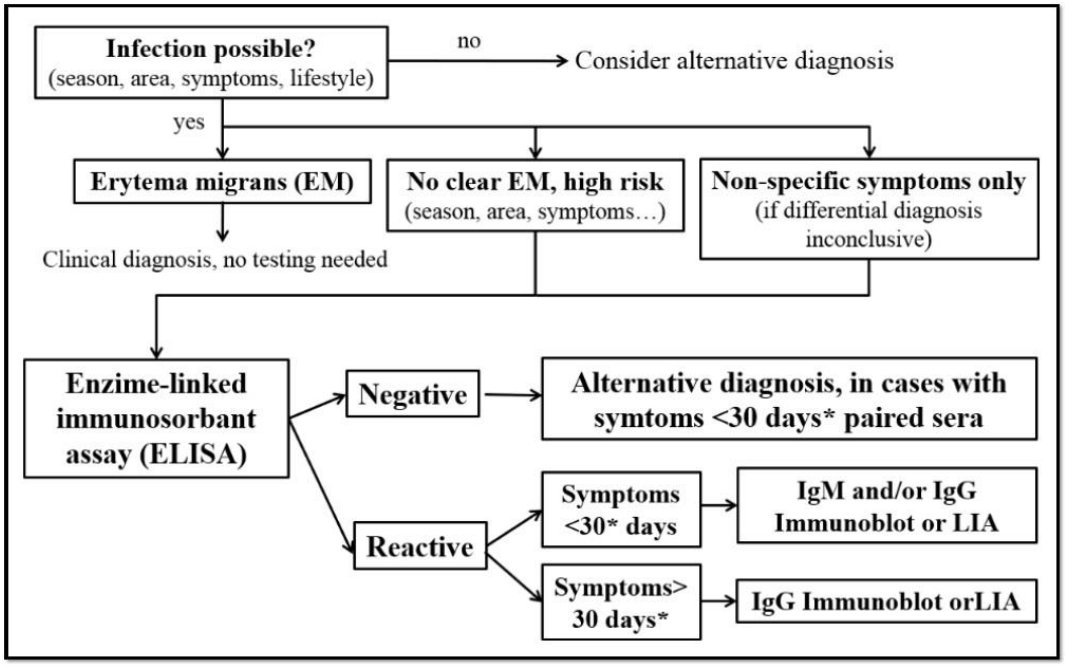

Figure 1. A diagnostic algorythm coherent with current evidence-based guidelines. The 30 days limit for the relevance of IgM reactivity suggested by CDC seemed to be overly rigorous in our practice. Especially in pretreated or immunocompromised patients IgM confirmation was considered for three months after exposure (if known) or after the beginning of the symptoms.

Results

Prevalence of symptoms

The most prevalent complaint among the indications for testing was a skin symptom with or without known tick exposure (196/539; 36,4%), followed by specific late symptoms (arthritis, neuropathy, acrodermatitis; 126/539; 22,8%). Symptoms characteristic for the early disseminated stage (cerebral nerve palsy, meningitis, encephalitis, carditis, flu-like symptoms -malaise, subfebrility, muscle pain without airway involvement following a tick-bite) were documented in 8,3% (45/539) of the cases. The detailed proportions are summarized in Figure 2.

Table 1. Characteristics of certain antigens.

Antigen	Antibody response	IgG ELISA		IgM ELISA		LIA	LIA score	
		Biomedica	Novatec	Biomedica	Novatec		IgM	IgG
p100	late	af.	af.	-	-	af.	5	5
VlsE	early and late	multiple	-	-	-	multiple	5	5
p58	early and late	-	-	-	-	gar.	4	4
p41	early and late	-	gar.	bav.	gar.	s.s.	1	1
p39	late	-	-	-	-	af.	4	5
OspA	late	-	-	-	-	af.	5	5
OspC	early	s.s., gar.	s.s., gar.	af., gar.	af., gar.	s.s., af., gar., sp.	8	5
p18	late	af.	af.	-	-	s.s., af., gar., sp., bav.	5	5

af.: *B. afzelii*; gar.: *B. garinii*; s.s.: *B. burgdorferi* sensu stricto; sp.: *B. spielmanii*; bav.: *B. bavariensis*

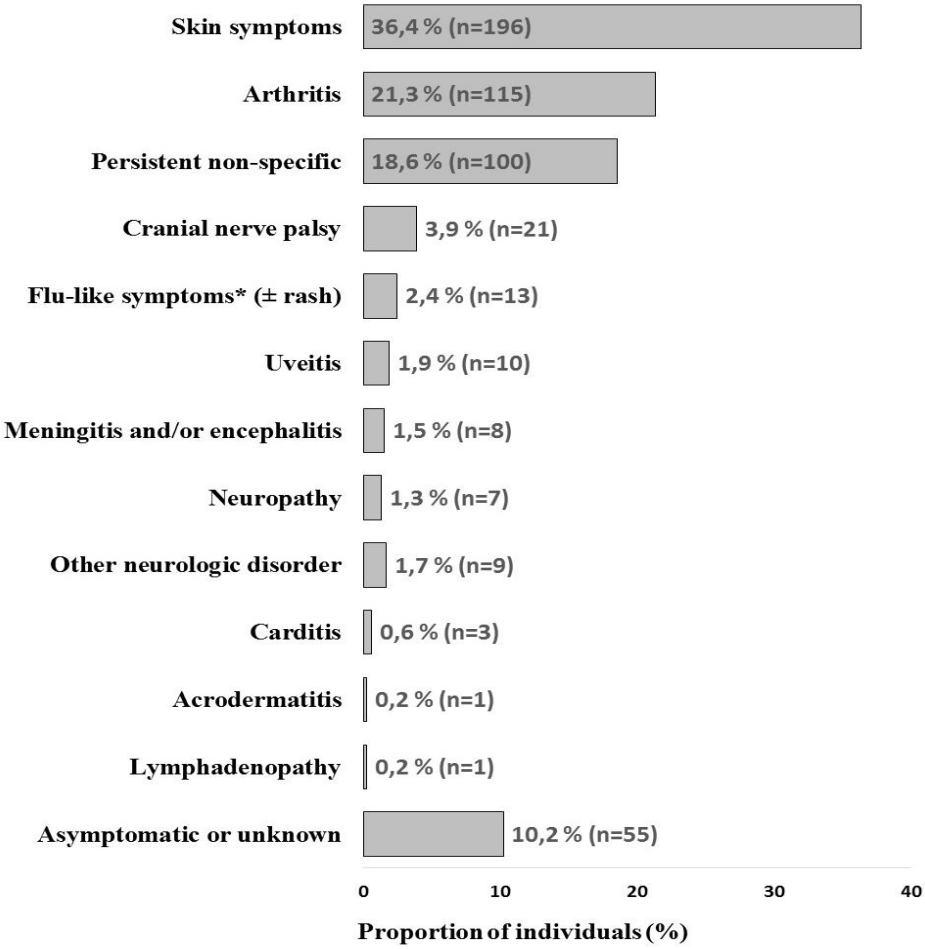

Figure 2. The prevalence of certain symptoms in our study. Flu-like symptoms indicate malaise, subfebrility and muscle pain without airway involvement after a tick bite.

Antibody characteristics

Confirmation with LIA was indicated in 373 cases. Although a follow-up study to characterize the sequential appearence of antigen-specific IgG antibodies was not feasible, we observed their stepwise entry with the rise of the total score. Anti-VlsE IgG almost uniformly formed the basis of seropositivity (present in 98% of all positive samples, Figure 3/A.), whereas the typical late antibodies „entered" gradually in concordance with the total LIA score (Figure 3/B.). It might also be of note that in 7,3% (14/193) of cases with ucertain early skin symptoms a lone IgG LIA positivity was detected in combination with a negative IgM ELISA screening.

Antibody patterns among patients with persistent symptoms

Among the 212 patients with persistent symptoms (arthritis, neuropathy, acrodermatitis, other neurologic or non-specific symptoms over three months) 107 showed IgG ELISA reactivity, and 73 proven positive, 22 borderline by LIA. The median LIA score of those displaying symptoms coherent with Lyme borreliosis (arthritis, neuropathy, acrodermatitis, other objective neurologic symptom after known exposure) was higher than of that those patients with non-specific symptoms (entirely subjective complaints, symptoms of other known pathology, persistent subfebrility) or uveitis, an extremely rare complication of Lyme borreliosis (non-specific vs. characteristic: median 6 points vs. 16 points; Mann-Whitney U-test, p=0.003; Figure 5). 101 of the 107 patients displayed IgG antibody positivity against any specific Borrelia antigen on LIA. The median LIA score of these samples was 15.

A significant difference was observed between the presence of a strong anti-VlsE IgG antibody response as well as the presence of any late antibodies (IgG against p18, p39, or p100) between the patients with a total LIA score reaching the group median and those with a score below 15 (88,2% vs. 30% and 100% vs. 38% respectively, Chi square test $p<0.0001$ for both comparisons; Table 2). Patients presenting with symptoms potentially coherent with ongoing Lyme borreliosis formed a majority within both subgroups, however, their proportion was significantly higher among patients with a LIA score reaching the group median (LIA score median and above vs. below: 88% vs. 64% respectively, Chi square test p=0.0086).

Table 2. Summary of statistical results.

	Below group median (n=50)	**Reaching group median (n=51)**	**Stat. significance (p)**
Gender (F/M)	27/23	19/32	n.s. (X^2)
Age (years)	53.3	55.1	n.s. (T-test)
Symptoms (coherent/incoherent)	32/18	45/6	0.0086 (X^2)
Strong anti-VlsE IgG	15 (30%)	45 (88,2%)	< 0,00001 (X^2)
anti-p100 IgG	5 (10%)	39 (76,5%)	< 0,00001 (X^2)
anti-p18 IgG	13(26%)	43 (84,3%)	< 0,00001 (X^2)
anti-p39 IgG	1 (2%)	32 (62,7%)	< 0,00001 (X^2)
Any late antibody	19 (38%)	51 (100%)	< 0,00001 (X^2)

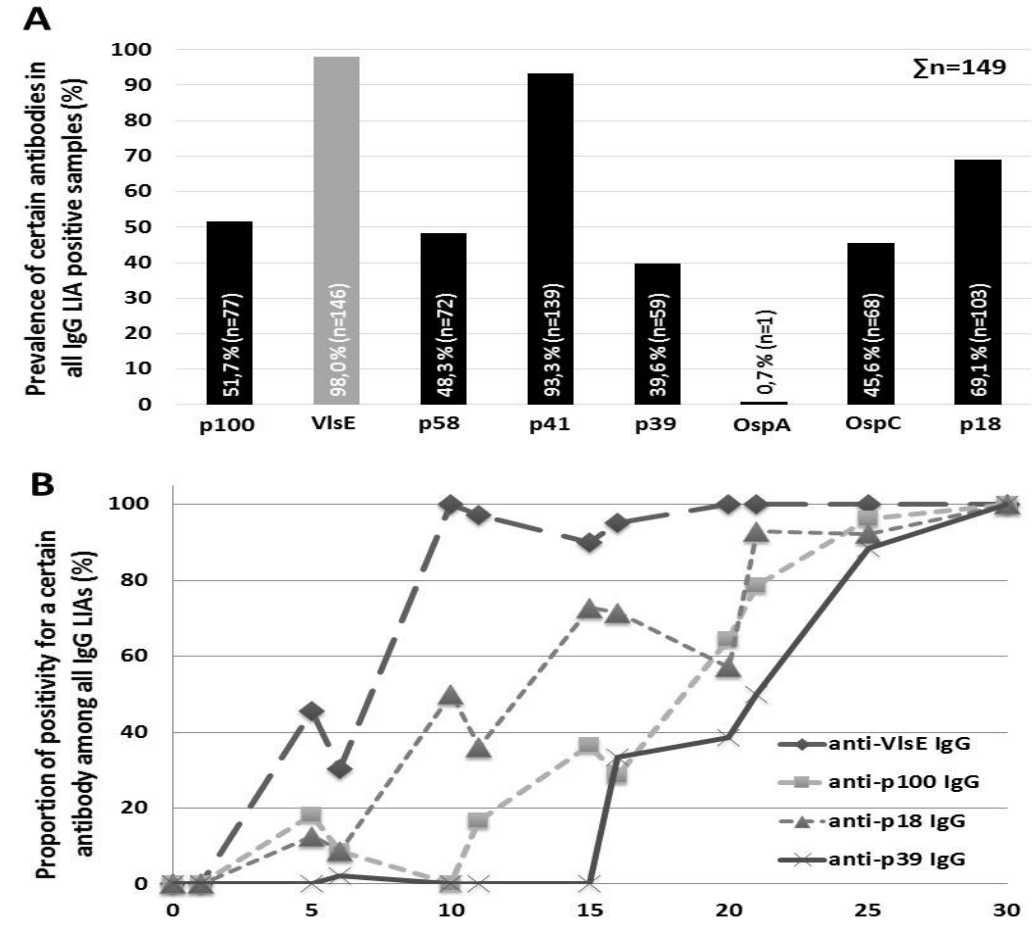

Figure 3. Fig. 3/A: The prevalence of certain IgG antibodies in LIA positive samples. Fig. 3/B: The proportion of samples positive for certain antibodies according to the total LIA score.

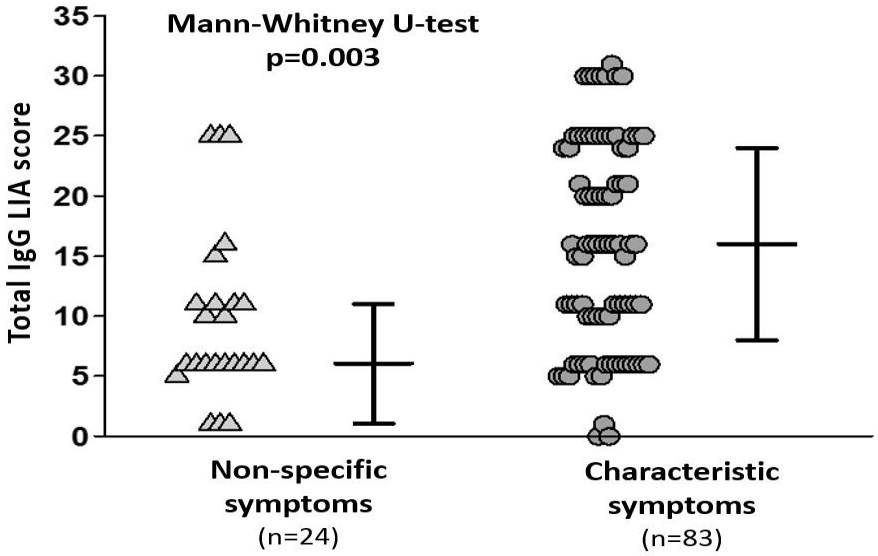

Figure 4. Patients with persistent non-characteristic symptoms (known other pathology, purely subjective complaints, monosymptomic subfebrility or uveitis) had a significantly lower LIA score than those presenting with symptoms coherent with postprimary Lyme-specific borreliosis (joint involvement, neuropathy or other objective nerulogic symptoms after tick exposure).

Discussion

Over one third of the reactive ELISA tests were indicated in patients with skin symptoms. The diagnosis in the early localised stage of Lyme borreliosis is principally clinical. Serological diagnosis should be reserved for the truly unclear cases, however, negativity is very prevalent (up to 60%) in this setting (10). Delayed treatment and comperative analysis of paired sera might help overcoming this problem. A lone IgM ELISA reactivity beyond primary symptoms should be interpreted with extreme caution, IgM class false reactivity is common (11). In contrast, the lack of detectable IgM in early cases does not rule out active infection (12). Antibodies (including IgM) might also persist for up to decades in certain cases (13). Although exceptional antibody profiles may exist (eg. in pre-treated cases or those receiving immunomodulatory medication), it might be necessary to state that adherence to evidence-based guidelines forms the basis of avoiding confusion. Laboratory practice unaware of the basic anamnestic data is neither acceptable from the medical nor from the ecological point of view.

Whereas in late stage disease seronegativity practically excludes active infection in immunocompetent, non-treated individuals (3), the clinical relevance of a weak seropositivity might often be uncertain. In the light of the observations reporting a background-seropositivity of up to 10% or above in asymptomatic individuals (4–6) weakly targeted testing of patients might result in a low positive predictive value of a test result, even with specific tests (14). Concordantly with other reports (7) anti-VlsE IgG formed the basis of seropositivity in our study population.

Our results underline the robust character and individual deteriortaion (8,9) of the IgG class anti-VlsE response and also suggest that its semi-quantitative strenght detected by LIA is coherent with the presence or absence of late antibodies. Although these results are not unexpected, they suggest the applicability of these observations in daily practice. It might be speculated that a weak or absent anti-VlsE IgG positivity, especially in combination with the lack of late antibodies, is inconsistent with persistent active infection in a case with suspected late postprimary symptoms and may justify further testing instead of overestimating the significance of a barely positive result. Factors, which might potentially increase specificity (in addition to self-restraint) might be an open-minded differential diagnosis.

Conclusion

Although the Western blot or LIA analysis of paired sera might come into consideration as the potentially most clear-cut method for ruling out seroprogression (thus ongoing infection), this might be time-consuming and is only advisable if applied in paralel with furter diagnostic tests. The analysis of antibody patterns in combination with the clinical presentation comes into consideration as a useful, inexpensive first step for chosing diagnostic preferences.

Limitations

The signal detected on LIA depends on the antigenicity and density of the recombinant protein applied. Altough our results are coherent with previously reported data, test characteristics might affect their reproducibility. As the evaluation criteria for certain tests are individually determined, our results cannot form the basis of the comparision of different tests. A reference test for the direct detection of borreliae was not used, thus we

cannot make conclusions regarding the sensitivity or specificity of certain markers. Our results highlight the relevance of certain antibodies in the differential diagnosis, but are yet insufficient to form the basis of non-individulaized algorythmic decisions.

Ethics Committee Approval: Yes
Informed Consent: NA
Peer-review: Externally peer-reviewed.
Conflict of Interest: No conflict of interest was declared by the author.
Financial Disclosure: The author declared that this study has received no financial support.

References

1. Eldin C, Raffetin A, Bouiller K, Hansmann Y, Roblot F, Raoult D, et al. Review of European and American guidelines for the diagnosis of Lyme borreliosis. Médecine Mal. Infect. 2019 1;49:121–32. doi: 10.1016/j.medmal.2018.11.011
2. Two-step Laboratory Testing Process | Lyme Disease | CDC [Internet]. 2018 21 [cited 2019 14];doi: https://www.cdc.gov/lyme/diagnosistesting/labtest/twostep/index.html
3. Lohr B, Fingerle V, Norris DE, Hunfeld K-P. Laboratory diagnosis of Lyme borreliosis: Current state of the art and future perspectives. Crit. Rev. Clin. Lab. Sci. 2018;55:219–45. doi: 10.1080/10408363.2018.1450353
4. Wilking H, Fingerle V, Klier C, Thamm M, Stark K. Antibodies against Borrelia burgdorferi sensu lato among Adults, Germany, 2008–2011. Emerg. Infect. Dis. 2015;21:107–10. doi: 10.3201/eid2101.140009
5. Zając V, Pinkas J, Wójcik-Fatla A, Dutkiewicz J, Owoc A, Bojar I. Prevalence of serological response to Borrelia burgdorferi in farmers from eastern and central Poland. Eur. J. Clin. Microbiol. Infect. Dis. 2017;36:437–46. doi: 10.1007/s10096-016-2813-7
6. Bušová A, Dorko E, Rimárová K, Diabelková J, Rovenská T, Feketeová E, et al. Seroprevalence of Lyme disease in Eastern Slovakia. Cent. Eur. J. Public Health 2018;26 Suppl:S67–71. doi: 10.21101/cejph.a5442
7. Jacek E, Tang KS, Komorowski L, Ajamian M, Probst C, Stevenson B, et al. Epitope-Specific Evolution of Human B Cell Responses to Borrelia burgdorferi VlsE Protein from Early to Late Stages of Lyme Disease. J. Immunol. Baltim. Md 1950 2016 1;196:1036–43. doi: 10.4049/jimmunol.1501861
8. 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 1;184:870–8. doi: 10.1086/323392
9. Peltomaa M, McHugh G, Steere AC. Persistence of the antibody response to the VlsE sixth invariant region (IR6) peptide of Borrelia burgdorferi after successful antibiotic treatment of Lyme disease. J. Infect. Dis. 2003 15;187:1178–86. doi: 10.1086/374376
10. Moore A, Nelson C, Molins C, Mead P, Schriefer M. Current Guidelines, Common Clinical Pitfalls, and Future Directions for Laboratory Diagnosis of Lyme Disease, United States. Emerg. Infect. Dis. 2016;22. doi: 10.3201/eid2207.151694
11. Seriburi V, Ndukwe N, Chang Z, Cox ME, Wormser GP. High frequency of false positive IgM immunoblots for Borrelia burgdorferi in Clinical Practice. Clin. Microbiol. Infect. 2012 1;18:1236–40. doi: 10.1111/j.1469-0691.2011.03749.x
12. Lakos A. [Lyme borreliosis--experience of the last 25 years in Hungary]. Orv. Hetil. 2009 19;150:725–32. doi: 10.1556/OH.2009.28576
13. Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC. Persistence of immunoglobulin M or immunoglobulin G antibody responses to Borrelia burgdorferi 10-20 years after active Lyme disease. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 2001 15;33:780–5. doi: 10.1086/322669
14. Lakos A, Reiczigel J, Solymosi N. The positive predictive value of Borrelia burgdorferi serology in the light of symptoms of patients sent to an outpatient service for tick-borne diseases. Inflamm. Res. Off. J. Eur. Histamine Res. Soc. Al 2010;59:959–64. doi: 10.1007/s00011-010-0209-1