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CLINICAL STUDY

Challenges of laboratory diagnosis of Lyme disease – Questionnaire survey results

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ABSTRACT

OBJECTIVE: The primary objective was to analyze the results of serologic testing used in the diagnostics of Lyme disease. Our second goal was to identify bacterial and viral co-infections occurring concurrently with Lyme disease. Furthermore, it was our intention to also analyze the correlation of laboratory testing with the occurrence of *erythema migrans* (EM).

BACKGROUND: The accuracy in diagnostic testing for Lyme disease in the early stages of infection is an important factor necessary for delivering proper treatment to patients.

METHODS: A total of 173 individuals with confirmed Lyme disease or with laboratory testing underway participated in the quantitative survey.

RESULTS: ELISA was the first test conducted in 51% of the respondents, 28% of whom yielded positive findings of both IgM and IgG antibody classes. The positivity of ELISA test findings was confirmed by Western blot in 100% of results. Negative results of ELISA were consistent with Western blot only in less than half of the patients. More than half of the respondents had not been tested for any bacterial or viral co-infections. The results of serological testing were not consistent with clinical findings in all cases, including those with clinically discernible skin manifestation of *erythema migrans*.

CONCLUSION: The comparison of results obtained by ELISA and Western blot revealed significant discrepancies. Simultaneous infections by vectors with several pathogens were detected *(Tab. 3, Fig. 2, Ref. 15)*. Text in PDF www.elis.sk

KEY WORDS**:** Lyme disease, *erythema migrans*, ELISA, Western blot, serologic testing.

Introduction

Borreliosis, also known as Lyme disease, is a vector-borne disease caused by the bacterium *Borrelia burgdorferi, s.l.*, which is transmitted to humans by the bite of infected ticks of the genus Ixodes. In Europe, Lyme disease is classified among the most common infections. Pathogenic genospecies within the *Borrelia burgdorferi, s.l.*, complex are capable of attacking a wide range of cells and tissues, inducing a variety of clinical symptoms (1). The most common early skin manifestation is *erythema migrans* occurring in about 60–70% of infected subjects (2). In addition to *Borrelia burgdorferi, s.l.*, ticks can transmit pathogens from genera such as *Rickettsia, Anaplasma, Coxiella, Francisella, Babesia*, tick-borne encephalitis virus, and other arboviruses. Therefore, when diagnosing tick-borne infections, it is essential to consider the possibility of multiple infections (3). According to the literature, up to 62% of patients diagnosed with Lyme disease reveal at least one concurrent infection (4).

The accuracy in diagnostic testing for Lyme disease in the early stages of infection is an important factor necessary for delivering efficient treatment to patients and avoiding complications resulting from absence of treatment. Infection with *Borrelia burgdorferi, s.l.,* does not produce bacteremia with abundant organisms in the bloodstream, consequently, the diagnostic tests through culture, microscopic examination, or PCR are currently not feasible. The cornerstone of laboratory diagnostics of Lyme disease worldwide lies in an indirect approach based on the determination of specific antibodies produced in response to the infection. Both, IgG and IgM antibody classes are detected using serological diagnostic methods (5). The Center for Disease Control and Prevention (CDC) currently recommends a two-step testing process for Lyme disease. The first step most commonly involves an enzyme-linked immunosorbent assay (ELISA). If the result is positive or indeterminate (equivocal), the second step should be performed, which is a diagnostic Western blot test (6). However, serological Lyme disease testing has several limitations. ELISA tests have relatively low sensitivity in the early stage of Lyme disease (35–50%) and inadequate sensitivity in particular later stages (75–90%) which may lead to false-negative results (7). Moreover, establishing a correlation between the antibody levels and the severity of the infection is not possible (8). Another

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drawback of serologic testing stems from the divergent interpretation of results between laboratories and cross-reactivity with other spirochetes (3). However, the most challenging concern seems to lie in significant discrepancy of ELISA tests compared to the reference Western blot, with a predominance of false-negative results (9).

Therefore, the main aim of our study was to analyze the results of serologic ELISA test and Western blot testing used in the diagnostics of Lyme disease in a sample of Slovak and Czech patients. Secondly, it has become our goal to identify bacterial and viral co-infections that commonly occur with Lyme disease, and thirdly, to analyze the correlation of laboratory testing with the occurrence of *erythema migrans* (EM).

Subjects and methods

A total of 173 subjects (151 women and 22 men) within age range of 18–45 years participated in the study. A quantitative survey with 17 questions related to the diagnostic process or results of the laboratory tests was used. It included individuals with Lyme disease diagnosis already confirmed, or at the time of our study, with the laboratory testing underway to confirm or rule it out. The sets of answers to each question within the questionnaire included an optional response of 'I do not know'. All subjects were randomly recruited members of a patient group comprised of citizens of Slovakia (45%) and the Czech Republic (55%). The obtained data were statistically processed.

The participation in our survey was voluntary and all participants were informed about the nature and the purpose of the study. Since no personal data related to the identity of respondents were collected, the data processing was conducted in a strictly anonymous manner.

Results

Detection of IgM and IgG antibodies by ELISA and Western blot

We investigated the distribution of serologic tests performed in our study group. The ELISA test for specific antibodies was conducted as the first laboratory test in 51% of respondents. Positive findings of both IgM and IgG classes in the ELISA test were identified in 28% of our respondents. Out of 48 respondents positive for both IgM and IgG antibody classes on ELISA test, 40 (83%) were not aware of having experienced *erythema migrans* and 37 (77%) of them were not conscious of being bitten by ticks or other insects. Even though IgM and IgG antibodies are formed in different phases of the infection, the analysis of respondents' answers showed that patients with suspicion of Lyme disease may reveal a positive concurrent finding of IgM and IgG antibody classes. In our survey, 60 (34.7%) respondents confirmed tick attachment. Of them, 18 respondents (30%) were confirmed positive by the ELISA test.

We have analyzed the results of ELISA tests in comparison with the results of Western blot tests. Out of all 173 respondents, the Western blot test was conducted in a group of 58 (33.5%), some of whom without undergoing prior ELISA test. Only 35 out of 173 respondents were familiar with their IgM results from both the ELISA and the Western blot (WB) tests. Of them, 22 (63%) were positive, and 13 (37%) were negative on ELISA test. The positive findings on ELISA test ascertained in the latter 22 respondents were confirmed by the WB test in 100% of cases. Of 13 cases with negative results on ELISA test, only 7 (54%) were confirmed by the WB test. In 6 (46%) cases, the negative result in the WB test was not confirmed and turned out to be positive (Tab. 1).

29 out of 173 respondents were familiar with their IgG results from both ELISA and Western blot tests (Tab. 2). Among them, 14 (48%) tested positive and 15 (52%) tested negative on ELISA test. The positive results (14) were confirmed by the WB blot in all cases (100%). The negative results (15) were confirmed in only 9 (64%) cases, while in 6 cases (36%), the negative result on WB test was not confirmed and turned out to be positive, i.e., presuming the confirmatory power of WB test given by its higher specificity and sensitivity, in 6 cases (36%), the results of ELISA test were falsely negative.

Investigation of cellular immunity, bacterial and viral co-infections

As chronic *Borrelia* infections are often accompanied by changes in cellular immunity, laboratory testing of the immune system status is commonly performed as part of the diagnostic process. In our study, 101 respondents (58.4%) stated that no examination of the immune system was performed in relation to Lyme disease examinations. The status of the immune system was examined in 30 respondents (17.3%) while 41 (24.3%) were not able to respond or provided other answers.

The diagnosis and treatment of Lyme disease can be influenced by associated bacterial infections. Most respondents, namely 94 (54%) stated that they had not been tested for any associated bacterial infections, while 65 (38%) of them had. The remaining 14 (8%) were not able to answer this question. In the group of 65 patients tested for bacterial co-infections, a total of 154 laboratory tests focused on detecting the specific types of bacterial infections were conducted. Most tests, specifically 60 (39%), were focused on the presence of *Chlamydia*. Figure 1 presents details (type, number,

Tab. 1. Comparison of ELISA test results in the IgM antibody class with Western blot results.

ELISA for IgM (n=35)	Confirmation by WB for IgM		Percentage of confirmed results
Positive 22	Positive 22	Negative 0	100%
Negative 13	Positive 6	Negative 7	54%

Tab. 2. Comparison of ELISA test results in the IgG class with Western blot results.

ELISA for IgG (n=29)	Confirmation by WB for IgG		Percentage of confirmed results
Positive 14	Positive 14	Negative 0	100%
Negative 15	Positive 6	Negative 9	64%

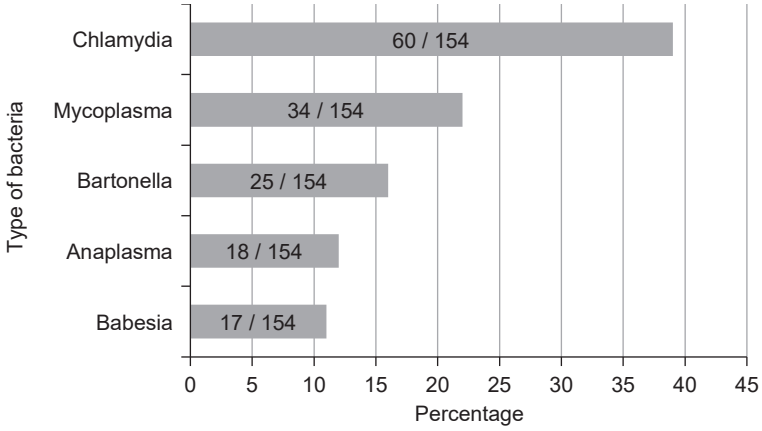

Fig. 1. Proportion of Lyme disease patients tested for bacterial co-infections.

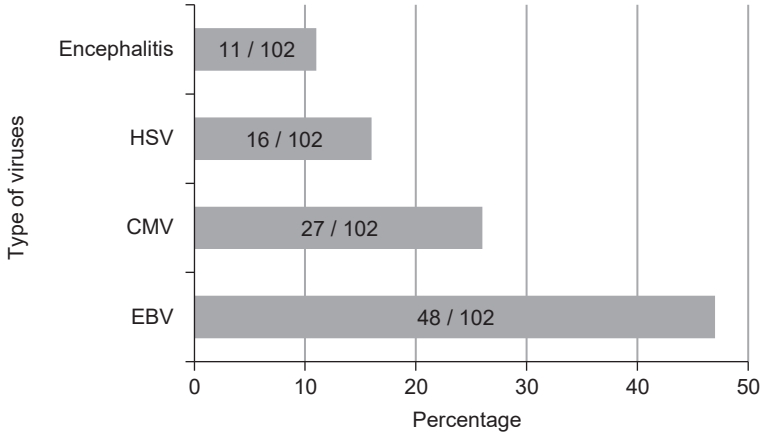

Fig. 2. Proportion of Lyme disease patients tested for viral co-infections. (EBV– Epstein–Barr virus, CMV – cytomegalovirus, HSV – Herpes simplex virus).

and percentage) of all 154 conducted laboratory tests for bacterial co-infections.

Most respondents, specifically 98 (56.6%), had not been tested for viral infections, 60 (34.7%) respondents had undergone testing for viral infections, and 15 (8.7%) respondents were unable to answer the question. For the group of 60 patients who had undergone testing for viral infections, a total of 102 laboratory tests were conducted to detect the type of viral infection. In Figure 2, the details (type, number, and proportion) of conducted laboratory tests for viral infections are presented.

Tab. 3. **Results of ELISA tests in subjects with and without *erythema migrans*.**

Respondents aware of experiencing *erythema migrans*	**n=36**
ELISA test positive for IgM and/or IgG	29 (81%)
ELISA test negative for IgM and/or IgG	7 (19%)
Respondents not aware of experiencing *erythema migrans*	**n=77**
ELISA positive for IgM	42 (55%)
ELISA negative for IgM	35 (45%)

Correlation of laboratory results with the occurrence of *erythema migrans*

Information diagnostically relevant to Lyme disease can be obtained from the history of tick attachment and subsequent manifestation of *erythema migrans*. In the group of 36 respondents who had been aware of experiencing EM in association with Lyme disease, 29 (81%) yielded a positive serological IgM or IgG finding. Despite the recognized presence of EM, 7 respondents (19%) tested negative for both IgM and IgG (Tab. 3). Our research shows that even in clinically clear cases in the presence of *erythema migrans*, there is no firm certainty for serological tests to validate Lyme disease by positive results. Such a situation can occur when the serological test is conducted prior to the initiation of antibody production in the patient. Out of 77 respondents without EM, serology confirmed the presence of of IgM antibody class in 42 respondents (55%) (Tab. 3).

Discussion

Our primary goal was to determine the distribution of tests used in the diagnosis of Lyme disease. In our survey, over half of respondents had an ELISA test conducted as a first test in relation to Lyme borreliosis. General practitioners for adults had most commonly requested this type of laboratory testing and examinations of antibodies in both IgM and IgG classes. IgM antibody production begins after 2–4 weeks, and that of IgG after 4–6 weeks. IgM antibodies are positive during the early phase of infection and decrease after three months. Some patients do not produce IgM antibodies at all (10). However, our survey showed that in 48 respondents (28%), the findings concurrently positive for IgM and IgG antibody classes were identified by way of the first ELISA test. Of them 40 (83%) did not confirm having experienced EM, and 37 (77%) were not aware of being bitten by a tick or other insects. In the diagnosis of Lyme disease, the information on tick attachment with subsequent manifestation of *erythema migrans* can be diagnostically significant. However, the lack of information about tick attachment cannot be considered as a factor indicative of excluding the diagnosis of Lyme disease (11). When establishing a diagnosis, the patient's information is indeed only one of the aspects considered by the diagnostician, and this information needs to be assessed in correlation with laboratory results.

Our findings confirm the statements of experts that the information on the absence of EM experience or tick attachment is not a valid reason to exclude the diagnosis of Lyme disease. In a significant number of patients, the information on tick attachment is missing; ticks or their less visible forms such as larvae or nymphs may have been overlooked (11). Out of the respondents who were aware of experiencing erythema and were knowledgeable of their

IgM and IgG antibody results on ELISA test, 81% had a positive finding of IgM or IgG antibody classes. Despite the experience of erythema, 19% of respondents tested negative for concurrent finding of both antibody classes.

Positive serological results of the ELISA test in the IgM antibody class were confirmed by the Western blot test in 100% of cases. Similarly, the positivity in the IgG antibody class was confirmed by WB in all 100% of positive ELISA results. False-positive results were not observed in any of our subjects. However, negative findings of IgM and IgG antibody classes by the ELISA test were confirmed by the Western blot test only in 54% and 64% of cases, respectively. The results of our research revealed cases of evidently false negativity on ELISA test. The discrepancies between the ELISA and Western blot results, manifesting as falsely negative outcomes can have serious clinical consequences in the progression of Lyme disease. If left untreated, the Lyme disease can affect more tissues and organs, impairing their functionality. At the same time, both the direct and the indirect healthcare costs increase (9). We believe that a deeper analysis of the effectiveness of two-step diagnostic examination in suspected cases of Lyme disease is warranted, as indicated by the results of comparing the findings of ELISA and WB tests. We are concerned that patients with false-negative serological results may not receive timely treatment, leading to disease progression. The early initiation of antibiotic therapy can influence the course of the disease and often determines its further development. The positive effect of treatment depends on the time and stage of infection, application of effective antibiotics, and adherence to the recommended duration of drug administration (12). Antibiotic therapy is successful when administered in the early stages of Lyme disease. The treatment is initiated based on suspicious clinical symptoms supported by positive results of laboratory tests. According to the analysis of respondents' answers, despite the positive finding of the IgM antibody class, 21.4% of respondents stayed not treated. These patients are at high risk of Lime disease progressing to further stages. According to a study conducted by Hündersen et al (13), up to 46.4% of respondents stated that, on average, the diagnosis of Lyme disease was established 8 years after the tick bite.

In European countries, on average, 0.4–67% of ticks are infected with pathogens such as *Ehrlichia* and *Anaplasma* (12). For *Anaplasma*, manifestations such as cranial nerve palsy, brachial plexopathy, demyelinating polyneuropathy, and bilateral facial nerve palsy have been confirmed. In our survey, 18 respondents underwent testing for the *Anaplasma* pathogen, accounting for 12% of laboratory tests for associated bacterial infections. Representatives of the *Bartonella* genus cause a wide range of infectious diseases in both humans and animals. In humans, the infection most commonly manifests as a recurrent fever and angioproliferative lesions. Serological tests for Bartonella may yield very unreliable findings when the pathogen is present in the patient's serum (14). Tests for *Bartonella* confirmed 25 positive results, accounting for 16% of laboratory tests for associated bacterial infections. Cross-reactions between *Chlamydia* with *Coxiella* are often observed, leading to false-negative/positive results (12).

Study limitations

We are aware of several limitations of the study which could have biased our findings. The primary limitation arises from the questionnaire-based nature of our epidemiological research which relies on accuracy of respondents' answers, particularly when responding to questions about laboratory test results. The respondents might not have had precise information, or they could have misinterpreted it. Another limitation lies in unequal representation of sexes among the respondents (13% men vs 87% women). This limitation stems from the nature of questionnaire completion by respondents on social networks, where women seem to be generally more active than men. Also, the overrepresentation of younger adults among respondents can be considered as a limitation of our study.

Conclusion

The currently employed methods of laboratory diagnosis of Lyme disease have numerous limitations. False-negative results are relatively frequent, leaving the subject deprived of necessary treatment and exposed to various progressive manifestations of the disease. No reliable tests confirming the actual presence and activity state of *Borrelia burgdorferi, s.l.*, spirochetes, are currently available (15). This poses a challenge to the accurate identification of the disease. In our study, noteworthy findings emerged from comparing the results of ELISA and WB tests. A significant discrepancy was revealed between cases with negative results on the ELISA test and the results on WB test. Furthermore, the majority of respondents had not been tested for any possible associated bacterial and viral infections. We suggest that when suspecting Lyme disease, it is essential to perform additional examinations for associated bacterial and/or viral co-infections.

In conclusion, in accordance with other authors, the results of our survey indicate that the currently employed diagnostic procedures may lead to a relatively high number of false-negative test results, leaving the patients with Lyme disease without treatment.

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