REVIEW | OPEN ACCESS

Medical Science

Lyme borreliosis - review of the diagnosis and management in the multidisciplinary tick-borne disease

Anna Salińska[1*], **Piotr Węgrzyn**[2], **Konstancja Węgrzyn**[2], **Agnieszka Góra**[3], **Marcin Wasilewski**[4], **Maciej Nowicki**[5], **Julia Skwara**[5], **Dawid Barański**[4], **Natalia Dąbrowska**[6], **Gustaw Laskowski**[2]

To Cite:
Salińska A, Węgrzyn P, Węgrzyn K, Góra A, Wasilewski M, Nowicki M, Skwara J, Barański D, Dąbrowska N, Laskowski G. Lyme borreliosis - review of the diagnosis and management in the multidisciplinary tick-borne disease. *Medical Science* 2024; 28: e93ms3399
doi: https://doi.org/10.54905/disssi.v28i150.e93ms3399

Authors' Affiliation:
[1]Mazowiecki Szpital Bródnowski, ul. Kondratowicza 8, 03-242 Warsaw, Poland
[2]Central Clinical Hospital of Medical University of Warsaw, 1a Banacha Str. 02-097 Warsaw, Poland
[3]Medical University of Warsaw, 61 Żwirki i Wigury Str. 02-091 Warsaw, Poland
[4]Jerzy Popiełuszko Bielański Hospital – Independent Public Healthcare Centre, 80 Cegłowska Str. 01-809 Warsaw, Poland
[5]National Medical Institute of the Ministry of the Interior and Administration, Wołoska 137, 02-507 Warsaw, Poland
[6]Szpital Dzieciątka Jezus, 4 Lindleya Str. 02-005 Warsaw, Poland

[*]**Corresponding Author**
Mazowiecki Szpital Bródnowski, ul. Kondratowicza 8, 03-242 Warsaw, Poland
Email: salinska.anna@gmail.com
ORCID: 0000-0001-7109-5738

Peer-Review History
Received: 20 May 2024
Reviewed & Revised: 24/May/2024 to 30/July/2024
Accepted: 03 August 2024
Published: 09 August 2024

Peer-review Method
External peer-review was done through double-blind method.

Medical Science
pISSN 2321–7359; eISSN 2321–7367

DISCOVERY SCIENTIFIC SOCIETY

ABSTRACT

Background: Lyme borreliosis is a tick-borne zoonotic disease caused by Borrelia burgdorferi spirochetes transmitted by Ixodes ticks. It is prevalent worldwide and spreads within one to two weeks, potentially causing erythema migrans and other symptoms. Diagnosing and treating Lyme borreliosis is challenging due to varied clinical presentations and diagnostic test limitations. *Diagnosis:* Diagnosis involves clinical symptoms and serological tests. Erythema migrans alone is diagnostic without further tests. The standard two-tiered testing approach, involving an initial ELISA followed by a confirmatory Western Blot, is commonly used but has low sensitivity in early-stage disease. Modified two-tiered testing aims to improve diagnostic accuracy but requires two stages for sufficient sensitivity and specificity. New diagnostic methods include lateral flow immunoassays, EliSpot techniques, biomarker-based approaches, pathogen culture, and PCR with modifications like immune-PCR. The methods mentioned each have their limitations. The lack of a gold standard method makes exploring a valid diagnostic algorithm necessary. *Treatment:* Treatment primarily involves antibiotics such as doxycycline, usually for no more than 28 days. Long-term antibiotic therapy is not supported by evidence and is not recommended for non-specific post-Lyme syndrome symptoms. *Conclusions:* In conclusion, advancements in Lyme borreliosis diagnostics continue, but challenges remain in developing a simple, cost-effective, and highly accurate diagnostic test. Improved diagnostics are crucial for timely treatment, preventing complications, and minimizing unnecessary antibiotic use.

Keywords: Lyme disease; Borrelia burgdorferi; tick-borne; diagnostic algorithm; antibiotic treatment

1. INTRODUCTION

Lyme borreliosis (LB, syn: Lyme disease) is a tick-borne, polymorphic, zoonotic disease caused by spirochetes Borrelia Burgdorferi (Bb), which are transmitted to humans by ticks in the genus Ixodes. Distribution of the disease is mainly related to the area where ticks are present, and most extend to North America, Europe, and Eastern Asia (Dong et al., 2022; Mead, 2022). In the human body, the spirochetes disseminate hematogenous or via lymphatics within one to two weeks (Wormser et al., 2005). The spirochetes can be eliminated by the effective immune system of the patient or remain alive and localize on the skin, causing characteristic lesions in the form of erythema migrans (EM). Due to the variety of clinical manifestations and the limitations of diagnostic tests, diagnosis of the disease is not always straightforward. Clinical manifestations of LB are presented in (Figure 1). The treatment method, which is most effective in the early phases, is based on antibiotic therapy. Early initiated and effective antibiotic treatment prevents the harmful complications of chronic LB. Our review attempts to compile knowledge on the diagnosis of LB and summarize current treatment options.

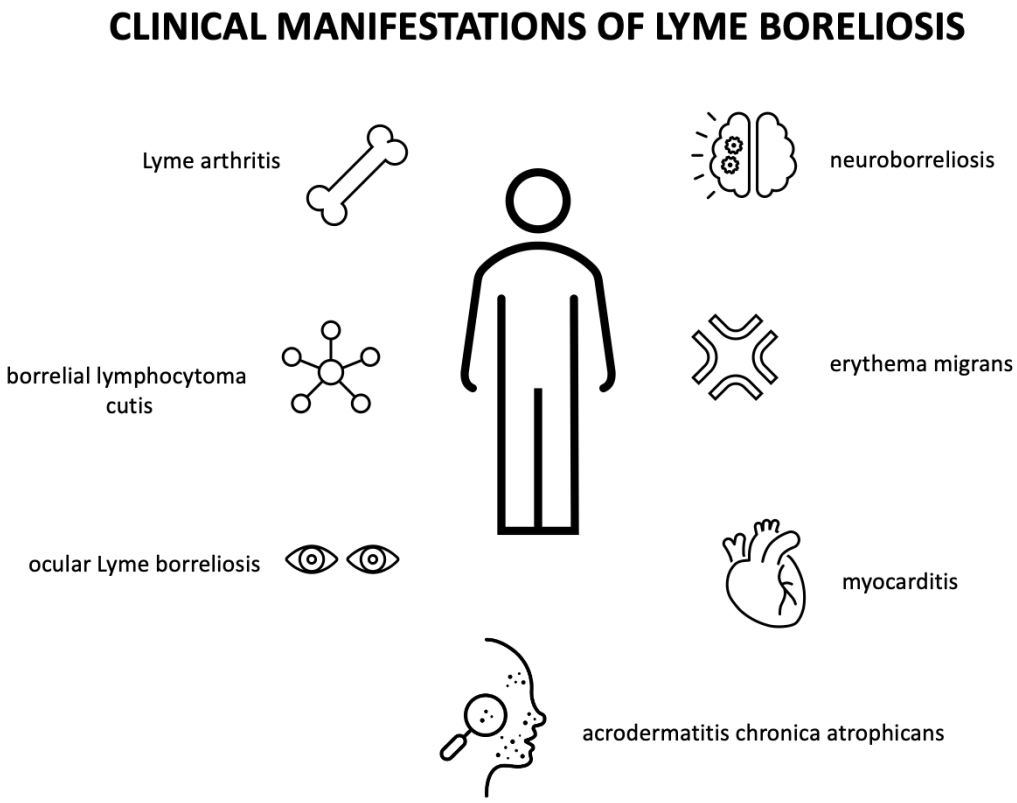

Figure 1 Clinical manifestations of Lyme borreliosis

2. METHODS

We performed an extensive literature search in the PubMed database to identify pertinent studies on the diagnosis and treatment of LB, including articles published up to June 2024. The search terms used were "Lyme borreliosis" OR "Lyme disease" AND "diagnosis" OR "treatment". Studies selected for the review had to meet specific criteria: they focused on diagnostic and therapeutic approaches or new diagnostic methods discovered in recent years, were published in peer-reviewed journals, and were available in English. Four reviewers independently extracted data, which included study characteristics, described diagnostic methods, test sensitivity and specificity, and key findings about the role of these methods in the diagnostic scheme of LB. A narrative synthesis was then conducted.

Findings were categorized according to the diagnostic issues addressed (indirect and direct diagnosis) and summarized to provide a comprehensive overview of LB diagnosis and treatment.

3. RESULT AND DISCUSSION

Indirect diagnosis

The diagnostic requirements for LB are (i) the identification of characteristic clinical symptoms and (ii) confirmation of infection by serological tests. Exclusively, the EM form of Lyme disease does not require any supporting tests for diagnosis. In Europe and the United States, a two-stage diagnosis is mandatory. The first stage is a quantitative screening test, for example, enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), and multiplex microsphere immunoassay (MMIA). A negative outcome indicates that the patient does not require any further investigation for LB. A positive or questionable result requires confirmation by the Western Blot (WB) test.

Standard two-tiered testing and modified two-tiered testing

Up-to-date recommendations for diagnosing LB include serologic tests, such as indirect diagnosis (Miraglia, 2016). Considering the limitations of serologic diagnosis in detecting early localized LB, early disseminated LB, or late-stage LB, typically proposed schemes include a standard two-tiered testing approach (CST). ST sensitivity, despite its recommendations, is poor in early localized infection (less than 50%), but in the late stages of infection, sensitivity approaches 100% (Waddell et al., 2016). In STTT, an ELISA test is performed first, and if the result is borderline or positive, a confirmatory WB test is required (Mead et al., 2019). The diagnostic algorithm based on STTT is presented in (Figure 2). The novel approaches for diagnosis of Lyme boreliosis is mentioned in (Table 1).

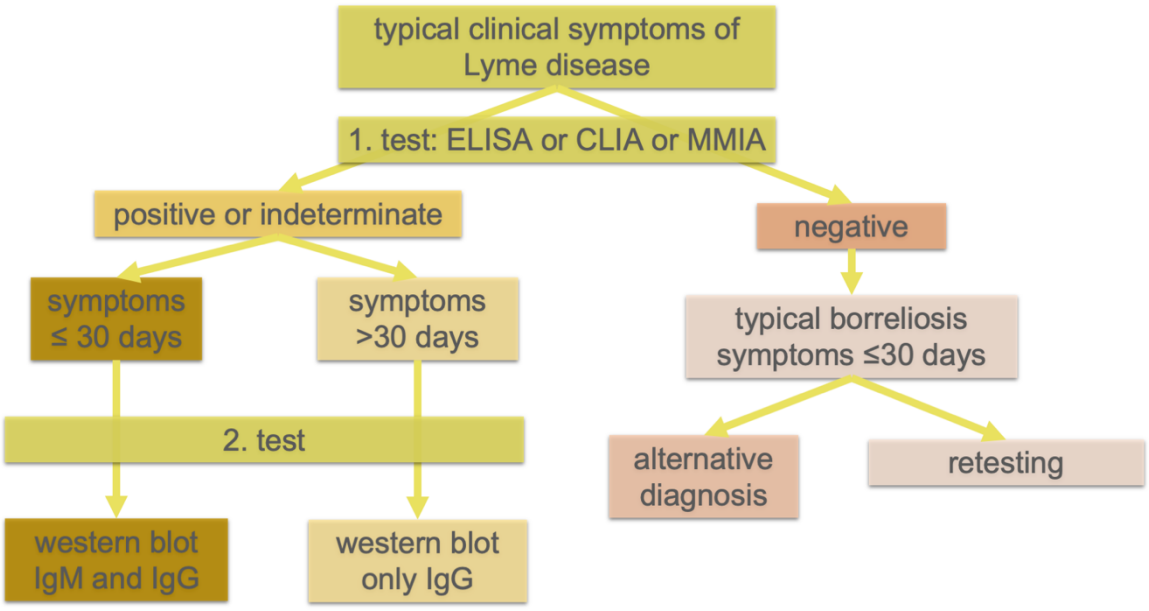

Figure 2 Diagnostic algorithm based on the standard two-tiered testing approach.

The ELISA method, commonly used in diagnostics, provides detection of immunoglobulin G (IgG) and, or immunoglobulin M (IgM). Antigens specific for Bb are coated on a plate, and patient serum samples are tested to identify these antigens. Products of the reaction induce a change in color or fluorescence, which is measured by the suitable detection system. The ELISA's reliability varies according to the antigen and its preparation. The first generation of ELISAs was based on spirochete lysates obtained by sonication, and its sensitivity was limited. Moreover, the first generation of ELISAs did not cover many antigens, such as VlsE or OspC (Kumaran et al., 2001; Nyman et al., 2006). The second generation of ELISAs uses purified, synthetic or recombinant antigens, such as the surface

lipoproteins OspC, OspA or VlsE, which are highly immunogenic and improve sensitivity (Kaiser and Rauer, 1999; Van-Burgel et al., 2011a). If a positive or borderline result is observed, WB is commonly performed. However, the method of this test is similar to the ELISA test. WB is usually based on Bb cell lysates and, or recombinant antigens.

The result is the detection of antibodies produced in response to infection by Bb. Separation of Bb antigens by SDS-PAGE, with a characteristic migration profile before detection, confirms the result, which increases specificity and validates the diagnosis (Mavin et al., 2009). However, sensitivity and specificity are the foremost considerations for this approach. Wojciechowska-Koszko et al. pointed out that actual testing generates many false-positive results, leading to misdiagnosis and unnecessary treatment. Authors tested the reactivity of sera from patients with Epstein-Barr virus or cytomegalovirus infection against Borrelia antigens used in serological tests. Several false-positive results have been obtained, probably related to the cross-reactivity of antibodies produced in response to the presence of viral superantigens (Wojciechowska-Koszko et al., 2022). Also, false-positive ELISA test results are possible due to cross-reactive antibodies to antigens common to other diseases i.e. Helicobacter pylori (Zóka et al., 2021).

Additional factors affecting the sensitivity and specificity of serological tests that should be considered are related to patients with early-stage LB. Generally, tests present a lower sensitivity than tests on patients with late-stage LB, because of the shorter time to develop an adequate antibody response. Effective antibiotic treatment in early-stage LB may also suppress the development of an antibody response, reducing the possibility of positive serological tests. In contrast, patients with late-stage LB, such as Lyme arthritis or neuroborreliosis, commonly present with a strong antibody response in serological tests (Quintero et al., 2021). A new concept for the diagnosis of LB is MTTT. It is suggested to replace WB with tests that can overcome the issues discussed previously (Branda et al., 2018). However, two-tier testing is still necessary as no test provides sufficient sensitivity and specificity.

Antibody-based diagnostic tests

There is an opening alternative for a simple screening test that can be performed simply on its own without the need for specialized laboratory equipment. The technology is called lateral flow immunoassay (LFIA), which is rapid, user-friendly, simple to interpret, and can be performed outside the laboratory. The premise of the test is similar to the ELISA method. The sample and migration buffer is applied to a paper carrier. After the sample migrates through the detection membrane, it reacts with antibodies directed against human IgG or IgM. Forming the complex provides a visible line, allowing the result to be interpreted - similar to the techniques used in screening tests during the COVID-19 pandemic. Commercial tests based on this technology for Lyme IgM or IgG antibodies are available, but they have varying sensitivity (30-100%) and specificity (38-100%) (Smit et al., 2015; Dickeson et al., 2016). However, current guidelines do not recommend them for routine diagnostic use, as they do not achieve a level of sensitivity and specificity comparable to traditional two-level tests (Eldin et al., 2019).

EliSpot as a potential new approach

Various studies indicate that Bb can enter endothelial cells and macrophages. In the same way as in viral infections, the activation of type I interferons intracellularly has an essential impact on infection by Bb (Livengood and Gilmore, 2006; Woitzik and Linder, 2021). Spirochete infection initiate an immune response via the release of cytokines and interferon γ (IFN-γ), and subsequently leads to an elevated expression of IL-4 by Th1 lymphocytes, which is associated with the non-chronic consequences of LB. However, persistent expression of IFN-γ may contribute to chronic manifestations of the LB (Oksi et al., 1996). Consequently, IFN-γ release can detect the presence of Bb antigens using EliSpot techniques - a quantitative test to determine the number of T cells producing IFN-γ. The sensitivity of this method is considerably higher than the ELISA test (Lehmann and Zhang, 2012). However, unlike conventional serology, EliSpot tests do not differentiate active from past infection (Van-Gorkom et al., 2018).

Biomarker-based assessment approach

Using transcriptomics, metabolomics, and inflammatomics approaches could help identify biomarkers or biosignatures of LB. Recent studies have shown that several molecules associated with the immune response can be used as biomarkers for diagnosis (Badawi, 2017). Chemokines and cytokines have a crucial role in the inflammatory process and the regulation of immune cells. At the diagnosis of Lyme neuroborreliosis (LNB), inflammatory changes in the cerebrospinal fluid (CSF), such as pleocytosis, blood-brain barrier damage, and increased immunoglobulin synthesis, can be expected. Laboratory diagnosis is based on measuring the antibody index by

comparing antibody levels in CSF and serum. Laboratory diagnosis is based on measuring the antibody index by comparing antibody levels in CSF and serum (Stanek and Strle, 2018).

CXCL13, a chemokine secreted by antigen-presenting cells, may be a promising diagnostic marker due to its high levels in CSF and rapidly decreasing levels after antibiotic treatment. However, CXCL13 is detectable before antibodies, whose levels can be deficient in the early stages of LNB (Senel et al., 2010; Yang et al., 2017). Also, its levels drop rapidly after antibiotic treatment, while pleocytosis in the CSF remains elevated, and antibody levels remain positive for years after treatment (Senel et al., 2010). Multiple methods can detect and quantify levels of this chemokine, with varying levels of sensitivity and specificity (Wagner et al., 2018; Van-Gorkom et al., 2021). Determination of CXCL13 levels as a marker of LNB can help make the diagnosis. However, CXCL13 is non-specific for LNB. Elevated CSF values have also been observed in patients with different neuro infections Dersch et al., (2015), as well as in patients who are immunocompromised or have an autoimmune disease (Van-Burgel et al., 2011b).

Direct diagnosis

Direct diagnosis to identify the pathogen for many infectious diseases is the gold standard for diagnosing the acute form of infection. Currently, it can be based on methods such as pathogen culture, microscopic observation, xenodiagnosis, pathogen DNA/RNA detection, and amplification.

Culture of Borellia burgfoderi

The culture of Bb is challenging on many points. Non-invasive blood, urine or skin biopsy sampling with EM may not be satisfactory and CSF, synovial membrane or myocardium may be required for culture (Trevisan et al., 2020). Furthermore, Borrelia culture is a long-term process, which makes this approach unsuitable for rapid detection of the pathogen. Culture of the Bb requires a specific and highly nutritious medium, i.e. Barbour-Stoenner-Kelly (BSK) - II or BSK-H supplemented with rabbit serum or modified Kelly-Pettenkofer (MKP) (Ružić-Sabljić et al., 2014). The growth rate varies from 8 to 12 hours at the appropriate temperature (30 to 35 °C), meaning it takes approximately 24 days for the pathogen to be detected in blood cultures and skin biopsy samples (Coulter et al., 2005). In addition, using a nutrient-rich medium makes the culture more sensitive to contamination by fast-growing bacteria.

To prevent bacterial contamination, an antibiotic is required. In addition, Borrelia culture requires long-term storage of samples for at least 8 to 12 weeks to confirm a negative result (Aguero-Rosenfeld et al., 2005). As mentioned earlier, despite Bb culture being considered the gold standard with a specificity close to 100%, the sensitivity of this method remains considerably low, depending on the clinical stage of the disease, the origin of the sample and the genogene responsible for LB (Kullberg et al., 2020). Samples frequently contain low numbers of viable bacteria, sometimes below measurable levels, and the authors highlighted the considerable variability in the amount of Bb collected in samples (Wormser et al., 2001). Consequently, a bacterial culture is a process exceeding the possibilities of most clinical laboratories and is, therefore, not suitable for routine diagnosis.

Microscopic observation

Direct microscopic observation of Bb is limited by the low concentration of bacteria in systemic fluids. This low concentration makes detection by light microscopy impractical, excluding its routine use in the diagnostic process. Additionally, the specificity of this method is moderate, as artifacts can lead to false-positive results, even with an experienced diagnostician. The study by Aase et al., (2016) showed that 85% of blood samples from a control group of healthy individuals were positive. In comparison, only 66% of those with Lyme disease were detected, making this approach impractical for diagnosis (Aase et al., 2016). Alternatively, the presence of infection can be determined by xenodiagnosis. This method involves placing and biting a patient suspected of being infected by an uninfected tick. The tick is then used to check for the presence of Bb using a polymerase chain reaction (PCR) test. Studies have been carried out on mouse models or rhesus macaques (Bockenstedt et al., 2002; Embers et al., 2012).

Tests based on antigen detection

Tests designed to detect the antigens have the same limitations as microscopic observation. There are few diagnostic tests for detecting Bb antigen Coyle et al., (1993), and their reliability in clinical practice is limited (Lohr et al., 2018). Considering the low concentration of bacteria in system fluids, depending mainly on the sample type and the severity of the disease, many methods have been developed to improve Bb antigens detection. These methods consider antigen concentrations, increasing the volume of the sample and improving

the binding activity of antibodies or peptides. Centrifugation increases the concentration of membrane proteins after bacterial lysis, allowing their detection even at low concentrations (Cheung et al., 2015). Other methods to increase antigen concentration are available, such as Nanotrap Magni et al., (2015) or hydrogel microparticle technology (Douglas et al., 2011). However, there is still a lack of appropriately sensitive and specific tests to introduce antigen-based assays into daily clinical practice.

Polymerase chain reaction tests

PCR is a well-known in vitro method for DNA amplification and detection. Due to the complexity of the Bb genome, multiple genes can be selected as target fragments of detected DNA (Lager et al., 2017). The sensitivity of the testing varies depending on the sample. The importance of early detection of Bb by PCR directly at the bite site has been demonstrated using a new non-invasive sampling device based on a micro-needle patch (Kight et al., 2022). However, one of the significant limitations of PCR testing is the difficulty in distinguishing between active infection and chronic LB, because Bb DNA remains present for a long time after antibiotic therapy (Lager et al., 2021). Consequently, the PCR method is not suitable for assessing the efficacy of treatment.

Improving traditional PCR techniques is an essential area of research in LB diagnosis. Studies have shown that a simple modification of the PCR procedure can enhance the sensitivity of tests, for example, by using a centrifugation step to concentrate the bacteria in the sample, using cDNA as a matrix, and eliminating erythrocytes (Lager et al., 2021). A novel method termed immuno-PCR (iPCR) allows to increase in the detection limit of conventional ELISA tests by up to 100-10,000- times (Adler et al., 2003). For LB diagnosis, this method uses a recombinant Bb protein antigen conjugated to magnetic beads to capture Bb-specific antibodies produced by the patient. This method, proposed by Halpern et al., (2013), Halpern et al., (2014) shows greater sensitivity than the traditional STTT method.

Potential co-infections should also be considered in the background of the PCR approach. The prevalent cases of co-infection reported include infection with more than one Borrelia species, as well as co-infection with Rickettsia spp. or Babesia spp. Raileanu et al. described the occurrence of more than one pathogen in 64.5 % of tested ticks 33 (Moniuszko et al., 2014; Raileanu et al., 2017). Co-infections can modify symptom severity and treatment outcomes. There is a need for multiplex testing for different strains of Borrelia and all tick-borne diseases. In this context, the development of multiplex PCR is crucial to increase the utility of this method in clinical practice and to ensure appropriate treatment.

Table 1 Key findings and novel approaches for diagnosis of Lyme boreliosis.

Diagnosis of Lyme borreliosis	
Indirect diagnosis	**Direct diagnosis**
Guidelines recommend serological diagnosis schemes based on standard two-tiered testing (Miraglia, 2016).	Lyme culture is a complicated procedure which makes it difficult to apply this method in daily routine (Coulter et al., 2005).
Guidelines do not recommend antibody-based tests in routine use - insufficient in sensitivity and specificity (Eldin et al., 2019).	Microscopic observation is not sufficiently sensitive and specific to be used in the LB diagnostic scheme (Aase et al., 2016).
New approaches, such as the Elispot, demonstrate excellent sensitivity but are limited in differentiating between the early and late forms of LB (Van-Gorkom et al., 2018).	Antibody-based diagnosis is problematic because of the low concentration of antigens in blood samples. However, new research is in progress (Lohr et al., 2018).
The potential for accurate diagnosis is offered by the use of biomarkers, but still requires further research (Van-Gorkom et al., 2021).	PCR offers the potential for precise diagnosis but has numerous limitations. The improvement of traditional PCR techniques is an area of research in LB diagnostics (Lager et al., 2017).

Treatment

Treatment of LB is based on the selection of appropriate antibiotic therapy. For many years, treatment options consisted of doxycycline, amoxicillin, cefuroxime axetil, ceftriaxone, cefuroxime, cefotaxime, penicillin G, and azithromycin. The choice of antibiotic and duration

of treatment is determined by many factors, i.e., the clinical presentation of the disease and of the patient's age. The first-choice antibiotic recommended for all forms of the disease is doxycycline. It is not recommended that the duration of antibiotic therapy exceeds 28 days. There is no evidence for the efficacy of chronic (multi-month) therapy or antiprotozoal drugs (Carriveau et al., 2019).

The use of antibiotics is not recommended for patients treated for LB who experience non-specific symptoms such as fatigue, pain, impaired memory, and concentration (post-Lyme syndrome). Comparative studies between this group of patients receiving antibiotics and the placebo group showed no benefit from antibiotic therapy (Fallon et al., 2008). For patients presenting with symptoms that suggest disease and positive serological results but without a precise diagnosis, oral antibiotic therapy may be considered. However, it is essential to remember that the effects may result from a placebo effect or a non-specific antibacterial effect of antibiotics (Leite et al., 2011; Lantos et al., 2021).

4. CONCLUSIONS

Being the most common tick-borne disease, diagnosis and treatment of LB remains a considerable challenge in daily practice. Despite multiple new approaches, the diagnosis pathway of this multidisciplinary disease is still suboptimal. Research on a simple, cheap, and effective diagnostic test is still being developed. With a highly sensitive and specific test, prompt and accurate diagnosis can be ensured, resulting in appropriate and early implemented treatment, therefore preventing patients from developing harmful complications. Furthermore, a precise diagnosis will reduce the rate of unnecessary antibiotic use, a global objective of responsible antibiotic use.

Ethical approval

Not applicable.

Authors' Contribution

Anna Salińska: Conceptualization, writing- rough preparation, methodology, investigation, project administration
Piotr Węgrzyn: Formal analysis, supervision
Konstancja Węgrzyn: Visualization, data curation
Agnieszka Góra: Conceptualization, data curation
Marcin Wasilewski: Methodology, data curation
Maciek Nowicki: Conceptualization, methodology, data curation
Julia Skwara: Resources, writing- rough preparation
Dawid Barański: Conceptualization, writing- rough preparation
Natalia Dąbrowska: Resources, investigation
Gustaw Laskowski: Writing - Review and editing, supervision
All authors have read and agreed to the published version of the manuscript.

Informed consent

Not applicable.

Funding

This study has not received any external funding.

Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

REFERENCES

1. Aase A, Hajdusek O, Øines Ø, Quarsten H, Wilhelmsson P, Herstad TK, Kjelland V, Sima R, Jalovecka M, Lindgren PE, Aaberge IS. Validate or falsify: Lessons learned from a microscopy method claimed to be useful for detecting Borrelia and Babesia organisms in human blood. Infect Dis (Lond) 2016; 48(6):411-9. doi: 10.3109/23744235.2016.1144931
2. Adler M, Wacker R, Niemeyer CM. A real-time immuno-PCR assay for routine ultrasensitive quantification of proteins. Biochem Biophys Res Commun 2003; 308(2):240–50. doi: 10.1016/s0006-291x(03)01364-0
3. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of lyme borreliosis. Clin Microbiol Rev 2005; 18(3): 484-509. doi: 10.1128/CMR.18.3.484-509.2005
4. Badawi A. The Potential of Omics Technologies in Lyme Disease Biomarker Discovery and Early Detection. Infect Dis Ther 2017; 6(1):85-102. doi: 10.1007/s40121-016-0138-6
5. Bockenstedt LK, Mao J, Hodzic E, Barthold SW, Fish D. Detection of attenuated, noninfectious spirochetes in Borrelia burgdorferi-infected mice after antibiotic treatment. J Infect Dis 2002; 186(10):1430-7. doi: 10.1086/345284
6. Branda JA, Body BA, Boyle J, Branson BM, Dattwyler RJ, Fikrig E, Gerald NJ, Gomes-Solecki M, Kintrup M, Ledizet M, Levin AE, Lewinski M, Liotta LA, Marques A, Mead PS, Mongodin EF, Pillai S, Rao P, Robinson WH, Roth KM, Schriefer ME, Slezak T, Snyder J, Steere AC, Witkowski J, Wong SJ, Schutzer SE. Advances in Serodiagnostic Testing for Lyme Disease Are at Hand. Clin Infect Dis 2018; 66(7):1133-1139. doi: 10.1093/cid/cix943
7. Carriveau A, Poole H, Thomas A. Lyme Disease. Nurs Clin North Am 2019; 54(2):261-275. doi: 10.1016/j.cnur.2019.02.003
8. Cheung CS, Anderson KW, Benitez KY, Soloski MJ, Aucott JN, Phinney KW, Turko IV. Quantification of Borrelia burgdorferi Membrane Proteins in Human Serum: A New Concept for Detection of Bacterial Infection. Anal Chem 2015; 87(22):11383-8. doi: 10.1021/acs.analchem.5b02803
9. Coulter P, Lema C, Flayhart D, Linhardt AS, Aucott JN, Auwaerter PG, Dumler JS. Two-year evaluation of Borrelia burgdorferi culture and supplemental tests for definitive diagnosis of Lyme disease. J Clin Microbiol 2005; 43(10):5080-4. doi: 10.1128/JCM.43.10.5080-5084.2005
10. Coyle PK, Deng Z, Schutzer SE, Belman AL, Benach J, Krupp LB, Luft B. Detection of Borrelia burgdorferi antigens in cerebrospinal fluid. Neurology 1993; 43(6):1093-8. doi: 10.1212/wnl.43.6.1093
11. Dersch R, Hottenrott T, Senel M, Lehmensiek V, Tumani H, Rauer S, Stich O. The chemokine CXCL13 is elevated in the cerebrospinal fluid of patients with neurosyphilis. Fluids Barriers CNS 2015; 12:12. doi: 10.1186/s12987-015-0008-8
12. Dickeson DJ, Chen SCA, Sintchenko VG. Concordance of four commercial enzyme immunoassay and three immunoblot formats for the detection of Lyme borreliosis antibodies in human serum: The two-tier approach remains. Pathology 2016; 48(3):251–6. doi: 10.1016/j.pathol.2016.02.004
13. Dong Y, Zhou G, Cao W, Xu X, Zhang Y, Ji Z, Yang J, Chen J, Liu M, Fan Y, Kong J, Wen S, Li B, Yue P, Liu A, Bao F. Global seroprevalence and sociodemographic characteristics of Borrelia burgdorferi sensu lato in human populations: a systematic review and meta-analysis. BMJ Glob Health 2022; 7(6):e007744. doi: 10.1136/bmjgh-2021-007744
14. Douglas TA, Tamburro D, Fredolini C, Espina BH, Lepene BS, Ilag L, Espina V, Petricoin EF 3rd, Liotta LA, Luchini A. The use of hydrogel microparticles to sequester and concentrate bacterial antigens in a urine test for Lyme disease. Biomaterials 2011; 32(4):1157-66. doi: 10.1016/j.biomaterials.2010.10.004
15. Eldin C, Raffetin A, Bouiller K, Hansmann Y, Roblot F, Raoult D, Parola P. Review of European and American guidelines for the diagnosis of Lyme borreliosis. Med Mal Infect 2019; 49(2):121-132. doi: 10.1016/j.medmal.2018.11.011
16. Embers ME, Barthold SW, Borda JT, Bowers L, Doyle L, Hodzic E, Jacobs MB, Hasenkampf NR, Martin DS, Narasimhan S, Phillippi-Falkenstein KM, Purcell JE, Ratterree MS, Philipp MT. Persistence of Borrelia burgdorferi in rhesus macaques following antibiotic treatment of disseminated infection. PLoS One 2012; 7(1):e29914. doi: 10.1371/journal.pone.0029914. Erratum in: PLoS One 2012; 7(4). doi: 10.1371/annotation/4cafed66-fb84-4589-a001-131d9c50 aea6
17. Fallon BA, Keilp JG, Corbera KM, Petkova E, Britton CB, Dwyer E, Slavov I, Cheng J, Dobkin J, Nelson DR, Sackeim HA. A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology 2008; 70(13):992-1003. doi: 10.1212/01.WNL.0000284604.61160.2d
18. Halpern MD, Jain S, Jewett MW. Enhanced detection of host response antibodies to Borrelia burgdorferi using immuno-PCR. Clin Vaccine Immunol 2013; 20(3):350-7. doi: 10.1128/CVI.00630-12
19. Halpern MD, Molins CR, Schriefer M, Jewett MW. Simple objective detection of human lyme disease infection using

immuno-PCR and a single recombinant hybrid antigen. Clin Vaccine Immunol 2014; 21(8):1094-105. doi: 10.1128/CVI.00245-14

20. Kaiser R, Rauer S. Advantage of recombinant borrelial proteins for serodiagnosis of neuroborreliosis. J Med Microbiol 1999; 48(1):5-10. doi: 10.1099/00222615-48-1-5
21. Kight E, Alfaro R, Gadila SKG, Chang S, Evans D, Embers M, Haselton F. Direct Capture and Early Detection of Lyme Disease Spirochete in Skin with a Microneedle Patch. Biosensors (Basel) 2022; 12(10):819. doi: 10.3390/bios12100819
22. Kullberg BJ, Vrijmoeth HD, Van-de-Schoor F, Hovius JW. Lyme borreliosis: diagnosis and management. BMJ 2020; 369: m1041. doi: 10.1136/bmj.m1041
23. Kumaran D, Eswaramoorthy S, Luft BJ, Koide S, Dunn JJ, Lawson CL, Swaminathan S. Crystal structure of outer surface protein C (OspC) from the Lyme disease spirochete, Borrelia burgdorferi. EMBO J 2001; 20(5):971-8. doi: 10.1093/emboj/20.5.971
24. Lager M, Faller M, Wilhelmsson P, Kjelland V, Andreassen Å, Dargis R, Quarsten H, Dessau R, Fingerle V, Margos G, Noraas S, Ornstein K, Petersson AC, Matussek A, Lindgren PE, Henningsson AJ. Molecular detection of Borrelia burgdorferi sensu lato - An analytical comparison of real-time PCR protocols from five different Scandinavian laboratories. PLoS One 2017; 12(9): e0185434. doi: 10.1371/journal.pone.0185434
25. Lager M, Wilhelmsson P, Matussek A, Lindgren PE, Henningsson AJ. Molecular Detection of Borrelia Bacteria in Cerebrospinal Fluid-Optimisation of Pre-Analytical Sample Handling for Increased Analytical Sensitivity. Diagnostics (Basel) 2021; 11(11):2088. doi: 10.3390/diagnostics11112088
26. Lantos PM, Rumbaugh J, Bockenstedt LK, Falck-Ytter YT, Aguero-Rosenfeld ME, Auwaerter PG, Baldwin K, Bannuru RR, Belani KK, Bowie WR, Branda JA, Clifford DB, Dimario FJ, Halperin JJ, Krause PJ, Lavergne V, Liang MH, Meissner HC, Nigrovic LE, Nocton JJJ, Osani MC, Pruitt AA, Rips J, Rosenfeld LE, Savoy ML, Sood SK, Steere AC, Strle F, Sundel R, Tsao J, Vaysbrot EE, Wormser GP, Zemel LS. Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 Guidelines for the Prevention, Diagnosis and Treatment of Lyme Disease. Clin Infect Dis 2021; 72(1):1–8. doi: 10.1093/cid/ciab049
27. Lehmann PV, Zhang W. Unique strengths of ELISPOT for T cell diagnostics. Methods Mol Biol 2012; 792:3-23. doi: 10.1007/978-1-61779-325-7_1
28. Leite LM, Carvalho AGG, Tavares-Ferreira PLF, Pessoa IX, Gonçalves DO, De-Araújo Lopes A, Dos-Santos Góes JG, De-Castro-Alves VC, Leal LKAM, Brito GA, Viana GSB. Anti-inflammatory properties of doxycycline and minocycline in experimental models: an in vivo and in vitro comparative study. Inflammopharmacology 2011; 19(2):99-110. doi: 10.1007/s10787-011-0077-5
29. Livengood JA, Gilmore RD Jr. Invasion of human neuronal and glial cells by an infectious strain of Borrelia burgdorferi. Microbes Infect 2006; 8(14-15):2832-40. doi: 10.1016/j.micinf.2006.08.014
30. Lohr B, Fingerle V, Norris DE, Hunfeld KP. Laboratory diagnosis of Lyme borreliosis: Current state of the art and future perspectives. Crit Rev Clin Lab Sci 2018; 55(4):219-245. doi: 10.1080/10408363.2018.1450353
31. Magni R, Espina BH, Shah K, Lepene B, Mayuga C, Douglas TA, Espina V, Rucker S, Dunlap R, Petricoin EF, Kilavos MF, Poretz DM, Irwin GR, Shor SM, Liotta LA, Luchini A. Application of Nanotrap technology for high sensitivity measurement of urinary outer surface protein A carboxyl-terminus domain in earlystage Lyme borreliosis. J Transl Med 2015; 13:346. doi: 10.1186/s12967-015-0701-z
32. Mavin S, Evans R, Milner RM, Chatterton JM, Ho-Yen DO. Local Borrelia burgdorferi sensu stricto and Borrelia afzelii strains in a single mixed antigen improves western blot sensitivity. J Clin Pathol 2009; 62(6):552-4. doi: 10.1136/jcp.2008.063461
33. Mead P, Petersen J, Hinckley A. Updated CDC Recommendation for Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep 2019; 68(32):703. doi: 10.15585/mmwr.mm6832a4
34. Mead P. Epidemiology of Lyme Disease. Infect Dis Clin North Am 2022; 36(3):495-521. doi: 10.1016/j.idc.2022.03.004
35. Miraglia CM. A Review of the Centers for Disease Control and Prevention's Guidelines for the Clinical Laboratory Diagnosis of Lyme Disease. J Chiropr Med 2016; 15(4):272-280. doi: 10.1016/j.jcm.2016.08.003
36. Moniuszko A, Dunaj J, Święcicka I, Zambrowski G, Chmielewska-Badora J, Żukiewicz-Sobczak W, Zajkowska J, Czupryna P, Kondrusik M, Grygorczuk S, Swierzbinska R, Pancewicz S. Co-infections with Borrelia species, Anaplasma phagocytophilum and Babesia spp. in patients with tick-borne encephalitis. Eur J Clin Microbiol Infect Dis 2014; 33(10):1835-41. doi: 10.1007/s10096-014-2134-7
37. Nyman D, Willén L, Jansson C, Carlsson SA, Granlund H, Wahlberg P. VlsE C6 peptide and IgG ELISA antibody analysis for clinical diagnosis of Lyme borreliosis in an

endemic area. Clin Microbiol Infect 2006; 12(5):496–7. doi: 10.1111/j.1469-0691.2006.01374.x

38. Oksi J, Savolainen J, Pène J, Bousquet J, Laippala P, Viljanen MK. Decreased interleukin-4 and increased gamma interferon production by peripheral blood mononuclear cells of patients with Lyme borreliosis. Infect Immun 1996; 64(9):3620–3623.
39. Quintero JA, Attah R, Khianey R, Capitle E, Schutzer SE. Arthritis and Diagnostics in Lyme Disease. Trop Med Infect Dis 2021; 6(1):18. doi: 10.3390/tropicalmed6010018
40. Raileanu C, Moutailler S, Pavel I, Porea D, Mihalca AD, Savuta G, Vayssier-Taussat M. Borrelia Diversity and Co-infection with Other Tick Borne Pathogens in Ticks. Front Cell Infect Microbiol 2017; 7:36. doi: 10.3389/fcimb.2017.00036
41. Ružić-Sabljić E, Maraspin V, Cimperman J, Strle F, Lotrič-Furlan S, Stupica D, Cerar T. Comparison of isolation rate of Borrelia burgdorferi sensu lato in two different culture media, MKP and BSK-H. Clin Microbiol Infect 2014; 20(7):636-41. doi: 10.1111/1469-0691.12457
42. Senel M, Rupprecht TA, Tumani H, Pfister HW, Ludolph AC, Brettschneider J. The chemokine CXCL13 in acute neuroborreliosis. J Neurol Neurosurg Psychiatry 2010; 81(8):929-33. doi: 10.1136/jnnp.2009.195438
43. Smit PW, Kurkela S, Kuusi M, Vapalahti O. Evaluation of two commercially available rapid diagnostic tests for Lyme borreliosis. Eur J Clin Microbiol Infect Dis 2015; 34(1):109-113. doi: 10.1007/s10096-014-2217-5
44. Stanek G, Strle F. Lyme borreliosis-from tick bite to diagnosis and treatment. FEMS Microbiol Rev 2018; 42(3):233-258. doi: 10.1093/femsre/fux047
45. Trevisan G, Bonin S, Ruscio M. A Practical Approach to the Diagnosis of Lyme Borreliosis: From Clinical Heterogeneity to Laboratory Methods. Front Med (Lausanne) 2020; 7:265. doi: 10.3389/fmed.2020.00265
46. Van-Burgel ND, Bakels F, Kroes ACM, Van Dam AP. Discriminating Lyme neuroborreliosis from other neuroinflammatory diseases by levels of CXCL13 in cerebrospinal fluid. J Clin Microbiol 2011a; 49(5):2027-30. doi: 10.1128/JCM.00084-11
47. Van-Burgel ND, Brandenburg A, Gerritsen HJ, Kroes ACM, Van-Dam AP. High sensitivity and specificity of the C6-peptide ELISA on cerebrospinal fluid in Lyme neuroborreliosis patients. Clin Microbiol Infect 2011b; 17(10):1495-500. doi: 10.1111/j.1469-0691.2011.03459.x
48. Van-Gorkom T, Sankatsing SUC, Voet W, Ismail DM, Muilwijk RH, Salomons M, Vlaminckx BJM, Bossink AWJ, Notermans DW, Bouwman JJM, Kremer K, Thijsen SFT. An Enzyme-Linked Immunosorbent Spot Assay Measuring Borrelia burgdorferi B31-Specif-ic Interferon Gamma-Secreting T Cells Cannot Discriminate Active Lyme Neuroborreliosis from Past Lyme Borreliosis: a Prospective Study in the Netherlands. J Clin Microbiol 2018; 56(4):e01695-17. doi: 10.1128/JCM.01695-17
49. Van-Gorkom T, Van-Arkel GHJ, Heron M, Voet W, Thijsen SFT, Kremer K. The Usefulness of Two CXCL13 Assays on Cerebrospinal Fluid for the Diagnosis of Lyme Neuroborreliosis: a Retrospective Study in a Routine Clinical Setting. J Clin Microbiol 2021; 59(9):e0025521. doi: 10.1128/JCM.00255-21
50. Waddell LA, Greig J, Mascarenhas M, Harding S, Lindsay R, Ogden N. The Accuracy of Diagnostic Tests for Lyme Disease in Humans, A Systematic Review and Meta-Analysis of North American Research. PLoS One 2016; 11(12):e0168613. doi: 10.1371/journal.pone.0168613
51. Wagner JN, Weis S, Kubasta C, Panholzer J, Von-Oertzen TJ. CXCL13 as a diagnostic marker of neuroborreliosis and other neuroinflammatory disorders in an unselected group of patients. J Neurol 2018; 265(1):74–81. doi: 10.1007/s00415-017-8669-7
52. Woitzik P, Linder S. Molecular Mechanisms of Borrelia burgdorferi Phagocytosis and Intracellular Processing by Human Macrophages. Biology (Basel) 2021; 10(7):567. doi: 10.3390/biology10070567
53. Wojciechowska-Koszko I, Kwiatkowski P, Sienkiewicz M, Kowalczyk M, Kowalczyk E, Dołęgowska B. Cross-Reactive Results in Serological Tests for Borreliosis in Patients with Active Viral Infections. Pathogens 2022; 11(2):203. doi: 10.3390/pathogens11020203
54. Wormser GP, Bittker S, Cooper D, Nowakowski J, Nadelman RB, Pavia C. Yield of large-volume blood cultures in patients with early Lyme disease. J Infect Dis 2001; 184(8):1070–2. doi: 10.1086/323424
55. Wormser GP, McKenna D, Carlin J, Nadelman RB, Cavallere LF, Holmgren D, Byrne DW, Nowakowski J. Brief communication: hematogenous dissemination in early Lyme disease. Ann Intern Med 2005; 142(9):751–5. doi: 10.7326/0003-4819-142-9-200505030-00011
56. Yang J, Han X, Liu A, Bao F, Peng Y, Tao L, Ma M, Bai R, Dai X. Chemokine CXC Ligand 13 in Cerebrospinal Fluid Can Be Used as an Early Diagnostic Biomarker for Lyme Neuroborreliosis: A Meta-Analysis. J Interferon Cytokine Res 2017; 37(10):433–439. doi: 10.1089/jir.2016.0101
57. Zóka A, Gönczi M, Kádár B, Steinhauser R, Baráth A, Bekő G. The potential impact of Helicobacter pylori seropositivity on

recombinant antigen-based Lyme serology. J Microbiol Methods 2021; 188:106298. doi: 10.1016/j.mimet.2021.106298