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ANALYSIS OF THE METHODS FOR DIAGNOSING BORRELIOSIS – LYME DISEASE

ANALIZA METOD UŻYWANYCH W DIAGNOSTYCE BORELIOZY – CHOROBY Z LYME

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Summary

Lyme borreliosis is a multi-organ disease transmitted by ticks, whose etiological factor is an anaerobic bacterium *Borrelia burgdorferi* sensu lato. The incidence of this disease has risen each year for more than 10 years now in all parts of the world where ticks are present. Due to the multiplicity of the clinical symptoms, the disease is difficult to diagnose as it resembles other illnesses. Further, its pathomechanism through which *Borrelia burgdorferi* spirochetes attack joints, heart, nervous system and skin is not fully understood. This leads to many problems, both diagnostic and therapeutic.

At present, there are two views on Lyme disease: one forwarded by the Polish Society of Epidemiology and Physicians of Infectious Diseases and the other one recommended by the International Lyme and Associated Diseases Society.

The following article presents clinical diagnostic procedures as well as additional laboratory, serological, histological, microbiological and genetic analyzes. It is an attempt to provide the most reliable diagnostic methods although it should be noted that all of them encounter difficulties. Accordingly, the diagnosis of Lyme disease is far from perfect and requires further research and standardization.

Keywords: Lyme disease, clinical diagnostics, laboratory diagnostics

Streszczenie

Borelioza jest wieloukładową chorobą przenoszona przez kleszcze, której czynnikiem etiologicznym jest bakteria beztlenowa *Borrelia burgdorferi* sensu lato. Od ponad 10 lat z roku na rok wzrasta zachorowalność na tą chorobę we wszystkich miejscach świata, tam gdzie występują kleszcze. Ze względu na złożoność objawów klinicznych jest ona trudna do rozpoznania imitując inne jednostki chorobowe. Nie do końca poznany jest patomechanizm, w którym krętki *Borrelia burgdorferi* atakują stawy, serce, układ nerwowy i skórę. Rodzi to wiele problemów zarówno diagnostycznych jaki i terapeutycznych.

Obecnie ścierają się dwa poglądy na boreliozę ten reprezentowany przez Polskie Towarzystwo Epidemiologów i Lekarzy Chorób Zakaźnych oraz ten sugerowany przez Międzynarodowe Towarzystwo ds. Boreliozy i Chorób z Nią Powiązanych.

Artykuł przedstawia diagnostykę kliniczną oraz badania dodatkowe w postaci analiz laboratoryjnych, serologicznych, histologicznych, mikrobiologicznych i genetycznych. Jest on próbą przedstawienia najbardziej wiarygodnych metod diagnostycznych z zaznaczeniem jednak, że we wszystkich napotyka się trudności i wnioskiem, że diagnostyka boreliozy wciąż jest niedoskonała i wymaga jeszcze wielu badań i standaryzacji.

Słowa kluczowe: choroba z Lyme, diagnostyka kliniczna, diagnostyka laboratoryjna

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Introduction

Poland has gathered more precise data on the incidence of Lyme disease since 2014 as the disease has also been subject to mandatory reporting since that time. It is due to a systematic increase in the number of reported patients in the past 10 years (10 times fewer cases were recorded in 2000 than in 2016). In 2016, the number amounted to 21 220 people, 56% more than the year before, when it stood at 13 625 [1].

Lyme borreliosis (Lyme disease, LD – Lyme Disease) is transmitted by arachnid vectors - ticks. In Poland these are, for example, *Ixodes ricinus* (the common tick) [2] and *Dermacentor reticulates* (the meadow tick) [3]. The disease is a worldwide problem though. It was first discovered in the United States but affects patients throughout Europe, Australia, Asia and America [4]. Its main vector in Australia is *Ixodes holocyclus* [5], in Asia, the infection is caused by *Ixodes persulcatus* (the taiga tick), whereas in the USA – by *Ixodes scapularis* in the east and *Ixodes paciflcus* in the west [6].

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The cause of the disease is *Borrelia burgdorferi* sensu lato. It comprises at least 12 genospecies. So far, several pathogens have been described for humans (*Borrelia afzelii*, *Borrelia spielmani*, *Borrelia garinii*, *Borrelia burgdorferi* sensu stricto, *Borrelia bissettii*, *Borrelia valaisiana*, *Borrelia lusitaniae*) [7,8]. *Borrelia burgdorferi* sensu stricto is predominantly present in the United States of America [9]. In Europe, the dominant genospecies are *Borrelia afzelii*, *Borrelia garinii* and *Borrelia burgdorferi* sensu stricto [10]. They are Gram-negative, intracellular, anaerobic bacteria in the order *Spirochaetales*, family *Spirochaetaceae*, genera *Borrelia* [11, 12].

The pathomechanism of the disease has not been thoroughly examined. Hence, it is difficult to define standardised diagnostic and therapeutic criteria. The diagnosis relies on laboratory tests; however, it is the presence or absence of clinical symptoms that determines the need for treatment.

Aim of the work: The purpose of the following paper is to analyze the diagnostic methods used in the process of Lyme identification. Clinical diagnostics as well as additional studies in the form of laboratory, serological, histological, microbiological and genetic analyzes will be presented.

Brief description of the status of knowledge

Clinical diagnostics

Lyme disease is a multi-organ disease with a non-specific set of symptoms that may suggest other conditions. These include skin lesions, heart disorders, neurological disorders, polyarthritis [13].

At early clinical stage – localised infection, lasting 1-8 weeks, skin lesions predominate, often accompanied by common mild flu-like symptoms.

Erythema migrans (EM)

It is a characteristic skin lesion that occurs at the site of a tick bite. Its presence does not require further diagnosis as it is an absolute indication for antibiotic therapy. Erythema migrans occurs around 3-30 days after the tick bite, when the body does not produce antibody against spirochetes in the patient's blood [14]. During that time, the diagnosis of Lyme disease is based entirely on the clinical picture. Erythema migrans occurs in about 50% - 80% of the persons bitten by the tick. It is classically above 5 cm in diameter and ends in a clear border. It expands circularly around the bite, leaving a light spot in the centre. It can be painful and itchy. If untreated, it disappears spontaneously after a few weeks or months. One can find spirochetes in skin samples taken from erythema migrans half a year after the tick bite [15]. This skin lesion is often accompanied by some common ailments – flu-like malaise, fatigue, headache, joint pain, fever or mild fever. An atypical form of erythema migrans is also described. This change is characterised by irregular outlines, bubbles, diathesis haemorrhagica, which tends to enlarge in diameter over 5 cm. It is recommended that the presence of erythema migrants on the skin at the tick bite site is a sufficient indication for treatment and does not need to be confirmed by laboratory diagnosis. This is an immediate therapeutic response to an infection with spirochetes. In such a short time after the bite, anti-tick antibodies in the patient's blood cannot be detected. Further, the recent research findings seem to indicate that not all erythema migrans lesions are a symptom of Lyme disease [16].

Borrelia lymphocytoma (BL) [17].

It is a blue-red painless nodule that is typically located on the ear lobe, nipple or scrotum. It occurs in about 2% of the patients, most often in children. It appears about 2 months after the bite by the tick carrying *Borrelia*. Then, a microscopic image of a lymphoma sample shows lymphocytic infiltrates with admixture of plasmocytes, macrophages and eosinophils [18, 19]. If untreated, lymphocytoma persists for several years and then disappears spontaneously.

Local limfadenopathy

Enlarged lymph nodes, often painful, sliding under the fingers during the test, with a reddened skin above, are located in the area of the tick bite. Some authors link the appearance of lymphadenopathy to simultaneous co-infection with various genospecies of *Borrelia burgdorferi* spirochete.

In its early stage – typically within 3-26 weeks, in addition to the skin changes, there appear symptoms in the nervous, circulatory and movement systems.

Multiple erytema migrans

The presence of an erythema similar to erythema migrans expanding in different places on the skin and of different diameters indicates blood-borne phase of spirochetes dissemination. These types of skin lesions are small, do not tend to enlarge, appearing and disappearing in turn. It is estimated that multiple erythema migrans is present in about 8% of the patients [21].

Lyme artritis (LA) [22]

This is a symptom characteristic both of the early and late stage of the disease. Pain, loss of joint function, frequent swelling, warmth and redness of the skin usually affect large joints such as knee or elbow joints (most often asymmetrically). Primary inflammation and pain last several hours and then recede. Further recurrence lasts for several days, with painless intervals. After receding, it will resume and last for several months. Pain episodes are weaker each time until they are completely gone, even when untreated. In some cases however, arthritis becomes chronic and lasts continuously or with short intervals for months or years. Then, large joints are painful and the condition can be both symmetrical and asymmetrical. Typically, the most painful are all large joints in one lower or upper limb. The symptoms that accompany arthritis include tendonitis, osteitis, myositis. Sometimes, there appears pain in phalanges, which is not an isolated symptom. Besides, a common symptom that comes along arthritis is fibromyalgia [23]. In the advanced phase of arthritis, hypertrophy develops. It is accompanied by deposition of connective tissue with mononuclear cells in an articular capsule. This leads to numerous erosions, as a result of which articular cartilage is destroyed. It appears that the presence of tissue-compatible antibodies – class II HLA-DR4, HLA-DR2, facilitates the development, spread and survival of the infection, aggravating arthritis.

Some centres use a joint fluid test in the diagnosis of Lyme arthritis. Such fluid shows the presence of several hundred to 50,000 cells/l, with predominance of neutrophils, and increased protein concentration (30-80 g/l). The test result is non-specific. Other joint fluid tests involve culture and spirochetes culture. However, breeding spirochete is extremely difficult to conduct. It is done only in few laboratories in the world. Spirochete DNA fragments can also be found in the PCR fluid. The negative result of this test does not exclude infection. Lyne arthritis is primarily caused by *Borrelia burgdorferi* sensu stricto spread mainly in the United States of America

Lyme carditis (LC)

It is a rare symptom of Lyme disease. It occurs most often in the early generalized phase about 20 days after infection. The symptoms last for 3 to 6 days and involve shortness of breath, limited physical activity, fatigue and chest pains. After a few weeks, they resolve spontaneously without treatment [14]. Cardiomyopathy with coronary artery disease is rarely observed. The second and third heart blocks require temporary electrostimulation.

Eyes

Another symptom that occurs in 1-3% of the patients is optic neuritis or oculomotor nerve paralysis. Also, erythema migrans in the eye area and conjunctivitis are more and more common.

Neuroboreliosis [24]

The neurological form of Lyme disease is particularly characteristic of our geographical latitude. Its cause is considered to be *Borrelia garinii* infection most commonly found in Poland. Spirochetes have the ability to adhere to glial cells, which impairs their functions. Histopathological changes in the brain and spinal cord affect mainly the white matter. Patients with peripheral nervous system disorders show a visible distal axonal loss. The symptoms in the nervous system are observed both in the early and late stages of Lyme disease. It is characterized by inflammation of nerves, nerve roots, spinal cord, lymphocytic meningitis. These symptoms are accompanied by inflammation of muscles and blood vessels. Common symptoms of neuroborreliosis include stroke, facial nerve inflammation, visual disorders, hearing loss, swallowing, paraesthesia. Besides, patients often suffer from root pain disorders in shoulders and lumbar spine. Other neurological symptoms include seizures, psychosis, paresis, extrapyramidal symptoms, cerebellar ataxia. Untreated neuroboreliosis progresses and destroys cognitive functions and memory (progressive encephalopathy).

Laboratory diagnosis of this form of Lyme disease relies on lumbar puncture during which fluid is collected for laboratory tests. There are several to more than 1000 mononuclear cells in 1µl in the MR fluid. A moderately elevated protein concentration (1-2 g/l) and the presence of specific anti-*Borrelia burgdorferi* IgM or IgG antibodies are also reported.

In the late stage of Lyme disease, occurring after six months or even a year after the tick bite, peripheral neuropathy develops. There might also appear chronic encephalitis and meningitis, atopic dermatitis (ACA) and arthritis (single or multiple).

Acrodermatitis Chronica Atroficans (ACA) [25]

The symptom appears a long time after the tick bite and infecting the human body with spirochetes - most commonly *Boreelia afzelii*. Changes in the skin in the form of edema, skin redness, hyperpigmentation and telangiectasia affect mainly the dorsal surface of palms and lower limbs. Most often, it is the limb where erythema

migrans develops after the tick bite. The skin changes are accompanied by pain, pruritus and hyperaemia. Acrodermatitis Chronic Atrophicans (ACA) begins after a few weeks or even years. It is characterised by dermal and epidermal thinning, superficial veins (a paper-thin skin syndrome), peripheral neuropathy, and degenerative changes in neighbouring joints.

The diagnosis of this form of borreliosis uses skin samples, from which spirochetes can be grown even 10 years after the infection. Histopathological examination reveals lymphocytic infiltrates (mainly T cells) with an admixture of plasma cells, macrophages and eosinophils.

Laboratory diagnostics

Indirect research

Indirect examination tests involve two-stage diagnosis that assesses the level of IgM (the early stage) and IgG (the late phase of the disease) antibodies produced in the patient's blood. EIA (immunoassay) tests and Western Blot or Immunoblot tests (as recommended by the Polish Society of Epidemiologists and Physicians of Infectious Diseases) are used. They have similar sensitivity although Western blot test is more specific. The International Lyme and Associated Diseases Society has extended its diagnostic criteria for using LTT (lymphocyte transformation test), CIC (circulating immune complexes), the C6 Elisa test, and others. Indirect Diagnosis is a late stage disease diagnosis. In the early phase, the organism does not produce *Borrelia* spirochetes antibodies. The test should be performed no earlier than 30 days after the contact with the tick [26]. Until then, confirmation of illness with the above presented tests is impossible in most cases.

EIA tests [27]

Among EIA tests, the ones most commonly used are ELFA (enzyme-linked fluorescent immunoassay) or ELISA (enzyme-linked immunosorbent assay). They are involved in the search of spirochetes antibodies in the biological material, mainly blood, cerebrospinal fluid and joint fluid. There exist several test methods: Direct ELISA, Indirect ELISA, Sandwich ELISA, Competitive ELISA (cElisa).

IgM antibodies appear in the blood 3-4 weeks after infection (peak 6-8 weeks) and disappear within 4-6 months. Their presence is evidence of fresh infection. Their low titre may persist for many months after successful treatment. Transmission of fresh infection into the late one results in an increase in IgG antibody titers, with lowering IgM. IgG antibodies it is detectable 6-8 weeks after infection and persists for many years (up to 10 years in patients treated with antibiotics). Assessing the presence and level of antibodies is efficient for finding IgG antibodies that are characteristic of late Lyme disease. As Schoen and Steere *et al*. [23,28] noted in their study, the vast majority of patients infected with *Borrelia* spirochetes show a strong IgG antibody response. Then, the positive immunoassay test result should be verified by Western blot. False positive results are due to other diseases such as syphilis, anaplasmosis, leptospirosis, some autoimmune diseases, bacterial endocarditis, *Helicobacter pylori* infection, Epstein-Barr virus.

WESTERN-BLOT (immunoblot) test

It is a test with sensitivity of up to 80%. It involves searching for *Borrelia* bacteria antibodies (it is positive, if there is confirmation of at least 2 positive IgM and 5 positive IgG results). The test uses antigens that come from different genospecies of *Borrelia* spirochete. However, it does not cover all of them. When choosing an immunoblot, it is important to make sure that it covers as many various bacterial genospecies antigens as possible [28].

The IgM antibodies are most commonly detected for the following antigens: VlsE and p39 (most commonly for *Borrelia burgdorferi* sensu lato), OspC – (for *Borrelia afzelii*, *Borrelia burdorferi* sensu stricto, *Borrelia garinii*, *Borrelia spielmani*). The presence of IgM anybodies, anti-IP41(flagellin), tends to produce false-positive results due to the fact that its protein occurs also in other pathogens, for example *Helicobacter pylori*.

The IgG antibodies are most often sought against the following antigens: VlsE - cell wall lipoprotein (mainly *Borrelia afzelii*, *Borrelia burgdorferi*, *Borrelia garinii*), lipids in cell-membrane (mainly *Borrelia afzelii*, *Borrelia burgdorferi*), p83 cell-wall associated protein, IP41 (flagelin), p39 (BmpA), *Borrelia burdorferi* BmpA outer surface protein, OspC, *Borrelia* antigens: (p18, p19, p20, p21, p58).

In the early stage of Lyme disease, most patients show a strong IgM reactivity with OspC protein. At the late stage, it is IgG antibodies that predominate.

CIC test (circulating immune complexes)

The test involves finding the presence of immune complexes between the antibody and *Borrelia* antigen. Immuneoglobulin complexes are not detectable by standard EIA or Western blot. Undetectable complexes give

false-negative results in patients with clinical signs of Lyme disease [29]. It is a test with sensitivity of 90%. Spirochetes antigens are detected after being released from antigen (spirochete). The test consists of 3 stages: the isolation of circulating immune complexes; the dissociation, or the "breakup" of the complexes; and the detection of antibodies released from the complexes with EIA or Western-Blot.

LTT lymphocyte transformation test, *Borrelia ELiSpot test*, *LTT MELISA* [30, 31, 32].

The *Borrelia ELiSpot* tests assess the presence of B or T lymphocytes in the blood of the patient with a *Borrelia* spirochete antigen. B lymphocytes are normally involved in humoral response and produce antibodies (immunoglobulins). T lymphocytes are responsible for the immune cell response. These tests are particularly useful in the diagnosis of seronegative Lyme disease in which for various reasons T lymphocytes stimulated by contact with spirochetes do not produce antibodies. Then, standard EIA and Western blot tests give false-negative results in patients with clinical signs of Lyme disease.

Lyme Trace ELISA IgG

The test detects antibodies to the VIsE protein specific for *Borrelia burgdorferi* spirochete. It assesses the IgG antibodies.

Test C6 Lyme Elisa [33].

The test searches for IgG and IgM antibodies produced against the C6 or IR6 peptide. These peptides are fragments of the VLsE protein characteristic of all *Borrelia* genospecies. Since the peptides are only present in *Borrelia* spirochete, the test excludes the appearance of cross reactions.

Direct research

Direct examination involves culture, PCR, histological examination, LUAT.

Inoculation - culture [34].

This is the most obvious method of direct diagnosis, which detects spirochetes rather than the reaction of the body. However, growing *Borrelia burgdorferi* spirochete takes about 3 months and the negative result does not exclude infection. Typically, bacteria grow on media very reluctantly, so after 3 months, DNA fragments are detected by PCR.

PCR (polymerase chain reaction), PCRrt (reverse transcriptase PCR) [35]

Polymerase chain reaction (PCR) is a technique to search for the DNA of *Borrelia* bacteria. The tested material includes blood, cerebrospinal fluid, synovial fluid, synovial membrane. Unfortunately, due to poor standardization of the test, its results are unreliable. A slightly better sensitivity is obtained by the RT-PCR assay (reverse transcriptase).

Histological examination

It involves the examination of skin samples if skin lymphocytic lymphoma and chronic atrophic dermatitis develop.

LUAT Lyme Urine Antigens [36]

The test detects spirochetes antigens in urine which are then assessed by PCR.

The International Lyme and Associated Diseases Society also recommends that the test be conducted for the search for co-infection of tick-borne pathogens disseminated by ticks with *Borrelia burgdorferi* spirochetes.

Discussion and conclusions

Attempts to standardize diagnostic tests have been made by several organizations, including the World Health Organization, the American Society for Infectious Diseases, the European Concerted Action on Lyme Borreliosis (EUCALB), the Polish Society of Epidemiologists and Physicians of Infectious Diseases, the International Lyme and Associated Diseases Society (ILADS) and Centers for Disease Control and Prevention (CDC).

Most of them, including the Polish Society of Epidemiologists and Physicians of Infectious Diseases, recommend two-stage indirect serodiagnosis [37]. Accordingly, the International Lyme and Associated Diseases Society has considerably extended its diagnosis by seeking confirmation of *Borrelia* spirochete infection by using both direct and indirect tests.

After the antibiotic therapy, Lyme disease often enters the chronic phase, during which time the symptoms are uncharacteristic. In some patients, they tend to gradually disappear along with the disappearing changes detected in laboratory. In other patients, clinical symptoms are aggravated by laboratory tests and further antibiotic therapy is required. There is also a large group of those patients whose clinical symptoms do not subside after treatment, although the results of standard laboratory tests have returned to normal and do not indicate infection [38]. Another group of patients concerns those whose post-treatment disease aggravates after an asymptomatic period, and the standard two-stage diagnosis proposed the Polish Society of Epidemiologists and Physicians of Infectious Diseases does not confirm the clinical status.

The general rule is not to undertake treatment in patients who, despite positive serological findings, have no clinical symptoms. The fate of such patients has not yet been fully understood and described. Will clinical manifestations occur in untreated patients with double strength after years being infected, or have these persons developed spirochete resistance and therefore never show a clinical form of the disease?

There is also a phenomenon of seronegative Lyme disease, which is not recognised by everybody. In such patients, despite full-blown clinical Lyme disease, serological tests show no infection. It concerns those who have undergone a long-term ineffective diagnosis for other diseases and, because of lack of laboratory evidence, remain untreated for any disease (including Lyme disease).

As there has appeared a need for more in-depth diagnosis of Lyme disease, doctors and scientists from the International Lyme and Associated Diseases Society have been looking for other diagnostic methods by analyzing the pathomechanisms that govern the *Borrelia burgdorferi* spirochete. Not all Lyme disease diagnostic methods are standardized and therefore reliable. This applies both to the methods of two-stage serological diagnosis proposed by the Polish Society of Epidemiologists and Physicians of Infectious Diseases, where each laboratory presents different results for the same patient, as well as the diagnostic methods proposed by the International Lyme and Associated Diseases, which have not been sufficiently tested in research on big reliable patient groups.

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