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DIAGNOSIS AND TREATMENT OF TICK-BORNE DISEASES RECOMMENDATIONS OF THE POLISH SOCIETY OF EPIDEMIOLOGY AND INFECTIOUS DISEASES

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Since many years, an increase in the prevalence of tick-borne diseases, especially: Lyme borreliosis, babesiosis, human granulocytic anaplasmosis and tick-borne encephalitis is reported in Europe, Asia and America. Geographical distribution and population of *Ixodes ricinus* ticks, present in Europe, are subject to systematic changes. The highest population of ticks is reported in forests, while the lowest in open meadows and pastures. In case of *Dermacentor reituculatus* ticks, the highest population is noted in meadows, open pastures and mid-forest glades in deciduous forests.

A risk of infection transmission to human is dependent on the geographical region and the prevalence of pathogens in animals and ticks. *Tijsse-Klasen* et al. estimate that the risk of local symptom appearance at the site of tick bite is 11.4%. A risk of borreliosis following one-time tick bite is assessed at below 1%, while in case of rickettsioses, ehrlichiosis or babesiosis it is below 0.5%. It is estimated that the probability of producing specific antibodies against *B. burgdorferi* following the tick bite amounts to 3–6%. Clinically overt disease occurs in 0.3–1.4% of cases bitten by ticks.

In Europe, the highest Lyme borreliosis incidence is reported in Germany, Austria, Slovenia, Sweden and Poland. According to the data of the National Institute of Public Health-National Institute of Hygiene (NIPH-NIH), the number of borreliosis cases is on the systematic increase in Poland – in 2012, a total of 8,794 infections were reported (incidence: 22.8/100, 000), while in 2013 – 12,763 (incidence: 33.12/100,000). The highest incidence rate in 2012 was registered in 2012 in podlaskie (81.4/100,000) and warmińsko-mazurskie provinces (47.9/100,000).

In Europe, tick-borne encephalitis (TBE) is the second most commonly reported disease transmitted by *I. ricinus* ticks. In 1990-2007, a total of 157,584 infections were registered in Europe, which accounted for 8,755 cases per year on average. In Poland, the number of TBE cases registered by the NIPH-NIH in 2012 was 189 (incidence: 0.49/100,000), while in 2013 - 225 (incidence: 0.58/100,000). The highest incidence is reported in podlaskie province. In 2012, a total of 101 cases (incidence: 8.5/100,000) were reported there, which accounted for 53.44% of infections registered in Poland, while in 2013 – 111 cases (incidence: 9.2/100,000), i.e. 49.33% of the total number of cases in Poland.

TICK-BORNE ENCEPHALITIS

Tick-borne encephalitis virus of the family *Flaviviridae* includes three subtypes: European subtype referred to as Western (W-TBEV) (*I. ricinus*), Siberian subtype (S-TBEV) and Far Eastern subtype (FE-TBEV) (*I. persulcatus*). All three subtypes may be present in one region (e.g. Estonia, Lithuania).

Infection is transmitted while being bitten by an infected tick (virus is found in the tick's salivary glands) or, less frequently, through food-borne route by consuming unpasteurized milk from infected goats, sheep or cows or diary products (yoghurt, cheese, butter). Pasteurization of milk entirely protects against infection.

On a rare basis, infection has also been reported to be acquired in laboratory settings (accidental needle-stick injury, damage) or through the air mode.

Incubation period for TBE ranges from 4 to 28 days following the tick bite and from 3 to 4 days in case of food-borne infection.

Course of infection. A typical biphasic course of disease is observed in 74-87% of patients. Abrupt onset of disease, influenza-like symptoms, fever, headaches, nausea and vomiting are indicative of the first phase

which lasts for 4 (1-8) days. Laboratory findings in this period reveal leukopenia, thrombocytopenia and increased concentration of albumin in CSF. For 13-26% of cases, infection terminates along with the first phase, while the remaining cases, usually after 8 (1-33) days of presenting good mood, experience the second phase, referred to as neurologic phase. Symptoms such as fever up to 40°C, headaches, nausea, vomiting, meningeal symptoms, myalgia and arthralgia recur. The following manifestations are distinguished: **meningitis**, of the mildest course, accompanied by symptoms typical of lymphocytic meningitis (ca 49% of patients); **meningoencephalitis**, of more severe course, accompanied by symptoms indicative of encephalitis such as ataxia, disturbances of consciousness and, sometimes, cranial nerve paralysis; **meningoencephalomyelitis,** of the most severe course (ca 10% of patients), accompanied by symptoms indicative of the damage of anterior horn of spinal cord and flaccid paralysis; involvement of medulla and brainstem exacerbates the prognosis; **meningoencephaloradiculitis** accompanied by meningeal symptoms, focal signs of CNS damage and injury of nerve roots and peripheral nerve.

Fatality. Fatality due to TBE amounts to 5%.

Complications. Prevalence of neurological complications is estimated at 20-50%, of whom the most prevalent are: paralysis or paresis of cranial nerves, polyneuropathies with paralysis of various muscle groups, particularly in the region of the shoulder girdle, flaccid paralysis, cerebellum damage (gait disturbances, speech disorders, nystagmus, intention tremor), epileptic attacks as well as intellectual disorders, concentration disturbances, attention deficit disorders and impairments of long-term and short-term memory.

Diagnostics. TBE virus may be isolated from serum and cerebrospinal fluid (CSF), and its genome may be detected, using RT-PCR (*reverse-transcriptase polymerase chain reaction*), in blood serum and CSF in the acute stage of infection. It is of marginal significance in routine diagnostics as in the neurologic phase, when patients are hospitalized, virus is not present in serum and CSF.

Diagnostics is based on the detection of IgM and/or IgG antibodies in blood serum and CSF by ELISA. IgM antibodies occur in serum after 10-14 days following infection and disappear after a few weeks. IgG antibodies are present after 3-4 weeks following infection with a peak noted at week 6. In symptomatic phase, antibodies of both classes may be detected in serum and CSF.

In the incubation period and the first, non-characteristic phase of disease, antibodies are not detectable in blood serum and CSF. In such case, test should be performed again in the successive 1-2 weeks.

In the initial phase, basic laboratory testing reveals thrombocytopenia and leukopenia, sometimes, increased activity of alanine and aspartate aminotransferases.

CSF examination reveals cytosis ranging from several dozen to over 100 cells in 1 μl with a predominance of granulocytes in the first four days of infection, and then lymphocytes predominate. Glucose and chloride are within normal levels while protein concentration may even exceed 100 mg/dl.

Treatment. Treatment consists in application of analgesics, anti-inflammatory drugs and drugs reducing intracranial pressure (20% mannitol). In specific situation, corticosteroids may be applied.

Prevention. Use of vaccinations. Vaccine schedule should be in line with the vaccine manufacturer's indications.

Basic vaccination comprises two doses of vaccine which are applied in an interval of 1-3 months and the third dose which is given 5-12 months following the second dose. Booster doses, dependent on the exposure to infection, are applied from 3 to 5 years following the last vaccination. There is also an accelerated schedule based on immunization on day 0, 7 and 21, and then after one year. Vaccines for children include a half of TBE virus dose intended for use in adults. Vaccine schedule, which is employed correctly, results in a sustained protective immunity in case of 98% of vaccinated persons.

Encepur and FSME IMMUN, vaccines against TBE, are effective, safe and highly immunogenic. They induce the production of neutralizing antibodies, presenting cross-reactivity to various virus strains in Europe and Asia (including Siberian and Far Eastern subtypes).

LYME BORRELIOSIS

Lyme borreliosis is a multi-organ disease caused by spirochaetes *Borrelia burgdorferi*, transmitted by *Ixodes* ticks, whose clinical picture is associated with the involvement of skin, joints, nervous system and heart.

A total of 18 genospecies of spirochaetes composing *Borrelia burgdorferi sensu lato* complex, present in wild animals and transmitted by ticks were identified. Of them, *B. burgdorferi sensu stricto*, *B. garinii, B. afzelii* and *B. bavariensis,* detected in 2009, are pathogenic for humans while *B. bissettii*, *B. valaisiana* (isolated from patients diagnosed with borreliosis), *B. lusitaniae* (unclear role, clinical symptoms do not correspond to well-known signs of borreliosis) and *B. spielmanii* (isolated from skin with *erythema migrans*) are potentially pathogenic.

Diagnosis

Diagnosis of Lyme borreliosis is made if at least one of the following clinical symptoms is present:

Borreliosis		Symptoms	Laboratory testing	PCR
Early stage	*Erythema migrans*	Erythema at the bite site up to a month after the bite, sometimes multiple erythema	No serological testing is recommended	Sample of skin from a margin of erythema
	Borrelial lymphoma	Livid red colour nodule on the ear lobe, scrotum, nipple. Past or current EM	ELISA Detection of IgG and/or IgM	
	Early neuroborreliosis	Meningoradiculitis, meningitis. Paralysis of nerve VII and, potentially, other cranial nerves	Lymphocytic pleocytosis in CSF. ELISA IgM and/or increased IgG titre in serum. Intradural production of antibodies against *B. burgdorferi*	Cerebrospinal fluid
	Lyme carditis	Past or current EM. Atrioventricular block. Cardiac arrhythmia, myocarditis or pancarditis	ELISA IgM + IgG antibodies (increasing IgG titre)	
	Lyme arthritis	Oedema, mobility restriction of joints, monoarthritis, oligoarthritis, poliarthritis	ELISA IgM and/or increased IgG titre in serum	Synovial fluid
Late stage	Neuroborreliosis	Encephalomyelitis, radiculoneuritis, meningitis, occlusive vasculitis, cerebral infarct	ELISA IgM and/or increased IgG titre in serum. Intradural production of antibodies, lymphocytic pleocytosis	Cerebrospinal fluid
		Peripheral polyneuropathies	IgG antibodies in serum	
	Lyme arthritis	Oedema, mobility restriction of joints, monoarthritis, oligoarthritis, poliarthritis	ELISA IgG antibodies in serum	Synovial fluid
	Acrodermatitis chronica athrophicans	Livid red colour skin, gradual skin atrophy	ELISA IgG antibodies in serum Histopathological examination of skin sample	Sample of changed skin

Erythema migrans (EM)

It is a typical marker of **early localization** of infection. It occurs between day 3 and 30 following the infection in ca 80% of patients. In case of adults, it is usually present on limbs and trunk while in children on head and neck. Erythema is spontaneously resolved within a few days to weeks (4 weeks on average) in persons who are not treated with antibiotics.

Diagnosis is based exclusively on the clinical picture. Initiation of treatment should not be dependent on serological testing.

Atypical manifestations of erythema of irregular shape, with ecchymosis or blisters, displaying the tendency to the enlargement of diameter (over 5 cm) should be treated as erythema migrans. In case of atypical manifestations, laboratory confirmation may be useful, however, only two weeks after the appearance of the lesion.

EM is confused with the lesions appearing shortly after the bite of insects, which are of high intensity and which resolve quickly without any antibiotic therapy.

Treatment of EM in pregnant women with β-lactam antibiotics is effective. It is not associated with a risk of complications to mother and newborns.

Serological diagnostics is not significant in typical cases of erythema migrans as the presence of characteristic skin lesion and history of tick bite are sufficient enough for diagnosis. Erythema of diameter over 5 cm is of diagnostic significance.

Treatment with doxycycline (2×100 mg) or amoxicillin (1.5–2.0 g/24 h) or cefuroxime axetil 2×500 mg lasts for 14-28 days.

Multiple erythema is an evidence of spirochetemia, and not multiple tick bites. It is recommended to treat the patients with multiple erythema, without involvement of nervous system or other organs, with the same antibiotics as in case of patients with single erythema.

BORRELIAL LYMPHOMA

It is a rare manifestation of Lyme borreliosis which occurs mainly in Europe. It is manifested by a single, painless nodule of livid red colour, which appears on the ear lobe, auricle, nipple or scrotum within 2 months following the infection. It occurs in ca 2% of patients, more frequently in children compared to adults.

It requires to be confirmed by serological testing. IgM antibodies and increased IgG titre are most commonly reported.

Treatment with doxycycline (2×100 mg) or amoxicillin (1.5–2.0 g/24 h) or cefuroxime axetil 2×500 mg lasts for 14-28 days.

LYME CARDITIS

Changes in heart muscle occur in the early stage of infection, around day 21 from the onset on average (from week 1 to month 7). Males are affected more frequently compared to females. Its typical features are: abrupt onset, atrioventricular conduction abnormalities: first degree, second degree or complete atrioventricular block, bundle branch block or block of electrical conduction system bundles. It is a rare phenomenon that lyme carditis results in myocarditis, pericarditis, heart failure or chronic congestive cardiomyopathy.

Serological testing most frequently reveals IgM antibodies and increased IgG titre.

Treatment with doxycycline (2×100 mg) or amoxicillin (1.5–2.0 g/24 h) or ceftriaxone (2,0/24 h) lasts for 28-30 days. Prognosis is good, in case of more than 90% of patients disorders resolve following treatment.

LYME ARTHRITIS

Lyme arthritis (LA) is a common manifestation of infection with *B. burgdorferi* in both **early disseminated stage** of borreliosis and its **late stage**.

In Europe, where borreliosis is more frequently caused by *B. garinii* and *B. afzelii* than *B. burgdorferi sensu stricto,* LA is observed in only 3–25% of patients.

Most frequently, ailments are restricted to large joints, especially knee joint, less often, shoulder, elbow, wrist, hip and ankle joints. Temporomandibular joints and small hand and leg joints are very rarely affected. Episodes of acute arthritis, accompanied by painful oedema, or less frequently, by increased temperature and redness, most commonly affecting asymmetric, single large joints, may persist for a few weeks. Very rarely, symmetric multiple joint inflammation is reported. Most often, disease is of self-limited course.

LA symptoms resolve following the oral administration of doxycycline for 1–2 months and intravenous therapy with ceftriaxone. If symptoms are still present, regardless of antibiotic therapy, it may suggest antibiotic-resistant arthritis. In such situation, further treatment with antibiotics is dependent on the identification of *B. burgdorferi* DNA in synovial fluid. If DNA of spirochetes is not detected, treatment with non-steroidal anti-inflammatory drugs or steroids should be considered as well as synovectomy.

In case of lyme arthritis in **early disseminated stage** of borreliosis, serologic testing most frequently reveals IgM antibodies and increased IgG titre. In the course of **late stage** borreliosis, IgG antibodies are detected while IgM antibodies are not of diagnostic significance.

Treatment with doxycycline (2×100 mg) or ceftriaxone (2.0/24 h) lasts for 28-30 days.

NEUROBORRELIOSIS

Neuroborreliosis (NB) is the most common manifestation of disseminated infection with *B. burgdorferi* in Europe; in patients in the USA, it is reported less frequently.

In the **early disseminated stage**, neuroborreliosis may be manifested by:

1. cranial nerve paralysis, most frequently of the facial nerve, which may be accompanied by inflammatory changes in cerebrospinal fluid (CSF). Paralysis of other cranial nerves, including abducent nerve and olfactory nerves occurs rarely. Each paralysis of nerve VII has to be treated with antibiotics in order to prevent further borreliosis progression. Oral administration of antibiotic is effective, while the use of steroids does not accelerate the regression of paralysis.
2. paralysis of nerve roots or single peripheral nerves,
3. meningitis, encephalitis or encephalomyelitis.

In the **late stage**, neuroborreliosis may proceed as encephalomyelitis of slow, progressive course with the involvement of white matter. In differential diagnosis, multiple sclerosis should be considered, which can be excluded based on CSF examination and the presence of antibodies against *B. burgdorferi* in blood serum.

Cerebral borrelial vasculitis is a rare manifestation of neuroborreliosis. It may result in cerebral infarction or stroke.

In the **late borreliosis**, neuroborreliosis may also proceed as peripheral neuropathy which is characterized by disturbances of sensation, paraesthesia, nerve root pain, and sometimes paresis.

Symptoms of neuroborreliosis are not specific, thus, it has to be confirmed by the presence of pleocytosis in CSF and intradural production of antibodies against *B. burgdorferi* of both classes, i.e. IgM and IgG.

Index of antibody production in the early stage of disease may be negative. Then, a criterion of inflammatory process is pleocytosis in CSF.

Intradural production of antibodies may persist even for a few months following the regression of inflammation.

Pursuant to the recommendations of the *European Federation of Neurological Societies* (EFNS), neuroborreliosis may be confirmed if the following criteria are met: patient presents neurological symptoms indicative of neuroborreliosis and pleocytosis in CSF and intradural

production of antibodies against *B. burgdorferi* are demonstrated. Possible diagnosis of neuroborreliosis may made if at least two of the following three criteria are met: presence of peripheral neuropathy, appearance of acrodermatitis chronica atrophicans of limbs (ACA) and identification of antibodies against *B. burgdorferi* in blood.

A lack of specific, intradural antibodies, however, does not exclude neuroborreliosis (testing in early stage of inflammation, temporary immunosuppression induced by co-infection with tick-borne pathogens, therapy with steroids). Then, identification of spirochete DNA in CSF may be of decisive nature.

Treatment:

- Paralysis of cranial nerves – doxycycline 2 x 100 mg for 14-28 days,
- Meningitis, radiculopathia, cerebral borrelial vasculitis - doxycycline 2×100 mg or ceftriaxone 2.0 g/24 h i.v. for 14-28 days,
- Encephalomyelitis, radiculoneuritis, meningitis, occlusive vasculitis, cerebral infarct - ceftriaxone 2.0g/24 h for 21-28 days.

ACRODERMATITIS CHRONICA ATROPHICANS

Acrodermatitis chronica atrophicans (ACA) is a red or livid red lesion which appears most frequently on the skin of distal parts of limbs, a few or several days following the infection (after 10 years on average). Skin lesions may also appear on face and trunk. Sometimes, ACA is accompanied by polyneuropathy. Then, patients complain about pain, pruritus and paraesthesia.

Serologic testing in ACA should confirm the presence of IgG antibodies.

Antibiotic therapy lasts for 14-21 days. Treatment with doxycycline 2×100 mg p.o., or ceftriaxone 2.0 g/24 h i.v., or amoxicillin 1.5–2.0 g/24 h p.o. or c*efuroxime axetil 2 × 500 mg p.o.* is effective.

REINFECTION

Infection with *B. burgdorferi sensu lato* does not provide sustained protective immunity. To confirm the reinfection, it is required to demonstrate the presence of erythema migrans or identify seroconversion between acute and convalescent stage of disease in serologic testing.

DIAGNOSIS

Diagnosis of Lyme borreliosis should be based on the criteria, of whom the most important are a history of tick bite and clinical symptoms. Laboratory diagnostics is based on 'two-tiered diagnostic protocol' which consists in detection of specific antibodies, using immunoenzymatic method and Western blot, most optimally with recombinant antigens (p100, p58, p41i, VlsE, OspC, DbpA), instead of cell lysate antigens. ELISA and Western blot display similar sensitivity, however, specificity of Western blot is higher, as interpretation consists in identification of specific immunoreactive bands. Irrespective of antibiotic therapy, seroconversion is usually present after 2 weeks from the onset.

False positive results in ELISA are associated with the presence of poorly reactive antibodies against 41-kDA and OspC antigens in serum of patients suffering from other infectious and non-infectious diseases. Such results may be present, inter alia, in patients diagnosed with syphilis or other spirochetoses, bacterial endocarditis, rheumatoid arthritis, infectious mononucleosis, autoimmune diseases, *Helicobacter pylori* infection. They apply mainly to IgM antibodies.

Specific IgM antibodies may persist for a few years following the treatment. Their presence in the late borreliosis is of not diagnostic significance. Neither it is indicative of active stage of infection nor it is an indication for treatment. IgG antibodies persist for many years, however, their titres in ELISA or the number of bands in Western blot show a tendency for slow decrease.

The majority of patients with early disseminated borreliosis are seropositive (strong IgM reactivity to OspC in Western blot). Patients with late borreliosis have high IgG antibody titre and many IgG bands in Western blot.

Practically, the lack of antibodies against *B. burgdorferi* in patients suspected of late Lyme borreliosis excludes this disease.

PCR TESTING

Nowadays, it is recommended to detect DNA of *B. burgdorferi* spirochetes by PCR, using skin sample with erythema migrans or acrodermatitis chronica atrophicans of limbs, synovial fluid and CSF. It is not indicated to perform PCR in blood sample. It is possible to perform PCR in CSF up to 6 weeks from the infection in a period when immunoserological tests are still negative.

A lack of standardization is a limitation of PCR in the diagnosis of Lyme borreliosis.

Methods which have not been approved to have diagnostic significance, employing the following processes, should not be applied in the diagnostics:

1. determination of CXCL13 chemokines and B lymphocytes,
2. searching for *B. burgdorferi* antigens in CSF and urine,

3. searching for cysts, spheroplasts or *B. burgdorferi* L-forms,
4. assessment of CD57+/CD3 lymphocyte subpopulation,
5. lymphocyte transformation test (LTT).

TREATMENT

Treatment of Lyme borreliosis lasts for at least 21 days. It is based on antibiotic therapy, which dependent on clinical manifestation of disease and patient's tolerance, includes mainly: doxycycline, amoxicillin, cefuroxime, ceftriaxone or cefotaxime.

A list of second-line antibiotics includes: clarithromycin, azithromycin and erythromycin. Macrolides are of lower effectiveness compared to tetracyclines. They are not recommended to be used in the first-line therapy. They may be applied if there is intolerance or contraindications to the therapy with the first-choice antibiotics.

It is not recommended to use the following drugs and regimens in the treatment of Lyme borreliosis:

- first-generation cephalosporins, fluoroquinolones, carbapenems, vancomycin, metronidazole, tinidazole, trimethoprim-sulfamethoxazole, benzathine penicillin or fluconazole,
- initiation of multi-month antibiotic therapy or its multiple repeating as well as use of combination therapy with a few antibiotics.

Prophylactic antibiotic therapy following tick bite is not recommended. It should be considered in individual, special cases when a person was bitten by tick repeatedly while staying in borreliosis endemic areas.

HUMAN GRANULOCYTIC ANAPLASMOSIS (HGA)

Human granulocytic anaplasmosis (HGA) is a zoonosis which occurs in temperate climate zone in the northern hemisphere in Europe, North America and Asia. First cases were reported in 1994 in the USA, and in Poland in 2001.

Etiological agent. *A. phagocytophilum* is a small Gram-negative bacterium of pleomorphic forms with a predominance of spherical or oval forms, most often granulomas. It is an intracellular bacterium with a tropism for neutrophil granulocytes.

Vectors are *Ixodes* ticks*: I. Ricinus* in Europe, *I. scapularis, I. pacificus, I spinipalpis* in North America*, I. persulcatus* and *I. ovatus* in Asia.

Wild ungulates (deer, roe-deer), small and large rodents are the reservoirs. Percentage of infected ticks in Europe, dependent on the region and method adopted, ranges from 1.1% to 19.5%, however, in the majority of countries it amounts to 2-3%.

Infection. *A. phagocytophilum* is most commonly transmitted through bite of infected ticks, however, perinatal and transfusion-induced infections may also occur.

Clinical picture. Infection in humans is of uncharacteristic course, ranging from asymptomatic, mild to severe which may result in patient's death.

Symptomatic infection is a fever disease of acute course, whose incubation period varies between 5 and 21 days (11 days on average). Typical symptoms include: fever up to 38-39 °C, headache, myalgia, arthralgia and malaise. Vomiting, nausea, stomach ache, diarrhoea and cough may also occur. Symptoms are often accompanied by splenomegaly and hepatomegaly with hepatocyte damage. Fever persists for 2-11 days (10 days on average). In the majority of patients, symptoms resolve within 30 days. In less than 10% of patients, maculopapular or petechial rash is present on the whole body, except for face, hands and feet soles.

In the majority of cases, *A. phagocytophilum* infection is of asymptomatic, self-limited course, which is confirmed exclusively by the presence of specific antibodies.

Infections occur mainly between April and October with a peak reported in July.

Injury of the central nervous system in the course of HGA occurs only in ca 1% of patients. Cases of brachial plexus damage, paralysis of facial nerve and other cranial nerves, polyneuropathies persisting for a few months were described. Examination of cerebrospinal fluid reveals lymphocytic pleocytosis and slight increase of protein concentration. In differential diagnosis, tick-borne encephalitis, Lyme boreliosis, *B. burgdorferi* co-infection and opportunistic infections should be taken into account.

Fatality in patients does not exceed 1%, however, in case of children with impaired immunity and elderly it ranges from 7 to 10%.

Severe clinical course is observed in patients at elder age, those treated with immunosuppressant drugs, with chronic inflammatory diseases or cancers.

Reinfection. HGA infection results in sustained protective immunity. Antibodies persist for a few years.

Laboratory diagnostics. In the first week of disease, leukopenia, thrombocytopenia, slight increase of aspartate (AspAT) and alanine (ALAT) aminotransferase activity as well as lactate dehydrogenase (LDH), alkaline phosphatase, sometimes increase of bilirubin, CRP protein, creatinine concentration and hyponatraemia are reported in 50% and 70% of adults and children, respectively.

Such changes resolve within 14 days after the onset of disease.

Blood smear. Peripheral blood smear, stained with May-Grünwald-Giemsa method is a quick diagnostic technique. Examination reveals the presence of *A. phagocytophilum* insertions (morulae) in neutrophil granulocytes. Blood smear has to be performed prior to the treatment with doxycycline as morulae disappear from blood within 24-72 hours following the initiation of therapy with this antibiotic. This test is of the highest sensitivity when it is performed within 7 days following the onset of fever.

PCR-based diagnostics. In the early stage of infection, *A. phagocytophilum* DNA may be detected in blood, using PCR. It is characterized by high specificity and sensitivity amounting to 67-90%. This test is of the highest sensitivity when it is performed in the first week of disease in bacteraemia.

Treatment with doxycycline should be initiated following the collection of blood for PCR testing, however, prior to achieving the test result (treatment with doxycycline hinders the detection of bacterium DNA).

Immunoserological diagnostics. Serological testing, using fluorescent immunoassay (IFA), is a gold diagnostic standard. IgM antibodies appear within 10-14 days following the infection. Positive result is demonstrated if there is a 4-fold increase of IgM antibody titres noted in two samples collected in an interval of 2-4 weeks or appearance of IgG antibodies. Immunoserological tests show the highest sensitivity in week 2-4 of disease.

HGA culture. Due to a long time, culture on media from human promyelocytic leukemia cells is not routinely applied.

Differential diagnosis. Early stage of disease is characterized by non-characteristic symptoms, thus, it is required to be differentiated, inter alia, with: influenza and other viral infections, respiratory tract infections, leptospirosis, tularaemia, sepsis, hepatitis. In case of patient with a history of tick bite and stay in endemic area, tick-borne encephalitis, Lyme borreliosis, human babesiosis and other infections caused by *Rickettsia* should be taken into account.

Treatment. It is recommended to initiate treatment with doxycycline for 5-14 days (contraindications to use doxycycline should be considered, especially in case of children <12 years old and pregnant women).

Rifampicin is the antibiotic of the second choice. In case of adults, it is used in the following dosage: 2 times for 300 mg p.o., while in children - 10 mg/kg (maximum 300 mg/dose). Treatment should be continued for 3 days following the regression of fever.

Fluoroquinolones, β-lactam antibiotics, cephalosporins, macrolides and aminoglycoside should not be applied.

It is not recommended to use antibiotics following the tick bite as a prophylaxis due to a very low risk of HGA infection.

BABESIOSIS

Babesiosis is a parasitic disease transmitted by *Ixodes* ticks. Out of 100 *Babesia spp., Babesia microti*, *Babesia divergens*, *Babesia venatorum*, *Babesia duncani* are pathogenic for humans.

Reservoirs are small rodents, cattle, sheep, deer, reindeer, horses, dogs.

Vector. *Ixodes* ticks transmit *B. microti* (North America, Europe, Asia) to humans and rodents, while *B. divergens* (Europe) to humans, cattle and deer.

Infection. Infection may be transmitted through tick bites, blood transfusion (blood donors with asymptomatic parasitemia) and placenta – newborn babesiosis.

Clinical picture. Symptomatic form is present within 1-6 weeks following the tick bite or 6-9 weeks after blood transfusion. Usually, it is of mild course. Influenza-like, non-specific symptoms predominate, including: apathy, fever, hyperhidrosis, chills, myalgia, arthralgia, headache, nausea and vomiting.

Severe course of *Babesia* spp. infection, resembling malaria, is usually associated with extensive parasitemia. Characteristic symptoms include: hepatomegaly, splenomegaly, progressive hemolytic anemia, jaundice, renal failure with haemoglobinuria, proteinuria, and even acute respiratory distress syndrome (ARDS) or heart and cardiovascular system complications. Hemolytic anemia may persist for a few days or months – usually in persons with a history of splenectomy or those at elder age.

Diagnostics. Laboratory testing. Complete blood count reveals: hemolytic anemia, thrombocytopenia and leukopenia. In urinalysis, haemoglobinuria, microhematuria and proteinuria are reported. Furthermore, increased aminotransferase activity is reported while renal failure is accompanied by increased urea and creatinine concentration.

Blood smear. **Microscopic analysis of stained blood smears (thin and thick blood smear) is a gold standard in the diagnosis of babesiosis.**

Within 12-17 days following the tick bite, blood smear, stained with May-Grünwald-Giemsa method reveals intraerytrocytic insertions of pear-shaped, oval, amebic or annular forms with blue cytoplasm and red chromatin. It is required to exclude infection with *Plasmodium*.

There is a necessity to determine the intensity of invasion – the percentage of infected erythrocytes (thin and thick blood smear). In case of low parasitemia, test

should be repeated in 2-3 days. Parasitemia may persist in untreated patients even for over a year.

Molecular testing. PCR allows for identification of *Babesia* spp. genetic material in blood in the early stage in patients with low parasitemia or without detectable parasitemia. It is required to be employed in the detection of protozoan in each case.

In molecular diagnostics of babesiosis, genes with highly conserved regions: *18S rDNA*, *β-tubulina* and *hsp70* are applied.

Positive test result by PCR has to be repeated, using product sequencing. It allows for determining parasite species and excluding false positive results.

Serological testing. Production of specific anti-*Babesia* antibodies begins within ca 14 days following the infection. It may be detected, using fluorescent immunoassay (IFA). Test result is considered to be positive if IgM and IgG antibody titres are 1:64, while higher titres (1:128 and 1:256) finally confirm *Babesia* spp. infection. These antibodies may be detected within 1-6 years following the infection.

Available standardized fluorescent immunoassay (IFA) tests for the presence of IgM and IgG antibodies against *B. microti* have limited usability in Europe as *Babesia microti* is not a predominant and only one etiological agent of human babesiosis.

Laboratory testing for babesiosis should be performed exclusively in case of justified suspicion of infection in patients living or coming from endemic areas with a history of tick bite and patients who have undergone blood transfusion within 6 months prior to the onset of symptoms and pregnant women bitten by ticks.

Treatment. The following antibiotics are used: azithromycin, clindamycin, quinine, atovaguone.

Empirical treatment should not be initiated if objective symptoms of disease and parasitemia in blood are not demonstrated!

Asymptomatic infection should not be treated until parasitemia persists for over 3 months (blood smear or PCR). Then, treatment with atovaquone or azithromycin should be considered.

Patients with positive serology, but negative blood smear and PCR test results should not be treated.

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