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RECOMMENDED MANAGEMENT OF *TOXOPLASMA GONDII* INFECTION IN PREGNANT WOMEN AND THEIR CHILDREN

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Toxoplasmosis is an infectious parasitic disease, whose acute stage is most frequently asymptomatic. Infection occurs worldwide – the percentage of seropositive persons ranges from 5% to 90%. For the Polish population, this percentage varies between 36.0% (małopolskie province) and 62.5% (pomorskie province). Globally, the percentage of seropositive women is in the range of 10.9% (Norway) - 90% or higher (France, Tahiti). Pursuant to the American data, a risk of primary infection in pregnancy is 0.1-1.0%. In the studies of the Institute of Mother and Child in Warsaw, infection was identified in 0.39% of women who were seronegative prior to the pregnancy; more frequently in women living in rural (1.1%) compared to urban (0.27%) areas.

A risk of transmission of this protozoan through placenta was estimated at ca 40% during the whole pregnancy; in Europe, it accounts for 29%. Percentage of transmission increases with the pregnancy week and amounts to 6-8% and even 80% in the week 10 and 38 of pregnancy, respectively. A risk of fetopathy is the highest if pregnant woman is infected prior to the week 24 of pregnancy and the higher the risk, the earlier the foetus is infected. Number of congenital toxoplasmosis in Poland was determined on a basis of the study conducted by the centre in Poznań at 1-2 cases per 2,000 live births on average.

ETIOLOGY, TRANSMISSION ROUTES AND SOURCES OF INFECTION

Infection is caused by an intracellular protozoan *Toxoplasma gondii (T. gondii*) which belongs to the coccidia.

Transmission routes and sources of infection

1. Food-borne – most commonly occurs by the consumption of raw or semi-raw meat and its products containing parasite cysts, by drinking water and consuming food contaminated with oocysts excreted by cat and through unwashed hands (contact with soil). Direct contact with cat does not increase the risk of infection.
2. Vertical (mother-to-foetus) – as a result of primary infection in pregnant woman or, unusually, from mothers who are seropositive prior to the pregnancy: reinfection with a more virulent strain of the protozoan (e.g. South American strains) or reactivation of chronic infection (non-immunocompetent women).
3. Post-transfusion – rarely.
4. Iatrogenic – sporadically.

LABORATORY DIAGNOSTICS

Provided the infection with *T. gondii* is suspected, it is required to confirm or exclude it based on the results of specific laboratory testing, including serologic and molecular methods. Results of such tests should allow for determining whether the person tested is infected, if it is acute stage of infection and when the person was infected.

1. Serologic testing (detection and differentiation of specific antibody classes: IgG, IgM, IgA).

   Seroconversion, i.e. a change from seronegative to seropositive status, is a certain criterion in the diagnosis of pregnant women. In the majority of persons tested, IgA and IgM may be present even 9 months following the infection, or even longer in case of IgM, which hinders estimating the time of infection. Thus, it is recommended to determine the avidity (maturity) of IgG in pregnant women. High avidity of IgG allows for excluding the infection acquired during the last 4 months. Initiation of treatment, however, may delay the maturation of antibodies which should be considered while interpreting the results. Considerable increase of IgG level is a marker of active toxoplasmosis in tests performed in an interval of 2-3 weeks, taking into account that treatment may reduce or inhibit the production of IgG. In case of pregnant women, both classes of antibodies should be determined simultaneously, i.e. IgG and IgM (eventually also IgA) and the avidity of IgG. In the diagnosis of congenital toxoplasmosis, a profile of mother's and child's antibodies are compared (IgG and/or IgM), using Western-blot; samples of umbilical cord blood and/or venous blood serum are tested.

2. Molecular testing (detection of genetic material of *T. gondii)*.

   Detection of *T. gondii* DNA substituted testing aimed at identifying the parasite (biological test, *in vitro* culture); it is a confirmation test. Molecular tests that are applied on a routine basis are not standardized, their sensitivity ranges from 65% to 100%. Identification of *T. gondii* genetic material in a sample of body fluid (anterior chamber fluid, cerebrospinal fluid, amniotic fluid) or blood confirms the infection with *T. gondii*.

Figure 1 presents the scheme of specific diagnostics of toxoplasmosis.

CLINICAL PICTURE OF TOXOPLASMOSIS

Toxoplasmosis in immunocompetent persons

1. Asymptomatic course (most frequently reported, ca 90% of cases) or influenza-like symptoms
2. Enlargement of lymph nodes (ca 10% of cases): most frequently occipital and cervical nodes which may even persist for a few months

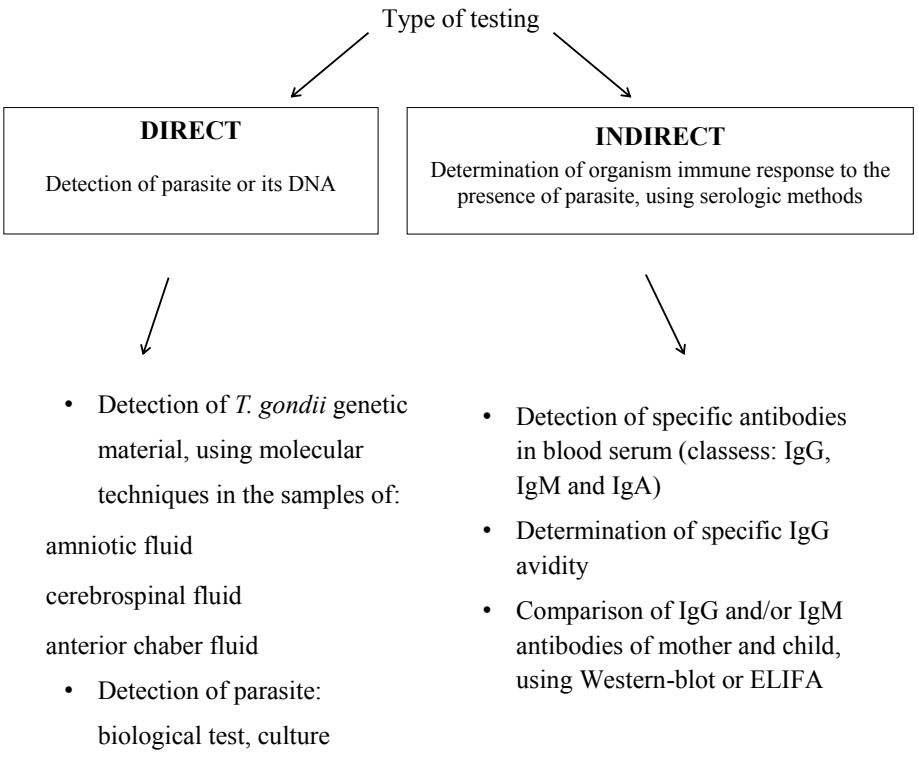

Fig. 1. Specific diagnostics of toxoplasmosis

3. Mononucleosis-like syndrome
4. Toxoplasmosis in pregnant women

Foetus may be infected due to primary infection acquired by pregnant woman, unusually due to reactivation (generally in immunosuppressed pregnant woman) or reinfection with another strain of the protozoan. Primary *T. gondii* infection in pregnant woman may result in spontaneous abortion, stillbirth, non-immune hydrops fetalis, preterm labour, intrauterine growth restriction or postpartum foetal death.

Specific serologic tests and IgG avidity test are applied to confirm the infection in pregnant woman. Foetal diagnostics includes detection of *T. gondii* genetic material in amniotic fluid. Postpartum diagnostics involves examining the placenta and serologic testing of umbilical cord blood for the presence of specific IgM (IgA) and IgG antibodies and comparing the profile of mother's and child's antibodies in blood serum, using Western-blot or ELIFA.

INTERPRETATION OF SEROLOGIC TEST RESULTS IN PREGNANT WOMAN:

1. IgM /+/, IgG /-/:
- probably false positive result; a control serologic testing is required in 2-3 weeks; further management depends on the result obtained: if the result is negative in both classes, management as in the point 3 should be initiated; provided the result is positive in both classes or IgG antibodies are exclusively present, management specified in the point 4 should be indicated.

2. IgM /-/, IgG /+/:
- probably past infection; a control testing is recommended in 2-3 weeks.

3. IgM /-/, IgG /-/:
- no infection; a serologic control until the end of pregnancy and compliance with prophylactic recommendations are required;
- detection of IgM, or IgM and IgG in the successive test suggests an active infection; it is recommended to determine IgG avidity, initiate chemoprophylaxis until the end of pregnancy and perform serologic testing every 2-3 weeks. In order to exclude/confirm mother-to-foetus transmission, amniotic fluid should be tested for the presence of *T. gondii* DNA.

4. IgM /+/, IgG /+/:
- probably active infection;
- it is recommended to determine IgG avidity. High avidity >30% (index > 300) indicates an infection acquired earlier than 4 months from the time of sample collection. Control serologic testing is suggested in 2-3 weeks. If IgG level in the second sample is comparable and avidity is high, it indicates that infection was acquired earlier than 2 months since the collection of the first sample. Low IgG avidity and considerable increase in IgG level in the second sample suggest that infection was acquired in a time shorter than 2 months since the collection of the first blood sample. Chemoprophylaxis should be initiated. Infection in foetus should be excluded or confirmed by molecular testing of amniotic fluid.

It would be optimal if women are tested prior to the pregnancy planned. Pregnant women who have not been tested for toxoplasmosis yet, should be examined as early as possible after they become pregnant. Provided serologic test result is negative, it is required to repeat serologic tests until the end of pregnancy, at least three-fold (at the beginning of pregnancy, in approximately week 24 of pregnancy and two weeks prior to the labour term) as well as inform them of transmission routes and measures which can be undertaken to avoid infection. Foetal diagnostics includes the examination of amniotic fluid (amniocentesis over week 18-21 of pregnancy). As severe clinical symptoms of congenital toxoplasmosis are present almost always when primary infection in mother was acquired in the first or second trimester of pregnancy, it is recommended to perform amniotic fluid test in week 21 of pregnancy. Until that time, spiramycin should be administered as to reduce the percentage of *T. gondii* transmission to the foetus. Week 21 was suggested due to an interval between the infection of mother and foetus and pregnancy week, after whom it is allowed to apply pyrimethamine and sulfadiazine in the treatment of infected foetus.

Imaging test of foetus (ultrasound, magnetic resonance imaging) should be adopted accordingly to the clinical condition of pregnant woman infected with *T. gondii.*

Neonatologist should obtain comprehensive data on diagnostic and therapeutic management of pregnant woman, and then initiate diagnostic procedure for congenital infection in foetus as to confirm or exclude it by serologic testing, using umbilical cord blood or venous blood and perform additional testing, including imaging tests and specialist consultations (ophthalmic, neurologic and others, according to recommendations). Moreover, further management should be planned following the discharge of child from neonatal ward.

Congenital toxoplasmosis

Symptomatic congenital toxoplasmosis occurs in ca 5%-10% of children in the following manifestations:

1. Triad of Sabin and Pinkerton (rarely at present): chorioretinitis, hydrocephalus or microcephaly, intracranial calcifications

2. Sepsis
3. Organ manifestations (ocular disorders, myocarditis, hepatitis, enteritis)

Furthermore, preterm delivery, hypotrophy, convulsions may also appear. Long-term complications include permanent impairment of visual and central nervous system – 3% while a risk of pre-or postnatal death is 2% or higher. In a group of children who do not present clinical symptoms in postpartum period (90%), long-term complications may appear in months or years with an estimated prevalence of 7%-15%.

Congenital toxoplasmosis is diagnosed on a basis of evaluation of anamnesis and results of paediatric, ophthalmic and neurological examinations as well as the findings of specific laboratory testing and imaging testing (US, CT, MRI).

Interpretation of specific serologic testing results in newborns and infants suspected of congenital toxoplasmosis is presented in Figure 2.

It should not be forgotten that specific IgG antibodies are passively transmitted through placenta from mother to foetus. A classic criterion allowing for a confirmation of passive antibody transmission is IgG disappearance up to month 11-12 of life. Passive transmission of antibodies may be also confirmed on a basis of compatible profile of mother's and child's antibodies, using Western-blot or ELIFA.

Ocular toxoplasmosis

Ocular disorders appear in the course of acquired or congenital infection. In both types of infection, chorioretinitis and effusion into the vitreous humour are present in acute stages. Usually, focal lesions are located in the posterior eye ball, presented as cream and white-coloured changes (cotton-wool spots). Inflammation resolves spontaneously in 2-6 weeks and a scar containing regrouped pigment remains. As the clinical picture of focal lesions in the course of acquired and congenital infection is identical, differential diagnosis based on the appearance of scar is not possible.

There is no correlation between specific antibody level and intensity of chorioretinitis.

In the diagnosis of ocular toxoplasmosis, besides serologic testing, the following examinations are also performed: fundoscopy (in each case), fluorescein angiography aimed at disclosing satellite inflammatory lesions, retinal tomography, ultrasound, examination of anterior chamber fluid, using PCR.

Toxoplasmosis in immunosuppressed women

In a group of patients with immunodeficiency resulting from e.g. HIV infection, immunotherapy or organ transplantation, primary infection and reactivation of chronic infection may be present. Suppression degree of immune system is measured by, inter alia, the number of CD4+ lymphocytes. In HIV-positive patients, with the number of CD4+ below 100 cells/µl, there is a risk of reactivation and possibility of foetus infection. Normal number of CD4+ (> 500 cells/µl) may constitute a protection against both reactivation and severe course of primary infection with *T. gondii*. It is considered that in immunosuppressed women parasitemia may persist longer.

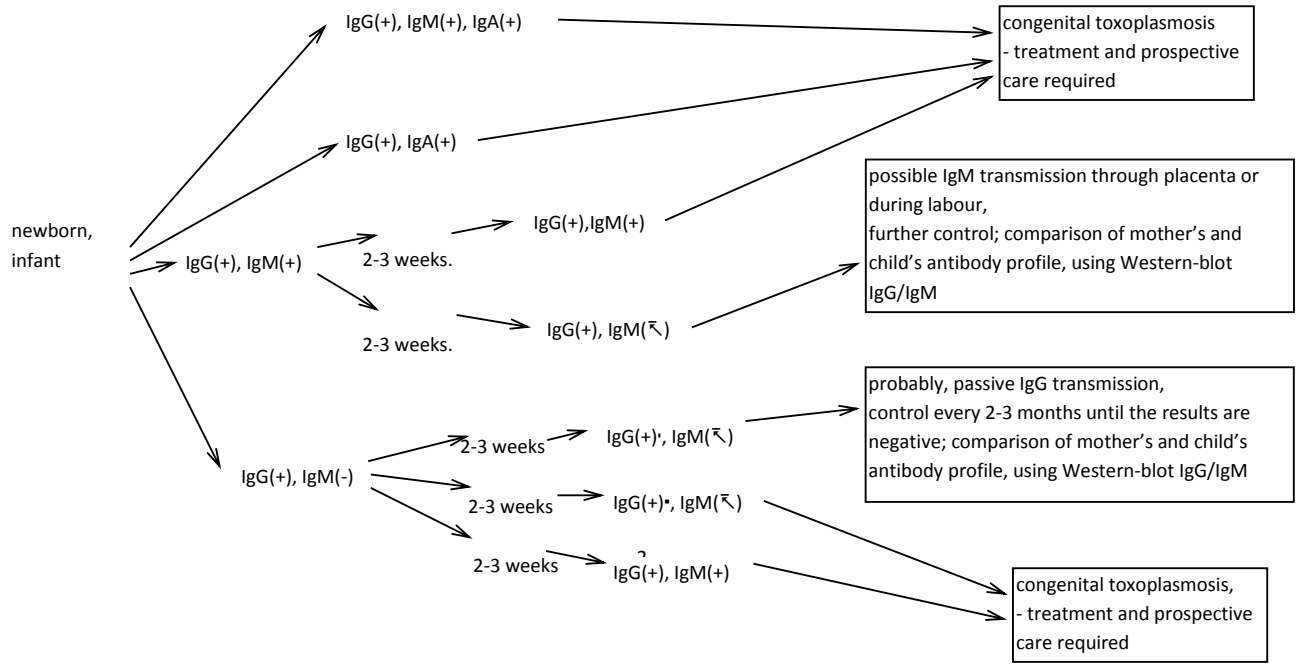

Fig. 2. Interpretation of specific serologic testing results in infants suspected of congenital toxoplasmosis

Due to possible impairment of synthesis of specific antibodies, regardless of an existing infection, molecular technique – PCR is a conclusive test in persons with immunodeficiency. Amniocentesis is recommended in both HIV-positive women who are effectively treated with antiviral drugs (undetectable HIV viremia) and HIV-negative women. Amniocentesis in HIV-positive women, who are not subject to antiviral treatment, may significantly increase the risk of HIV transmission to foetus. In each case, test for HIV should be performed prior to amniocentesis in women whose HIV status is unknown.

In case of pregnant women under the week 18 of pregnancy with impairment of immune response, spiramycin should be initiated in treatment. Once the infection of foetus is confirmed, pyrimethamine and sulfadiazine should be applied as well as folinic acid on a daily basis during treatment with pyrimethamine and one week following its discontinuation. If active *T. gondii* infection is diagnosed in women over the week 18 of pregnancy, it is recommended to initiate treatment with pyrimethamine and sulfadiazine and folinic acid from the very beginning.

In case of HIV-positive women with the number of CD4+ lymphocytes below 200 cells/µl, presenting serologic markers of *T.gondii* infection, it is recommended to initiate secondary prophylaxis, using co-trimoxazole of 480 mg per day. Secondary prophylaxis of toxoplasmosis should be also considered in HIV-positive pregnant women, presenting high level of specific toxoplasma antibodies.

All seronegative women with immunosuppression are recommended to strictly comply with prophylactic measures against *T. gondii* infection and undergo specialist medical care.

TREATMENT OF TOXOPLASMOSIS

Indications for treatment:

- congenital toxoplasmosis – symptomatic and asymptomatic manifestation
- primary toxoplasmosis in pregnant women
- acquired toxoplasmosis with involvement of vital organs
- active chorioretinitis

Once the diagnosis of primary infection or reactivation, in the acute stage of infection, is made, it is of importance to quickly initiate treatment as all drugs have parasite-static action, inhibiting parasite proliferation; neither drugs act as parasiticides nor penetrate to tissue cysts.

Drugs

- **chemotherapeutics:** acting synergystically: pyrimethamine (daraprim) and sulfonamides (sulfadiazine being a drug of choice). In case of allergy to sulfadiazine, it is recommended to substitute it with antibiotic, i.e. clindamycin or continue the treatment with pyrimethamine only.
- **antibiotics:** having parasite-static action: spiramycin (rovamycine), clindamycin, azithromycin, clarithromycin and others.
- **combination drugs**: Fansidar (pyrimethamine with sulfadoxine), co-trimoxazole (trimethoprim with sulfamethoxazole).
- **glucocorticoids**: applied in specific indications, always simultaneously with antiprotozoan treatment.
- **folinic acid**: in the treatment with antifolates, it is recommended to apply folinic acid (Calcium folinate, Leucovorine, Lederfolate) – not folic acid!

Method of treatment is dependent on the clinical manifestation of infection.

Table 1 presents drug regimen for children and adults.

Treatment of toxoplasmosis in pregnant women

Standardization of chemoprophylactic management in pregnancy is a very difficult task. Time of diagnosis of infection in pregnant woman and her child has an impact on the method of treatment. Having selected the method of treatment, it should not be forgotten that:

- **pyrimethamine is not applied in the first trimester of pregnancy**
- amniocentesis is performed in the week 18-21 of pregnancy in women diagnosed with active toxoplasmosis
- it should be individually considered whether infected woman over the week 24 of pregnancy should be

Tab. 1. Dosage of antiparasitic drugs applied in prophylaxis and treatment of toxoplasmosis.

Drug	Children	Adults
pyrimethamine	2 mg/kg/day for 2 days, and then, 1 mg/kg/day in 1 dose (max.25 mg)	2x50 mg for 2 days, and then, 50 mg/day, in 1 dose
sulfadiazine	50-100 mg/kg/day, in 2-3 doses	3 g/day, in 2 doses
Fansidar (pyrimethamine with sulfadoxine)	1 tablet per 20 kg of body weight, once a week	2 tablets, once a week
folinic acid	5-10 mg/dose, 3 times in a week (higher dose in case of bone marrow suppression)	5-20 mg, once a day (higher dose in case of bone marrow suppression)
spiramycin	150-300 thousand IU/kg/day in 2-3 doses	9 million IU in 3 doses
clindamycin	20-30 mg/kg/day in 4 doses	600 mg 4 times a day

Tab. 2. Recommended multi-specialist clinical examination and laboratory testing in children with congenital toxoplasmosis observed prospectively (up to 2 years of life)*

Term of child's examination - age in weeks and months	Week 1-4	Week 5-8	Week 9-12	Month 6	Month 9	Month 12	Month 24
anamnesis, paediatric examination	x	x	x	x	x	x	x
specific serologic testing	x	x	x	x	x	x	x
complete blood count, blood chemistry panel**	x	x	x	x	x		
transfontanelle ultrasonography***	x		x		x		
electroencephalogram *				x		x	
Neurologist	x		x			x	x
Ophthalmologist	x		x	x		x	x
Audiologist	x****		x	x			x
Psychologist				x		x	x

* frequency of examinations may be modified, depending on the child's condition
** complete blood count – white blood cells (with smear), platelets; in case of treatment with pyrimethamine on a daily basis, a control morphology every 7-10 days, blood chemistry panel - urea, creatinine, ALT
*** computed tomography or brain MRI following fontanelle coalescence or earlier, if indicated
**** hearing screening

administered exclusively spiramycin until the end of pregnancy or subject to amniocentesis. Based on the examination of amniotic fluid, using PCR, treatment with pyrimethamine and sulfadiazine should be initiated following the confirmation of infection in the foetus.

Recommended therapeutic management:

1. Until the week 18-21 week of pregnancy (until the time of amniocentesis), or until the end of pregnancy, provided no infection of foetus was determined: spiramycin (rovamycine) of 9 mln. IU per day in 3 divided doses
   Remark! Spiramycin reduces the risk of protozoan transmission from mother to foetus, but it does not penetrate into placental barrier; it is used as chemoprophylaxis.
2. Once the infection is identified in foetus, based on amniotic fluid examination by PCR, it is recommended to apply:
   pyrimethamine: loading dose of 50 mg every 12 hours for 2 days; since day 3 - 50 mg once a day, until the end of pregnancy, with sulfadiazine: 3.0 g per day in 2 divided doses, until the end of pregnancy.
3. Folinic acid 5-20 mg on a daily basis until the end of pregnancy
   In the time of treatment with pyrimethamine, it is required to perform complete blood count, hepatic and renal function panel and urinalysis every 7-10 days, or less frequently, provided normal parameters were determined earlier. In case of treatment with sulfonamides, adequate hydration of patient is required.

Treatment of congential toxoplasmosis

1. Infants diagnosed with severe toxoplasmosis should be administered pyrimethamine with sulfadiazine on a daily basis for 6 months, and then, they are subject to 4-week cycles staggeringly with spiramycin, or Fansidar every 7 days, until the month 12 of life or longer depending on the clinical condition.
2. In a milder manifestation, treatment with pyrimethamine and sulfadiazine on a daily basis lasts for at least 2 months, and then, aforesaid drugs are applied staggeringly with spiramycin in 4-week cycles, or Fansidar is applied every 7 days, until the month 12 of life or longer depending on the clinical condition.
3. In subclinical or asymptomatic infection, pyrimethamine with sulfadiazine is administered for 4 weeks, staggeringly with spiramycin for 6 weeks (high doses) or Fansidar one time every 7 days (low doses) until the month 12 of life.

In case of treatment with pyrimethamine, it is required to apply folinic acid and control complete blood count with blood smear and platelet count, hepatic and renal function panel and urinalysis every 7-10 days, or less frequently, provided normal parameters were determined earlier. During treatment with sulfonamides, adequate hydration of child is required.

In the presence of indications (high concentration of protein in cerebrospinal fluid >1.0 g/dl; active chorioretinitis), it is possible to apply glucocorticoids until protein concentration in cerebrospinal fluid would be normalized and active chorioretinitis would be resolved. Use of glucocorticoids should be applied exclusively in reference centres; in case of eye changes, it should be always accompanied by ophthalmologist consultation.

In each confirmed case of congenital toxoplasmosis, treatment should be continued through infancy period, in both symptomatic and asymptomatic infection.

Children with congenital toxoplasmosis should be subject to a programme of multi-specialist prospective examinations (see Tab. 2); a range and frequency of examinations depend on the clinical condition. Ophthalmic examination on an annual basis is obligatory due to

relatively high risk of inflammatory lesion recurrence in the region of retina.

Complex rehabilitation

Generally, long-term, severe complications of congenital toxoplasmosis, resulting from post-inflammation injury of the central nervous system, include: spastic tetraplegia with hydrocephalus and/or microcephaly, epilepsy which may occur even during the first year of life, mental retardation and injuries of sense organs, especially eye or, less frequently, ear.

Movement rehabilitation should be initiated in the first three months of child's life. Exercises should be adjusted individually and according to child's neurological condition, i.e. the presence of incorrect movement patterns such as persistent tonic reflex, excessive Moro reflex, axial hypotonia, tendency to opisthotonus, strong palmar grasp reflex. Children with congenital toxoplasmosis frequently present increased rather then decreased muscle tension.

NDT Bobath and the Vojta method are commonly applied in the rehabilitation of infants. In case of elder children, proprioceptive neuromuscular facilitation (PNF) and the Peto method are also employed. Of importance is also sensory integration (SI).

Rehabilitation of children with congenital toxoplasmosis is hindered by visual and hearing impairments, presence of valve system or drug-resistant epilepsy.

In case of elder children with increased spasticity, often it is required to apply muscle relaxants, botulinum toxin or perform orthopaedic surgeries, e.g. the Achilles tenotomy. There may be a necessity to use orthoses, orthopedic shoes and provide rehabilitation equipment.

A role of psychologist is also significant, who should not only be a support in difficult life situation, but may also have an effect on parents' attitude, their motivation and collaboration within active, complex, long-term process of child's rehabilitation.

Treatment of acquired toxoplasmosis in immunocompetent persons

1. manifestation, involving vital organs: pyrimethamine and sulfadiazine or aforesaid drugs are applied staggeringly with rovamycine, for 4-6 weeks, or longer; treatment should be continued for 2 weeks following the disappearance of clinical symptoms
2. glandular manifestation does not usually require treatment; if lymphadenopathy is accompanied by other organ lesions or simultaneously, another chronic disease is diagnosed, then management should be as specified above

Treatment of ocular toxoplasmosis

Irrespective of the fact whether it is congenital or acquired manifestation, primary infection or its recurrence, treatment of active lesions should be initiated. Treatment should be combined and continued for 4-6 weeks; treatment should be continued for two weeks following the disappearance of active chorioretinitis. Decision on treatment initiation is made by ophthalmologist or with his assistance. Fansidar may be applied in the treatment of eye changes; effectiveness of treatment with clindamycin and azithromycin was also demonstrated.

Administration of glucocorticoids in the treatment of active chorioretinitis in the course of *T.gondii* infection may be controversial and should be adopted exclusively in reference centres.

PREVENTION

- avoidance of consuming raw or semi-raw meat and its products
- proper washing of hands and objects used during meat processing
- washing of vegetables and fruits prior to consumption
- protection of food against cockroaches and flies transmitting parasites
- drinking of boiled water and milk
- proper washing of hands following the contact with soil or working, using protective gloves
- avoidance of contact with objects that could be contaminated by cat's faces
- pregnant women living in countries of low risk of infection should avoid travelling to high risk countries.

SUMMARY

Aforesaid recommendations for the management of *T.gondii* infection, elaborated by the group of experts, are intended for physicians of various specialties in order to standardize and facilitate diagnostic and therapeutic management.

Early diagnosis of congenital toxoplasmosis, both symptomatic and asymptomatic, in neonatal period, initiation of adequate treatment and long-term, multispecialist monitoring, including multi-organ rehabilitation of children may prevent or reduce the complications of congenital toxoplasmosis.

Health education, whose role is often underestimated, should be targeted mainly on girls and women at reproductive age as to prevent from infection during pregnancy.

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