Abstract. – Objectives: Congenital toxoplasmosis is a particular manifestation of Toxoplasma gondii infection, which may present as a mild or severe neonatal disease. This pathology remains a difficult challenge in terms of therapy for the pediatrician and gynecologist. In this article we have set ourselves the objective to provide an overview of the main aspects of the disease, with particular attention to the treatment, based on the information in the literature.

Results: Two kinds of treatment are currently available: prenatal and postnatal. When pregnant women seroconvert, spiramycin is administered in order to prevent the mother-to-child transmission. When the fetal infection is confirmed the association of pyrimethamine and sulfadiazine is prescribed. After birth the specific therapy is based on the administration of pyrimethamine and sulfadiazine. However, to date, there is not strong evidence on the effectiveness of treatment, whether prenatal or postnatal.

Conclusions: The studies undertaken so far have not given satisfactory answers. Double-blind randomized controlled trials would be required, but for obvious ethical reasons they cannot be achieved.

Key Words: Congenital toxoplasmosis, Pyrimethamine, Sulfadiazine, Spiramycine, Randomized controlled trial.

Introduction

Toxoplasmosis is an important zoonotic parasitic disease worldwide. Toxoplasma gondii (T. gondii) is a coccidiod protozoan that multiplies only in living cells. Its life cycle includes sexual reproduction in the definitive host, the cat, and asexual division in intermediate hosts which include man, birds and rodents.

The human infection may be acquired by ingestion of oocysts excreted by cats and contaminating soil or water, or by eating tissue cysts that remain viable in undercooked meat of infected animals and it may generate devastating damage in fetuses, newborns and immunocompromised patients.

Most human infections with this protozoon are asymptomatic, although a minority may present malaise, low grade fever and lymphadenopathy. The immunocompromised patient can face a severe, life-threatening infection.

Mother-to-child transmission of the parasite occurs only when infection is acquired for the first time during pregnancy. Infection may be transmitted to the fetus transplacentally or during vaginal delivery.

Congenital toxoplasmosis (CT) may present as a mild or severe neonatal disease. There is a wide variety of manifestations of congenital infection, ranging from fetal hydrops and perinatal death to small size for gestational age, prematurity, peripheral retinal scars, persistent jaundice, mild thrombocytopenia, cerebro-spinal fluid (CSF) pleocytosis, and the characteristic triad of chorioretinitis, hydrocephalus, and cerebral calcifications. Retinal disease, which may be unilateral, follows a relapsing and remitting course into adult life, often with severe impairment of vision.

Epidemiology

There are considerable geographic differences in prevalence rates. In Europe the prevalence of infection is higher in France (54.3%) than elsewhere in the continent (46%), probably attributed to a preference for consumption of undercooked meat.

One recent French study, based on serology data collected during national perinatal surveys conducted between 1995 and 2003 to estimate toxoplasmosis prevalence, indicates that although it decreased over time, toxoplasmosis prevalence remained higher than 43.8%.

Cultural habits regarding food are probably the major cause of the differences in frequency of T. gondii infection from one country to another, from one region to another in the same country, and from one ethnic group to another in the same region.

The mother-to-child transmission rates rise from 7% in the first trimester, to 24% in the second, to 59% in the third, while the incidence of severe fetal infection falls from 75% to a negligible risk in late pregnancy.

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**Diagnosis**

Where maternal toxoplasmosis is suspected, appropriate assays for testing maternal serum samples include the latex agglutination test or the direct agglutination test followed by referral, where indicated, to a reference laboratory for the dye test, indirect haemagglutination test, ELISA for IgG and IgM, the immunosorbent agglutination assay (ISAGA) for IgM and IgA, the enzyme-linked immunofiltration assay (ELIFA) or immunoblotting (IB) for the detection of IgG or IgM. A positive result requires that a repeated maternal serum sample be requested to confirm identification and results of the previous sample.

For the serological evaluation and diagnosis of primary maternal infection during pregnancy we can use the “case definition”, proposed by the European Research Network on Congenital Toxoplasmosis, based on probability of infection (Table I)\(^1\).

Other useful diagnostic tools to determine the infection of the fetus are the polymerase chain reaction (PCR) and ultrasound. The amplification of *T. gondii* DNA in amniotic fluid should be done at 18 weeks of gestation or later. If done at the 18\(^{th}\) week, this test has an overall sensitivity of 64%, a negative predictive value of 88%, and a specificity and positive predictive value of 100%\(^1\). Its sensitivity and specificity for amniotic fluid obtained before 18 weeks of gestation have not been studied. In addition, the procedure done early in gestation is associated with a higher risk to the fetus and is likely less useful.

Concerning the ultrasound, this procedure is recommended for women with suspected or diagnosed acute infection acquired during or shortly before gestation. It may reveal the presence of fetal abnormalities, including hydrocephalus, brain or hepatic calcifications, splenomegaly, and ascites.

After the birth, diagnosis of CT in the postnatal period is problematic. Current assays often fail to detect IgM in the neonatal serum and passively acquired IgG makes interpretation of routine serology difficult. Following identification of a toxoplasma-positive serum in the primary laboratory, that serum and antenatal booking and current sera from the mother should be investigated at one of the reference laboratories. When maternal infection cannot be excluded, serial serum specimens from the infant should be tested until toxoplasma antibodies disappear.

**Treatment**

To treat CT two types of regimen are available: prenatal and postnatal. The first is aimed at preventing the infection of the fetus and, in case of documented infection, hasten the treatment before birth, while the second is designed to treat the infection and to prevent the sequelae.

**Prenatal Treatment**

With the advent of prenatal diagnosis, attempts are being made to treat the infection during the gestational period. The aim of this approach is to decrease the risk of mother-to-child transmission of the infection and possibly anticipate its treatment before birth.

The key drugs which are administered are: Spiramycin – a macrolide antibiotic, with an antibacterial spectrum comparable to that of erythromycin; Pyrimethamine – a substituted phenylpyrimidine antimalarial drug; and Sulfadiazine – a sulfonamide antibiotic.

Once it has been established that the mother’s serological test results are consistent with a recently acquired infection and that acquisition of the infection during the first 18 weeks of gestation or shortly before conception cannot be excluded, a treatment with spiramycin should be administered in order to prevent vertical transmission of the parasite.

If fetal infection is confirmed by a positive result of PCR on amniotic fluid at 18 weeks of gestation or later, treatment with pyrimethamine, sulfadiazine, and folinic acid is recommended\(^1\). Af

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**Table I. Criteria for evaluation of maternal serology.**

<table>
<thead>
<tr>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG avidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG –</td>
<td>IgM –</td>
<td>IgA –</td>
<td>IgG avidity &lt; 15%</td>
</tr>
<tr>
<td>IgG + (&lt; 200 UI)</td>
<td>IgM –</td>
<td>IgA +</td>
<td>IgG avidity &lt; 15%</td>
</tr>
<tr>
<td>IgG + (&gt; 300 UI)</td>
<td>IgM +</td>
<td>IgA –</td>
<td>IgG avidity &gt; 30%</td>
</tr>
<tr>
<td>IgG + (&lt; 300 UI)</td>
<td>IgM +</td>
<td>IgA –</td>
<td>IgG avidity &gt; 30%</td>
</tr>
<tr>
<td>IgG + (&gt; 300 UI)</td>
<td>IgM +</td>
<td>IgA –</td>
<td>IgG avidity &gt; 30%</td>
</tr>
<tr>
<td>IgG –</td>
<td>IgM +</td>
<td>IgA –</td>
<td>Natural IgM</td>
</tr>
</tbody>
</table>

These are the criteria for the serological evaluation and diagnosis of primary maternal infection during pregnancy, proposed by the European Research Network on Congenital Toxoplasmosis, based on probability of infection.


### Table II. Guidelines for treatment of *T. gondii* infection in the pregnant women.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosage</th>
<th>Duration of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant woman with</td>
<td>Spiramycin 1 g (3 million U) every 8 h</td>
<td>Spiramycin treatment should be continued until delivery in women with low suspicion of fetal infection or those with documented negative results of amniotic fluid PCR and negative findings on ultrasounds at follow-up</td>
</tr>
<tr>
<td>suspected or documented</td>
<td>(for a total of 3 g or 9 million U per day)</td>
<td></td>
</tr>
<tr>
<td>infection (&lt; 18th week of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gestation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant women with</td>
<td>Pyrimethamine Loading dose: 100 mg per day in two divided doses for 2 days, then 50 mg per day</td>
<td>Until birth</td>
</tr>
<tr>
<td>confirmed infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;18th week of gestation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>Loading dose: 75 mg/kg per day in two divided doses (maximum 4 g per day) for 2 days, then 100 mg/kg per day in two divided doses (maximum 4 g per day)</td>
<td>Until birth</td>
</tr>
<tr>
<td>Leucovorin</td>
<td>10-20 mg daily</td>
<td>During and for 1 wk after pyrimethamine therapy</td>
</tr>
</tbody>
</table>


After the eighteenth week of gestation, it’s necessary to administer the combination pyrimethamine-sulfadiazine immediately, because the rate of mother-to-child transmission is so high as to render useless the use of spiramycin (Table II).

Obviously it’s important to take account of possible side effects of pyrimethamine. It inhibits the dihydrofolate reductase, which is important in the synthesis of folic acid, thus producing reversible and usually gradual bone marrow depression.

In fact, during this period of drugs administration, the mother is carefully monitored for development of hematologic toxicity. If significant toxicity appears, despite treatment with folinic acid, the drug combination is discontinued until the hematologic abnormalities are corrected and the drug regimen is then restarted.

Although this treatment is in use for a long time and many studies have been conducted over the years, our knowledge on its efficacy is not entirely clear. Concerning spiramycin, the results of observational studies showed the potential of this antibiotic to prevent vertical transmission of the parasite. Moreover, it may reduce the severity of infection in a fetus because it delays transmission to a later time in gestation, when transmission is associated with less severe manifestations of infection.

Instead, the combination of pyrimethamine and sulfadiazine is highly active against *T. gondii* and is widely used as a treatment to reduce the risk of clinical manifestation in infected children.

Currently the debate about the effectiveness of this treatment is becoming more heated. Many studies have set themselves the goal of finding strong evidence for the efficacy of these drugs.

### Table III. Guidelines for treatment of CT in the infant.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosage</th>
<th>Duration of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrimethamine</td>
<td>Loading dose: 2 mg/kg per day for 2 days, then 1 mg/kg per day for 2 or 6 month, then this dose every Monday, Wednesday, Friday.</td>
<td>1 year</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>100 mg/kg per day in two divided doses.</td>
<td>1 year</td>
</tr>
<tr>
<td>Leucovorin</td>
<td>10 mg three times weekly</td>
<td>During and for 1 wk after pyrimethamine therapy</td>
</tr>
</tbody>
</table>

Evidence that could solve this Gordian knot. Probably, the most comprehensive overview of the complex situation, we are talking about, is provided by the work of SYROCOT study group in 2007. It's a systematic review and individual data meta-analysis of 20 European cohort studies, in which universal screening for toxoplasmosis in pregnancy was performed. In 1438 treated mothers identified by prenatal screening, the European researchers found weak evidence that prenatal treatment significantly reduced the risk of clinical manifestations. Moreover, they found weak evidence for an association between early treatment and reduced risk of CT. Analyzing the data from this study it is obvious that there is a need to change the way of approach to evaluating the effectiveness of treatment. Indeed, the evidence obtained from observational studies is not suitable to make this kind of assessment.

Moreover, the lack of appropriate knowledge and clear data, has led to consider other drugs as possible candidates for prenatal treatment. Indeed, we must not forget that spiramycin is unable to eradicate existing fetal infection and administration of the combination pyrimethamine-sulfadiazine is not without risks (potential toxicity and teratogenicity). In fact an ideal drug would show an efficient penetration and concentration in the placenta, should be effective against different stages of the parasite and not having any kind of fetal toxicity.

Examples include atovaquone or fluoroquinolones that have shown activity against T. gondii in vivo and in vitro, but that cannot be used during pregnancy because the potential harmful effects on the embryo and fetus have not been well evaluated.

Others are azithromycin, that showed to be capable to inhibit the vertical transmission of T. gondii in mice model of CT, or thiolactomycin analogues that demonstrated anti-T. gondii in vitro activity.

Finally we must emphasize that encouraging results were observed using spiramycin-cotrimoxazole association, primarily for its safety and also because it showed effects on reducing the mother-to-child transmission rate and on preventing the risk of clinical sequelae.

**Postnatal Treatment**

Concerning the postnatal treatment, it’s recommended to administer specific therapy in every case of CT, symptomatic or not, even though there are no sufficient data to evaluate properly treatment in the asymptomatic infected infant. That treatment of such infants should be undertaken with the aim of preventing the late untoward sequelae.

Another important consideration is that the agents which can be recommended for specific therapy at present are beneficial against the tachyzoite form, but none has been shown to effectively eradicate the encysted form, especially from Central Nervous System and from eye.

The specific postnatal therapy is based on the same two drugs also essential in the prenatal: pyrimethamine and sulfadiazine.

The simultaneous use of both drugs is 8-fold more active than either pyrimethamine or sulfadiazine alone, and has been the “gold standard” to which other antimicrobial agents, alone and in combination, have been compared.

This regimen, described more accurately in the Table III, has been associated with resolution of signs of active CT, usually within the first weeks after initiation of therapy. We must, however, keep in mind the pyrimethamine toxic effects, which had already talked about. This is why all patients treated with this drug should have a peripheral blood cell and platelet count twice each week and moreover folinic acid (in the form of leucovorin calcium) administration, as described in Table III.

Two recent studies have been published to support this type of treatment. The first evaluated the outcomes of treatment in 120 infants with CT between 1981 and 2004. It affirmed that treatment of infants without substantial neurologic disease at birth with pyrimethamine and sulfadiazine for 1 year resulted in normal cognitive, neurologic, and auditory outcomes for all patients. Treatment of infants who had moderate or severe neurologic disease at birth resulted in normal cognitive, and auditory outcomes for >72% of the patients, and none had sensorineural hearing loss. 91% percent of children without substantial neurologic disease and 64% of those with moderate or severe neurologic disease at birth did not develop new eye lesions. So it seems clear in this study that almost all of these outcomes are markedly better than outcomes reported for children who were untreated or treated for 1 month in earlier decades.

Similar results have been obtained in a prospective longitudinal observation of a cohort of 132 children, treated during their first year of life with...
pyrimethamine, sulfadiazine and leucovorin, and evaluated for chorioretinal involvement\textsuperscript{25}.

Although, as the prenatal, concerning the postnatal treatment, it was developed many years ago and the studies to determine its effectiveness cannot yet give us satisfactory answers. The debate is still open on which drugs are the best, what kind of regimen should be adopted, and finally what the ideal duration of treatment should be. This becomes evident if we observe the different regimens adopted by the various centers of CT treatment. The main cause of this situation is the lack of adequate evidence. In fact, studies conducted until now have several limitations: first, the comparison with historical studies, rather than appropriate control groups. This limits their validity.

So, the general opinion is that children born with symptomatic \textit{T. gondii} infection should be treated, but the benefit of treatment in children without symptoms at birth is presently under intense discussion, because there is a lack of evidence.

Moreover, opinions regarding postnatal treatment vary considerably in terms of drugs and regimens. New drugs suitable for treatment of CT have been studied, although there are still insufficient data enabling usage.

The most promising drug in the treatment of \textit{T. gondii} is atovaquone, already proposed for toxoplasmosis during pregnancy, which has potent \textit{in vitro} activity against the tachizoiote and the cyst forms\textsuperscript{26,27}. Other studies about atovaquone in animal models have also reported significantly increased survival and reduction in brain cyst burden\textsuperscript{26,29}.

The same can be said for azithromycin, that has also been found to have a partial effect on \textit{T. gondii} tissue cysts\textsuperscript{30}.

## Conclusions

After having completed this overview on treatment of CT, in agreement with other Authors, many questions are still to be solved, regarding the management of patients (both for mother and child) and the choice of a specific therapy.

Available studies are either observational studies of groups of referred patients or observational studies with historical controls accrued over many years, often decades. In fact we have to keep in mind that in the past, diagnosis of disease and patient care were different: for example, prenatal diagnosis or ultrasound were not available.

It was also impossible to perform therapeutic maneuvers to change the survival of the patient (hospitalization in intensive care units or shunt procedures), as the current ones. Then, only well-conducted randomized trials evaluating new treatment regimens or strategies will allow a collection of data which could be more significant. The potential for bias and unmeasured confounding factors in cohort studies, coupled with the moderate treatment effects expected, limits the value of information from observational studies. Confounding factors can only be avoided by randomization and biases can only be avoided by high quality, standardized procedures\textsuperscript{31}. The ideal approach would be double-blind randomized controlled trials.

However, it’s realistically impossible to achieve this kind of studies for obvious ethical reasons. It would be unlikely that a mother aware of current treatment, even if of dubious effectiveness, could accept the risk of receiving a placebo or not being treated.

Thus, although methodologically not correct, comparison with historical data of CT studies in pre-treatment era, remains the only option that can be followed.

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