Options for clinical trials of pre and post-natal treatments for congenital toxoplasmosis

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Clinical trials comparing different drug regimens and strategies for the treatment of congenital toxoplasmosis and its clinical manifestations in the liveborn child in different clinical settings should aim at formally evaluating the net benefit of existing treatments and at developing new therapeutic options. Currently, there is no ideal drug for congenital toxoplasmosis; future research should focus on the screening of new active drugs and on their pre-clinical and early clinical development, with a focus on pharmacokinetic/dynamic studies and teratogenicity. For the prenatal treatment of congenital toxoplasmosis, a trial comparing spiramycine to pyrimethamine-sulphadiazine and placebo would allow a formal estimation of the effect of both drugs in infected pregnant women. In newborn children, the net benefit of pyrimethamine-sulphadiazine should also be formally assessed. These trials will be implemented in settings where prenatal screening for Toxoplasma gondii *is currently implemented. Trials should be carefully designed to allow for translation to other settings and modelling tools like cost-effectiveness analysis should be used to provide clinicians and founders with the best available evidence to establish recommendations.*

Key words: congenital toxoplasmosis - pyrimethamine - sulphadiazine - spiramycine - randomised controlled trial

In the fall of 2005, a European consensus conference on the prevention and management of congenital toxoplasmosis and its consequences (European Commission Contract No QLG4-CT-2002-30262 http://eurotoxo.isped.u-bordeaux2.fr/) concluded that there was no evidence that current prenatal treatment had a clinically important effect on the risk of transmission and that the variation in the observed effects of various prenatal treatment regimens on the occurrence of clinical symptoms could be explained by biases (SYROCOT Study Group 2007). Moreover, there was a lack of evidence on the effect of postnatal treatment in children, especially for pyrimethamine/sulphonamides (Stanford et al. 2003).

The impressive volume of available information reviewed in the setting of this consensus conference unambiguously called for the development of new treatment strategies to achieve lower rates of transmission and clinical symptoms than are possible with currently recommended treatment. It was explicitly recommended to use randomised clinical trials to test new options and generate hard evidence compels clinicians and founders to change their practices (Petersen 2007). This conclusion is also shared by our colleagues from North America (Montoya & Remington 2008) and all efforts should be made in the next 10 years to complete a large randomised clinical trial that screens new drugs. In this paper, we summarise the current practices for pre and post-natal treatment of congenital toxoplasmosis, lessons learned from observational studies, the rationale for conducting clinical trials and possible options for such trials. Finally, we discuss the type of information that is still needed to inform the design of a trial.

What are the current practices?

In countries where prenatal screening is implemented, spiramycine is generally prescribed for the prevention of mother-to-child transmission of *Toxoplasma gondii*. If foetal infection is confirmed during the second or third trimester, a combination of pyrimethamine, sulphadiazine and folinic acid is recommended. This combination is also recommended during the same period of gestation to prevent mother-to-child transmission of the parasite (Montoya & Remington 2008). Nevertheless, these practices differ widely by country. There is no recommendation for prenatal treatment in the United Kingdom, where there is no policy of prenatal screening (Gilbert & Peckham 2002), nor in Denmark, where neonatal screening is offered (Schmidt et al. 2006). In Austria, however, the combination of pyrimethamine and sulphadiazine is prescribed regardless of the timing of gestation (Aspöck et al. 1994).

These practices are based on the results of observational studies showing the potential of spiramycine to prevent vertical transmission of the parasite (Desmonts & Couvreur 1974, 1979, Couvreur et al. 1988, Hohlfeld et al. 1989, Forestier 1991).

The combination of pyrimethamine and sulphadiazine is highly active against *T. gondii* and is the most widely used treatment for prenatal infection (Hohlfeld et al. 1989, Couvreur et al. 1993, Villena et al. 1998). Moreover, this combination is assumed to have a higher potential than spiramycine in reducing the risk of clinical manifestations in infected children (Foulon et al. 1999, Gras et al. 2005, SYROCOT Study Group 2007).

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In the postnatal period, antibiotic treatment is used to reduce the duration and severity of symptoms such as acute intraocular inflammation, the risk of permanent visual impairment and the risk of recurrent episodes (Stanford et al. 2003). A large number of drugs, such as spiramycine, pyrimethamine-sulphadiazine and atovaquone or cotrimoxazole, are used, but there is no clear evidence of their efficacy.

No randomised clinical trial has been performed to show the net benefit of either spiramycine or pyrimethamine combined with sulphadiazine for decreasing the rate of toxoplasmosis transmission or its effect on infected children (Wallon et al. 1999). More recently, analyses of the largest database of available observational cohorts were unable to provide clear evidence of an effect of prenatal treatment on transmission or the occurrence of retinochoroiditis or brain lesions (EMSCT 2003, SYROCOT Study Group 2007).

What have we learned from observational studies?

Although observational studies are not designed to provide a valid estimation of the value of current treatments, they have yielded important information regarding the frequency of vertical transmission, clinical symptoms, their severity and the major determinants of these conditions. These data help to inform the design of future clinical trials for populations that are especially at risk, or define the magnitude of clinically relevant differences that need to be achieved with new treatment options or strategies.

In France, maternal the first time acquisition of *T. gondii* during gestation is responsible for one-10 cases of either congenital toxoplasmosis in the liveborn child or death of the foetus per 10,000 pregnancies. This frequency of mother-to-child transmission varies according to the time during gestation when a susceptible mother becomes infected: 15% of the children will be infected if a susceptible woman acquires the infection in the first trimester of gestation, 44% in the second trimester and 71% in the third trimester (SYROCOT Study Group 2007). As a consequence of this trend, the evaluation of different treatment strategies on the rate of transmission needs to compare groups with similar infection timings (Thiebaut et al. 2005). On the other hand, early maternal infection results in more severe infection in the foetus, causing death *in utero* and abortion. Therefore, a new treatment option to reduce the number of severe infections in the foetus would have to target women in the first months of gestation, whereas treatment interventions to reduce the overall number of infections would need to target women in the later months of gestation. Nevertheless, we would hypothesise that an intervention that lowers the overall transmission of infection would also lower the number of severe infections, provided that the drug works well enough and adequately penetrates to the foetal tissues.

Most children with congenital toxoplasmosis are developmentally normal (Salt et al. 2005), but up to 4% die or show evidence of permanent neurological damage or bilateral visual impairment during the first years of life (Salt et al. 2005). After prenatal toxoplasmosis, the risk of ocular lesions in the first year of life varies from 15-45%, and the risk of intracranial lesions varies from 10% when detected by cranial ultrasound to 50% when detected by computed tomography scan (SYROCOT Study Group 2007). More specifically, the risk of retinochoroiditis may reach 12% by two years of age (Kieffer et al. 2008) and 20% up to six years (Guerina et al. 1994, Dunn et al. 1999, Lebech et al. 1999). New lesions can even occur for the first time in adolescence (Koppe et al. 1986, Wallon et al. 2004).

Moreover, observational studies have shown that the timing of the infection is associated with whether women are treated and the type of treatment received (EMSCT 2003, Thiébaut et al. 2006, SYROCOT Study Group 2007). As a consequence, interpretation of the treatment effect is much more complex in the absence of randomisation, since the repartition of major confounding factors is unbalanced between the treatment comparison groups. For instance, a crude analysis of the rate of infection in untreated women, who are more likely to have seroconverted at the end of the pregnancy (i.e. when the risk of transmission is highest), compared to women treated with spiramycine would artificially favour spiramycine because gestational age at seroconversion acts as a confounding factor (Thiébaut et al. 2006). In the SYROCOT study, among treated women, a prenatal treatment initiated within the first three weeks after seroconversion tended to reduce the risk of transmission compared to a treatment initiated later on (SYROCOT Study Group 2007). However, this effect might be due to the selection of women treated late after seroconversion, with those at higher risk of transmission tending to be followed more closely. This is the same kind of caveat that led to the exclusion of untreated women from analyses of the effect of prenatal treatment on the transmission of infection.

Why do we need clinical trials?

Congenital toxoplasmosis is still the cause of a number of severe conditions in children, some of which lead to irreversible damage. In the next century we should aim to reduce the number of new infections as much as possible. Only well-conducted randomised trials that evaluate new treatment regimens or strategies will allow for the development of the best possible treatment options. 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 randomisation and, biases can only be avoided by high quality, standardised procedures.

Moreover, treatment regimens may have benefits but also cause harm, and only a randomised trial may yield a valid and robust estimation of the benefit/risk ratio of a new treatment strategy, especially in the context of especially fragile populations like pregnant women and newborn children.

Finally, given that infection is usually asymptomatic in the mother and liveborn child, a new treatment regimen or strategy would not only impact guidelines for the management of susceptible pregnant women or children

but would also inevitably impact the choice for a screening policy in each country. It is, therefore, a matter of public health policy to provide hard evidence for clinicians and funders to change practices where needed.

Options for clinical trials of pre and post-natal treatment for toxoplasmosis

Prenatal treatment - Searching for new drugs and evaluating existing treatment regimens - Given the uncertain efficacy of current treatment options and concerns about adverse effects and the infrastructure needed to implement prenatal screening, large clinical trials should aim to achieve the lowest possible rate of transmission and the lowest possible rate of clinical symptoms in children. Since a clinical trial will be difficult to implement, it would be important to prioritise trials that evaluate the efficacy of existing regimens (i.e. compared to placebo) or the superiority of new treatments over existing regimens.

An ideal drug would show effective penetration and concentration in the placenta and the transplacental passage, parasiticidal properties against the different parasitic stages, penetration into cysts, distribution in the main sites of foetal infection and limited foetal toxicity and teratogenic effects (Derouin 2001). Such a drug could be evaluated in susceptible women throughout the whole duration of pregnancy to assess either the rate of transmission or the incidence of clinical symptoms in children.

Since no such drug has been identified, further research should screen for existing or new drugs that fulfil these criteria. In this respect, pharmacokinetic/pharmacodynamic studies should help define appropriate dosages in specific populations and tolerability studies should also be performed before entering into comparative trials.

Only a few drugs are known to be active against *T. gondii* and information on their toxicity or teratogenicity is lacking (Derouin 2001). Among macrolides, which are parasitostatic drugs, data on concentrations in the foetus and transplacental passage have only been reported for spiramycine, which exhibits good tolerability.

The activity of pyrimethamine, an inhibitor of folic acid synthesis, is limited to the tachyzoite. It is parasiticidal only above a certain concentration and should be prescribed in combination with another drug, given the high intra-individual variability of serum concentrations. Given its mode of activity, this drug is toxic on bone marrow and patients should receive folinic acid supplementation to prevent thrombocytopaenia, anaemia and neutropaenia. Among drugs of the same class, only trimethoprim seems to have activity against *T. gondii*, although 10- 100 fold lower than pyrimethamine.

Sulphamides, like sulphadiazine, sulphamethoxazole or sulphadoxine, exert parasitostatic activity by inhibiting folate synthesis. They are recommended in combination with another drug, given their lack of activity against cysts.

Finally, atovaquone has parasiticidal activity and could be useful against cysts, but no data exist on its tolerability in pregnant women.

Some combinations of drugs may also be considered, such as pyrimethamine plus one drug from the sulphamide class. Sulphamides act on different sites of folate synthesis and are synergic and parasiticidal. A lower dose of pyrimethamine can therefore be used to limit haematotoxicity. Three different combinations can be used: pyrimethamine-sulphadiazine, pyrimethamine-sulphadoxine and trimethoprim-sulphamethoxazole (cotrimoxazole).

Information on pyrimethamine-sulphadiazine is the most comprehensive in terms of pre-clinical and clinical data in immunocompromised patients. All studies show a high activity for this combination and good penetration in tissues like the brain.

The combination of pyrimethamine and sulphadoxine has high synergic activity against *T. gondii*. However, preclinical and clinical data are sparse compared to the previous combination (Peters et al. 2007). Finally, trimethoprim-sulphamethoxazole is widely used in the primary or secondary prevention of toxoplasmosis reactivation in immunocompromised hosts, is effective and well tolerated (Derouin et al. 2000). However, the in vitro activity of trimethoprim is lower than that of pyrimethamine.

In the absence of new drugs, at least two questions could be resolved in a large clinical trial: (i) Is spiramycine (or pyrimethamine-sulphadiazine) effective in preventing prenatal infection? The comparison to a placebo would provide an estimate of the net benefit of both drugs on transmission. The size of such a trial would be in the range of 90 (80% power) to 120 (90% power) infected pregnant women per arm to show at least a 50% reduction in the rate of transmission (i.e. from an average rate of transmission of 40-20%). (ii) Is pyrimethamine-sulphadiazine more effective than spiramycine when infection is confirmed during the second or third trimesters of gestation? The size of such a trial would be in the range of 260 (80% power) to 350 (90% power) infected pregnant women per arm to show at least a 30% reduction in the rate of transmission (i.e. from an average rate of transmission of 40% to 28%).

It would be ideal to respond to both questions within the same trial. However, in certain settings, priority could be given to question 2. If pyrimethamine-sulphadiazine proves to be superior to spiramycine, this would prevent performing a placebo-controlled trial (assuming that spiramycine does not increase the risk of transmission compared to placebo). On the other hand, if the two drugs do not differ from each other, this would mean they are either similarly effective or similarly ineffective. Only a placebo-controlled trial would then determine the net effect of both drugs.

Such trials would need highly standardised conditions for *T. gondii* screening during pregnancy in countries where screening is already implemented (France, Austria, Italy, parts of Spain, Switzerland and Belgium) (Bénard et al. 2008). However, the generalisation of the results will remain questionable.

Postnatal treatment: who should be treated and when?

Though there is some evidence that cotrimoxazole reduces the recurrence of toxoplasmic retinochoroiditis in infancy and adulthood (Silveira et al. 2002), it is not clear who should be treated or for how long. Given that

even long-term follow-up studies have shown prenatal treatment has no or little effect on the development of ocular lesions (Binquet et al. 2003, Freeman et al. 2008), it is also desirable to evaluate existing drugs and develop new potentially active drugs by the same model described for prenatal treatment.

Since the effectiveness of starting treatment early in newborns children with clinical symptoms is unknown, it is even more important to evaluate potentially active options when infection is not confirmed to outweigh the adverse effects. A large reduction in risk must be expected, because the trial needs to be large and have a long follow-up.

Postnatal treatment options should be evaluated in randomized clinical trials with a masked outcome evaluation to avoid contamination between groups over a long-term follow-up period and ensure comparability between groups through the end of the trial. Such trials should consider the treatment of acute retinochoroiditis as well as the prevention of recurrence, the type of lesion and several treatment duration. These interventions should target high-risk patients (Binquet et al. 2003) because the indication of long-term therapy should be balanced with the risk of adverse effects.

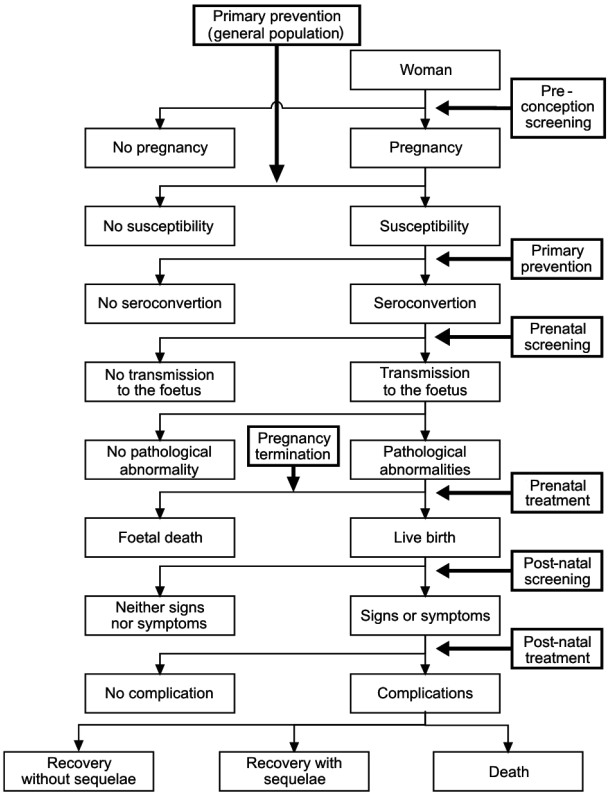
How to inform future trials and recommendations?

This is where cohorts will remain highly valuable for providing adequate and updated descriptions of the prognosis during pregnancy as well as for the long-term follow-up of infected children to adolescence and even adulthood. There will only be a few trials and each attempt to answer one major question. Observational studies can complement trials and are important to inform their design. These data can identify high-risk populations that should be targeted by specific interventions and estimate the rate of outcomes that can be anticipated in the control arm of a trial.

The generalisation of results will be especially difficult for prenatal interventions, as they depend on several important factors that may influence the results. The results may vary according to the virulence of strains (Jamieson et al. 2008), the variability of drug concentrations or the genotypic characteristics of the population.

Moreover, if a drug is effective, a cost-effectiveness analysis at the population level could be used to synthesise the data from multiple cohorts and trials into a form that is more explicit for decision makers (Cooper et al. 2007). In such a model, factors like cost, demographics, the availability of appropriate tests, the incidence of toxoplasmosis infection in pregnant women and of clinical symptoms in children must be taken into consideration to evaluate future options and recommendations (Ades 2005, personal communication at the EuroTOXO consensus conference). Such an analysis could also be done systematically during the preparation of a randomised trial to assess whether different results yield contrasting conclusions and policy decisions. Finally, a cost-effective analysis may identify areas of uncertainty in the data and help prioritise research that is needed before a clinical trial is performed (Figure).

In summary, identifying new treatment options for the prenatal treatment of congenital toxoplasmosis is a high research priority. Also of great priority, is ensuring the most adequate pre-clinical and early clinical development of new drugs to inform the design of a future randomised trial. This is even more important as the epidemic is of alarming proportions in certain parts of the world, like South America (Neto et al. 2000, Bahia-Oliveira et al. 2003), and populations urgently need effective treatment. Meanwhile, in the next decade, currently available pre and post-natal treatment strategies should be formally evaluated to estimate their net benefit where possible.



Flow chart of congenital toxoplasmosis and potential interventions.

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