Congenital Toxoplasmosis: Value of Antenatal Screening and Current Prenatal Treatment

Frédérique Martin

Introduction

Mrs M., a 30 year-old lecturer in psychology is expecting her second baby. Her first baby was born while she was on sabbatical in France. During that pregnancy she was screened for Toxoplasmosis, found to be non-immune and had repeated monthly screening throughout her pregnancy. The consensus in France is to screen all pregnant women for toxoplasmosis at the first visit, and to offer repeated screening to those women who are found to be non immune because of the generally accepted idea that early maternal treatment leads to improved foetal and infant outcome in terms of mortality and severe sequelae in later life. She is concerned that she was not offered screening again during her second pregnancy. She wants to know why screening is not routine in Ireland and if there is any real benefit to be gained from it.

Due to her anxieties about Toxoplasmosis she is screened and found to be non-immune at 8 weeks, but at 16 weeks has a Toxoplasma IgM of 1 in 512. She now needs to know what the implications of this result are, and what should be done. To answer Mrs M.'s questions, we will review in this article the consequences of acquiring toxoplasmosis in pregnancy, the criteria to be fulfilled by a good screening programme and the possible benefits and problems related to screening for toxoplasmosis in Ireland.

To determine the best approach to the treatment of Mrs M., information about the current management of toxoplasmic infection in pregnancy was sought from a consultant in the Rotunda Hospital. Although the incidence of toxoplasmosis is thought to have increased over the last few years, it is still quite rare in Ireland and no precise protocol exists concerning the approach to adopt. RCOG guidelines were not available from their web site and the opinion of a French obstetrician was sought regarding the topic. The current recommendation in France is to start all mothers suspected of being infected during, or shortly before, the pregnancy on spiramycin to decrease the transmission to the foetus. Prenatal diagnosis is then carried out by PCR on an amniotic fluid sample and infected foetuses are treated in utero by administration of a combination of pyrimethamine and sulfadiazine to the mother. This regimen is subsequently given to the newborn (with overt or latent infection) until one year of age. To determine the value of this approach, a search of the literature was conducted using Medline and the Cochrane Database. The keywords used included toxoplasmosis, Toxoplasma, pregnancy, congenital, treatment, drug therapy, prenatal diagnosis, chorioretinitis, amniocentesis and PCR. About half of the recent publications on the topic had been made outside the UK-USA area and were not available from the Dublin medical libraries. Of those, three publications had to be obtained directly from France.

Toxoplasmosis: Transmission And Clinical Features

Toxoplasmosis is an infection caused by the protozoan *Toxoplasma gondii*, a world-wide parasite of animals and birds. Felines are the definitive hosts and excrete oocytes in their faeces. In humans infection occurs via ingestion of contaminated under-cooked food (especially meat) or transplacentally during acute infection in pregnancy, leading to congenital toxoplasmosis. In the immunocompetent host, Toxoplasma infection is usually asymptomatic but can produce a mild self-limiting illness with lymphadenopathy similar to infectious mononucleosis. Congenital infection, however, is a very serious condition with a lethal prognosis in about 10% of cases and a high proportion of disabling sequelae. Toxoplasmosis is a lifelong condition but the foetus is only at risk of congenital disease when acute infection occurs in pregnancy. Placental contamination is a prerequisite to congenital infection and occurs almost only following primary infection when there is maternal parasitaemia. The infected placenta then acts as a reservoir from which the parasite can spread to the fœtus, leading to a multisystemic disease. When the mother is chronically infected by *T.gondii,* the parasite is dormant in the maternal tissues and there is no parasitaemic phase. Only rarely has congenital infection been reported from a chronically infected immunocompromised mother with a reactivation of toxoplasmosis. Foetal transmission can occur

immediately after maternal infection or be delayed by several weeks. Spiramycin can be effective in sterilising the placenta and can thus be used to prevent foetal infection when there is a delay between the placental contamination and the actual transmission to the foetus.[1] Transplacental transmission is more likely to occur in later pregnancy with reported rates ranging from 10% in the first trimester to 80% after 34 weeks when no treatment is given to the mother.[2]

Infection in early pregnancy can lead to miscarriage or intra-uterine death. In those foetuses who survive the lesions observed are predominantly cerebral, caused by cerebral vasculitis and necrosis. The necrotic lesions can later calcify and if located near the aqueduct they can cause obstruction and hydrocephalus. The earlier the infection occurs in pregnancy, the worse the outcome is for the foetus, both in term of survival and sequelae. A recent study shows that among 60 foetuses infected between 17 and 23 weeks gestation the infection was sub-clinical in 37 (61.7%) and 21 (35%) had severe intracranial calcifications, while among 56 foetuses infected between 24 and 36 weeks the infection was sub-clinical in 40 (71.4%) and only 12 (21%) had severe intracranial calcifications.[3] Although the classical manifestations of congenital toxoplasmosis in infancy are a triad of hydrocephalus, brain calcifications and chorioretinitis, most symptomatic infants present with a combination of fever, microcephaly or hydrocephalus, hepatosplenomegaly, jaundice, chorioretinitis and seizures. Pneumonitis, myocarditis, thrombocytopenia, mono- and lympho-cytosis and a maculopapular rash also occur. Sequelae such as chorioretinitis, mental retardation, seizures and nerve palsies are common but they may be delayed, sometimes for years, the child being totally asymptomatic during the neonatal period. Chorioretinitis in particular may not occur before adolescence, which emphasises the importance of long-term follow-up for children with congenital toxoplasmosis. The prognosis depends mainly on the timing of the infection during the pregnancy, but up to 65-85% of all infected infants develop ocular sequelae in later life.[4]

Criteria for Screening

Screening during pregnancy is aimed ultimately at preventing congenital infections and at diagnosing all infected infants, so as to offer them the earliest and best possible care. As most congenitally infected infants appear normal at birth a policy of screening for toxoplasmosis would imply either the identification of all the women who are non-immune at the beginning of their pregnancy or a serological screening of all new-borns. Prenatal screening requires that mothers susceptible to infection are identified. Screening for the presence of antibodies allows primary prevention of toxoplasmosis infection where eating habits and hygiene practices have been clearly identified as risk factors. Ideally all women of child-bearing age should know their serological status before conception. Once the maternal serological status is known, screening for maternal then foetal infection during pregnancy is necessary, as is the availability of adequate in utero and postnatal care for the infected infants.[5] As maternal infection is most often clinically silent, repeated serological testing is the only way to diagnose all acute infections occurring during pregnancy. Diagnosis is based on maternal seroconversion to IgM anti-Toxoplasma or on a four-fold rise in IgG titre in two serum samples taken three weeks apart. The absence of both antibodies virtually rules out the diagnosis of toxoplasmosis. Mothers who tested positive before the pregnancy do not need monitoring because foetal infection from a chronically infected mother is exceptional. Mothers who tested negative should be monitored until term to avoid missing a clinically silent infection in the days following delivery. Although symptoms of toxoplasmosis in adults are not specific, if serology suggests a recent infection careful retrospective questioning may be a reliable indication of the time of infection. Once acute maternal infection is diagnosed, prenatal diagnosis of foetal infection is necessary in order to avoid exposing healthy foetuses to antibiotics that are not without side-effects on the neonatal gut flora in particular. Prenatal diagnosis is based on the detection of the parasite or its constituents by techniques of molecular biology. Foetal blood sampling has now been abandoned at the expense of amniocentesis with polymerase chain reaction (PCR) and mouse inoculation of amniotic fluid.[6]

The Situation In Ireland

Toxoplasmosis is quite a rare disease in the British Isles when compared to most of continental Europe. Population seropositivity to *T.gondii* was reported to be as high as 70% in some regions of France but it is only around 19% (Dublin) to 40% (midlands) in Ireland. This is the main reason why routine screening is worthwhile in France and not in Ireland. The positive predictive value of screening tests, even when they are quite specific, falls dramatically when the incidence of the disease in a population is very low, with a parallel increase in the number of false positive cases. In Ireland, the rate of seroconversion to *T.gondii* in pregnancy is estimated to be around 2 per 1000, leading to the birth of 50 to 100 infected children per year. Even if serology testing had a false positive rate of only 1%, routine screening would wrongly label 500 mothers per year as infected and 500 healthy foetuses would be exposed to invasive exploration (amniocentesis), leading in average to 5 miscarriages. Among the infants born to infected mothers, 50% overall are infected in utero and 25% suffer severe damage. Screening a population of pregnant women for a disease they are unaware might be transmitted to their baby generates a great burden of anxiety. This should be taken into account when establishing a screening programme, as should the anxiety caused by a positive prenatal diagnosis when the prognosis for the foetus

might be difficult to determine. However, screening remains the only way to diagnose infection in neonates with a subclinical form of the disease and in whom crippling late sequelae could be effectively prevented by treatment. Screening also allows primary prevention and health education for seronegative women at risk of infection. The availability of the antenatal diagnosis techniques is also a point to consider, as amniocentesis is now available in the Dublin maternity hospitals but it may not be in rural areas where toxoplasmosis is more prevalent. Many cases would then need referral to specialised centres, which dramatically increases the cost of the programme.

When the topic of routine screening was last reviewed by the Royal College of Obstetricians and Gynaecologists (RCOG) in the early 1980s, it was concluded that routine screening should not be instituted in Ireland because the disease is so rare. It would not be cost effective and the benefits of treating infected infants in utero would be outweighed by the negative effects of screening, notably the exposure of too many healthy foetuses to invasive testing and the distress for so many parents told that their baby might be infected. It is interesting to note that the last RCOG guidelines where published at a time when amniocentesis testing for toxoplasmosis was not widely available and where the samples had to be sent to reference laboratories in the United Kingdom to be analysed. Furthermore, there has recently been suggestion of an increasing incidence of Toxoplasma in Ireland. If this is confirmed and develops to such an extent that the benefits outweigh the risks associated with screening, then routine screening should be reconsidered.

Another specificity of the Irish situation when compared to France, or indeed to many other European countries, is that in France termination of pregnancy is carried out, on request from the parents, when the infection has occurred early in pregnancy (before 10 weeks) because the neurological outcome is very poor or when major brain lesions are diagnosed on ultrasound scan.[7] This option is not available in Ireland. An alternative to antenatal screening in Ireland might be neonatal screening. Most congenitally infected infants are asymptomatic at birth (80%) but of those another 80% will develop sequelae in later life (mainly chorioretinitis). The incidence and severity of the sequelae are very significantly reduced by early neonatal treatment and routine screening for neonatal IgM, for example from a Guthrie card sample, may be of great value. Further research is still needed to determine the value of neonatal screening but this practice may prove of great benefit. Antenatal treatment by spiramycin and pyrimethamine-sulfadiazine has been shown to reduce significantly the sequelae and the severity of congenital toxoplasmosis. Mrs M. should be told that although toxoplasmosis is too rare a disease in Ireland to make routine screening worthwhile, selective screening is performed in people who are concerned about the disease or who have definite risk factors or when there is suggestion that the mother or the baby might be infected. She should also be reassured that antenatal diagnosis and treatment are available if the screening test is positive.

Benefits of Screening

Several prospective studies published since the end of the 1980's have shown conclusive evidence that antenatal anti-parasitic treatment reduces very significantly the rate and the severity of sequelae among infants born to infected mothers and to date there is no doubt that infected foetuses should be treated in utero. The most interesting article recovered from the literature search was published in April 1999 in Presse médicale and reviewed the problem of congenital toxoplasmosis and its evolution over the last four decades, based on the experience of a specialised toxoplasmosis centre in Paris (Institut de puériculture). This article offers an overview of the situation of congenital toxoplasmosis in France and describes the current recommended approach to management when a mother is infected during pregnancy.[8] In the following paragraphs, we will review this article and compare how it correlates with the case of Mrs M.

Prospective screening for women seronegative to toxoplasmosis before their pregnancy has been mandatory in France since 1978. Prevention of foetal transmission by spiramycin, possibility of in utero diagnosis and the availability of in utero treatment in cases of recognised foetal infection have led to a dramatic change in the pattern of the disease. Institution of prenatal diagnosis was a major advance in the management of congenital toxoplasmosis. It was based at first on parasitologic, serologic and biologic examination of a foetal blood sample obtained by cordocentesis. Disadvantages included a false negative rate of up to 15% and 4 to 6 weeks were necessary to obtain parasitology results (mouse inoculation). PCR detection of *T.gondii* genome in amniotic fluid obtained by amniocentesis is now the method of choice with a specificity of 100% and a sensitivity of 97.4% (foetal blood analysis has a comparable sensitivity but a specificity of only 89.5%). It is vital that the laboratory processing the samples is experimented in PCR techniques and has rigorous protocols. PCR is performed from 18 weeks gestation onwards and at least two months after the seroconversion. Mouse inoculation from the sample is still performed as a control and to study the different serotypes of the parasite.[9]

The institution of prenatal diagnosis and follow-up of infected infants allowed to establish precisely the risk of foetal infection: 40% overall, but reduced by half if treatment by spiramycin is given. With spiramycin treatment, the risk of foetal infection is 2% if the infection occurred between the 3 and 10 weeks gestation, 7% between 11 and 14 weeks, 12% between 15 and 18 weeks, 21% between 19 and 30 weeks and 50% after the 30th week. It could be as high as 90% in the last 2 or 3 weeks of the pregnancy.

The risk of serious disease with cerebral lesions is high during the first weeks of the pregnancy but decreases thereafter and is minimal if the infection occurred after 26 weeks. So far, nine cases have also been reported where congenital toxoplasmosis was due to maternal infection in the few weeks preceding the conception. It may be wise to delay pregnancy for about 6 months in women with recent infection. Careful pre- and post-natal follow-up of the infants is also recommended when the mother was infected shortly before the pregnancy. The classical syndrome described in the 1940's when the entity was first recognised is now exceptional and most congenital infections are subclinical at birth (70%). The incidence of severe disease with hydrocephalus or neurological signs has fallen dramatically from 45% of all cases in the 1949-60 period to 5% between 1984 and 1992. Furthermore, about half of the severe cases reported in the 1980's were in infants born to mothers who had spent their pregnancy outside France, hence who had not been screened for toxoplasmosis. In 30 years, the incidence of toxoplasmosis in France has declined from around 80% in Paris in 1965 to 54.3% overall average (38.2% to 68.3%) in 1995. The incidence of seroconversion during pregnancy is 1.48%.

Ultrasound in Management

Foetal ultrasound is also essential to the management of gestational infection and its role is both diagnostic and prognostic. Signs of foetal infection are present in about 65% of pregnancies when infection occurred in the first trimester and in 20% in the second trimester.[7] Ultrasound cannot be used as a screening tool as it detects only major signs of infection and in those foetuses the clinical syndrome of congenital toxoplasmosis would be obvious at birth. It can be extremely valuable however in pointing towards a toxoplasmic infection in countries like Ireland where routine screening is not performed. Ultrasound signs of foetal infection after a negative amniocentesis is also of value in indicating a second sampling as a few foetuses are infected after the time of prenatal diagnosis, even when the mother has correctly taken her spiramycin.[7] Following a negative amniocentesis, scans should be performed monthly. When amniocentesis is positive scans should be performed fortnightly.[2] Repeated scans are mandatory during an at risk pregnancy. Cerebral ventricular dilatation, usually symmetrical and bilateral, is the most common and characteristic sign. It starts in the posterior horns of the lateral ventricles and leads, sometimes in the space of a few days, to hydrocephalus. It is a poor prognostic sign but its absence cannot be interpreted in terms of prognosis as major brain lesions may be present without involvement the periaqueductal area. When infection occurred during the first half of the pregnancy, cerebral ventricular dilatation may appear after a negative amniocentesis hence the need for careful repeated scanning.[2] Intracranial densities are found less frequently. They correspond to the intracranial calcifications observed after birth. They may be difficult to identify on ultrasound although higher quality probes and a better standardisation of the ultrasonic examination have lead to a more accurate diagnosis. Intracranial densities are now well correlated with the presence of chorioretinitis at birth. In a study of 133 infected neonates of 37 with intracranial densities 9 (24.3%) had chorioretinitis at birth, while of 96 without intracranial densities only 7 (7.3%) had chorioretinitis at birth. Non cerebral signs of foetal infection should also be looked for. Hyperechogenic foetal bowel is a marker of foetal infection and when present screening not only for toxoplasmosis but also for CMV, cystic fibrosis, foetal karyotyping and amniotic digestive enzymes assays is worth doing.[2] Placental thickening with a "frosted glass" aspect, hepatosplenomegaly and hepatic densities, pleural and pericardic effusions and ascites should also be looked for and signify the multisystemic nature of the disease. These may be transient but are often associated with neurologic and ocular disease. Most foetuses however are not severely damaged by the infection and cannot be identified by ultrasound examination.[2, 7]

Treatment

Prenatal treatment consists of a combination of pyrimethamine 50 mg/kg/day and sulfadiazine 3 g/day with folinic acid supplementation (50 mg twice weekly) and regular FBCs. This regimen is given for four weeks alternating with two weeks of spiramycin throughout the pregnancy.[8] When the PCR is negative, it is important for the mother to continue taking spiramycin because of the risk of late transmission to the foetus. Data from the Institut de Puériculture published in 1991 show that in treated infants Toxoplasma is less frequently isolated from the placenta (42% vs 77% in the non treated group). The incidence of seropositivity of Toxoplasma-specific IgM is also lower (17% vs 69%). It will be necessary to follow those infants prospectively to determine whether in utero treatment adds to post-natal treatment in preventing late reactivations of the disease (chorioretinitis for example).[8]

Neonatal infection is diagnosed by the presence of specific IgM, by the synthesis of IgA or IgG antibodies, by a positive mouse inoculation from the placenta and by the presence of clinical signs (chorioretinitis, brain calcifications). It is not uncommon for all these examinations to be negative. Sufficient time has to be allowed to obtain completely negative serologic studies before follow-up can be interrupted. Subclinical congenital toxoplasmosis carries a risk of up to 80% of late reactivation and this justifies post-natal treatment. Spiramycin has never been shown to cure toxoplasmosis and should be stopped after birth while treatment is continued with pyrimethamine and sulfadiazine. The best regimen post-natally is not yet established. Courses of 3 to 4 weeks repeated 3 to 4 times during the first year of life have been shown to prevent the development of ocular sequelae.[8] However, recent evidence from trials conducted

on cerebral toxoplasmosis on AIDS patients and from the Chicago Collaborative Treatment Trial suggest that much more intensive regimens may be indicated.[10] It seems difficult to impose too strict a regimen when most cases are sub-clinical because of side-effects of the treatment and for compliance reasons. Sulfadoxine may be an alternative to sulfadiazine although more research is necessary on that matter.[11]

The appearance of chorioretinitis in a child is an emergency and pyrimethamine and sulfadiazine should be started immediately. This combination is very effective and lesions usually resolve within two weeks although treatment should be continued for at least 4 to 8 weeks and for up to 6 to 12 months if a relapse is likely. Serological rebounds (a secondary increase in antibody titres) are frequent, occurring in up to 70% of treated infants.[8] They often occur around two or three years of age, but can occur up to the age of 12 and they are more frequent and more marked if the treatment regimen has been intense. Their significance is not known and they are not statistically associated with secondary reactivation of chorioretinitis. When they occur after two years of age, they may raise the suspicion of parasitic proliferation and may warrant treatment for six months. In the future, more research is necessary to determine alternative regimens for cases where intolerance to pyrimethamine and sulfadiazine develop and to determine more effective combinations or treatments that could act on the parasites dormant in tissue cysts. Vaccination against *T.gondii* might also be an advance in the future.

Although most data in this article were obtained from a single centre, the Institut de puériculture de Paris, several other studies, particularly a multicentre European trial, have been published which confirm the role of antenatal treatment for toxoplasmosis and the efficacy of the pyrimethamine-sulfadiazine combination. In the European study, published this year in the American Journal of Obstetrics and Gynaecology, spiramycin was not shown to decrease the rate of foetal-maternal transmission.[12] Giving the mothers spiramycin should remain the standard management until further conclusive evidence is available.

Implications for Mrs M.

The appearance of a significant IgM antibody titre in a previously seronegative patient implies that Mrs M. has been infected by *T.gondii* at some time between 8 and 16 weeks pregnancy and that her foetus is now at risk of developing congenital toxoplasmosis. The benefits of treating a pregnant patient recently infected by *T.gondii* seem obvious from these data and Mrs M. should be started immediately on spiramycin. It should be explained to her that because she has been infected between 8 and 16 weeks and because she is now on treatment, her risk of transmitting the infection to her baby is about 7 to 12%. 16 weeks is too early to perform an amniocentesis but she should be offered this examination later in her pregnancy, probably around 24 weeks to allow for a two months lapse if she has been infected closer to 16 weeks. Amniocentesis is available in the Dublin maternities and the sample should be send to a laboratory competent to perform a reliable PCR on it, either in Dublin or in a reference laboratory in the UK. It carries a lower mortality and morbidity than cordocentesis and should be used preferably wherever possible. If the PCR results are negative, Mrs M. should be kept on spiramycin until the end of her pregnancy to prevent a late infection of her baby. If the PCR is positive then the foetus is infected and she should be started on a combination of pyrimethamine, sulfadiazine and folinic acid as recommended. The potential risks for her baby should be explained to Mrs M. but she should be reassured that with treatment the chances of her baby not being affected are much greater than the chances of her baby being affected. The choice of the post-natal treatment may be more difficult: she was infected at a time where the risk of neurological sequelae in the child is high and a more intensive regimen than the one recommended may be necessary if the baby is symptomatic at birth. The importance of compliance should be emphasised and it should be explained that long-term follow-up of the child will be necessary, certainly up to one year of age and probably up to the teenage years if the infant shows evidence of latent infection. This is because of the high risk of developing chorioretinitis in later childhood despite treatment.

The use of fortnightly ultrasound examination may not be of evident benefit in the Irish context. The appearance of rapidly evolutive cerebral lesions on ultrasound (particularly rapidly evolutive hydrocephalus) which have been constantly associated with a poor neurological outcome lead to discussion with the parents of the possibility of termination of the pregnancy. This is of course not an available option in Ireland and performing routine scans to establish a neurological prognosis would not add a great benefit in term of management. Periodic ultrasound can be used to assess the development of signs of infection in a foetus with a negative PCR and may lead to performing a second amniocentesis if this is thought appropriate.

Conclusion

In conclusion, toxoplasmosis remains a serious disease although recent advances in diagnosis and treatment have greatly ameliorated the prognosis for the affected infants. Routine screening is currently being discussed in several European countries because of the well proven efficacy of the treatment. In Ireland, the incidence of the disease is probably too low to justify routine screening but recent data are lacking and a re-evaluation of the situation may lead to different conclusions. When infection in utero is documented, using PCR on an amniotic fluid sample, the mother should be started on a combination of pyrimethamine and sulfadiazine with folinic acid supplementation. Infected infants should be treated postnatally up to one year of age with the same drugs,

whether the infection is overt or latent and follow-up is important up to adolescence. The best regimens of post-natal treatment are not yet established and new more effective combinations may be used in a near future. The possibility of a Toxoplasma vaccine is also promising although it is still at its embryonic stages.

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