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# Diagnostic problems and postnatal follow-up in congenital toxoplasmosis

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***Aim.*** **In order to assess the consequences of different clinical approaches in the prenatal management of congenital toxoplasmosis, we retrospectively reviewed 58 pregnant women with Toxoplasma seroconversion and prospectively enrolled their 59 infants, referred to us from 1999 to 2004.**

***Methods.*** **Data on clinical, laboratory and demographic characteristics of the pregnant women were collected. Their children were entered into a 48-month follow-up programme in which clinical, instrumental, ophthalmologic and serologic evaluations were carried out at birth, at 1, 3, 6, 9, 15, 18, 24, 36 and at 48 months of life. Paediatric treatment with Spiramycin alone or alternated with Pyrimethamine-Sulphadiazine was administered according to the different clinical cases.**

***Results.*** **Time of infection was dated in the first trimester for 24 women (41%), in the second trimester for 18 women (31%) and in the third trimester for 16 (28%). In the first trimester of pregnancy 20 of the 24 infected women had undergone amniocentesis, while the test had not been performed on any of the women infected in the third trimester. Serological follow-up revealed that 11 (19%) of the infants had been infected. An alternating regimen with Pyrimethamine-Sulphadoxine was administered to the infected children. All the infants were clinically asymptomatic, and the instrumental follow-up revealed specific toxoplasmosis anomalies in 4/11 infected children.**

***Conclusion.*** **Our results highlight issues and problems concerning current prenatal diagnostic tests and the therapeutic approach based on PCR testing of amniotic fluid alone. The incidence of ocular-cerebral lesions observed in children born to women with seroconversion in the third trimester raises questions about the diagnostic and therapeutic approach for these women and their offspring. Paediatric therapeutic protocol, with alternating Pyrimethamine-Sulphadiazine regimen, applied also to asymptomatic children born to women with inadequate prenatal diagnostic management, could prevent severe sequelae.**

Key words: **Toxoplasmosis, congenital - Toxoplasmosis diagnosis - Toxoplasmosis, drug therapy.**

Congenital toxoplasmosis results from foetus *Toxoplasma gondii* (TG) infection during pregnancy. Transplacental transmission

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occurs in 10-80% of maternal infections, depending on gestational age at the time of maternal infection, and prenatal treatment received.[1, 2] Although foetal involvement is most severe when infection occurs at the beginning of pregnancy, severe neonatal toxoplasmosis has also been observed after third trimester maternal infection.[3] Prenatal monitoring of susceptible pregnant women through gestation and delivery would allow early identification and treatment of the women and their offspring. Nevertheless, prenatal diagnosis is difficult and requires careful consideration, as maternal serological investigations exhibit intersite variability in the estimated time of infection and PCR testing of amniotic fluid shows different sensibility and/or specificity ranges.[4-6] Moreover, the management of toxoplasmosis must be guided by the trimester of maternal infection, which affects the diagnostic and therapeutic approach to be adopted. In the absence of uniform therapeutic and diagnostic care, the evaluation of infants, including those that are asymptomatic, requires a thorough, long-term follow-up.[7]

The aim of the study was to compare prenatal management of congenital toxoplasmosis with definitive diagnosis in the infants, obtained by follow-up serological tests during the first year of life.

## Materials and methods

### *Study design*

We retrospectively evaluated 58 pregnant women who met the diagnostic criteria for definite TG infection, and prospectively enrolled their 59 infants in a 48-month follow-up.

### *Patients*

Mother-child data were inserted in a computerized database (Eureka), an *SQL-based* system, capable of data porting, which can be interfaced with any other research system. The relevant obstetric notes were reviewed, and data were collected on clinical, laboratory and demographic characteristics, private or public hospital assistance, educational level and occupation of the pregnant women.

The children's files contained information on sex, gestational age and a follow-up schedule with clinical, neurological, serological evaluations at birth, at 1, 3, 6, 9, 15, 18, 24, 36 and at 48 months of life.

### *Definition of cases*

In accordance with the European case definition for TG infection in immunocompetent pregnant women, we identified as "definite" TG infection all cases with: 1) seroconversion (appearance of specific anti-*Toxoplasma* immunoglobulin IgG and IgM) during pregnancy both samples taken after conception; 2) positive culture from maternal blood; 3) confirmed congenital infection in offspring.[8]

Congenital infection was diagnosed on the basis of persistence of TG-specific IgG antibodies during the first year of life.[8]

### *Diagnostic methods*

#### MATERNAL SAMPLE

*Serologic test.*—*Toxoplasma*-specific IgM and IgG antibodies were detected by 2 commercial enzyme immunoassay tests (ETI TOXOK-M reverse, ETI TOXOK-G PLUS [DiaSorin, Saluggia, Italy], and Toxo-IgM ISAGA, Toxo-IgG VIDAS, Toxo-IgG avidity VIDAS [BioMerieux, Marcy-l'Etoile, France]).

All tests were performed by strictly following the instructions in the manufacturer's package insert. In accordance with NCCLS recommendations, both label kits report sensibility and specificity >95% and >98-99% respectively.[9] All the mothers were double tested, with an intra-assay coefficient of variation (CV) <2%. Each serum sample was tested at least twice on different days and different plates. The serological tests were performed in various authorised clinical laboratories in Palermo.

*PCR.*—Amniotic fluid was drawn for prenatal diagnosis by PCR (35-fold-repeated first gene B1; sensitivity, 97.4, negative predictive value 99.7 [10]). The PCR analysis was performed in various authorised clinical laboratories in the Palermo area.

*Paediatric sample.*—All infants with suspected infection were tested for *Toxoplasma-*

TABLE II.—*Management of 58 infected women and postnatal diagnosis of children.*

| Prenatal diagnosis (No.) | Prenatal treatment (No.) | Infected infants (No.) | OR (IC 95%) P^ |
|---|---|---|---|
| Women without prenatal diagnosis (33) | Spiramycin 26 | 6* | 0.40 (0.05-3.13) |
| | Untreated 7 | 3§ | P=0.27 |
| Women with positive prenatal diagnosis (6) | Spiramycin 4 | 1 | |
| | Combined therapy 2 | 0 | P=0.66 |
| Women with negative prenatal diagnosis (19) | Spiramycin 17 | 1 | |
| | Untreated 2 | 0 | P=0.89 |

* 3 infants showed sequelae; § 1 infant showed sequelae; ^ Fisher's exact test.

immune response. Only 2/58 (3.4%) had undergone specific pre-pregnancy serological tests. None of the cases of maternal-foetal infection resulted in pregnancy termination.

Time of infection was dated in the first trimester for 24 (41%) of the pregnant women, in the second trimester for 18 (31%) and in the third trimester for 16 (28%) women. Maternal-foetal transmission rate was 17.7%. Chi-square test for trend of vertical transmission (1/24 in the first trimester, 4/18 in the second and 6/16 in the third trimester; odds ratio=1, 6.57 and 13.80 respectively) was significant (P<0.05). Prenatal diagnosis by PCR testing of amniotic fluid was performed in 25/58 (43%) women, in most cases at the 22nd week of gestation (range between 18 and 33 weeks). Twenty of the 24 women (83%) with primary TG infection in the first trimester of pregnancy had undergone amniocentesis, while the test had not been performed on any of the women infected in the third trimester.

TG DNA was detected in the amniotic fluid of 6/25 (24%) of the women tested.

Among fifty eight women, only 49 (84%) women with certain infection received treatment: 47 were treated with Spiramycin, and 2 with PYR-SDX.

Prenatal ultrasound examination did not reveal any of the anomalies normally associated with an infected fetus (such as ventricular dilatation, increased thickness of the placenta, intracranial calcification, ascites).

We prospectively enrolled in the study 59 children (male/females 0.96) born to the sample of women with certain TG infection. Serological follow-up showed that 11 (19%) of the infants had been infected: 1 was born to a mother infected during the first trimester of pregnancy, 4 to mothers infected in the second trimester, and 6 to women infected in the third trimester; 5/11 (45%) had specific IgM and IgG antibodies at birth.

Table II compares prenatal diagnosis and treatment with final diagnosis in infants. Prenatal PCR results were discordant with post-natal serological follow-up in 6 out of 25 tested cases (24%). We found no statistically significant difference between infected and uninfected newborn of treated and untreated women; moreover, after adjustment for trimester of maternal infection no significant association was found. The association between maternal risk factors (contact with cats, eating raw/undercooked meat) and infant infection was tested and no statistically differences were detected.

We observed a greater number of infected infants among those born to mothers who had not undergone PCR testing of amniotic fluid compared to children born to mothers with prenatal diagnosis (9 *versus* 2). The analysis for trimester of seroconversion showed an association between infection in the third trimester and infection in offspring (P<0.05).

At birth, none of the infected infants

of maternal infection and the beneficial effects of continuous Spiramycin maternal treatment.[16] At present, there are no randomized controlled trials to assess the effect of antenatal treatment with Spiramicyn or PRY-SDX; when maternal infection is diagnosed, Spiramycin with or without PYR-SDX [17] remains the only recommended treatment. Although prenatal treatment of congenital toxoplasmosis has been used for almost 30 years, an increasing numbers of papers underline the uncertainty of its effectiveness particularly on vertical transmission. Because maternal-foetal transmission vary greatly with gestational age, a strict stratification of studied population is necessary, however at date it seem could be justified start directly with a PYR-SDX association, also without a confirm of foetal infection.[18]

In our sample, the infected children were asymptomatic and only cerebral instrumental monitoring revealed typical lesions. This confirms the importance of a careful and long-term instrumental follow-up, as most of these children were apparently normal at birth, developing toxoplasmosis-associated injuries later in life, ocular lesions in particular. Our paediatric therapeutic protocol, with alternating PYR-SDX regimen, applied also to asymptomatic children born to women who had not undergone amniocentesis, offers an effective way of preventing severe neonatal disease, especially in areas with an inadequate diagnostic protocol. However, it is difficult to evaluate the efficacy of our program in preventing sequelae without an untreated control group.

Only 2/58 pregnant women underwent serological testing for TG before pregnancy. Identification of risk factors for adverse pregnancy outcome is a main component of preconception care, but requires adequate time and knowledge: pre-conception testing is uncommon in our region, despite the fact that it has been shown to be an effective preventive diagnostic method. The integration of preconception care services within a larger maternal and child health continuum of care is well aligned with a prevention-based approach to enhancing global health. There is no standard and/or homogeneous approach to preventing congenital toxoplasmosis in Italy, so the approach is often a direct expression of the different local realness; in Sicily, for example, screening for toxoplasmosis is offered free of charge to all susceptible pregnant women, but this practise is not adequately promoted.

The impact of screening programs and the cost-benefit question need to be evaluated; moreover the cost of long-term follow-up to prevent sequelae in children later in life needs further consideration. Cost-effectiveness of optional screening (no screening, pre-conception or neonates screening, frequency of test during pregnancies) depends on local factors: incidence of congenital toxoplasmosis, available diagnostic and therapeutic services and the population compliance with the screening. Is important to promote public as well as professional knowledge regarding the disease, in order to prevent, diagnose and treat congenital toxoplasmosis in order to minimize the risk of infection.

Neonatal screening has been implemented in several countries (*e.g.*, Denmark, Massachusetts, USA) [19] and thanks to these programmes as many as 80% of infected newborns have been identified: of course, neonatal screening is feasible for diagnosing children with congenital toxoplasmosis at birth just in low endemic areas.

Despite great progress in clinical and basic science research, many unresolved issues in toxoplasmosis have still to be addressed: our results highlight issues and problems concerning the prenatal diagnostic tests currently available and the therapeutic approach based on PCR testing of amniotic fluid alone. In the future, the standardization of quantitative PCR methods may be particularly valuable for the prognostic evaluation of congenital toxoplasmosis.[20]

Congenital toxoplasmosis continues to be a tragic outcome of a preventable and treatable infection. Education of patients, physicians and health policy makers on the primary and secondary preventive measures of the disease, and their execution, will undoubtedly result in lower incidence, morbidity, and mortality rates from congenital disease due to Toxoplasma.

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