In Utero and at Birth Diagnosis of Congenital Toxoplasmosis

Use of Likelihood Ratios for Clinical Management

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Background: The results of the ante- and neonatal diagnostic tests for congenital toxoplasmosis influence the decision to treat the newborn immediately after birth. Here, we estimate the positive and negative likelihood ratios (LRs) and the probabilities of congenital infection according to PCR and IgM-IgA tests results.

Methods: The study concerned 767 children born between 1996 and 2002 and followed-up for 1-year at Croix-Rousse hospital, Lyon, France. The LRs and the post-test probabilities were estimated conditionally on gestational age at maternal infection using a logistic regression approach.

Results: For the PCR and the IgM-IgA tests, the positive LRs were high. In children with one positive test when only one test was done, the probability of infection reached 90% when the maternal infection occurred at 18-weeks gestation or later. This probability was close to 100% when the 2 tests were positive, whatever the gestational age. The negative LRs of the 2 tests moved closer to 0 at later gestational ages. However, when the tests were negative, the probability of infection remained greater or equal to 10%, except in early maternal infection. When the 2 tests were discordant, the probability of infection was about 50% in early maternal infection.

Conclusion: Providing reliable probabilities of congenital infection, the PCR and IgM-IgA tests guide clinical management and counseling of parents.

Key Words: congenital toxoplasmosis, antenatal and neonatal diagnosis, sensitivity and specificity, likelihood ratios, posttest probability

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Toxoplasma gondii infection during pregnancy can have serious consequences on the fetus, ranging from fetal loss to severe neurologic or ocular lesions. In other cases, infected newborns are asymptomatic at birth, but are at risk for developing retinal diseases during childhood or adolescence on *Toxoplasma* reactivation.[1] Because maternal infection is usually asymptomatic, the diagnosis of *T. gondii* infection relies on serologic tests. On a national level, those tests are either mandatory as in France and Austria[2,3] or highly recommended as in Italy and Slovenia.[4,5] On an individual level, the tests are increasingly performed at the request of the obstetricians or of the pregnant women themselves.

In a pregnant woman, the diagnosis of an acute infection usually leads to amniocentesis in search for *Toxoplasma* DNA by PCR technology. In case of a positive result, fetal ultrasound surveillance is intensified, and treatment with pyrimethamine and sulfadiazine (PS) is prescribed. Because the risk of fetal infection varies with the gestational age at maternal seroconversion, the value of an antenatal diagnosis varies according to the trimester of pregnancy. Early detection and treatment of congenital toxoplasmosis is believed to reduce infection sequelae, though the exact benefits are still debated.[6]

In some settings, the diagnosis of the disease relies on cord blood testing[7] or only on the presence of clinical signs of infection in the newborn. Neonatal diagnosis consists mainly of serologic tests. Because *Toxoplasma*-specific IgG antibodies cross the placenta, their presence in the newborn blood cannot be considered as a marker of congenital infection. On the contrary, *Toxoplasma*-specific IgM or IgA antibodies do not cross the placenta; thus, their presence is considered as a good marker of congenital infection. Gestational age at maternal infection affects the diagnostic performances of IgM and IgA tests.[8,9] The results of the neonatal and antenatal tests, when available, influence the decision to treat the newborn immediately after birth. The physician should thus have an adequate knowledge of the performance and the limitations of the diagnostic tests.

The accuracy of diagnostic tests is usually described by their sensitivity and specificity. The likelihood ratios (LRs) of the test results are more useful.[10] The LR of a positive result quantifies the knowledge gained from the test about presence of the disease. The higher the LR, the greater the disease probability. By contrast, the LR of a negative result quantifies the knowledge gained through the test about absence of the disease. The more the negative LR is near to 0, the smaller the disease probability. A test with a positive and a negative LRs equal to 1, provides no useful information.

In the present study, we estimated the positive and negative LRs of the *Toxoplasma* PCR test on amniotic fluid and of the combination of specific IgM and IgA tests at birth in a cohort of children born to mothers who seroconverted during pregnancy. The probabilities of congenital infection according to the gestational age at mother infection and to the result of ante- and neonatal tests were also estimated.

MATERIALS AND METHODS

Study Population

The study comprised a cohort of 767 consecutively included children born between January 1996 and December 2002 to mothers whose toxoplasmosis infection during pregnancy was serologically confirmed by the laboratory of our department. All children were managed according to a standard protocol. Assessment at birth included funduscopic examination and cranial ultrasonography in addition to tests for *Toxoplasma*-specific IgM, IgA, and IgG on cord or neonatal blood samples. Pediatric check-ups, assessment of neurologic development and IgG and IgM determinations were scheduled every 2 to 3 months for at least 1 year. The

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gold standard for congenital toxoplasmosis was persistence of specific IgG beyond 1 year of age[11] as detected by indirect immunofluorescence (Toxo-Spot IF, Biomérieux, Marcy l'Etoile, France). Congenital infection was excluded if specific IgG declined below undetectable levels in a child who had received no treatment.

Antenatal Diagnosis by PCR

Antenatal diagnosis was based on monthly fetal ultrasound monitoring and on amniocentesis for PCR on amniotic fluid. In 481 children, an amniocentesis was performed allowing an antenatal diagnosis by qualitative PCR (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A332). Amniotic fluid was sampled after the 17th week of gestation and at least 4 weeks after the estimated date of infection.

The PCR methodology has been previously detailed.[12] Amplification targeted the *B1* gene of *T. gondii*. The sensitivity of the PCR was monitored using an internal artificial DNA control; this included a decontamination step with uracil DNA glycolase to prevent carry-over contamination. The results were simply expressed as positive or negative.

Neonatal Diagnosis by IgM and IgA Tests

These tests were performed on cord blood samples at birth or on peripheral blood samples during the very first days after birth. In 452 children, the tests were performed on peripheral blood and among them, 320 children had the tests also on cord blood (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A332).

Specific IgM antibodies were detected by an immunosorbent agglutination assay (Toxo-ISAGA, Biomérieux, Marcy l'Etoile, France). The results were expressed on a 0 to 12 scale and considered positive for any value but 0.

Specific IgA antibodies were detected by the Toxo IgA test using an ELISA technique (Clonatec SFRI, Saint Jean d'Illac, France). The test result was considered positive for values equal to or greater than 0.6 IU/mL. The combined IgM-IgA test was considered positive when at least 1 of the 2 tests was positive and negative when both tests were negative.

Antenatal PCR and Neonatal Tests on Peripheral Blood

Of the study population, 265 children had an antenatal diagnosis by qualitative PCR and a neonatal diagnosis by IgM-IgA on peripheral blood (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A332). These data were used to estimate the diagnostic accuracy of the IgM-IgA test in peripheral blood in case of positive PCR result and in case of negative PCR result.

Factors That Affect the Diagnostic Accuracy of the Tests

In all tests, we studied the effect of gestational age at maternal infection. In neonatal tests, we studied the effect of the origin of the blood sample using the group of children who had tests on cord and peripheral blood. The effect of the delay between the date of birth and the date of blood sampling was also studied in all tests carried out on peripheral blood. Concerning PCR, we studied the effect of the delay between the time of maternal infection and the time of amniocentesis.

Statistical Analysis

In a first step, the sensitivity and the specificity of the antenatal PCR and of the IgM-IgA test were estimated whatever the gestational age at maternal infection and for each trimester of gestational age. Their exact 95% confidence intervals were calculated.

In a second step, the sensitivity and specificity of the IgM-IgA test performed on cord blood and on peripheral blood were compared using McNemar test.

In a third step, the sensitivity, the specificity, and the positive and negative LRs (LR+ and LR−) were estimated conditionally on gestational age at maternal infection and on the other above-cited factors using a logistic regression approach developed by Janssens et al.[13] In particular, LR+, LR−, sensitivity, and specificity were estimated at 3 times of maternal infection (6, 18, and 30 weeks gestation), and the confidence intervals were obtained using a bootstrap method. The 90% confidence intervals were calculated to obtain a 5% probability for the true LRs to be under the lower limit for LR+ estimates and over the upper limit for LR− estimates.

The posttest probabilities of congenital infection were calculated combining the probability of congenital infection according to the gestational age at maternal infection to the LR+ when the test was positive and the LR− when the test was negative. A logistic regression with a modeling of the effect of gestational age by a second-order orthogonal polynomial, allowed determining the probability of congenital toxoplasmosis according to gestational age at maternal infection.

RESULTS

Population Characteristics

Of the 767 children included in the study, 396 were males (52%). The median gestational age at birth was 38 weeks (first and third quartiles: 36 weeks 6 days and 38 weeks 6 days, respectively) and the median birth weight was 3.32 kg (first and third quartiles: 2.97 kg and 3.62 kg, respectively). These values were nearly the same in children with congenital infection and in children without congenital infection.

Maternal infection occurred during the first trimester of pregnancy in 46.4% of cases (356/767), during the second trimester in 28.4% of cases (218/767), and during the third trimester in 25.2% of cases (193/767). A total of 93% of the mothers (717/767) received treatment during their pregnancy. Among them, 86% (617/717) were treated with a macrolide only and 14% (100/717) received PS.

About 22% (171/767) of the children had a congenital infection. The risk of congenital infection was 3.4% when the infection of the mothers occurred during the first trimester (12/356), 21.6% when that infection occurred during the second trimester (47/218), and 58% when it occurred during the third trimester (112/193). The risk of congenital infection was estimated at 2.2%, 23%, and 56% for maternal infections occurring at 6, 18, and 30 weeks gestation, respectively. A treatment was started at birth for 176 children. Among them, 168 had a congenital infection.

Antenatal Diagnosis by PCR

The median delay between gestational age at time of maternal infection and amniocentesis was 8 weeks (first and third quartiles: 6 weeks and 11 weeks, respectively). The sensitivity of the PCR was estimated at 69% (95% Confidence Interval [CI]: [59%–79%]) and its specificity at 99.2% (95% CI: [98.2%–100%]). The sensitivity of the PCR tended to increase with later gestational ages at the time of maternal infection (Tables 1, 2). Its specificity was high, close to 100% (Table 1) with a slight trend toward a decrease with later gestational ages at maternal infection (Table 2). The estimation of specificity at the third trimester was based on 31 children only and the lower limit of the confidence interval was compatible with a slight decrease in specificity (Table 1). The PCR positive LR was high whatever the gestational age at mother infection, due to the high specificity (Table 2). It tended to get closer to 1 for later

gestational ages at mother infection, but this trend was not statistically significant (Table 2). There was no statistically significant effect of the delay between maternal infection and amniocentesis on the LRs (data not shown).

Neonatal Diagnosis by IgM and IgA Tests in Cord or Peripheral Blood Samples

Among the 646 children with blood samples, 452 were tests of peripheral blood (70%). The median day of sampling was the fourth day after birth (first and third quartiles: third and fifth day after birth, respectively).

Among the 320 children with available cord and peripheral blood samples, 105 had congenital infection. In these children, the sensitivity of the IgM-IgA test was estimated at 71.4% (95% CI: [63%–80%]) in peripheral blood and at 69.5% (95% CI: [61%–78%]) in cord blood. The difference between these 2 sensitivities was not statistically significant ($P = 0.77$). The specificity of the IgM-IgA test was estimated at 99.5% (95% CI: [98.6%–100%]) in peripheral blood and at 80.5% (95% CI: [75%–85.6%]) in cord blood. It was significantly higher in peripheral blood than in cord blood ($P < 0.001$). Because of this higher specificity, the following results concern only the population of children who had tests on peripheral blood samples.

The sensitivity of the IgM-IgA test in peripheral blood increased with later gestational ages at maternal infection and its specificity, very close to 100%, tended to decrease slightly (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A333). The ability of the test to identify infected children was very high with a LR+ estimated at 248 for a gestational age at mother infection of 6 weeks (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A333). The LR+ tended to get significantly closer to 1 at later gestational ages, but remained high at 30-weeks gestation. The test was not accurate to eliminate congenital infection with a LR− estimation of 0.63; ie, not far from 1, at 6 weeks gestation. It tended to get significantly closer to 0 with later gestational ages (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A333).

The positive LR tended to get closer to 1 and the negative LR closer to 0 when the delay between birth and the day of blood sampling increased, but this effect was not statistically significant.

Neonatal Diagnosis by IgM and IgA Tests on Peripheral Blood When the Result of the PCR Test is Known

In comparison with the absence of PCR result, the gain of knowledge provided by the IgM-IgA test was smaller when the PCR result was available: the positive and negative LRs were closer to 1 (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A333).

Congenital Infection Probability According to the Test Results

In children with one positive test when only one test was done (PCR or IgM-IgA on peripheral blood), the probability of congenital infection reached 90% when the maternal infection occurred at 18 weeks gestation or later (Table 2 and Table, Supplemental Digital Content 2, http://links.lww.com/INF/A333). In children with one negative test when only one test was done, the probability of congenital infection remained greater or equal to 10%, except when the maternal infection occurred early during pregnancy; it was around 1% when the maternal infection occurred at 6 weeks gestation (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A333).

In children with 2 positive tests (PCR and IgM-IgA test on peripheral blood), the probability of congenital infection was very high, close to 100%, whatever the gestational age at maternal infection. In children with 2 negative tests, the probability of infection remained close to 10% when maternal infection occurred during the third trimester of gestation (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A333).

When the results of the PCR and the IgM-IgA tests were discordant, the probability of congenital infection reached 90% when the maternal infection occurred at 18 weeks gesta-

TABLE 1. Estimation of the Sensitivity and the Specificity of *Toxoplasma* PCR in Amniotic Fluid and IgM-IgA Test in Peripheral Blood According to the Trimester of Maternal Infection During Pregnancy

Test and Trimester	Sensitivity		Specificity	
	% (n/d)*	95% CI†	% (n/d)*	95% CI†
PCR				
First	57.0 (4/7)	(14–86)	99.6 (226/227)	(98.7–100)
Second	67.0 (26/39)	(51–82)	98.6 (141/143)	(96.5–100)
Third	73.5 (25/34)	(59–88)	100.0 (31/31)	(89.0–100)
IgM-IgA test in peripheral blood				
First	56.0 (5/9)	(22–89)	100.0 (179/179)	(98.0–100)
Second	43.0 (12/28)	(25–61)	99.0 (90/91)	(96.7–100)
Third	78.0 (77/99)	(70–86)	100.0 (46/46)	(92.0–100)

*n indicates the number of true positives for sensitivities and true negatives for specificities; d is the number of children with congenital infection for sensitivities and without congenital infection for specificities.

†CI indicates Confidence interval.

TABLE 2. Estimation of the Sensitivity, the Specificity, and the Positive and Negative Likelihood Ratios (LR+, LR−) of *Toxoplasma* PCR Test According to the Gestational Age at Mother Infection, and Evolution of the Probability of Congenital Infection According to the PCR Result

	Gestational Age at Time of Mother Infection		
	6 wk	18 wk	30 wk
Sensitivity % (95% CI)	57.8 (10.4–80.6)	63.3 (50.8–75.4)	77.4 (64.8–88.6)
Specificity % (95% CI)	99.3 (97.2–100)	99.1 (98.0–99.9)	98.2 (95.8–99.9)
Pretest probability of congenital infection %	2.2	23.0	56.0
Positive likelihood ratio (90% CI)	79 (29–>1000)	69 (34–>1000)	43 (20–>1000)
Probability of congenital infection with a positive test % (90% CI)	64.0 (39.0–100)	95.4 (91.0–100)	98.2 (96.2–100)
Negative likelihood ratio (90% CI)	0.43 (0.10–0.78)	0.37 (0.25–0.48)	0.23 (0.12–0.36)
Probability of congenital infection with a negative test % (90% CI)	1.0 (0.2–1.7)	10.0 (7.0–12.5)	22.6 (13.2–31.4)

CI indicates Confidence interval.

tion or later, and was about 50% for very early (6 weeks gestation) contaminations (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A333).

DISCUSSION

Maternal *Toxoplasma gondii* infection consistently causes anxiety and feelings of guilt in future parents. The physician faces the difficult tasks of assessing the clinical status of the fetus or the newborn through antenatal and neonatal tests and of prescribing drugs to reduce the risk of malformation and to counsel the parents about the best choices. The practitioner should thus have a precise knowledge of the risk for the fetus or the newborn to have congenital infection.

Several studies on the performance characteristics of ante- and neonatal tests for the diagnosis of congenital toxoplasmosis have been published; these studies were usually multicentric with no standardization of the tests and no clear inclusion criteria.[12,14–18] Here, we present results obtained in a reference laboratory and based on a large sample of consecutive documented seroconversions, at various gestational ages, managed according to a standardized protocol with at least 1-year follow-up of the infant. Seroconversion during the first trimester of pregnancy requires specific diagnostic strategies to determine whether infection occurred before or after the beginning of pregnancy. Mothers with early seroconversion are more often referred to our department than mothers with later seroconversion, which could explain the high proportion of first trimester seroconversions we observed.

The fetal loss rate attributable to amniocentesis is about 0.13%.[19] Thus, the decision to perform such an investigation should rely on strong evidence of its benefit. The sensitivity of *Toxoplasma* PCR increased and its specificity tended to decrease slightly during the course of pregnancy. The explanations given by Thalib et al[17]—low fetal cell concentration in amniotic fluid during the first trimester or delayed transmission[20]—are not in agreement with our results because the positive and negative LRs did not significantly change according to the delay between contamination and amniotic fluid sampling. Instead, our results are in agreement with those of Romand et al[12] and Gilbert et al.[21]

Combining the pretest probability of vertical transmission and LRs, we were able to determine the risk of fetal infection. Because of the low pretest probability of congenital infection (2%) in case of a first trimester maternal infection, one frequent question is whether amniotic fluid should be sampled. In such early infections, a positive result would significantly shift the probability of infection to 64%, offering the opportunity of early PS treatment and a careful ultrasound monitoring. Some authors have advocated pregnancy termination in case of a positive PCR result. In agreement with previously reported favorable outcomes of children born with a congenital toxoplasmosis due to an early maternal infection,[22,23] we favor a conservative policy unless there are ultrasound signs of fetal lesions. A negative PCR result, the most frequent situation, greatly helps reassuring parents even if ultrasound monitoring remains necessary.

In second trimester maternal infections, a positive PCR result corresponds to a probability of congenital infection of greater than 95% compared with a pretest probability of 23%. Such a positive prenatal diagnosis is an argument to change therapy from spiramycin to PS, is the latter being expected to prevent long-term sequelae.[24] A negative result alleviates parents' anxiety until child birth by reducing the probability of congenital infection from 23% (pretest) to 10% (posttest). Nevertheless, a negative test cannot exclude an infection and should not stop ultrasound monitoring or treatment.

For third trimester maternal infections, fetal infection is very likely. One option is to start PS treatment immediately without performing amniocentesis. In our study, this option would lead to unnecessary treatment of 44% of noninfected children. Amniotic fluid testing allows an adequate management of the newborn in case of positive PCR test. The PCR result provides important information by doubling or halving the probability of fetal infection in case of positive or negative results, respectively.

In the present study, the PCR results were obtained with the 35-fold repeated *B1* gene.[25] Like many other laboratories, we have switched now to the 200- to 300-fold repeated AF146527 sequence. These 2 PCR techniques are not statistically different in terms of sensitivity and specificity[26]; thus, our conclusions regarding the PCR test are unlikely to be changed by the current use of the *AF146527* gene as a target gene.

Because we did not have reliable information on mothers' adherence to taking medication, we did not investigate the effect of antenatal treatment on the PCR performance characteristics. In agreement with other authors,[14,16,17] we have already reported no effect of prenatal treatment on PCR sensitivity. One possible explanation is that, before sampling, women were usually given spiramycin treatment that did not reach parasiticidal plasma concentrations.[27]

Regarding perinatal work-up, the benefit of IgM and IgA detection has been convincingly demonstrated.[9] In the same children, the specificity of the IgM-IgA test was higher with peripheral blood than with cord blood. Our results are not in agreement with those of Gilbert et al[18] who found no significant improvement in the specificity of the test on peripheral blood versus cord blood. With cord blood samples, there is a risk of false positive results because of contamination with maternal blood[9]; this might explain the higher specificity and LR+ with peripheral blood versus cord blood. For 75% of the children who had an IgM-IgA test in peripheral blood, blood sampling was performed during the first 5 days after birth. We did not find a low specificity for the IgM and IgA tests as stated by Pinon et al[28] during the first 10 days after birth and attributed to leakage of IgM and IgA from mother to child. For these reasons, we recommend performing serologic tests on peripheral blood.

As observed for the PCR, the sensitivity of the IgM-IgA test was higher in third trimester infections, whereas its specificity remained constant or slightly decreased. This effect of gestational age has been reported previously.[9,18] The low sensitivity for detecting fetuses infected early in pregnancy could be due to a short-lasting primary response disappearing at the time of delivery rather than to an inability of fetuses to produce immunoglobulins during the first trimester.[29]

Whatever the sampling day (third, fourth, or fifth day of life), early infections were associated with high positive LR, whereas late maternal infections were associated with the highest estimates of sensitivity and posttest probability of infection.

As for PCR, maternal treatments were not taken into account but had no impact on IgM-IgA sensitivity.[18]

At birth, when PCR results are not available, IgM and IgA tests on peripheral blood perform well in diagnosing infection. Due to high positive LRs, the probability of infection reached 84.8% in first trimester infections and exceeded 98% in late maternal infections. Negative results are reassuring because they reduce the probability of fetal infection by approximately 50%, but these results must be confirmed by IgG monitoring during the first year of life.

When available, the results of prenatal and neonatal diagnostic tests should both be taken into account. The probability of infection is greatly decreased whenever both these tests are nega-

tive. The probability of infection in the infant increased up to 100% when both tests were positive or even, in second and third trimester infections, when only one test was positive. Discordant results in early infection are more critical because the posttest probability of infection remained close to 50%. IgG and IgM western blots of mother-infant pairs on samples taken one week later should be performed before making a decision to treat.[30]

In yielding reliable probabilities of *Toxoplasma* congenital infection, the results of PCR on amniotic fluid and of IgM-IgA test at birth guide clinical management and parent counseling. Nevertheless, the follow-up of the child is important because the presence or absence of specific IgGs at 1 year remains the criterion for confirming or ruling out congenital toxoplasmosis. When toxoplasmosis is not clearly diagnosed in an asymptomatic newborn, it would be better to withhold presumptive treatment and wait until serologic tests indicate a congenital infection. When ante- or postnatal work-ups are negative, apparently healthy infants should be followed-up at least quarterly until specific IgG detection turns negative.

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