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Early Neonatal Diagnosis of Congenital Toxoplasmosis: Value of Comparative Enzyme-Linked Immunofiltration Assay Immunological Profiles and Anti-*Toxoplasma gondii* Immunoglobulin M (IgM) or IgA Immunocapture and Implications for Postnatal Therapeutic Strategies

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Diagnostic strategies for congenital toxoplasmosis have changed profoundly in recent years. Immunological diagnostic methods, long considered disappointing, can now be used at a very early stage. Over a 3-year period, 1,050 infants at risk of congenital toxoplasmosis (born to 1,048 mothers infected during pregnancy) were monitored for a minimum of 12 months and a maximum of 7 years. More than 6,000 serum specimens were analyzed by comparative mother-infant immunological profiles (CIPs) based on an enzyme-linked immunofiltration assay (ELIFA) and an immunocapture method for the detection of specific immunoglobulin M (IgM) and IgA. IgG antibodies were also titrated. One hundred three cases of congenital toxoplasmosis were demonstrated. The CIP-ELIFA method had a better diagnostic yield (sensitivity, 90%) than specific IgM and/or IgA detection by immunocapture assay (sensitivity, 77%). By using a combination of these tests, congenital infection was diagnosed in the first month and the first 3 months of life in 90 and 94% of infants with toxoplasmosis, respectively, with a specificity of 99.8% and a positive predictive value of 99% at 8 months of age. This dual diagnostic approach (ELIFA and IgM-IgA immunocapture) is highly efficient and has important implications for therapy. Indeed, early postnatal diagnosis based on objective evidence enables therapy with pyrimethamine-sulfadoxine to be started immediately for 24 months, while spiramycin (which used to be given preventively for 9 to 12 months to all infants at risk) can be stopped after the first 3 months of life.

Toxoplasmosis is a ubiquitous protozoan infection; it is generally asymptomatic in healthy adults, but the fetus or newborn is highly susceptible to serious complications when it is infected during pregnancy. About 50% of French women of childbearing potential have immunity to this pathogen (2), but the obligatory monthly screening tests for the remaining women is a major burden for the welfare system. The maternal seroconversion rate during pregnancy is about 1%, and maternal infection accounts for one to four cases of congenital toxoplasmosis per 1,000 births (18). In the absence of treatment, congenital toxoplasmosis can be catastrophic during childhood, the main dangers being neurologic damage and toxoplasmic retinochoroiditis with a risk of blindness (14, 30). These complications can be prevented by early treatment of the baby with pyrimethamine-sulfonamide combinations (4). As a result, the diagnosis must be made as soon as possible after birth (25).

In these children at risk, quantitative tests for anti-*Toxoplasma gondii* immunoglobulin G (IgG) based on classical methods (dye test, indirect immunofluorescence, agglutination, and enzyme-linked immunosorbent assay) become significant at a relatively late stage because of passive transfer of specific maternal IgG and their gradual disappearance in the circulation of healthy infants (3). In contrast, detection in the infant's serum of specific IgM, IgA, or IgE, which do not normally cross the placenta, is the hallmark of congenital toxoplasmosis.

The first aim of the study described here was to determine the performance of a combined qualitative and quantitative approach for the diagnosis of toxoplasmosis at birth or during the first few months of life. We analyzed specific IgG and IgM by comparative mother-infant immunological profiles (CIPs) by an enzyme-linked immunofiltration assay (ELIFA) and quantified specific IgM and IgA antibodies by an immunocapture (IC) method. A multidisciplinary group assessed the therapeutic, preventive, and legal implications of our findings.

MATERIALS AND METHODS

Samples. One thousand fifty children were monitored prospectively by regular clinical, radiologic, ophthalmologic, and laboratory studies from birth until the age of 12 months to 7 years. Their mothers (n = 1,048) were infected with *T. gondii* during pregnancy (seroconversion or an increase in IgG antibody titers [>2 dilutions] with the presence of IgM and/or IgA). All of the mothers (n = 101) who transmitted the parasite to their babies had been treated with spiramycin until delivery; none had received pyrimethamine-sulfadoxine or pyrimethamine-sulfadiazine during the pregnancy.

Samples of maternal serum, cord blood, and neonatal serum were obtained at

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TABLE 1. Performances of the CIP-ELIFA method and IC tests for IgM and IgA in the diagnosis of 103 cases of congenital toxoplasmosis

Age at diagnosis	No. (%) of positive children						
	CIP-ELIFA			IC			CIP-ELIFA plus IC
	IgG	IgM	IgG + IgM	IgM	IgA	IgM + IgA	
0–30 days	67 (65)	66 (64)	83 (80.5)	72 (70)	64 (62)	76 (74)	93 (90)
31–60 days	69 (67)	66 (64)	85 (82.5)	73 (71)	64 (62)	77 (75)	95 (92)
61–90 days	71 (69)	67 (65)	87 (84.5)	73 (71)	64 (62)	77 (75)	97 (94)
>3–<9 mo	77 (75)	69 (67)	93 (90)	75 (73)	65 (63)	79 (77)	103 (100)

birth. The infant's sera (100 to 500 μl) were then studied monthly for 4 months and then at 5 to 6, 7 to 9, and 12 months to follow the disappearance of maternal-specific IgG antibodies. Thereafter, we only monitored (every 4, 6, and then 12 months) children with confirmed congenital toxoplasmosis who were given specific treatment (pyrimethamine-sulfadoxine twice monthly for 12 months or monthly courses of daily pyrimethamine-sulfadiazine alternating with spiramycin for a total of 1 year). Patients on the two treatments also received folic acid or folinic acid (5 mg/week by mouth). More than 6,000 serum samples were thus studied, and aliquots were stored at −70°C.

A total of 103 children developed congenital toxoplasmosis. Among the 72 children in whom follow-up was particularly intensive, there were 14 cases of chorioretinitis (1 of which was bilateral), 1 case of uveitis, and 5 children with brain lesions (two cases of intracranial calcification, 1 case of macrocephaly, 1 case of microcephaly, and 1 case of meningitis).

Specific immunological study. The CIP-ELIFA method was carried out as described in detail elsewhere (19, 20). Briefly, in a coimmunoelectrodiffusion procedure, the soluble toxoplasmic antigen (10 μl; obtained from the RH strain after freezing-thawing and sonication with 0.01% Tween 20) and three adjacent serum samples (15 μl each) migrated on the same microporous cellulose acetate membrane for 2.5 h. The precipitation arcs thus formed were revealed by immunofiltration (through the membrane) of alkaline phosphatase-labeled anti-IgG and anti-μ antibodies (BI 2515 and BI 2519, respectively; Biosys, Compiègne, France). The part of the membrane corresponding to the field of antigen-antibody migration was cut out and placed in an ELIFA cell controlled by an automat (MATEOCE, La Lande, France; Orkys, Reims, France) which programmed the washing, labeling, and impregnation steps in revelation buffer. After 30 min of immunofiltration, the membrane was placed in the relevant substrate for revelation. The three comigrated serum samples were compared by establishing the CIP from the following four sets of data: (i) the number of precipitation arcs detected, (ii) the isotype (IgG or IgM) in each precipitation arc, (iii) the comparative specificity by continuity (coalescence) of the arcs present in two serum samples revealing the same antigen involved in the precipitate, and (iv) relative quantification of antibodies with the same specificity in each sample. In the case of specific IgG, the mother-child CIP-ELIFA method yielded three types of results for cord blood or neonatal serum (see Fig. 1): (i) specific antibodies present in neonatal serum but absent from maternal serum, (ii) antibodies with identical specificities present in the neonate and mother, but at a higher concentration in the neonate (precipitating arc closer to the antigen deposit), and (iii) antibodies with the same specificities as maternal antibodies present in the neonate at the same concentration as that in the maternal serum (passively transmitted maternal antibodies with no pathological significance).

The first two situations (IgG neoantibodies) or ELIFA detection of specific IgM reveal antibody synthesis by the fetus or neonate. Each is a positivity criterion for congenital toxoplasmosis by the ELIFA method. This method is applied identically to samples from older children (by comparing their own sera taken at different times).

IC-M and IC-A methods. IgM and IgA IC methods (IC-M and IC-A, respectively) were applied to maternal serum at delivery, neonatal serum (not cord blood), or infant serum as described previously (21, 23). Briefly, microplates were sensitized with a monoclonal anti-μ and anti-α antibody (11-140 and 11-137, respectively; Argène-Biosoft, Varilhes, France). After 24 h at 4°C, the plates were washed and saturated. Each sample was diluted (1/100 for adult serum, 1/25 for neonatal and infantile serum up to the age of 4 months) and deposited in three adjacent wells, and the plate was then incubated for 2.5 h. After washing, a suspension of formolized tachyzoites (obtained in our laboratory from the RH strain and treated with trypsin) was added in volumes of 100, 150, and 200 μl to the three wells, respectively. Sedimentation was read after 12 h; a score of 0 corresponds to full sedimentation, while complete agglutination as a film is considered positive (score of 4). The cumulative value of the three wells for a given sample can thus range from 0 to 12. To avoid subjectivity, we developed an automated method on a microplate reader (MR 7000; Dynatech, Guyarcourt, France) coupled to appropriate software (Orkys); this automated reading technique was also applied to an anti-*T. gondii* IgE screening test (22).

In infants, IgM titers are significant if they are equal to or greater than 1. Beyond 6 months of age, the cutoff is increased to 6, given the risk of interference by natural IgM (8). The corresponding cutoff values for IgA are 1 for neonates and 2 for infants over 6 months of age (21). Positive results for IgM or IgA at birth must be confirmed on a second sample between 5 and 10 days of life when the mother also has these specific isotypes at delivery. If the positive results are due to maternal antibodies, negative results are obtained at the second test because of their short half-lives (3 to 5 days) (8, 21, 23).

HSDA. All of the samples (treated with 2-mercaptoethanol) were tested in the presence of a tachyzoite suspension to detect specific IgG (titers expressed in units per milliliter). The reaction can be read visually or automatically, as in the IC-M and IC-A tests. The persistence of IgG in the high-sensitivity direct agglutination (HSDA) assay (cutoff value, 6 U/ml) at 12 months of age was used as the reference diagnostic method for congenital toxoplasmosis.

RESULTS

HSDA detection of IgG antibodies at the age of 12 months. Among the 1,050 children at risk, we diagnosed 103 cases of congenital toxoplasmosis (patients 1 to 103), with IgG (HSDA, ≥6 U/ml) persisting until the age of 12 months or a reincrease after the cessation of therapy. The complete disappearance of antibodies (HSDA method) in the other 947 children strongly suggested that they were uninfected.

CIP-ELIFA and IC-M and IC-A tests. In the 1,050 children studied by CIP-ELIFA and the IC-M and IC-A tests, the results were in concordance with the changes in HSDA IgG titers in the 103 patients (patients 1 to 103) with congenital toxoplasmosis and in 945 negative subjects, but they disagreed in 2 patients (patients 104 and 105). Patients were classified blindly on the basis of serological results. The values are reported by age category, as follows (Table 1): (i) 0 to 30 days (not all first samples were obtained at birth), (ii) 31 to 60 days, (iii) 61 to 90 days, and (iv) beyond 91 days.

IgM was detected by the IC method in 72 children during the first month of life. Detection of specific IgA was less sensitive, because only 64 children were found to be positive by that method. We excluded from these data positive results at birth which were not confirmed on day 5 to day 10. By combining the results for the two isotypes, we identified 76 infected children between day 1 and day 30 of life. The CIP-ELIFA method was positive for 83 patients. By combining the three tests, congenital toxoplasmosis was diagnosed during the first month of life in 93 patients (patients 1 to 93), i.e., 90% of the 103 infected children.

During the second month of life, IC revealed specific IgM in an additional child (patient 94), increasing to 77 the number of children with IgM and/or IgA. CIP-ELIFA revealed neoantibodies in two children (patients 94 and 95), one of whom (patient 94) was IgM positive by the IC method. Overall, the diagnosis of congenital toxoplasmosis was made in 95 patients (92% of all patients) less than 60 days after birth.

During the third month, two further cases of congenital toxoplasmosis were diagnosed (patients 96 and 97) by CIP-ELIFA. In one patient (patient 96) the diagnosis was suspected at birth on the basis of IgG positivity by the CIP-ELIFA method, but the diagnosis could not be confirmed because of a lack of samples; at 3 months the diagnosis was confirmed on the basis of persistent IgG neoantibodies and an IgM precipitation arc. In the second patient (patient 97), the emergence

TABLE 2. Results of immunological tests (HSDA, CIP-ELIFA, and IC) in six patients with congenital toxoplasmosis

Patient no.	Age at diagnosis (mo)	Changes in IgG antibodies (HSDA method)	CIP-ELIFA[b]		IC[b]	
			IgG	IgM	IgM	IgA
98[a]	4	Falling	+	−	+	−
99[a]	5	Stable	+	−	−	−
100	4	Stable	+	−	−	−
101	5	Rising	+	−	−	−
102	7	Rising	+	+	+	+
103	8	Rising	+	+	−	−

[a] Children not tested before the ages of 4 and 5 months.
[b] +, positive; −, negative.

of IgG neoantibodies by the ELIFA method also confirmed the diagnosis at 3 months. Thus, at the end of the first 3 months of life, a positive diagnosis of congenital toxoplasmosis had been made in 97 of 103 patients (94%).

Diagnoses of six cases of congenital toxoplasmosis were made later (patients 98 to 103) (Table 2). Two patients (patients 98 and 99) underwent their first immunological tests at the ages of 4 and 5 months, respectively. The presence of IgG neoantibodies by ELIFA was diagnostic in these two patients, one of whom (patient 98) was also positive for IgM by the IC method. Three other children (patients 100, 101, and 103) were diagnosed at 4, 5, and 8 months of age, respectively, on the basis of the emergence of IgG neoantibodies by the ELIFA method, together with an increase in the HSDA IgG titer in two patients (patients 101 and 103) and the ELIFA IgM titer in one patient (patient 103). The diagnostic delay in the fourth patient (patient 102) was apparently due to the treatment (pyrimethamine-sulfadiazine) prescribed for the chorioretinitis that was diagnosed at 2 months; the diagnosis was confirmed at 7 months on the basis of the emergence of neoantibodies by the ELIFA method and IgM and IgA by the IC method after treatment had been suspended because of neutropenia.

In two patients (patients 104 and 105) the ELIFA results were not in concordance with those of the HSDA IgG at 12 months. In the first patient (patient 104) the diagnosis was based on ELIFA IgG at birth. In the other patient (patient 105), the diagnosis was based on the emergence of ELIFA IgM at 5 months and was confirmed 1 month later. In both patients the specific IgG titer was 6 U/ml at 11 months and was below the cutoff at 1 year, with no further rise. The result for the first patient classified as falsely positive and that for the second patient was classified as indeterminate (see Discussion).

Among the different ELIFA precipitation arcs, one (arc B [arc 2 in Fig. 1]) was more frequent than the others. Arc B consisted of an immune complex composed of an antigen recognized by an anti-66/70-kDa monoclonal antibody. This specific antibody is detected very frequently and is important for the diagnosis of congenital toxoplasmosis (Fig. 1). Moreover, its titer increases (greater than twofold, with displacement of the precipitating arc toward the antigen deposit) during immunological reactivation. This situation was detected in 83% of the infected infants in this series when the 1-year treatment course (pyrimethamine-sulfadoxine or pyrimethamine-sulfadiazine) was stopped for 1 to 3 months; it was also the case for two infants (patients 33 and 54) during treatment.

Table 3 shows the chronological progression of the sensitivity, specificity, and positive and negative predictive values of the three methods for the diagnosis of congenital toxoplasmosis.

At 1 month, the sensitivities were 80.5, 74, and 90% by the ELIFA, the IC test, and the ELIFA-IC tests, respectively, and the negative predictive values were 97.9, 97, and 98.9%, respectively. At 6 months, the sensitivity of ELIFA-IC test was 98% and the negative predictive value was 99.8%.

DISCUSSION

The present study confirms the value and complementary nature of ELIFA and IC detection of specific IgM and IgA for the early neonatal diagnosis of congenital toxoplasmosis in infants at risk. During the first month of life we identified 80% of the 103 patients with congenital toxoplasmosis in this series by one of the three tests. By combining the results of the three tests, the diagnostic yield reached 90% and then reached 94% at the end of the first 3 months of life. The complementarity of the tests is due to the detection of multi-isotypic markers, i.e., IgM, IgA, and IgG; it also benefits from the wide diversity of antibodies detected (antimembrane antibodies by the IC method and antibodies against both membrane and soluble cytoplasmic components by the ELIFA method) (8, 20, 21). Each method only identified 93 and 79 of the 103 cases of congenital toxoplasmosis, respectively, that were diagnosed by combining the results of the three tests before the age of 9 months, with the persistence of a positive HSDA test result at 12 months. This dual analytical and quantitative approach performs very well relative to other methods for the neonatal diagnosis of congenital toxoplasmosis (3, 5, 25); furthermore, closer monitoring of some infants during the first few weeks of life would no doubt have increased the diagnostic yield.

IC detection appears to be reliable for the postnatal diagnosis of congenital toxoplasmosis. The poor sensitivity of indirect immunofluorescence for the detection of anti-*T. gondii* IgM fully justified the development of an IC test for IgM (8) (eliminating competition between IgG and IgM) and its extension to IgA and IgE (21, 22). In the case of IgM, Desmonts et al. (8) obtained a sensitivity comparable to that reported here. The sensitivity of IC detection of IgM or IgA reached 74% at 1 month and plateaued at 77% after 8 months. Testing for IgE did not significantly improve the diagnostic yield (22, 31). The

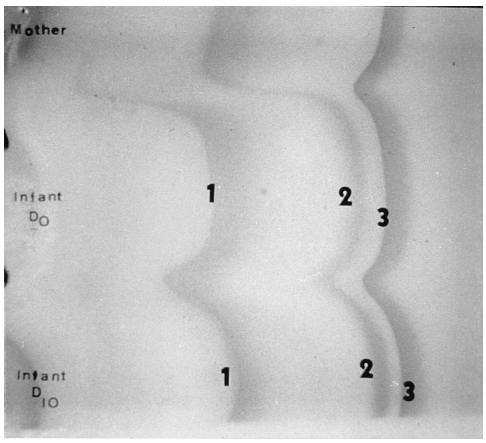

FIG. 1. CIP-ELIFA method: patterns reflecting congenital toxoplasmosis after immunoelectrodiffusion (on the left, are three serum samples: from the mother at delivery, from the infant at birth [day 0], and from the infant at 10 days of life (day 10); on the right is a soluble antigen deposit) and immunofiltration with alkaline phosphatase-labeled anti-IgG antibodies. Note the presence of IgG neoantibodies (points 1) synthesized by the fetus or neonate (absent from the maternal serum), passively acquired maternal IgG antibodies associated with neoantibodies (points 2) of the same specificity synthesized by the fetus or neonate (arc B), and passively acquired maternal IgG antibodies at the same concentration in the mother and the infant (points 3).

TABLE 3. Chronological progression of the sensitivity, specificity, and positive and negative predictive values of the CIP-ELIFA (IgG or IgM) and IC (IgM or IgA positivity) methods for the diagnosis of 103 cases of congenital toxoplasmosis among 1,050 infants at risk[a]

Age (mo)	Sensitivity (%)			Specificity (%)		PPV (%)		NPV (%)		
	ELIFA	IC	ELIFA + IC	ELIFA	IC	ELIFA	IC	ELIFA	IC	ELIFA + IC
1	80.5	74	90	99.9	100	98.8	100	97.9	97	98.9
2	82.5	75	92	99.9	100	98.8	100	98.1	97.3	99.2
3	84.5	75	94	99.9	100	98.8	100	98.3	97.3	99.4
6	88	76	98	99.8	100	98.9	100	98.7	97.4	99.8
8	90	77	100	99.8	100	98.9	100	99	97.5	100

[a] Of the 1,050 infants at risk, 103 had congenital toxoplasmosis and 945 were healthy. There was one false-positive result (patient 104) and one indeterminate result (patient 105). PPV, positive predictive value; NPV, negative predictive value.

detection of specific IgM (or IgA) at birth appears to be less efficient if the mother is treated with pyrimethamine-sulfonamides during the pregnancy (6). This in utero treatment of the infected fetus can shorten the IgM-IgA response, and at birth, the validation of a positive antenatal diagnosis is difficult without these specific markers; the only immunological sign of infection in certain neonates is IgG neoantibodies detected by the ELIFA method. The specificities of the IC-M and IC-A tests were excellent, because no false-positive results were observed (Table 3). It should be noted that confirmatory testing is mandatory between 5 and 10 days after birth, especially when the positive neonatal sample was cord blood (7, 28), because these isotypes are also detected at birth in about 1 to 3% of uninfected children. First reported in 1986 (21), the value of the IC-A test for the diagnosis of congenital toxoplasmosis has since been largely confirmed (1, 7, 16, 28). In our study of children born between 1987 and 1990, specific IgA was paradoxically detected less frequently than specific IgM (1, 7, 28). With the introduction of automated plate reading in 1991, the detection of specific IgA has become more reliable (10), but it remains dependent on the date of maternal infection (1, 7, 10).

One of the original qualities of CIP-ELIFA is that it can distinguish between IgG antibodies present in two samples. This analytical approach is thus particularly suited to comparing samples from the mother and the infant. Classical quantitative methods cannot be used to determine the maternal or mixed (maternal-fetal) origin of anti-*T. gondii* IgG detected in a neonate or an infant. The study of immune IgG loads is not always explicit (3). In contrast, Western blot (immunoblot) analysis can be used to compare mother-infant antibodies (11, 24), but it involves three steps (electrophoresis, transfer, and immunolabeling) and is not suited to determining the relative concentrations of IgG with the same specificity in two samples. Antibodies with different specificities can recognize antigens with similar or identical molecular masses, making it difficult to interpret the results unless the isotypes are different (IgG, IgM, IgA, or IgE). We used the ELIFA method to detect only IgG and IgM. Although IgA and IgE can be detected by this method (20), the IC method is more sensitive for the IgA and IgE isotypes (21, 22, 31). For the diagnosis of congenital toxoplasmosis during the first, second, and third months of life, ELIFA had sensitivities of 80, 82, and 84%, respectively; this value rose to 90% at 8 months. The bulk of this diagnostic yield was based on the detection of IgG neoantibodies, while specific IgM, IgA, and IgE were no longer synthesized, given their shorter kinetics. The specificity of the CIP-ELIFA results was doubtful in two patients (patients 104 and 105). In the first patient the mother seroconverted during the first 3 months of pregnancy; the diagnosis of congenital toxoplasmosis was based on the detection of IgG by ELIFA by comparison of cord blood and maternal serum samples obtained on the day after delivery. The samples and the ELIFA pattern were blindly reassessed several times and were always considered characteristic of congenital infection. During treatment (sulfamethoxazole-trimethoprim [Bactrim] in this case), we observed a rapid regression of IgG, with no immunological rebound effect. We were unable to confirm any of the possible explanations for this situation, such as maternal transfusion at delivery leading to hemodilution, the possibility that the "maternal" serum tested on day 1 was not actually that of the child's mother, and atypical disappearance of IgG upon treatment with sulfamethoxazole-trimethoprim. This result was classified as false positive. In the second patient, a child who was on spiramycin, the diagnosis was made at 5 months and was based on the emergence of IgM by the ELIFA method; treatment was continued with pyrimethamine-sulfadoxine until the age of 30 months. The IgG titer by HSDA at 12 months was below 6 U/ml, but it was not unequivocally negative by the dye test (1 IU/ml at 1 year, 3 IU/ml at 40 months). Similar observations have been made in infected children on treatment (3), and we classified this result as indeterminate.

In the case of immunological reactivations 1 to 3 months after the end of treatment, regardless of the infant's age, CIP-ELIFA revealed IgG neoantibodies or an increase in preexisting IgG. These variations, which can involve a limited number of precipitating antibodies, are often undetectable simultaneously by the HSDA method. The 66/70-kDa antigen, located in rhoptries by electron microscopy (15), is involved in these reactivations, as well as seroconversions and the synthesis of neoantibodies at birth. These reactivations indicate that the parasite "escaped" from chemotherapeutic suppression independently of the infant's humoral immune response capacity.

In the second phase of the study, a multidisciplinary group assessed the impact of these findings on postnatal monitoring, medico-legal considerations, and treatment (17, 27). Even if the sensitivity, specificity, and predictive values of the methods used were acceptable, each child at risk must still be monitored for at least 12 months; in contrast, inoculation of placental tissue (which is less efficient and more time-consuming) now appears to be unnecessary (5). For these children at risk, when the diagnosis of congenital toxoplasmosis is not made during the first few days of life, spiramycin should be prescribed as preventive therapy, even though its efficacy against congenital toxoplasmosis is controversial (26, 29). In our study, this compound did not prevent the emergence of immunological signs of congenital toxoplasmosis in seven of the children who we studied; in addition, reactivation on pyrimethamine-sulfadoxine withdrawal occurs with the same time lag and frequency whether or not spiramycin is given subsequently (17, 27). So, for merely medico-legal reasons, spiramycin should be prescribed, but only up to the age of 3 months; indeed, 94% of the

cases of congenital toxoplasmosis had been diagnosed by this time in our study, and the classical protocol (12, 29) would have required 947 children to receive spiramycin for 9 to 12 months for a benefit theoretically limited to six children.

In the case of a positive neonatal diagnosis, we immediately start curative treatment with pyrimethamine (1.25 mg/kg of body weight for 15 days) plus sulfadoxine (25 mg/kg for 15 days) and folinic acid (5 mg/week by mouth), even in premature infants. The fact that two immunological reactivations occurred at 7 and 12 months in infants (patients 33 and 54, respectively) on pyrimethamine-sulfadoxine led us to study 45 children (17) to check that twice-monthly dosing maintained an adequate concentration of pyrimethamine and sulfadoxine in serum; assays of sera obtained before the two paradoxical reactivations showed that the problem was in fact poor compliance. Finally, since 1991, given the frequency (83%) of reactivations after a 1-year course of treatment, we have recommended the combination of pyrimethamine-sulfadoxine and folinic acid for 24 months to minimize the risk of complications, especially of an ocular nature, which have been described during this period (4, 5, 12, 13).

Conclusion. The combination of CIP-ELIFA and the IC-M and IC-A tests had a high diagnostic yield for congenital toxoplasmosis. The analytic and quantitative study of antibodies synthesized by the fetus (detectable at birth), neonate, or infant provided a positive diagnosis in 90% of the patients within 1 month of birth and 94% of the patients by the end of the first 3 months of life. These results led us to develop a management strategy taking into account surveillance tests and legal and therapeutic matters. When congenital infection is not demonstrated at a very early age, we recommend daily spiramycin administration until the age of 3 months, with immunological monitoring until 12 months of age. When congenital infection is demonstrated, we recommend treatment with pyrimethamine-sulfadoxine twice monthly for 24 months, together with weekly administration of folinic acid.

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