Lack of Value of Specific IgA Detection in the Postnatal Diagnosis of Congenital Toxoplasmosis

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To improve the performance of the postnatal diagnosis of congenital toxoplasmosis, we assessed the detection of IgA antibodies to *Toxoplasma gondii* by ELISA, compared with that of IgM by ELISA, ISAGA, and IFAT and neosynthesized antibodies using Western blot. From 1993 to 1996, IgA antibodies were detected using the Toxo IgA test (SFRI, Société Française de Recherches et d'Investissements, Bordeaux, France), in 195 serum and cord blood samples from 63 infants born to mothers who seroconverted during pregnancy. Eighteen infants had proven congenital toxoplasmosis (confirmed by the presence of IgG after 12 months of life) and 45 had no congenital toxoplasmosis (nega-

tivity of IgG after 6–12 months of life). The sensitivity of IgA detection by ELISA on serum and cord blood samples was 38.9 and 54.5% respectively, which is low when compared with the sensitivity of IgM detection by ISAGA (66.7% on serum samples, 90.9% on cord blood), ELISA (61.1% on sera, 81.8% on cord blood) and Western blot (83.3% on sera, 72.7% on cord blood). IgA antibodies were never detected by ELISA earlier than IgM or neosynthesized Ig (antibodies synthesized by infants). Thus, the detection of IgA antibodies by Toxo IgA is not useful in improving the diagnosis of congenital toxoplasmosis. J. Clin. Lab. Anal. 13:27–30, 1999. © 1999 Wiley-Liss, Inc.

Key Words: congenital toxoplasmosis; specific IgA detection; postnatal diagnosis

INTRODUCTION

Maternal infection with *Toxoplasma gondii* during pregnancy may result in congenital infection (1). In the United States, the incidence of congenital toxoplasmosis is estimated to be 1 in 1,000 births (2). Most infants have no symptoms at birth but, if they are not treated, they may develop retinal or neurological diseases in the second decade of their lives (3). Recently, advances in early diagnosis coupled with early and prolonged treatment have appeared to modify the course of infection (4–6).

In France, the diagnosis of congenital toxoplasmosis includes a postnatal follow-up, often preceded by a prenatal diagnosis (7), based on the date of the mother's seroconversion. At birth, three samples are studied: cord blood, the newborn's blood, and placenta. The cord blood and the newborn's blood are tested for IgM and IgG titers but also for neosynthesized IgG, IgM, and IgA antibodies determined in comparison with those of the mother's blood (8). In the placenta, parasites are detected by in vitro and in vivo cultures, while parasitic DNA is detected by polymerase chain reaction (PCR). However, the serological follow-up of infants remains necessary to definitely conclude the presence or ab-

sence of congenital toxoplasmosis (9). During the first year of life, the increase in specific IgG and the presence of specific IgM and/or neosynthesized IgG, IgM, or IgA are the arguments in favour of congenital toxoplasmosis (10). All the newborns of mothers infected during pregnancy are treated with Spiramycine. When one of the biological postnatal criteria is positive, treatment with Pyrimethamine-Sulfadoxine or Pyrimethamine-Sulfadiazine can be initiated.

To improve the sensitivity of diagnosing congenital toxoplasmosis, different tests (ELISA, ISAGA) have been developed to detect IgA (11–13). Several studies showed that IgA antibodies could be detected earlier than IgM and that their detection was more sensitive than the IgM detection on samples collected at birth (14–17). To evaluate the importance of IgA detection in the diagnosis of congenital toxoplasmosis, we have tested the commercially available kit Toxo IgA (SFRI, Société Française de Recherches et d'Investissements, Bordeaux, France). The

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detection of IgA antibodies was compared with that of IgM and neosynthesized IgG, IgM, and IgA antibodies in the cord blood and serum samples of newborns born to mothers infected during pregnancy.

MATERIALS AND METHODS

From 1993 to 1996, 63 cases of acquired *Toxoplasma* infection in previously seronegative pregnant women were diagnosed. The children born to these mothers infected during pregnancy were classified into two groups. Group 1 was comprised of 18 infants (49 serum and 11 cord blood samples) with proven congenital toxoplasmosis (CT+). The conclusive criterion for the presence of CT was the lasting presence of specific IgG antibodies after 12 months of life or the presence of *T. gondii* in amniotic fluid and/or fetal blood (7, 8). Group 2 was comprised of 45 infants (105 serum and 30 cord blood samples) without congenital toxoplasmosis (CT-). The conclusive criterion for the absence of CT was the negativity of specific IgG antibodies after 6–12 months of life.

All the samples were collected at birth (D₀ and/or D₅) and during the next two years, which gave a total of 154 sera and 41 cord bloods. The results of the detection of anti-*T. gondii* IgM, IgA and neosynthesized antibodies were compared with one another in these 195 samples. IgM antibodies to *T. gondii* were detected by enzyme-linked immunosorbent assay (immunocapture ELISA) (VIDAS, VITEK system: bioMérieux, Lyon, France) (18), immunosorbent agglutination assay (immunocapture ISAGA) (bioMérieux) (19), and immunofluorescence antibody test (IFAT) (conjugate bioMérieux, antigen prepared in our laboratory from ascite of toxoplasmosis infected mice) (20). The ELISA, ISAGA, and IFAT cutoffs used were as follows: index 0.65, index 6 for newborns, and a titer of 1/40, respectively. Western blot (WB) was used to detect newly synthesized anti-*T. gondii* IgG, IgM, and IgA (8). This technique combined electrophoresis of toxoplasmic antigens, an electrotransfer, and a specific antibody test, permitting the comparison of immunological patterns of mothers and infants.

IgA antibodies to *T. gondii* were detected by immunocapture ELISA using the Toxo IgA kit (SFRI). The polystyrene microtitration plates were sensitized with mouse antihuman IgA monoclonal antibodies. One hundred microliters of serum diluted 1:100 positive and negative controls were distributed in three wells of the microtitration plate. After 30 min of incubation at room temperature and washing, 100 µl of mixture of *T. gondii* antigen and conjugate (peroxidase-labeled monoclonal antibody against *T. gondii*) were added and incubated for 2 hr in the reaction wells. The monoclonal antibody used was specific for a 30,000 molecular weight major protein (P 30) located at the surface of the tachyzoites. After washing, presence of the enzyme was revealed by addition of 50 µl of chromogenic substrate (Ortho phenylene diamine). The reaction was stopped after 15 min and the optical

densities were read at 492 nm on a spectrophotometer. The indices were calculated from the sample's optical density and those of the positive and negative controls. The cutoff was set at 0.7, with an indeterminate zone between 0.5 and 0.7.

RESULTS

The data obtained from the 154 serum samples, cord blood excepted, are presented for each infant in Tables 1 and 2. IgA antibodies were detected by ELISA in 38.9% of CT+ cases, which was lower than the IgM detection by ELISA (61.1%) or ISAGA (66.7%) and lower than that of neosynthesized IgG and IgM detection (72.2% and 83.3% respectively). However, IgA detection was more frequent than IgM detection by IFAT (22.2%) and neosynthesized IgA detection (27.8%). The specificity of the tests was in the 93.3–100% of range (Table 2). In three serum samples collected from three infected newborns (Table 1: cases 2, 16, and 17), IgA antibodies were detected by ELISA, though no IgM antibodies were detected, but the Western blot results were positive for IgG, IgM or IgA. On the contrary, IgA antibodies were never detected by ELISA in the absence of detection of newly synthesized antibodies by Western blot. In seven serum samples collected from five infected newborns (Table 1: cases 5, 7, 8, 9, and 10), IgM antibodies were detected, though no IgA antibodies were detected by ELISA. Furthermore, in the 18 cases of CT+, IgA antibodies were never detected by ELISA earlier than with the other methods. When we compared the IgA detection by ELISA and Western blot, the Western blot was negative whereas the ELISA was positive in four serum samples collected from four infected newborns (cases 4, 16, 17, and 18). On the contrary, in two serum samples (cases 2 and 15), the ELISA IgA was negative whereas the Western blot detecting IgA was positive. In group 2 (CT-), only one serum (case 62) was positive with ELISA IgA while it was negative with Western blot.

Table 3 shows the results obtained on the 41 cord blood samples. The IgA detection by ELISA (54.5%) was less sensitive than the IgM detection by ELISA (81.8%) and ISAGA (90.9%), except by IFAT (27.3%). In two infected newborns (cases 3 and 4), IgA antibodies were detected by ELISA in the absence of newly synthesized IgA. Moreover, in case 3, the IgA antibodies were detected by ELISA in the absence of newly synthesized antibodies, but the IgM antibodies were positive with ELISA and ISAGA. The sensitivity of all the methods was higher on cord blood than on serum samples, except for the neosynthesized IgG and IgM antibodies detected by Western blot.

DISCUSSION

In our study, the sensitivity of IgA detection using ELISA was low on serum (38.9%) and cord blood (54.5%) samples, compared to the other immunological tests: IgM detection by ELISA (61.1% on serum samples, 81.8% on cord blood),

TABLE 1. Results of Serologic Tests in Post-Natal Follow-Up (From D₀ to One Year) of 18 Infants With Proven Congenital Toxoplasmosis—Group 1: 49 Serum Samples

Case no.	IgM detection ^a			Neosynthesized Ig detection			IgA detection by ELISA
	ELISA	ISAGA	IFAT	IgG	IgM	IgA	
1	— ^b	—	—	+	—	—	—
2	—	—	—	+	+	+	+
3	—	—	—	+	+	—	—
4	+	+	—	—	+	—	+
5	+	+	+	+	+	—	—
6	—	+	—	—	+	—	—
7	+	+	—	—	+	—	—
8	+	+	+	+	+	—	—
9	+	—	—	+	—	—	—
10	+	+	—	+	+	—	—
11	—	—	—	—	—	—	—
12	+	+	—	+	+	+	+
13	—	+	—	+	+	—	—
14	—	—	—	+	+	—	—
15	+	+	—	+	+	+	+
16	+	+	+	+	+	+	+
17	+	+	—	+	+	+	+
18	+	+	+	—	+	—	+
No. of positive cases (%)	11/18 (61.1)	12/18 (66.7)	4/18 (22.2)	13/18 (72.2)	15/18 (83.3)	5/18 (27.8)	7/18 (38.9)

^aELISA, enzyme-linked immunosorbent assay; ISAGA, immunosorbent agglutination assay; IFAT, immunofluorescence antibody test.

^b—, all the samples have negative results; +, at least one sample, among the sera sampled from each infant during the first year of life, has a positive result.

ISAGA (66.7% on serum samples, 90.9% on cord blood), Western blot (83.3% on serum samples, 72.7% on cord blood), and neosynthesized IgG detected on serum by Western blot (72.2%). In other studies, the sensitivity of IgA detection was described to be greater. Decoster et al. (14) reported a sensitivity of 76% on serum samples, and Ecochard et al. reported (16) 93% on cord blood samples. Such conflicting results may be explained by the different conditions of the studies. The 76% sensitivity (14) was obtained by testing another kit, the Platelia-Toxo IgA kit (Diagnostics Pasteur, Marnes-la-Coquette, France). Moreover, that study tested children with suspected congenital toxoplasmosis, but the diagnosis was not confirmed. The 93% sensitivity on cord blood samples was obtained by other authors (16) with the Toxo IgA, but

the cutoff was set at 0.37, which explains the higher sensitivity but the lower specificity (87% in this study), compared to our results.

The sensitivity of IgM detection using IFAT was very low (22.2% on serum samples, 27.3% on cord blood). In fact, this test is not useful for the postnatal follow-up of the infants but for dating seroconversion in pregnant women. Moreover, the performance of the neosynthesized IgA antibody detection using Western blot is lower than that of ELISA and the specificity of the IgG and IgM detection by Western blot is not absolute (95.6 and 93.3%, respectively). The detection of neosynthesized IgA antibodies is used to confirm diagnosis; the presence of neosynthesized IgA antibodies confirms congenital toxoplasmosis (8).

TABLE 2. Results of Serologic Tests in Post-Natal Follow-Up (From D₀ to One Year) of 45 Infants With No Congenital Toxoplasmosis—Group 2: 105 Serum Samples

Case no.	IgM detection ^a			Neosynthesized Ig detection			IgA detection by ELISA
	ELISA	ISAGA	IFAT	IgG	IgM	IgA	
19→57	— ^b	—	—	—	—	—	—
58	—	+	—	—	—	—	—
59	—	—	—	+	—	—	—
60,61	—	—	—	—	+	—	—
62	—	—	—	—	—	—	+
63	—	—	—	+	+	—	—
No. of negative cases (%)	45/45 (100)	44/45 (97.8)	45/45 (100)	43/45 (95.6)	42/45 (93.3)	45/45 (100)	44/45 (97.8)

^aELISA, enzyme-linked immunosorbent assay; ISAGA, immunosorbent agglutination assay; IFAT, immunofluorescence antibody test.

^b—, all the samples have negative results; +, at least one sample, among the sera sampled from each infant during the first year of life, has a positive result.

TABLE 3. Results of Serologic Tests on 41 Cord Blood Samples

		No. of positive samples/ total number of CT+ ^a samples	No. of negative samples/ total number of CT- samples
IgM detection by	ELISA ^b	9/11 (81.8%)	30/30 (100%)
	ISAGA	10/11 (90.9%)	28/29 (96.6%)
	IFAT	3/11 (27.3%)	29/29 (100%)
Neosynthesized Ig detection	IgG	4/11 (36.4%)	30/30 (100%)
	IgM	8/11 (72.7%)	28/30 (93.3%)
	IgA	4/11 (36.4%)	30/30 (100%)
IgA detection by	ELISA	6/11 (54.5%)	29/30 (96.7%)

^aCT+, proven congenital toxoplasmosis; CT-, absence of congenital toxoplasmosis.

^bELISA, enzyme-linked immunosorbent assay; ISAGA, immunosorbent agglutination assay; IFAT, immunofluorescence antibody test.

On serum or cord blood samples, IgA detection using ELISA was never positive when the other techniques were negative. Opposite results have been reported by Decoster et al. (14), who have shown that IgA antibodies were always present in infected children in the postnatal period. In addition, positive IgA detection by ELISA was never found earlier than with the other tests. Thus, it appears that the diagnosis of congenital toxoplasmosis cannot be established earlier with the Toxo IgA system, contrary to results described elsewhere (14).

In our study, all serological tests performed on cord blood samples were more sensitive than those performed on serum samples, except for neosynthesized IgG and IgM antibodies detected by Western blot. In fact, the IgG, IgM, and IgA detected in cord blood may be maternal antibodies which are transferred passively at the time of delivery. This could account for the increased sensitivity and, in some cases, the decreased specificity. This is why serological tests must be performed on serum samples at day 5, which increases the specificity of the diagnosis.

In conclusion, anti-P30 IgA antibody detection using the commercially available assay Toxo IgA is not helpful in improving the diagnosis of congenital toxoplasmosis, in comparison with ISAGA and ELISA for IgM antibodies, and Western blot for neosynthesized IgG, IgM, and IgA antibodies. This technique was less sensitive on serum and cord blood samples than the other techniques in cases of congenital toxoplasmosis confirmed by the presence of infant's IgG after 12 months of life or the presence of *T. gondii* in amniotic fluid and/or fetal blood.

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