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Assessment of the IgA Immunosorbent Agglutination Assay for the Diagnosis of Congenital Toxoplasmosis on a Series of 145 Toxoplasmic Seroconversions

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A retrospective analysis of 145 medical records from our teaching hospital laboratory showed an overall specificity of greater than 97% for the IgA immunosorbent agglutination assay (ISAGA A) performed on the sera of babies to diagnose congenital toxoplasmosis (CT). These actualized data emphasize the ability of this test to confirm a diagnosis of congenital toxoplasmosis.

Measuring IgA is considered useful for the postnatal detection of congenital toxoplasmosis (CT) (1, 2). IgA assessment was introduced into hyperspecialized tests used for the detection of congenital toxoplasmosis because it is more sensitive, and IgA is detectable for a longer period in newborns than is IgM (contrary to the situation in adult acute toxoplasmosis) and because of IgA's inability to permeate the placental barrier (2–5). To detect IgA, an IgA immunosorbent agglutination assay (ISAGA A) was shown to be more sensitive than enzyme-linked immunosorbent assay (ELISA) techniques (4, 6). However, focused evaluations of IgA and especially the ISAGA A remain scarce and were mainly published more than 15 years ago (4, 6–8). Moreover, more recent results from a European multicenter study were contradictory, showing close sensitivities of the ISAGA and ELISA and a relatively poorer specificity (Sp) of the ISAGA (91%) (9). In the current study, we retrospectively analyzed our daily routine records to determine if IgA assessment in babies is still of interest today and to provide an update on the performances of the commercial tests used in our laboratory.

The medical records of 157 mothers who had experienced acute toxoplasmosis during or just before pregnancy were investigated. These patients were followed up from January 2006 to September 2012 in either the Grenoble teaching hospital or a peripheral center. All the samples were analyzed in the clinical laboratory of the Grenoble teaching hospital. For each newborn, the usual biological protocol was applied. Briefly, serum samples were taken 3 to 5 days after birth (the serum samples were not taken at birth to decrease the risk of detecting IgM transmitted by leakage) and, when possible, at 1 and 3 months of life to monitor the variations of antibody levels. Cord blood samples were also analyzed when available. To assess the levels of IgA, an ISAGA (Toxo ISAGA IgA; bioMérieux, Marcy l'Étoile, France) was prospectively performed according to the manufacturer's recommendations. Scores of ≥ 6 were considered positive, and scores of < 3 were considered negative. The specificity and sensitivity (Ss) were calculated by comparing the ISAGA A results (positive or negative) with the final diagnoses, i.e., the presence or absence of congenital transmission, based on the complete radiological, clinical, and biological analyses of the cases. Biological interpretations were based upon the analyses of each sample with the following assays: Vidas Toxo IgG II and Toxo IgM (bioMérieux), IgG and IgM in-house immunofluorescence (10), comparative immunoblotting (Toxoplasma WB IgG IgM; LDBio, Lyon, France), and Toxo ISAGA IgM (bioMérieux). Additionally, specific quantitative PCR targeting rp529 and inoculation of mice were performed on amniotic fluid and on placenta when sampled (11).

Among the 145 babies who were tested with the ISAGA A using at least one serum sample, 26 were children with biologically proven CT, 40 were children with a biologically proven absence of CT (i.e., disappearance of anti-*Toxoplasma gondii* antibodies), and 79 did not show any biological or clinical evidence of CT but were not followed long enough to observe full disappearance of antibodies. Table 1 shows, for each type of sample, the numbers of true-positive, false-positive, true-negative, and false-negative results and the sensitivity (Ss) and specificity (Sp) values of the ISAGA A test.

In our data set, IgA was never detected earlier than other biological elements indicative of congenital toxoplasmosis. In one case, IgA was positive (score, 6) at 1 month of life without any detection of IgM at birth or at 1 month of life, while at 7 months the ISAGA A score reached 12 and the ISAGA M score only reached 3; however, the diagnosis of CT had been made during pregnancy on the basis of a positive PCR in amniotic fluid, and IgA was the only serological confirmation of the disease. In 2 other cases of CT, IgA remained positive for a longer time than IgM, leading to samples with IgA but with no more IgM; in these cases, IgA was not required for diagnosis. Consequently, in our experience, ISAGA A does not provide a faster diagnosis than other biological tests.

The main result of this study was the intrinsically high level of Sp of the ISAGA A at any time of baby blood sampling; indeed, the Sp values were greater than 97% in all situations and reached

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TABLE 1 Performance parameters of the ISAGA A for detecting congenital toxoplasmosis according to age at sampling

Performance parameter[e]	Cord blood	Patient data at the age of sampling			
		Birth	1 mo	3 mo	All ages
No. of samples	94	135	90	69	294[a]
No. of children with CT ($n = 26$)	17	23	22	24	69
No. of children with no evidence of CT ($n = 79$)	47	74	34	18	126
No. of children with proven absence of CT ($n = 40$)	30	38	34	27	99
No. of true positives	9	11	13	7	31
No. of false positives[b]	1	2	0	0	2
No. with gray zone with CT	1	1	0	3	4
No. with gray zone with no CT[b]	1	1	0	0	1
No. of true negatives (no evidence of CT, proven absence of CT)	75 (45, 30)	109 (71, 38)	68 (34, 34)	45 (18, 27)	222 (123, 99)
No. of false negatives	7	11	9	14	34
Sensitivity (%)[c,d]	52.9 (42.9–62.7)	47.8 (39.6–56.2)	59.1 (48.8–68.7)	29.2 (19.8–40.8)	44.9 (39.3–50.6)
Specificity (%)[c,d]	97.4 (91.9–99.2)	97.3 (93.0–99.0)	100.0 (95.9–100)	100.0 (94.7–100)	98.7 (96.6–99.5)

[a] Cumulative number of samples, including several samples per child.
[b] Only children with no evidence of CT (no child with proven absence of CT is included in these categories).
[c] Shown in parentheses are 95% confidence intervals calculated according to reference 17.
[d] Calculations were made with the hypothesis that congenital transmission was absent in children with no evidence of CT and in children with a proven absence of CT, indistinctly.
[e] Gray zone, ISAGA A scores ranging from 3 to 5.

100% when IgA levels were assessed after 1 and 3 months of life. Only 3 false positives were detected for 2 patients (for one child, the cord blood was positive in addition to the serum at birth). For each of these cases, the mother's seroconversion had occurred during the periconceptional period, and there was no biological or clinical argument for congenital toxoplasmosis in the child. A similar phenomenon was described by Patel et al., with one false-positive ISAGA (i.e., an Sp of 94.1%; $n = 17$ noninfected children) observed in a child 2 days after delivery (6), and by Decoster et al. in one sample taken at birth and tested with the Platelia ELISA (Sp, 96.7%; $n = 30$ noninfected children) (7). Foudrinier et al. observed nine similar cases at birth using two techniques (ISAGA [Sp, 98.3%] and a home-made ELISA [Sp, 98.3%]; $n = 228$ noninfected children) (4). As with these authors, we suppose that the few false positives in our study corresponded to contamination at delivery by maternal blood (which showed a maximal ISAGA A score of 12) via a placental defect, even though the samples were taken at 4 days of life. Contrary to Robert-Gangneux et al., we did not observe lowered specificity in cord blood (12).

In our study, the Ss values of the ISAGA A hardly exceeded 50%, particularly in the samples taken 3 months or more after birth, which is consistent with previous findings that IgA levels are higher in newborns than in older infants, which may be due to IgA kinetics (13). Using a prototype of the test that we used, Patel et al. found a Ss of 71% in a series that included 17 positive patients (exclusively sampled at birth). In tests described by Foudrinier et al., Ss values reached 68% for first-month sera and only 16% for sera taken between the second month and 10 years of life (4); the same team later found that Ss values reached 62% in early months, which were lower than those of the analogous IgM assay (8); however, values as low as 1 were considered significant in these two studies. Similar results were obtained by Bessières et al., who observed that 60% of the 42 serum samples of infected infants were detected, compared with 50% of them detected by IgM tests (14). In a study comparing analytic strategies, Pinon et al. (15) found that ISAGA A sensitivity was superior to that of a Bio-Rad Pasteur ELISA (62% versus 55%, respectively). Accordingly, other techniques were not found to be much more sensitive than the ISAGA and were sometimes found to be less sensitive. Sensitivities measured 64% ($n = 58$ samples) for the Platelia ELISA targeting anti-p30 IgA (7), 54.5% and 54% for the SFRI ELISA using cord blood ($n = 41$ and 83, respectively), and 38.9% and 60% for the SFRI ELISA using sera ($n = 154$ and 83, respectively) (1, 16). The highest sensitivity was found by Olariu et al. in a retrospective analysis of the Palo Alto Medical Foundation laboratory database, with a value of 77.4% for their in-house ELISA, but the population may have been different from those of the previously cited studies, since this study focused on newborns who were not treated for toxoplasmosis and whose mothers had not been treated during their pregnancy (13).

Originating from a retrospective analysis of the usefulness of the ISAGA A test in our daily routine over more than 5 years, we believe that the current study provides an actualized and significant reflection of the performances of this assay under the conditions of use in a reference laboratory. Given the moderate values of sensitivity, IgA detection cannot be used alone and cannot replace IgM detection, but it appears to be of interest in completing a panel of tests detecting IgG and IgM.

We validated with actualized data the high specificity of this test and conclude that, based on these data, a positive ISAGA A at 1 or 3 months of life confirms the diagnosis of congenital toxoplasmosis. It possibly can be considered a second-line assay in a sequential strategy as a confirmation test when positive IgG and/or IgM are detected by very sensitive tests.

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