The use of an IgM immunosorbent agglutination assay to diagnose congenital toxoplasmosis

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Summary. An IgM immunosorbent agglutination assay (ISAGA) was compared with a standard ELISA IgM test for the diagnosis of congenital toxoplasmosis. It was more sensitive, detecting all of five mothers of infected babies whereas the IgM ELISA was positive in two of three mothers tested at delivery and neither of two mothers referred 10–12 months after delivery. Five women infected in a previous pregnancy had IgM detectable by ISAGA in a subsequent pregnancy. The assays were comparable when sera from patients with past infection were tested or following toxoplasma-associated miscarriage or abortion. Four cord sera from congenitally-infected babies were positive by the ISAGA but only three of these were positive by ELISA for IgM. The ISAGA also detected IgM in another four sera from congenitally-infected babies referred late (10–18 months old); none were IgM positive by ELISA. The increased sensitivity of the ISAGA is an improvement in the diagnosis of congenital toxoplasmosis.

Introduction

Infection with *Toxoplasma gondii* is a recognised cause of congenital disease (Remington and Desmonts, 1976). Approximately 50% of actively infected women transmit the parasite to their fetus (Desmonts and Couvreur, 1974). Although most infected babies are symptomless at birth, up to 90% may later develop sequelae (Koppe *et al.*, 1986). Since treatment may reduce or prevent such damage (Wilson *et al.*, 1980), it is important to identify infected infants.

Ultrasound and fetoscopy techniques can confirm fetal infection during pregnancy (Daffos *et al.*, 1988). However, primary infection is often asymptomatic (Desmonts and Couvreur, 1974) and, in the absence of antenatal screening, rarely detected during pregnancy (Joss *et al.*, 1988). The detection of specific IgM in the mother, neonate or baby remains the mainstay of diagnosis of infection. Although its presence in maternal serum does not predict the delivery of a damaged child, specific IgM in the baby's serum may confirm congenital infection (Remington and Desmonts, 1976). However, up to 25% of infected babies have negative results for specific IgM (Naot *et al.*, 1981). This may be due to an inability of some babies to

produce antibody or to the insensitivity of current tests.

An IgM immunosorbent agglutination assay (ISAGA) has been described as more sensitive and specific than the indirect immunofluorescence test (Desmonts *et al.*, 1981). A modification of this technique has been widely used in France and is now available in Britain. We have assessed this test on various panels of sera to determine if its increased sensitivity is of value in the diagnosis of congenital toxoplasmosis.

Materials and methods

Assays

The ISAGA (Toxo-ISAGA, API-bioMérieux, Charbonnières les Bains, France) was used according to the manufacturer's instructions. Sera, diluted 1 in 100 (1 in 20 for neonatal specimens) in phosphate-buffered saline pH 7.2, were incubated for 2 h at 37°C in a humid chamber on strips precoated with anti-human IgM monoclonal antibody. After washing, toxoplasma antigen was added and the strips were incubated overnight at 37°C. Wells were read for the degree of agglutination on a scale 0–4; total sedimentation scoring 0 and complete agglutination read as 4. In the preliminary screening test sera were tested with 100 µl and 150 µl of antigen, agglutination scores were combined and sera scoring ≥6 retested at three antigen concentrations (100 µl, 150 µl,

and 200 μ l). The final reading for each serum was calculated from the combined scores of the three wells and interpreted as: <5, negative; 6–8, borderline; and 9–12, positive. All plates were read independently by at least two operators.

Specimens were also tested by indirect haemagglutination (HA; Thorburn and Williams, 1972) or ELISA IgG (Joss *et al.*, 1988) followed by a micromodification of the dye test (DT; Williams *et al.*, 1981) with titres expressed in International Units (IU)/ml. Specific IgM was investigated initially by indirect immunofluorescence and occasionally sucrose gradient fractionation followed by HA and DT on relevant fractions. For comparison with the ISAGA, most sera were tested with an ELISA IgM (Toxonostika IgM, Organon Teknika, Durham, NC, USA) which was performed according to the manufacturer's instructions.

Serum samples

Mothers. Sera were selected from those submitted to the laboratory for routine testing and others were collected for a prospective antenatal survey (Joss *et al.*, 1988). Samples from 26 women were tested by the ISAGA and ELISA IgM methods. For analysis, patients were classified into 5 groups according to clinical information and previous serology results (DT titres, specific IgM). *Group 1* consisted of five mothers of congenitally-infected babies. Two had DT titres ≥ 300 IU/ml and specific IgM in specimens taken at delivery, a third had seroconverted during pregnancy. The other two sera were only tested 10–12 months after delivery. The seven mothers in *group 2* had serological results suggestive of recent infection but delivered apparently healthy babies. Six of the sera had DT titres ≥ 300 IU/ml and three of these were also weakly positive for specific IgM. The seventh mother had shown low-level seroconversion during pregnancy. *Group 3* consisted of five pregnant women who had serologically-proven toxoplasmosis in a recent (≤ 30 months) pregnancy (specific IgM positive or seroconversion). The three women in *group 4* had a toxoplasma-associated miscarriage or abortion. All three samples were specific IgM positive by sucrose gradient fractionation and two showed DT titres ≥ 2000 IU/ml. The final six sera (*group 5*) were from pregnant women who had serology consistent with past toxoplasma infection (low positive DT titres and no specific IgM).

Babies. Sera from 39 babies were also tested. These were classified into three groups, again according to clinical information and previous serology data. *Group 1* consisted of eight babies with symptoms compatible with congenital toxoplasmosis and persistent, significant DT titres; four were tested at delivery and four 10–18 months after delivery. The five babies in *group 2* were apparently healthy but were born to mothers thought to be recently infected. All had DT titres at birth reflecting maternal serology with, when available, follow-up specimens at 6 months showing a decrease in DT titres. *Group 3* consisted of 26 neonatal sera which were used as controls. Ten samples were jaundiced or haemolysed and, of these, four

were DT positive. As testing was retrospective (some neonatal sera in groups 1 and 2 had been stored for up to 4 years), eight control sera of similar storage time were included.

Results

Pregnant women

Sera from all pregnant women with evidence of past toxoplasma infection gave negative results by ISAGA and all from cases of miscarriage or abortion associated with toxoplasma gave positive results. Apart from one positive ELISA IgM in serum from a case of past infection, the ISAGA results agreed with the standard assay (table I). Neither test could distinguish toxoplasma-infected women who gave birth to healthy babies from mothers of congenitally-infected babies; the ISAGA was consistently positive whereas the ELISA IgM gave negative results in a proportion of both groups. All five women who had serologically-proven toxoplasmosis in a recent pregnancy were ISAGA positive but only one had a positive result by ELISA IgM.

Babies

Sera from all eight toxoplasma-infected babies were positive by ISAGA but the ELISA IgM failed to detect antibody in samples from the four older children and one newborn (table II). One positive result in both assays was found among samples from the five babies in whom congenital infection was thought unlikely. We believe that this result was due to a placental leak. One control serum from a jaundiced baby with negative toxoplasma serology was initially borderline positive by ISAGA but negative on repeat. The remaining 25 control specimens gave negative results. Two control sera were positive by ELISA IgM. Storage of serum for prolonged periods did not affect the results of the ISAGA assay.

Discussion

Whether testing serum from antenatal patients, neonates or babies, a more sensitive and specific assay for IgM will improve the diagnosis of congenital toxoplasma infection. The sensitivity of the ISAGA was superior to that of the ELISA IgM. The ISAGA identified all five mothers of congenitally-infected babies whilst the ELISA IgM detected only two of the three mothers investigated at or before delivery and none of the mothers

Table I. ISAGA and ELISA IgM results in 26 women

Patient classification*	Number of women tested	Number of sera tested by				
		ISAGA		ELISA IgM		
		Positive	Negative	Positive	Negative	Not done
Group 1. Mothers of congenitally-infected babies						
—confirmed at birth	3	3	0	2	1	0
—confirmed 10–12 months after birth	2	2	0	0	2	0
Group 2. Mothers of healthy babies	7	7	0	3	3	1
Group 3. Women positive in recent pregnancy	5	5	0	1	3	1
Group 4. Women with a miscarriage or abortion	3	3	0	3	0	0
Group 5. Women with evidence of past infection	6	0	6	1	4	1

*Detailed classification in *Materials and methods*.

Table II. ISAGA and ELISA IgM results in 39 babies

Patient classification*	Number of babies tested	Number of sera tested by				
		ISAGA		ELISA IgM		
		Positive	Negative	Positive	Negative	Not done
Group 1. Infected baby						
—tested at birth	4	4	0	3	1	0
—tested at 10–18 months	4	4	0	0	4	0
Group 2. Mother infected, baby not infected	5	1	4	1	3	1
Group 3. Control group	26	0	26	2	23	1

*Detailed classification in *Materials and methods*.

examined 10–12 months after delivery. All eight congenitally-infected babies were detected by ISAGA; the ELISA IgM identified only three of the four infected newborns. The ISAGA was sufficiently sensitive to detect low levels of antibody and identify the four congenitally-infected babies who presented late. However, this sensitivity may present a problem in the management of pregnant women, as a positive maternal IgM is taken as an indication for treatment or fetal investigation (Desmonts *et al.*, 1985). By relying on ISAGA results in our groups of pregnant women, 18 would have needed monitoring but only three gave birth to a damaged child, and three had a miscarriage or abortion. Ten were identified by ELISA IgM, including two of the three mothers who delivered

infected babies and all three who had miscarriages or abortions. The use of the ISAGA would mean the need to monitor and follow-up more women, but this would result in a small increase in the detection of congenital infection.

The specificity of the ISAGA was better than ELISA IgM when assessed on 26 sera from the selected panel of babies. All were negative by the ISAGA but two sera were positive by ELISA IgM. Both these were haemolysed specimens, common among neonatal sera but not recommended for use in this test. The ELISA IgM assay has shown that specific IgM persists for up to 10 months (Wieland *et al.*, 1983). The ISAGA, when testing sequential maternal sera, consistently remained positive beyond this period and after the ELISA IgM had

become negative. The sensitivity of the ISAGA in detecting low antibody levels reduces its usefulness in diagnosing current infection; five of the 11 women known to be past the acute stage of infection were positive by ISAGA, but only two of nine were positive by ELISA IgM. The failure of an assay to exclude patients without disease limits its application. It must be used in conjunction with other tests, particularly when investigating a single serum sample. The interpretation of discrepant ISAGA and ELISA IgM results would depend on the level of specific IgG. For sera with a positive ISAGA and negative ELISA IgM, high levels of specific IgG would imply infection had occurred some months previously whereas the absence of specific IgG would indicate an early primary infection.

The ISAGA does not require specialised equipment or technical skill. If used according to the manufacturer's guidelines, 2 days are required to obtain a positive result. This can be reduced by testing with three concentrations of antigen at one time but at a greater cost in reagents. In the present study, all plates were read independently by at least

two workers and although interpretations varied it was rarely to the extent of moving a specimen between classifications. Incorporation of a colour plate of reference wells might help to standardise reading but an automated reading system would be preferable. For the comparison of ISAGA and ELISA IgM tests, borderline results were taken as positive. This affects the performance of a test by increasing sensitivity.

Investigation of congenital disease is not confined to determining the causative agent after the birth of a damaged child. In our experience, the majority of congenital-toxoplasmosis cases which are referred are over 10 months old. The ISAGA was sufficiently sensitive to detect antibody in the serum of such children and was thus helpful in reaching a presumptive diagnosis. When the presence of specific IgM is itself diagnostic, as in neonates, the ISAGA represents an important improvement in serological diagnosis.

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