Epidemiol. Infect. (1990), **105**, 409–414
Printed in Great Britain

Selective antenatal screening for toxoplasmosis and the latex agglutination test

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(*Accepted 17 May 1990*)

SUMMARY

Recent publicity concerning congenital toxoplasmosis has generated a demand for serological assessment of pregnant women. Many laboratories are requested to undertake primary screening in these cases. We assessed the latex agglutination test (LAT) findings in 158 specimens with detectable toxoplasma specific IgM derived from pregnant women. The LAT titres ranged from 16 to $\geqslant 4000$ reflecting the variable antibody response observed in acute toxoplasmosis. We recommend that non-reference laboratories test specimens from pregnant women using the LAT at a screening dilution of 1:16 and select all reactive samples for detailed investigation.

INTRODUCTION

Increased awareness of the sequelae of toxoplasmosis in pregnancy has lead to a debate over the role of antenatal screening for this infection [1, 2]. Currently there is no provision of resources in the UK for a general screening programme. A number of pregnant women consult their general practitioner or obstetrician and request toxoplasma screening. After careful consideration of the individual's risk of infection and the management response to laboratory findings, the clinician may decide selected cases require serological assessment. In some regions the reference laboratory may have sufficient resources to accept all samples derived from pregnant women. However, many district hospital laboratories will be asked to undertake primary screening of sera with referral of selected samples to a reference centre.

The factors to consider in any screening programme are well defined [3] but the optimum approach to toxoplasma screening during pregnancy is not established. Few sera derived from pregnant women will contain toxoplasma specific IgM and the sensitivity and specificity of many commercial assays for the measurement of these antibodies have not been defined although comparisons of some test findings have been undertaken [4]. Consequently, most non-reference laboratories elect to perform IgG assessment. This approach allows the laboratory to offer serological testing in other clinical settings, such as transplantation and the immunosuppressed, where IgM measurement is of limited value [5, 6]. Any assay used in a screening programme must be simple to perform, rapidly applicable to a large population, reproducible and valid. Although a number of methods are available

for toxoplasma-specific IgG assessment the latex agglutination test (LAT) is the assay most widely used by centres referring samples to our unit. The value of LAT as a general screening test has been established [7] but the application of this assay to the investigation of pregnant women has not been studied. In particular, the screening dilution used in LAT to select samples for subsequent IgM assessment has not been defined. The manufacturers consider LAT reactivity at a sample dilution of 1:16 as a positive result. However, some laboratories prefer to screen antenatal sera at a higher initial dilution, often 1:64, in order to reduce the numbers of samples sent to reference centres. We have reviewed our findings using this test for samples from pregnant women in order to establish the appropriate criteria for the rational selection of sera requiring more detailed examination.

METHODS

The results of toxoplasma serological examination of pregnant women during a period of 30 months were considered. All patients having detectable IgM were studied in detail. The latex agglutination test (LAT) findings for the initial sample of serum only were recorded when serial samples were received. Toxoplasma-specific IgM was measured using a μ-chain capture double sandwich enzyme linked immunosorbent assay (DS-ELISA) and an immunosorbent agglutination assay (ISAGA) as previously described [8]. The LAT was performed according to the manufacturer's instructions [9].

Serological findings from the investigation of children delivered to women with detectable specific IgM during pregnancy were documented. Congenital infection was diagnosed by the persistence of toxoplasma specific IgG in the infant's circulation at 12 months of age with or without detectable IgM production during the first year of life. Details of the clinical examination of congenitally infected babies were recorded. In cases of therapeutic termination of pregnancy, products of conception were investigated for the presence of *Toxoplasma gondii* by intraperitoneal inoculation into laboratory mice.

RESULTS

During the period of study 1522 pregnant women were investigated and 347 found to have an LAT titre $\geqslant 16$. A total of 158 pregnant women were found to have detectable toxoplasma specific IgM. Of these 158 women, 63 were investigated during referral for foetal blood sampling. All samples producing a positive or borderline DS-ELISA result also reacted in the ISAGA. In contrast, 85 specimens producing a positive or borderline ISAGA result were found to be negative by DS-ELISA. Three samples were of insufficient volume to allow complete analysis. The LAT titres recorded from sera of these 158 women ranged from 16 to > 4000. No sample associated with detectable IgM produced a LAT titre result below 16. The results of LAT and DS-ELISA examination of ISAGA positive/borderline positive samples are presented in Table 1.

Complete laboratory and clinical findings were available for 11 confirmed cases of congenital toxoplasmosis and 11 infants found not to be infected. The results of 15 of these patients have been reported previously [10]. Maternal antenatal

Table 1. *Results of double sandwich enzyme linked immunosorbent assay (DS-ELISA) and latex agglutination test (LAT) examination of immunosorbent agglutination assay (ISAGA) positive or borderline positive samples derived from pregnant women*

	DS-ELISA result				
LAT result (titre)	Positive	Borderline positive	Negative	Not performed	Total
16	0	0	1	—	1
32	0	0	0	—	0
64	6	0	4	1	11
128	2	3	9	—	14
256	14	1	14	2	31
512	15	0	20	—	35
1000	10	3	18	—	31
2000	11	0	8	—	19
⩾ 4000	3	2	11	—	16
Total	61	9	85	3	158

samples were reactive by ISAGA and DS-ELISA in 10 cases of congenital infection. Of the 10 infants delivered 6 showed congenital abnormality. In one instance the maternal antenatal samples were reactive by ISAGA alone and the resulting infant was clinically well at birth. A detailed description of this case is in preparation. Of the remaining 136 pregnancies, 107 are under continuing investigation and 14 were subject to incomplete evaluation. Termination of pregnancy was performed in 15 cases and *T. gondii* was isolated from three products of conception.

DISCUSSION

The design of the study described does not permit an accurate investigation of the seroprevalence of acute and chronic toxoplasmosis infection associated with pregnancy. The relatively large number of cases of acute toxoplasmosis included in the study was due, in part, to the investigation of women with suspected toxoplasma infection referred for consideration of foetal blood sampling. In addition stored sera was investigated retrospectively in cases when a clinically abnormal infant was delivered or recognized in the early postnatal period. In consequence, the acutely infected proportion of pregnant women is likely to be represented in excess when compared to the population of pregnant women as a whole. The non-specific signs and symptoms of toxoplasma infection make diagnosis based on clinical examination unreliable [11]. Limited availability of serum samples showing recent seroconversion and the failure of Western blots or parasite isolation to define recent infection lead to considerable difficulties in the assessment of IgM assays [8]. The absence of a definitive reference has resulted in commercial toxoplasma immunoglobulin-M assays being made available without quantitative assessment of the sensitivity and specificity of the technique. Reference centres attempt to overcome these problems by the use of several IgM tests with comparison of findings. In our experience the ISAGA is significantly more sensitive than DS-ELISA for the detection of toxoplasma specific IgM but the specificity of the two assays are comparable [8]. Our findings of 85 ISAGA

reactive sera, negative on examination by DS-ELISA reflects the comparative sensitivity of the assays. When a pregnant woman is found to have toxoplasma specific IgM detectable by ISAGA but not by DS-ELISA the risk of foetal infection is difficult to define, but we have observed congenital infection in one of these cases. Although 5 of the 11 congenitally infected children were asymptomatic on initial examination the prognosis for these infants remains uncertain. Long term follow-up of congenitally infected infants, unaffected at birth, has shown that the majority of these individuals develop visual defects in later life [12]. Anti-parasitic therapy is administered to such children in an attempt to prevent ocular sequelae but there are no studies assessing the effectiveness of this management. Evaluation of the predictive value of LAT results compared to ISAGA findings is likely to minimize the numbers of pregnancies at risk left undetected. An alternative to initial assessment of toxoplasma specific IgG status alone in the primary laboratory would be to incorporate initial IgM assessment, either as a second test for IgG reactive samples or in place of IgG investigation. Unless IgG and IgM levels are measured it is not possible to identify those pregnant women who have passed through the stage of acute toxoplasma infection and who can be reassured. Should IgM assessment be performed as an adjunct to IgG testing, the characteristics of the assays employed must be understood. When a highly sensitive assay, such as ISAGA, is used the number of specimens selected for referral will be greater than if a less sensitive test, such as DS-ELISA was utilized. Our findings indicate twice as many sera would be referred after testing by ISAGA compared to DS-ELISA. Conversely, use of DS-ELISA in the primary laboratory may result in a failure to identify some women who subsequently deliver infected infants. One such case was identified during the present study but the total number of congenitally infected children included was small. Further investigation of this important aspect is required.

When performing an initial assay to select samples for more detailed investigation, the most important criteria is to maximize sensitivity so that 'at risk' samples are not discarded in error. A high degree of specificity is desirable but some false positive reactions can be accepted as these specimens will be subjected to further testing. Previously we have found the sensitivity and specificity of LAT to be 99% and 81% respectively, using the dye test as a reference [7]. Consequently a number of LAT results will represent false positive reactions. Using primary LAT screening and secondary confirmation by other tests these discrepant findings would be identified and these women would not be falsely reassured. The women erroneously classified as having had no previous exposure to toxoplasma (false negative reactions) would be offered health education and would be correctly diagnosed when subsequent testing was performed. Antenatal screening for toxoplasmosis in France specifies repeat testing of women found to be susceptible to infection at the initial assessment.

We have shown that the LAT findings in IgM positive samples are highly variable but that all had titres of $\geqslant 16$. The LAT uses disrupted toxoplasma trophozoites as an antigen source. In consequence, the assay detects antibodies with affinity for cytoplasmic and membraneous antigens of *T. gondii* [8]. Western blot studies have shown wide variation in the individuals response to toxoplasma infection, both in the number of antigens recognized and the relative amount of

antibody directed to different antigens [13]. In view of these factors the variable LAT titres recorded are not unexpected. The majority of the samples included in this study were derived from patients attending our own hospital or referred from peripheral laboratories without prior testing. However, the results of the present study could have been biased by initial screening of sera in primary laboratories so that specimens producing low titre LAT results were not sent for IgM assessment. Previously we were unable to demonstrate ISAGA or DS-ELISA reactivity amongst 50 sera producing LAT titres < 16 [8]. Nor have we detected toxoplasma-specific IgM in LAT negative samples taken from AIDS sufferers or transplant recipients. We are not aware of reports of an IgG response measured by LAT preceding the appearance of toxoplasma specific IgM during acute infection of the immunocompetent. Therefore it is appropriate to undertake toxoplasma investigation of a pregnant woman by primary IgG assessment with subsequent IgM measurement in selected cases. This approach permits the differentiation of acute infection, chronic latent infection and the absence of previous exposure to toxoplasma. Women found to be in chronic infection can be reassured, those not exposed given appropriate health education to avoid subsequent infection and acutely infected individuals offered specialized management [14]. These conclusions apply only to a screening programme involving selected antenatal patients. The methods advocated may not be applicable to the very large numbers of samples which would require investigation should universal antenatal screening for toxoplasmosis be introduced.

We recommend that sera selected for antenatal toxoplasma investigation be tested by LAT at a screening dilution of 1 : 16 and those producing a positive result be subject to further examination. The application of a higher initial screening dilution in an attempt to reduce the number of samples requiring further investigation is inappropriate as this would lead to some IgM positive specimens being discarded. There is a need to define similar criteria for other IgG assays and to establish the performance characteristics of IgM tests promoted for selective or universal toxoplasma antenatal screening.

ACKNOWLEDGEMENT

We are grateful to Mrs I. Crossman for the preparation of the typescript.

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