Strategies for detecting toxoplasma immunity

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ABSTRACT

A strategy for identifying toxoplasma immunity in pregnancy must provide good evidence of immunity but not falsely reassure; that for immunocompromised patients should identify immunity and also the risk of reactivated toxoplasmosis. Using sera from both of these patient groups, the performance of an in-house IgG EIA and two commonly used commercial assays (Abbott AxSYM Toxo-G and Eiken latex test) were compared with the dye test. False-positive results were obtained using the IgG enzyme immunoassay (EIA) and AxSYM Toxo-G, and false negatives using all three screen tests. During pregnancy, positive results may falsely reassure, and patients should be tested for toxoplasma-specific IgM to differentiate between current infection and immunity. In immunocompromised patients, positive results indicate immunity but negative results do not exclude it; these should be tested by dye test. Despite these reservations, we have demonstrated that immunity screening can be performed within a district general hospital.

KEY WORDS: Immunity. Immunocompromised host. Pregnancy. Toxoplasma.

Introduction

Immunity screening for *Toxoplasma gondii* is important in two groups of patients: pregnant women and the immunocompromised.[1] In both groups, the consequences of toxoplasma infection can be severe.[2,3] For pregnant women it must provide good evidence of immunity but not give false reassurance of immunity;[4] for the immunocompromised it must accurately identify past infection and the risk of toxoplasma reactivation.[5,6] The demand for immunity screening is increasing and the needs of both patient groups must be met within the toxoplasma testing scheme.

In the UK, sera are usually screened in local district general hospital laboratories and all positive and some negative samples are referred (when there is particular clinical concern) to a reference laboratory. Local laboratories use a wide variety of commercial toxoplasma tests, the sensitivity and specificity of which are often assessed on a group of sera without taking the individual patient's condition into account.[7]

While the need for immunity screening is well recognised, there is much debate about what constitutes a positive result.[8,9] Previously, we compared the performance of different assays for the diagnosis of current toxoplasma infection and found considerable variation.[10] This led to the development of a testing strategy for the diagnosis of current toxoplasma infection.[11] The study also highlighted the problem in immunity screening of false-positive and false-negative results in sera from pregnant women and immunocompromised patients.[10]

In response to our users' requests, this study aims to determine the best approach to screening for toxoplasma immunity.

Materials and methods

Sera

Two groups of sera were selected from those received by the Scottish Toxoplasma Reference laboratory (STRL) between January and December 2001. Group 1 (pregnant) consisted of 150 samples referred for immunity screening or investigation of symptoms (e.g., stillbirth, miscarriage, fetal abnormality). Group 2 (immunocompromised) consisted of 153 samples from patients who were immunocompromised due to HIV/AIDS, organ or bone-marrow transplantation, or malignancy. Sera were sequentially selected on the basis of dye test result. In each group, 50 negative (<2 iu/mL) samples were included and the remainder chosen to represent a range of positive dye-test results (2-4000 iu/mL). Sera were stored at –20°C and anonymised before testing.

Dye test

Sera were tested by a micromodification of the Sabin-Feldman toxoplasma dye test.[4,12,13] This is the gold-standard test for toxoplasma serology but is only available in reference laboratories because of the need for live tachyzoites.[9] The end-point titre was considered to be the serum dilution that produced 50% killing of the toxoplasma tachyzoites in doubling dilutions of serum. Results were expressed in iu/mL relative to the international standard anti-toxoplasma serum provided by the National Institute of Biological Standards and Controls (NIBSC; Potters Bar, Hertfordshire, UK). The dye test detected all immunoglobulin classes, and a value >4 iu/mL was regarded as positive.[9]

IgG enzyme immunoassay

An in-house IgG enzyme immunoassay (EIA) has been in routine use as a screening test in this laboratory since 1989,[14] and is similar to commercially available EIA tests. Microtitre

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plates were coated with a water-soluble extract of toxoplasma RH tachyzoites and antibody was detected with commercial alkaline phosphatase-conjugated anti-human IgG.[14] The threshold of the IgG EIA was 8 iu/mL, which was calibrated to the NIBSC international standard.

AxSYM Toxo-G
The AxSYM Toxo G assay (Abbott Diagnostics, Maidenhead, Berkshire, UK) is a microparticle enzyme immunoassay (MEIA) and is the second most widely used toxoplasma test in UK laboratories according to UK NEQAS. The assay was calibrated to standards used to produce a quantitative value of toxoplasma- specific IgG for each sample tested. Calibrators were referenced to the World Health Organization international standard for anti-toxoplasma antibody. A negative value was defined as <2 iu/mL, an equivocal result as ≥ 2 and <3 iu/mL, and a positive result as ≥ 3 iu/mL.

Eiken latex
The Toxoreagent 'Eiken' (Eiken Chemical Company, Japan) is a latex particle agglutination assay, and the most widely used toxoplasma test in UK laboratories according to UK NEQAS. The end-point titre was considered to be the serum dilution that produced 50% agglutination of the latex particles in doubling dilutions of serum. A negative value was defined as a titre <1 in 16, a weak positive as a titre of 1 in 16 and a positive as a titre ≥ 1 in 32. The test detected all immunoglobulin classes.

IgM assays
Sera from HIV/AIDS patients with dye-test results ≥ 2 iu/mL were tested for IgM by the Toxo ISAGA (bioMerieux, Marcy Etoile, France). All other sera were tested by an in-house IgM EIA,[15] and positive results confirmed by Toxonostika IgM II EIA (bioMerieux).

Statistics
Statistical analysis was performed using the χ^2 test.

Results

Group 1
IgG EIA gave no false-positive results in group 1 (pregnant) patients (Table 1) but two samples (dye test: 2 and 4 iu/mL) were negative. However, these results were below the threshold for this assay. Two sera gave false-positive results with AxSYM Toxo-G, and the two samples negative by IgG EIA were also negative by AxSYM Toxo-G

No false-positive results were produced by the Eiken latex test; however, five negative results were found in sera with dye-test results ranging from 2-125 iu/mL. Two of these had low levels of toxoplasma-specific antibody (2 and 4 iu/mL), two had levels of 8 and 15 iu/mL and one had a level of 125 iu/mL.

Further investigation of the last sample (125 iu/mL) revealed that it was IgM-positive by screening and confirmatory IgM EIA. This indicated that it was from a patient with a current infection. The Eiken latex test was repeated on this sample and the result remained negative.

Results of the IgG EIA, AxSYM Toxo-G and Eiken latex were identical for sera with dye-test results of 250-4000 iu/mL inclusive (Table 1).

Group 2
IgG EIA gave three false-positive results in group 2 (immunocompromised) patients (Table 2). Of the 14 sera negative by IgG EIA, four had a dye-test result of 2 iu/mL, seven were 4 iu/mL, two were 8 iu/mL and one was 15 iu/mL. AxSYM Toxo-G gave one false-positive result (Table 2). Of the 18 false-negative AxSYM Toxo-G results, four had dye test results of 2 iu/mL, seven were 4 iu/mL, five were 8 iu/mL and two were 15 iu/mL.

The Eiken latex test gave no false-positive results but 19 false-negative results: four had a dye-test result of 2 iu/mL, six were 4 iu/mL, four were 8 iu/mL, four were 15 iu/mL and one was 30 iu/mL.

Results of the IgG EIA and Eiken latex test were either below or within one dilution of the threshold. Identical results were achieved in all tests of sera with a raised dye-test result (≥ 250 iu/mL).

Fewer group 1 (pregnant, n=17) than group 2 (immunocompromised, n=33) sera were found in the dye-test range 2-15 iu/mL. Nevertheless, using all three tests, significantly more false-negative results were found in group 2 than in group 1 (P<0.05).

Discussion

Growing awareness among women of the consequences of toxoplasma infection during pregnancy, and an increase in the number of immunocompromised patients has significantly increased the immunity screening workload in routine laboratories.[16] Previously, we compared the IgG EIA, AxSYM Toxo-G and Eiken latex tests with the dye test in sera from all patient groups. Overall, they performed well but both false-positive and false-negative results were recorded.[10]

Table 1. Comparison of dye test, in-house ELISA-G, Abbott AxSYM Toxo-G and Eiken Toxoreagent results in sera from pregnant women

Dye-test result (iu/mL)	*n*	IgG EIA			AxSYM Toxo-G			Eiken latex	
		-	±	+	-	±	+	-	+
<2	50	50	0	0	48	1	1	50	0
<8 (2-4)	2	2	0	0	2	0	0	2	1
8-125	92	0	1	91	0	4	88	3	89
250-4000	6	0	0	6	0	0	6	0	6

Table 2. Comparison of dye test, in-house ELISA-G, Abbott AxSYM Toxo-G and Eiken Toxoreagent results from sera of immunocompromised patients

Dye-test result (iu/mL)	*n*	IgG EIA			AxSYM Toxo-G			Eiken latex	
		-	±	+	-	±	+	-	+
<2	50	47	1	2	49	1	0	50	0
2-4	11	11	0	0	11	0	0	10	1
8-125	90	3	4	83	7	2	81	9	81
250-4000	2	0	0	2	0	0	2	0	2

In the current study, which looked specifically at toxoplasma immunity, false-positive (IgG EIA, AxSYM Toxo-G) and false-negative results were found with all three screening tests (Tables 1 and 2). This has implications for their suitability for the determination of immunity.

The Sabin-Feldman dye test is the gold standard for serological testing of *T. gondii* infection. A multicentre European study, which included input from the UK Toxoplasma Reference Laboratory, showed that the dye test plays an important role as a reference test and in validating commercial toxoplasma assays.[9] Although there has been disagreement about the level of antibody that indicates toxoplasma infection, a consensus in Europe[9] and in America[17] suggests that >4 iu/mL or a serum dilution of 1 in 16 is appropriate.

Users of screening tests should be aware of the thresholds used in each commercial assay. These are usually established against an international reference serum. The IgG EIA has a threshold of 8 iu/mL, while that of AxSYM Toxo-G is 2-3 iu/mL. The Eiken latex threshold is expressed as a titre of 1 in 16. However, using dilutions of serum with well-characterised dye-test results, we have determined that the Eiken latex threshold titre of 1 in 16 is equivalent to approximately 15 iu/mL in the dye test.

Toxoplasma infection during pregnancy may result in fetal death, severe damage to the fetus and long-term sequelae. However, unless a woman is severely immunocompromised, transmission of toxoplasma to the fetus is almost always limited to those infected during pregnancy.[18] Therefore, an accurate diagnosis of immunity prior to conception can be used to reassure against congenital infection.[4]

Using AxSYM Toxo-G, two false-positives would have resulted in false reassurance of immunity. In order to avoid this, it is recommended that a higher threshold – equivalent to 15 iu/mL IgG in the dye test – be used to establish immunity.[4] This is the approximate threshold of the Eiken latex test, which gave no false-positive results in group 1 (Table 1).

The Eiken latex test did give three false-negative results, two of which had dye test results around the threshold (8-15 iu/mL). However, the third had a dye test result of 125 iu/mL and was also positive for toxoplasma-specific IgM; thus, it is of particular concern that it was not detected by the Eiken latex test, which measures total antibody.

An immunocompromised state may result following transplantation, malignancy, immunosuppressive therapy or infection (e.g., HIV/AIDS).[19] Following solid organ transplantation (i.e., kidney, heart, heart-lung and/or liver), primary infection results from transplantation from a seropositive donor to a seronegative recipient.[20] Reactivated infection can also occur but is less common.[6] Conversely, toxoplasmosis in bone-marrow transplant recipients is usually a result of reactivated infection, which is serious and can be fatal.[3,20,21]

In HIV/AIDS patients, the most frequent manifestation of toxoplasmosis is encephalitis, also usually as a result of reactivated infection.[22] It is estimated that 30-50% of toxoplasma-specific antibody-positive HIV/AIDS patients will develop toxoplasma encephalitis.[22,23] Consequently, determination of toxoplasma immunity is a management requirement and should be performed when an immunocompromised state is diagnosed.[6]

In immunocompromised patients, any amount of specific antibody indicates toxoplasma infection; thus dye test results ≥ 2 iu/mL are considered positive. In the present study, false-positive reactions occurred with IgG EIA and AxSYM Toxo-G tests, and false-negatives occurred with all tests (Table 2). More than half of the false-negatives had dye test results lower that the test threshold. The remainder had dye test results ≥ 8 iu/mL and therefore should have been detected (except perhaps with Eiken latex)

As 14/18 of the AxSYM false-negatives had dye test results ≥ 4 iu/mL, and the AxSYM Toxo-G threshold is 2-3 iu/mL, this might indicate that it is less sensitive for use with sera from immunocompromised patients. Furthermore, as these sera were negative for specific IgM, the contribution of toxoplasma-specific IgM to dye-test results was not a factor. Although it is possible that the low-level dye-test results were non-specific, we do not believe this to be the case as previous doubts about the specificity of the dye test have proved unfounded.[9]

Compared with the dye test, each of the other tests produced more false-negative results in group 2 (immunocompromised) than in group 1 (pregnancy) ($P<0.05$). In particular, these were in sera with dye-test results in the 2-4 iu/mL range, highlighting the difficulties associated with diagnosis of immunity in this patient group. Thus, it is essential that sera from immunocompromised patients – especially the bone-marrow transplantation and HIV/AIDS groups – be tested with the dye test, which is both sensitive and specific.[20]

The aim of immunity screening in pregnancy is different. Here, it is important to be able to provide reassurance of immunity but not falsely reassure.[4] As a positive result on a single serum sample using any test does not differentiate between immunity and current infection, testing for toxoplasma-specific IgM is indicated.[1,4] Although a negative screening test result suggest that the patient is susceptible to

toxoplasma infection, further tests may be required if they are symptomatic.[20]

In immunocompromised patients, the aim of immunity screening is to identify those at risk of reactivated infection. A positive result indicates past infection and further testing is only indicated in symptomatic patients. As false-negative results are obtained with all screening tests, and this is an elective test for future management, referral for dye test should be available.[20]

In conclusion, all tests used in this study performed well, as one might expect of well-established and widely used technology. The study highlighted the different thresholds of each test and the difficulty of equating positive and negative results with immune or susceptible status. If used within a given strategy for immunity testing, all should perform well. However, we cannot explain why the Eiken latex test was negative in a pregnant woman who was IgM-positive and had a dye test result of 125 iu/mL. Nonetheless, we have demonstrated that effective strategies for detecting toxoplasma immunity are available to routine laboratories in the UK, using current testing schemes. □

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