Do IgA, IgE, and IgG avidity tests have any value in the diagnosis of toxoplasma infection in pregnancy?

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Abstract
Aim — To determine the value of tests for specific IgA, IgE, and IgG avidity in diagnosing Toxoplasma gondii infection during pregnancy.

Methods — In a retrospective study, current serological tests (dye test and three IgM assays with different sensitivities) were compared with immunosorbent agglutination assays (ISAGA) for specific IgA and IgE and an IgG avidity enzyme linked immunosorbent assay (ELISA). Patient group 1 comprised six women with definite or probable infection during pregnancy determined by congenital toxoplasmosis or laboratory results. Group 2 comprised seven women infected during or before 11 pregnancies (two consecutive pregnancies in two patients and three in a third).

Results — One patient in group 1 seroconverted during pregnancy. IgA ISAGA and avidity confirmed acute infection when confirmatory IgM ELISA remained negative. In five of six patients from group 1, IgA and IgE ISAGA and avidity confirmed acute infection. In group 2, the dye test titre was raised in seven of 11 pregnancies (six of seven patients). Specific IgM and IgA were positive during all 11 pregnancies. IgE ISAGA was positive in only four of 11 pregnancies (three of seven patients), but negative results in the remainder may exclude acute infection. High avidity antibodies indicative of past infection were found in four of 11 pregnancies (two of seven patients).

Conclusions — Each test improved diagnosis or timing of infection but no single test was ideal. The IgA ISAGA was sensitive and detected seroconversion. Positive IgE ISAGA and low avidity both confirmed infection, whereas negative IgE may exclude acute infection. High avidity diagnosed past infection but persistence of low avidity reduced its value to differentiate acute and past infection. Further studies with larger patient groups are needed to determine the optimum diagnostic strategy. These techniques are valuable in complementing existing tests.

Keywords: immunoglobulin avidity; toxoplasmosis; pregnancy

When a mother acquires primary Toxoplasma gondii infection during pregnancy, congenital infection may result, with severe consequences for the fetus. However, as the risk of fetal infection is very small when maternal infection occurs before conception, it is important to make a precise estimation of the timing of maternal infection. Serological diagnosis of toxoplasma infection is usually based on fourfold increases in IgG titre and demonstration of specific IgM. As rising titres are seldom demonstrated outside an antenatal screening programme, detection of specific IgM is often used to indicate acute infection. However, detection of IgM may be protracted, and therefore interpretation of results is dependent on the sensitivity of the IgM technique, and with the use of current tests it may not be possible to determine the onset of maternal infection accurately.

In order to overcome these difficulties several new techniques have been developed. Immunoglobulin A (IgA) is produced as part of the acute phase response, and has been found to be of value in the investigation of cytomegalovirus infection. Toxoplasma specific IgA has been measured both by enzyme linked immunosorbent assay (ELISA) and immunosorbent agglutination assay (ISAGA), and in the sera of patients with acquired primary infection. Following infection it is claimed that specific IgA production parallels that of specific IgM, or lags slightly behind IgM. Toxoplasma specific IgE is also detected early after infection. Results suggest that the duration of detectable specific IgE may be shorter than IgM or IgA. While measurement of specific IgA and IgE may prove useful in improving the assessment of timing of infection, there is wide patient to patient variation in their detection. Measurement of IgG avidity represents a different approach to antibody studies, assessing the interaction between antibody and antigen. In early infection low avidity antibodies predominate, whereas later high avidity antibodies are more common. Measurement of IgG avidity has been applied to several viral infections, and to toxoplasma infection, in which it has been used to time maternal infection during pregnancy.

In this study, to determine whether newer techniques have any value in the diagnosis of toxoplasma infection during pregnancy, IgA and IgE ISAGAs and an IgG avidity assay based on an in-house IgG ELISA were used. The results were compared with those obtained using current tests (dye test, two different IgM ELISAs with different sensitivity, and an IgM ISAGA).

Methods
In a retrospective study, toxoplasma antibodies were measured in 43 sera from 17 pregnancies in 13 pregnant women. Sera had been tested using
current tests: in-house IgG\(^2\) and IgM\(^3\) ELISAs, and a micromodification of the dye test.\(^2\) Toxo-
plasma specific IgM positive results were con-

confirmed using a commercial assay, Toxonostika
ELISA-IgM (Organon Technika, Cambridge,
UK). Those that were negative in the screening
test or not confirmed were also tested using the
more sensitive Toxo-ISAGA IgM (BioMerieux,
Marcy l’Etoile, France). Patients were divided
into two groups, determined by the presence of
congenital toxoplasmosis and the results of cur-
rent serological tests.

Group 1 consisted of six patients with
definite or probable infection during preg-

nancy. The outcome of pregnancy was con-
genital toxoplasmosis in three cases. In two of
the three cases there were rising dye test titres,
raised titres (> 250 IU/ml) in the third, and
IgM was confirmed positive in all. Two other cases
were included on the basis of raised dye
test titres and confirmed IgM. One pregnancy
had a normal outcome, but was included
because the mother had cervical lymphaden-

opathy, a raised dye test titre, and specific IgM
detectable in the latter stages of pregnancy.
This combination of clinical and laboratory
data indicates infection during pregnancy. The
second pregnancy ended in fetal death which
may have been caused by toxoplasma, but no
tissues were available for confirmation. The
remaining patient seroconverted during preg-
nancy, but again the outcome was normal. In
this patient IgM was not confirmed by Toxon-
ostika ELISA IgM, but was positive by the
more sensitive Toxo-ISAGA.

Group 2 comprised seven women with past
or possible acute infection during 11 pregnan-
cies (two consecutive pregnancies in two
women, and three in a third). These were fur-
ther subdivided into groups 2a and 2b.

Group 2a comprised women with only a sin-
gle pregnancy or the first of two or three
consecutive pregnancies. Raised dye test titres
were measured in all four single pregnancies
and during the first pregnancy in two other
women. Toxonostika ELISA IgM was also
positive in two of four single pregnancies and in
all three first pregnancies, but toxoplasma spe-
cific IgM was detected by one or more of the
methods used in all group 2a pregnancies.
Specific IgM was detected early in all four sin-
gle pregnancies and—in the absence of symp-
toms or rising dye test titres—it was therefore
not possible to determine whether infection
occurred before or after conception.

Group 2b included the second pregnancy in
three women and the third in one of these.
Although the dye test titre was normal in the first
specimens taken during each of the consecutive
pregnancies, the Toxonostika ELISA-M was
positive. Thus group 2 shows that similar
laboratory results may be obtained in both past
infection and possible acute infection.

All sera were tested using in-house immuno-
sorbent agglutination assays for specific IgA\(^4\)
and IgE.\(^2\) Briefly, round bottomed microtitre
plates (Immulon 1, Dynex Technologies, Bill-
inghurst, UK) were coated with capture anti-
body (Dako, Copenhagen, Denmark), anti-
human IgA (α chain specific; 2 µg/ml) or anti-
human IgE (ε chain specific; 2.4 µg/ml) for the
IgA and IgE ISAGA, respectively. Patients’ sera
(100 µl) diluted 1/100 in phosphate buffered
saline (PBSpH 7.2, were added to triplicate
wells. After incubation for two hours at 37°C
wells were washed with PBS plus 0.05% Tween
20 (pH 7.2) (PBST) and then with PBS alone.
Formaldehyde fixed tachyzoites (1.25 × 10\(^7\)/ml)
were added to each of the triplicate (100,
150, and 200 µl) wells and incubated overnight
at 37°C. Results were scored 0–4 in each well
relative to negative (no agglutination) and
positive (agglutination) controls. The scores of
triplicate wells were accumulated to produce
the ISAGA matrix (maximum 12). A matrix of
< 2 was negative; 3–4 equivocal; 5–9 positive,
and ≥ 10 strong positive.

IgG avidity was measured using the in-house
IgG ELISA under conditions similar to a pub-
lished method.\(^9\) Flat bottomed microtitre
plates (Virion B, Shield Diagnostics, Dundee,
UK) were coated overnight with toxoplasma
antigen optimally diluted in a 10% solution of
ELISA coating buffer (Don Whitley Scientific,
Shipley, W Yorkshire, UK). After aspirating the
plates, they were dried at 37°C for four hours
and stored at −20°C until use. Patients’ sera
were diluted with PBST in fourfold steps to
produce five dilutions: 1/50, 1/200, 1/800,
1/3200, 1/12 800. The four highest dilutions
(1/200–1/12 800) were added (100 µl per well)
to rows A–D and the four lowest to rows E–H.
After incubation at room temperature for one
hour, the plates were washed as follows: rows
A–D, 3 × 5 minutes with PBST; rows E–H 3 ×
5 minutes with urea wash solution (6 M urea
in PBST). All wells were then washed a further
three times with PBST. Conjugate—

anthi-human IgG–alkaline phosphatase (Sigma,
Poole, Dorset, UK)—was added (100 µl per
well) at optimal dilution. After incubation for
one hour at room temperature, plates were
washed with PBST and substrate added
(Sigma phosphatase substrate) diluted in
ELISA substrate buffer (Don Whitley Scien-
tific). After 30 minutes, the optical density was
measured at 405 nm using a Dynatech
MR-5000 plate reader (Dynex Technologies).
Dilution curves were plotted for the urea
washed and PBST washed wells and avidity
calculated as the ratio of titres measured
(urea:PBST) at the threshold of 0.2 absorb-
ance units. Low, borderline, and high avidity
was < 15%, 15–30%, and > 30% respectively.

Results

Group 1 comprised six women with definite or
probable evidence of T gondii infection during
pregnancy. In one patient, who seroconverted
during pregnancy, toxoplasma specific IgM
was detected only by the BAM-ELISA and
Toxo-ISAGA. Positive IgA ISAGA results and
low avidity IgG were also measured, but IgE
ISAGA failed to diagnose acute infection in
this patient. In the other five patients from
group 1, positive results in the IgA ISAGA, IgE
ISAGA, and low avidity IgG (table 1) con-

firmed a diagnosis of acute infection.

In group 2a and 2b, the IgA ISAGA was
positive during all pregnancies (table 1). The

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IgE ISAGA was positive in only four of 11 pregnancies from three of seven patients; three of these were during the first or a single pregnancy (group 2a) and the fourth from a consecutive pregnancy (group 2b). Negative results could possibly have been used to exclude acute infection in four of seven and three of four group 2a and 2b pregnancies, respectively. Avidity measurement was less useful, as high avidity antibodies were measured in only three of seven group 2a patients and in one of four group 2b pregnancies. Furthermore, IgG avidity did not mature from low to high avidity in two patients between their first pregnancy in group 2a and their second or third in group 2b. In contrast to group 1 patients, simultaneous positive results for IgM, IgA, IgE, and IgG antibodies of low avidity were measured in only one group 2a patient during her first pregnancy. These results suggest that infection occurred during this pregnancy. Therefore if all of the results from all tests were used, a correct timing of the infection could be achieved.

Discussion

Protracted detection of raised levels of specific IgG makes timing infection in pregnancy difficult. Diagnosis of infection during pregnancy is therefore often based on comparison of results from two or three IgM tests. In this laboratory, the less sensitive Toxonostika ELISA IgM assay is most useful in the confirmation of onset of infection within a period ≤ 1–2 trimesters. Patients were divided into two groups according to the likelihood of infection. Thus it was possible to evaluate the three relatively new tests on their ability to improve on the existing IgM based strategy.

It has been claimed that specific IgA is detected early after infection but also disappears rapidly. In our study specific IgA was detected in the first post-seroconversion sample in one patient, confirming its rapid appearance after infection. However, the IgA ISAGA was also positive in all other pregnancies from patients in both groups. Persistence of raised levels of specific IgA has been reported previously. Our results of patients followed through consecutive pregnancies (group 2) confirm that, like specific IgM, specific IgA positive results may persist for prolonged periods. Therefore the IgA ISAGA was not able to differentiate between acute and past infection. This may reflect the enhanced sensitivity of ISAGA over ELISA methods; use of a less sensitive IgA ELISA may be more appropriate to improve timing of infection and should be investigated.

In other studies, detection of specific IgE was associated with toxoplasma infection during pregnancy in 33 of 37 women. In our study, toxoplasma specific IgE was detected in five of six patients from group 1, but not in the patient who seroconverted, confirming the results of the Toxonostika ELISA IgM. In our study, there was no evidence of congenital toxoplasmosis in this patient and therefore negative IgE ISAGA results may indicate a favourable outcome of infection during pregnancy. Further testing of more patients is necessary to confirm the prognostic value of a negative IgE ISAGA result. Specific IgE was not detected in eight of 11 pregnancies from five of seven patients from group 2. It has been suggested that specific IgE remains detectable for less than four months after infection. Therefore, negative results may be useful to exclude acute infection. However, because IgE assays may lack sensitivity, results of the IgE ISAGA should be interpreted in conjunction with results of other tests.

Measurement of IgG avidity has previously been applied to determination of duration of infection and to identifying pregnancies at risk of congenital toxoplasmosis. One study included 11 women who seroconverted during pregnancy. All had low avidity antibodies, which in 10 of 11 women matured to high avidity within six months. The remaining patient still produced low avidity antibodies after 11 months. This is different from the second study in which the mean avidity result was low both in sera collected less than 20 weeks and between 20 to 52 weeks after infection. The discrepancy between these studies is
probably because in the first, results were correlated with the time elapsed since the last seronegative sample compared with the estimated time since infection. Nevertheless, in both studies high avidity was measured in past infection. In our study, all patients from group 1, including one who seroconverted, had low avidity antibodies. Low avidity antibodies were also measured in five of 11 pregnancies from three of seven patients in group 2. As three of these were from group 2b, and infection was therefore before pregnancy (second pregnancies of two patients and the third pregnancy of another), the value of low avidity antibody measurement to confirm acute infection may be less than previously suggested. Further more persistence of low avidity antibody, like persistent IgM, may complicate diagnosis.

These findings may indicate variation in immune response. Persistent borderline avidity antibody has also been reported in non-pregnant patients with lymphadenopathy present for over six months. High avidity antibodies in four pregnancies, three of which were confirmed as IgM positive, were used to diagnose past infection. Thus the IgG avidity ELISA has proved less useful in our studies to diagnose past infection. Thus the IgG avidity ELISA has proved less useful in our studies to diagnose past infection. Therefore the value of specific IgA, IgE, or IgG avidity tests is to augment rather than replace the current tests.

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