REVIEW

Toxoplasma gondii infection in pregnancy: opportunities and pitfalls of serological diagnosis

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ABSTRACT

Because of its life cycle, the recovery of *Toxoplasma gondii* from biological samples is often impracticable. Consequently, a serological diagnosis represents the first and the most widely used approach to defining the stage of infection. The detection of IgG, IgM, IgA, IgE and IgG avidity by different methods offers this opportunity. However, the results may be affected by difficulties in interpretation, as the same antibody pattern may have a different valency, contingent upon subjects and clinical settings, e.g., pregnant women vs. neonates, and treated vs. untreated patients. This review describes the various factors that should be taken into account when performing serological tests for *T. gondii*, as well as the pitfalls that may be encountered during the interpretative process.

Keywords   Diagnosis, interpretation, pregnancy, review, serological tests, *Toxoplasma gondii*

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INTRODUCTION

Approximately one-third of the world's population is infected by *Toxoplasma gondii*, an obligate intracellular protozoan belonging to the phylum Apicomplexa, subclass Coccidia. The infection can be acquired by eating raw meat containing tissue cysts, or food and water contaminated by oocysts. The clinical manifestation is usually benign in immunocompetent hosts, but can be life-threatening in an immunocompromised patient. Diagnosis of toxoplasmosis can be achieved by demonstrating the parasite in biological samples or by detection of specific antibodies. Molecular diagnosis by PCR has reduced greatly the time required to determine the presence of parasites when compared with the time required following mouse or tissue culture inoculation. PCR amplification of the 35-fold repetitive B1 gene has been used successfully to diagnose congenital infection. Real-time PCR and other gene targets will probably be used in the future, but serological tests to determine specific anti-bodies are currently the first-line method of diagnosis for current, recent or past infection. Since the medical history may not be informative, all test results must be considered to represent parts of a puzzle, where each piece has its own special significance. Some pieces often seem out of place, so that the final interpretation can be achieved only when the puzzle is considered as a whole.

The diagnosis of primary infection during pregnancy and the diagnosis of congenital infection are the most challenging situations. First, the pitfalls hidden in the serological responses can make the interpretation problematic. Simultaneous testing for specific IgG and IgM in serial serum samples collected at an interval of 3 weeks is the initial approach in screening for *T. gondii* infection. Successive tests, and the conclusive diagnosis, will depend on these initial results. Second, the immunological markers can vary depending on the trimester of infection, and maternal and neonatal therapeutic treatment during pregnancy can block or retard the immune response of the neonate. An approved guideline for the interpretation of serological tests was published by CLSI (formerly NCCLS) in 2004 [1], and updates of recent developments in the

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diagnosis and management of toxoplasmosis in different clinical settings have also been published [2,3]. The present review considers the occurrence and significance of different serological patterns in pregnant women and in congenitally infected neonates.

IgG- AND IgM-NEGATIVE

Pregnant women

An individual is considered susceptible to infection when specific antibodies are not detected in serum samples. Therefore, women of childbearing age are at risk for acquiring primary infection, and pregnant women require regular checks for seroconversion. The frequency of these checks varies, depending on the screening programmes adopted in different countries. Maternal monitoring usually ceases during the third trimester or just before delivery. However, infection could be acquired at the very end of pregnancy, with the mother still seronegative at delivery. The rate of transmission from mother to foetus is >70% during this period [4], and the neonate will be infected without any clinical symptoms at birth. In the absence of prompt and appropriate therapeutic treatment, the neonate can develop late sequelae [5–8]. Armstrong *et al.* [9] reported a case of severe neonatal toxoplasmosis, demonstrated by positive IgG, IgM and placental tissue PCR results, in a neonate whose mother remained IgG- and IgM-negative in repeated serum samples. This finding was intriguing, given that there was no evidence of maternal illness as a possible cause of this negative serology [9]. In our own experience [10], a woman aged 32 years, who was IgG- and IgM-negative until the eighth month of pregnancy, was found to be IgM-positive by ELISA at 10 days before delivery. Three days later, IgM was positive by an immunosorbent agglutination assay (ISAGA), with a positive IgA result by ISAGA at delivery. Ten days after delivery, IgG appeared in serum (50 IU/mL). Congenital toxoplasmosis was diagnosed in the neonate (IgM- and IgA-positive by ISAGA at birth, and an IgG titre of 40 IU/mL after 8 days). The infant, who was completely asymptomatic, received appropriate treatment until aged 1 year. At present, our protocol recommends a further serological check after delivery for all seronegative pregnant women.

Congenital infection

A negative serological pattern can be a transitory phenomenon in congenital toxoplasmosis as a consequence of maternal and neonatal treatment, particularly when the maternal infection occurred during the first two trimesters of pregnancy [11,12]. In such cases, the initial diagnosis of congenital toxoplasmosis should not be questioned, and treatment and routine monitoring should be continued. Frequently, the transitory negative period is followed by a serological rebound, often after the cessation of therapy. The consequent rise in antibody titres has not been associated with increased risk for the child, and additional courses of treatment and enhanced ophthalmological surveillance do not seem to be warranted [13].

IgG-NEGATIVE AND IgM-POSITIVE

Pregnant women

IgM antibodies are characteristic markers of acute infection. They appear at the onset of infection and persist for variable periods, but their time-dependent detection is determined by the sensitivity of the test. Numerous tests are now available commercially. In immunocompetent subjects, IgG production follows IgM production at different times, according to the diagnostic methods used. When seroconversion occurs, the diagnosis of primary infection is confirmed.

Pregnant women are sometimes seropositive only for IgM, and IgG may not be detected during the serological follow-up. These natural IgM antibodies are believed to react with toxoplasma antigens in the absence of infection. Natural antibodies are predominantly of the IgM class [14], and only occasionally of the IgG class [15], and vary greatly following electrophoretic examination [16]. They are found rarely in neonates and infants aged <6 months. In pregnant women they can be present for the whole gestation [17], or for only a limited period [18]. In such cases, because of the slow increase in IgG titres observed with conventional ELISAs, it is advisable to employ additional tests with the use of the whole parasite as an antigen, e.g., dye tests, indirect immunofluorescence assays or agglutination tests [19].

A sudden IgM seropositivity during the course of pregnancy should alert the physician to start

therapeutic treatment before confirmation of the infection (seroconversion) in an attempt to prevent transmission to the foetus. However, therapeutic treatment, particularly with pyrimethamine and sulphadiazine, may block the production of IgG (personal unpublished results). In such a case, the serological pattern may remain unchanged.

It is worth mentioning that a peak of IgA antibody production occurs following primary infection, shortly after the detection of IgM. Since IgA is not considered to be naturally occurring, the concurrent presence of IgM and IgA denotes primary infection.

Congenital infection

As discussed above, detection of IgM, but not IgA, can occur at birth in the case of very late maternal infection during pregnancy. IgA and IgG are usually detected in subsequent serum samples. In the case of IgM and/or IgA detection in the neonate, the test should be repeated *c.*10 days after birth in order to exclude contaminating maternal antibodies [20].

IgG-POSITIVE AND IgM-NEGATIVE

Pregnant women

Detection of IgG without IgM defines the classical serological pattern of past infection. An immunocompetent pregnant woman with serologically demonstrated chronic (or latent) infection at the beginning of pregnancy is not considered to be at risk for giving birth to a neonate with congenital infection [5]. In the third trimester, a negative IgM titre probably reflects past infection, but does not exclude an acute infection early in pregnancy. This can occur in some patients exhibiting a rapid decline in IgM titre. In such cases it is advisable to use other tests, e.g., IgA and IgG avidity, as described below. A few cases of infected offspring born from previously immune mothers have been described [21–26], but these are regarded as exceptions. Interestingly, in some cases, IgA antibodies were also present. Since these immunoglobulins are produced during the digestive phase of acute infection, it has been suggested that reinfection was probably linked to accidental ingestion of oocysts from a different or partic-

ularly virulent strain following maternal contact with kittens [21–23].

As mentioned above, naturally occurring IgG can be present, albeit rarely. Our own experience has included five probable cases of natural IgG. IgG was detected in three of these during the first trimester, in one during the second, and in one during the third. Several serial serum samples were collected for the detection of IgM and IgA (ISAGA test) and for the IgG avidity test. IgM and IgA antibodies were always negative, and the IgG avidity value was low in two cases, intermediate in one case and high in the remaining two cases. Three women were treated with spiramycin. Following birth, the neonates showed the same western blot profile and IgG levels as their respective mothers. All children were seronegative at the last check (aged 1 year) without any treatment (personal unpublished results).

Congenital infection

At birth, the detection of IgG without any other positive serological marker is indicative of either: (i) congenital infection after maternal infection during the first or second trimester of pregnancy; (ii) infection of neonates whose mothers have been treated during pregnancy; or (iii) non-infection. In the case of early maternal acquisition, the neonate could be at the end of the sub-acute stage of infection, at which time the production of IgM and IgA has already occurred. In the case of maternal infection during the second trimester, IgA can be detected in the absence of IgM because of the longer persistence of IgA compared with IgM in the neonate (contrary to what is observed in adults). However, it is advisable to test serum samples from the neonate for IgA, given the higher sensitivity of this class of immunoglobulin as a marker for congenital infection [27–30]. In the case of IgA detection in the neonate, the test should be repeated *c.*10 days after birth in order to exclude contaminating maternal antibodies [20].

In the case of suspected maternal infection and consequent therapeutic treatment, the fetal immune response can be blocked or retarded, with a consequent delay in the production of predictive serological markers. Persistence or increase of IgG during the first year of life remains the most reliable way of diagnosing congenital infection, but this procedure is

time-consuming. If IgG antibodies only are detected, it is crucial to distinguish whether they are of maternal origin and have been transmitted passively, or whether they represent de-novo synthesis by the congenitally infected foetus. In the latter case, therapeutic treatment should be initiated as soon as possible following birth to prevent late sequelae.

The immunoblotting method has been used successfully to compare antibody reactivity in mother and child for diagnosis of congenital toxoplasmosis [31]. This method combines electrophoresis of toxoplasma antigens under denaturing conditions with a specific antibody test. The study of *T. gondii* antigens by western blotting has allowed the identification of immunodominant antigens, as well as the identification of stage-specific antigens for use in serological assays [32–34]. The strain-specific antigenic differences detected help to explain the different electrophoretic patterns observed among different subjects [35]. The definition of positivity is based on the presence of at least one IgG-reactive antigen in the sample from the child, and its absence in the corresponding sample from the mother. A moderate variability has been observed among studies. However, the method has not been standardised, and antigen preparation, gel conditions, dilutions of patient sera, and sources of secondary antibody are not yet defined. Nevertheless, some applications have been semi-automated by a computer program, which makes reading the intensity of the bands easier and gives greater reproducibility. The best results in terms of sensitivity have been obtained when the method has been used for detection of IgM and IgA, as well as IgG, in combination with standard methods.

Chumpitazi *et al.* [36] evaluated the immunoblot method in comparison with other conventional tests, including mouse inoculation and in-vitro culture of the parasite, for pre-natal, at-birth and after-birth diagnosis of congenital toxoplasmosis. It was concluded that for at-birth diagnosis, immunoblot analysis of IgG, IgM and IgA reached a sensitivity of 92.6%, with a specificity of 89.1%. Analysis of the electrophoretic bands showed antibodies directed against antigens with high and low molecular sizes (18 000–185 000 kDa), although most were in the 18 000–135 000 kDa range [36]. Gross *et al.* [37] focused their attention on IgG detection, reporting a

sensitivity of 82.4%, a specificity of 93.0%, a positive predictive value of 73.7%, and a negative predictive value of 95.7%. The immunodominant *Toxoplasma* antigen SAG1/P30 was not recognised preferentially in child serum samples. In nearly all cases of congenital infection, the child developed IgG against at least two antigens, and all of the IgG-reactive antigens were consistently >30 kDa. It was suggested that two samples should be collected, at birth and 4–6 weeks later, to confirm the diagnosis [37]. In a collaborative study for post-natal diagnosis of congenital infection, Pinon *et al.* [38] compared immunoblotting and enzyme-linked immunofiltration assay (ELIFA) with standard methods for IgG, IgM and IgA detection during the first year of life. In the case of treatment *in utero*, and after treatment of the child for 1 year with pyrimethamine and sulphonamides, congenital infection was not detected. In these individuals, immunological markers were detected only when treatment was withdrawn, owing to a rebound of anti-*Toxoplasma* antibodies. Combining commercial western blot assays with conventional serological analyses at birth and within the first 3 months, 94% of congenital infections were detected [39]. A two-dimensional immunoblotting method to improve the differentiation of mother and child IgG profiles has been proposed [40].

IgG- AND IgM-POSITIVE

Pregnant women

One of the most challenging situations occurs when IgG and IgM are positive and the serological status before pregnancy is unknown. The collection of a second serum sample after 3 weeks is recommended, but meaningful differences in IgG and IgM titres are observed rarely. IgG titres can show great variability among individuals, and even a high titre, e.g., IgG ≥ 300 IU/mL in a dye test, cannot be used as a diagnostic criterion for recent primary infection because of its low sensitivity [41]. A positive IgM result can be interpreted as: (i) a true-positive result indicating recently acquired infection; (ii) a true-positive result indicating past infection; or (iii) a false-positive result [20]. Many commercial products for IgM detection have been studied extensively and compared for sensitivity and specificity [42–45], but since there is no accepted reference

standard, parameters of test accuracy are poorly defined. The specificity and positive predictive value of new assays are related directly to the prevalence of negative and positive samples, respectively, as detected by the reference test. Therefore, the selection of sera used for evaluation influences the accuracy of the test markedly [46]. Several groups of investigators are working to find specific antigens and/or to produce recombinant antigens to improve the performance of IgM assays [47–53].

IgM can be detected for a long period following the acute infection, and therefore a true-positive result cannot discriminate between acute, recent and past infection. Timing the onset of the infection is crucial in pregnant women, especially because post-conceptional acquisition represents a risk for the foetus. For this purpose, other diagnostic tools can be utilised, such as IgA and IgG avidity detection. IgA antibodies appear shortly after IgM and persist for some time (usually 6–7 months) after the onset of infection. IgA-ISAGA is the more sensitive assay [54–57]. However, IgA has been detected for >1 year in some subjects, but is never detected in a small percentage of acute infections [58]. Therefore, a negative IgA result does not exclude an acute infection, and a positive IgA result does not necessarily indicate an acute infection.

IgE antibody detection has been proposed as a way of defining the stage of infection [59–62], because IgE is found only in serum samples from patients with acute infection, and the duration of IgE seropositivity is shorter than that of IgM and IgA. Enzyme immunoassays (EIAs) and ISAGA tests have been used for IgE determination, but these assays are home-made and have been applied in only a few reference centres. However, Foudrinier *et al.* [63] showed limited (a few months) IgE seropositivity in 85.7% of asymptomatic seroconverters, and long persistence at a very high titre in 100% of seroconverters with overt toxoplasmosis. Furthermore, IgE emerged concomitantly with the increase of IgG during reactivation [63].

Since its introduction [64], many studies have been published concerning the use of the IgG avidity test to discriminate between recently acquired and past infections by employing natural [65,66] or recombinant antigens [67], as well as its adaptation to automated systems [68,69]. The functional affinity of specific IgG antibodies is initially low after primary antigenic challenge, but increases during subsequent weeks and months by antigen-driven B-cell selection. Protein-denaturing reagents, including urea, are used to dissociate the antibody-antigen complex. The avidity value is determined by using the ratios of antibody titration curves for urea-treated and untreated samples. The maturation of IgG avidity has been studied by monitoring subjects with seroconversion or with typical clinical manifestations: high-avidity results exclude *Toxoplasma* infection during the preceding 3–5 months.

The length of time required for conversion from low- to high-avidity antibodies depends on the method used. In pregnant women, a high-avidity test is highly predictive of past infection if performed in the first trimester. Low or borderline IgG-avidity antibodies are known to persist for >1 year and, for this reason, are not reliable for diagnosis of recently acquired infection. Furthermore, it has been suggested that antibiotic treatment can modify the maturation of IgG avidity [70,71], although discordant results have been reported occasionally [72].

Congenital infection

IgM detection in neonatal serum samples is indicative of congenital infection. However, the test should be repeated 10 days after birth to exclude contaminating maternal antibodies. An ISAGA is more sensitive than an EIA in defining congenital infection. This serological pattern presumes that maternal infection was acquired in the third trimester if IgA is also detected, or in the last month of pregnancy if IgA is still not present.

IgE has been found in serum samples from congenitally infected neonates, but the sensitivity of IgE detection is lower than that of IgM and IgA [73]. Emergence of specific IgE during post-natal treatment is considered to be a sign of poor adherence or inadequate dosing [63]. Nevertheless, simultaneous measurement of IgM, IgA and IgE improves the diagnostic yield. As for IgM and IgA, a positive test should be repeated *c.*10 days after birth to exclude contaminating maternal antibodies.

IgG avidity is generally not evaluated in the neonate, because it is similar to that of the mother. However, it has been observed that, in the absence of maternofetal transmission, the avidity index remains stable until the disappearance of

Table 1. Serological patterns in pregnant women infected with *Toxoplasma gondii*

Serological pattern	Interpretation	Comments	Advice	References
IgG- IgM-	Susceptibility	Risk for primary infection	Risk for maternal infection at the end of pregnancy	[7-9]
IgG- IgM+	(a) Onset of infection	Primary infection. Risk for congenital infection	Collect second serum sample 2-3 weeks apart and test in parallel to demonstrate seroconversion	[1-3,19]
	(b) Natural antibodies	No risk for congenital infection	Collect serial serum samples and test in parallel to exclude seroconversion	[14-18]
	(c) False-positive	No risk for congenital infection	Collect serial serum samples and test in parallel to exclude seroconversion	[1-3]
IgG+ IgM-	Past infection	No risk for congenital infection	Take natural IgG antibodies into account Rare congenital infections	[15] [21-26]
IgG+ IgM+	(a) Past or recently acquired infection	Risk for congenital infection	Take gestation period into account. Test for IgA, IgE and IgG avidity in a reference laboratory	[1-3]
	(b) False-positive	No risk for congenital infection	Test for IgA, IgE and IgG avidity in a reference laboratory	[1-3]

+,–, indicates the presence or absence of antibodies.

Table 2. Serological patterns in neonates infected with *Toxoplasma gondii*

Serological pattern	Interpretation	Comments	Advice	References
IgG- IgM-	(a) Susceptibility	No congenital infection		
	(b) Transient seronegativity	Congenital infection	Take maternal and/or in-utero treatment and possible serological rebound after therapy cessation into account	[11-13]
IgG- IgM+	(a) Maternal IgM	No congenital infection	Collect second serum sample 10 days after birth to confirm contaminating maternal antibodies	[20]
	(b) Neonatal IgM	Congenital infection after very late maternal infection	Collect serial serum samples to demonstrate seroconversion	[5]
IgG+ IgM-	(a) Maternal antibodies	No congenital infection	Serological follow-up for 1 year to confirm IgG negativity	[5]
	(b) Maternal and neonatal antibodies	Congenital infection after maternal infection in the first or second trimester	Test for IgA, more lasting than IgM. Differentiate maternal and neonatal IgG by WB or ELIFA	[27-30] [31-40]
IgG+ IgM+	(a) Maternal antibodies	No congenital infection	Collect second serum sample 10 days after birth to confirm contaminating maternal IgM antibodies. Test in parallel maternal and neonatal IgG by WB or ELIFA. Serological follow-up for 1 year to confirm IgG negativity. Check for stable IgG avidity index	[20] [31-40] [5] [72,74]
	(b) Maternal and neonatal antibodies	(b) Congenital infection after maternal infection in the third trimester (IgA+) or in the last month (IgA−)	Collect second serum sample 10 days after birth to exclude contaminating maternal IgM antibodies. Test in parallel maternal and neonatal IgG by WB or ELIFA. Serological follow-up for 1 year to demonstrate IgG persistence. Check for increased IgG avidity index	[20] [31-40] [5] [72,74]

+,–, indicates the presence or absence of antibodies.
WB, western blot; ELIFA, enzyme-linked immunofiltration assay.

passively transmitted maternal antibodies. In contrast, the IgG avidity index shows a significant increase in congenitally infected children. In addition, long-term therapy with pyrimethamine-sulphonamide, as opposed to treatment with spiramycin alone, was found to slow the progression of the avidity index [72]. A delayed maturation of IgG avidity in congenital toxoplasmosis has been reported by Buffolano *et al.* [74]. These authors demonstrated the feasibility of performing the test on antibodies eluted from dried blood spots (Guthrie cards), making it possible to detect at birth a maternal primary infection acquired during the second or third trimester of gestation, and to evaluate retrospectively the risk for congenital infection when suspicion of congenital infection arises during late infancy [74].

Tables 1 and 2 summarise the serological patterns discussed in the previous sections.

CONCLUSIONS

Important progress has been made in acquiring knowledge concerning the antigenic and genomic structure of *T. gondii*. Adjunctive tests are now available that will allow a better definition of the stage of infection. Pelloux *et al.* [75] show that definition is very easy in the presence of seroconversion or persistent negative serology, but the challenge is to interpret the detection of specific IgM in pregnant women. The demonstration of a significant rise in antibody titres in serial serum samples obtained at least 3 weeks apart and run in parallel is probably the standard, but precious

time can be lost in initiating therapeutic treatment to prevent transmission and/or to limit damage to the foetus. At this time, the results of new serological tests should be added to the puzzle and referred to a reference laboratory. The benefits derived from this strategy have been demonstrated by Liesenfeld *et al.* [76], who reported a decrease in unnecessary abortions for *c.*50% of women with positive IgM tests performed at peripheral laboratories, once confirmatory tests had been performed in a reference laboratory and the correct interpretation given to the patient's physician. However, serology represents only a branch of the complex immunological response of the host to *T. gondii* infection.

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