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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 antibodies 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