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Review Article

Current and Emerging Techniques for Diagnosis of Toxoplasmosis in Pregnancy: A Narrative Review

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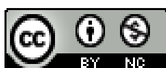
Abstract

Toxoplasma gondii is an intracellular parasite capable of crossing the placenta in pregnancy and infecting the developing fetus, leading to various congenital anomalies and even abortion. Acute *Toxoplasma* infection is responsible for almost all cases of congenital toxoplasmosis in immunocompetent pregnant women. Prenatal screening for acute toxoplasmosis primarily involves maternal serology and fetal ultrasound imaging. When serological or ultrasound findings suggest acute infection, further diagnostic tests are necessary to confirm fetal infection. Currently, molecular methods to detect the parasite's DNA, including polymerase chain reaction-based methods, on amniotic fluid are the gold standard tests for the diagnosis of congenital toxoplasmosis. In this review, we aim to discuss various aspects of screening and diagnostic methods for toxoplasmosis in pregnancy, including (i) current serological assays, screening approaches, and future perspectives; (ii) the role of imaging techniques, with an emphasis on ultrasound; (iii) principles and recent advances in diagnostic molecular methods; (iv) emerging techniques, such as point-of-care-based tests and biosensors, and microRNAs as novel biomarkers of acute infection; and (v) an overview of screening programs in different countries, important epidemiological determinants, and recommendations for *Toxoplasma* screening health policies.



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Introduction

Toxoplasma gondii is an intracellular protozoan transmitted to humans by consuming raw or undercooked meat from animals harboring tissue cysts or food and water contaminated with cat feces (1). Although the infection in healthy individuals is commonly asymptomatic or presents with mild systemic symptoms, it harbors a significant health issue in pregnant women and immunocompromised patients (1, 2). *T. gondii* poses a serious risk during pregnancy due to its ability to cross the placenta and cause congenital toxoplasmosis (CT), which can lead to severe fetal abnormalities or miscarriage (3). Women acutely infected with *T. gondii* during pregnancy are asymptomatic or have mild nonspecific symptoms, such as cervical or generalized lymphadenopathy, low-grade fever, myalgia, pharyngitis, hepatosplenomegaly, and rash (4).

The global prevalence of latent toxoplasmosis among pregnant women ranges from 32.9% to 33.8%, while acute infections are reported at 1.1% to 1.9% (5, 6). Acute *Toxoplasma* infection in pregnant women is highest in the Eastern Mediterranean region and lowest in Europe (6). Each year, CT affects approximately 200,000 newborns, contributing to a global burden of about 1.20 million disability-adjusted life years (DALYs) (6, 7). This significant health impact, combined with the severe consequences of CT for fetuses, underscores the importance of effective prenatal screening and diagnostic programs for *T. gondii*. Although advances in early laboratory diagnosis of CT have been made, many assays still have limitations, and comprehensive prenatal screening programs are not yet widely adopted in many countries. This review aims to summarize current diagnostic approaches for acute toxoplasmosis during pregnancy and to explore the challenges and future directions of these methods.

Diagnosis of maternal T. gondii infection

Accurate diagnosis of maternal *T. gondii* infection is crucial for timely intervention to prevent adverse effects on both the mother and the fetus (Fig. 1). Effective screening tests, combined with advanced diagnostic methods, are essential for managing the infection appropriately. Serological assays identify specific antibodies in the mother to determine the infection stage. Molecular assays, including PCR on amniotic fluid (PCR-AF) from amniocentesis, detect *T. gondii* DNA to confirm fetal infection. Imaging techniques, especially ultrasound (US), are used to screen for fetal anomalies associated with CT. This integrated approach ensures accurate diagnosis and optimal care for both mother and fetus.

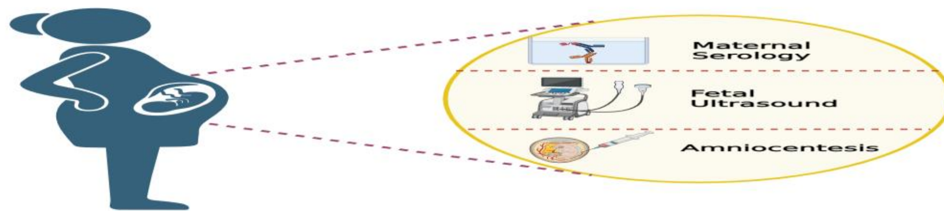

Fig. 1: Diagnostic approaches for toxoplasmosis in pregnancy. Serological testing identifies specific antibodies in maternal blood to determine the infection stage. Ultrasound is the primary imaging technique for screening fetal anomalies associated with congenital toxoplasmosis. Amniocentesis can confirm fetal infection when indicated (Original figure)

Serological testing

Serological tests are critical for detecting *T. gondii* infections by identifying specific antibodies. The detection of immunoglobulins specific to *T. gondii* can be achieved through various serological techniques that provide both qualitative and quantitative results (8). Commonly used methods include enzyme-linked immunosorbent assays (ELISA) and chemiluminescence immunoassays (CIA) to detect *T. gondii*-specific IgG and IgM (9, 10). If routine serological tests indicate or do not definitively confirm an acute *T. gondii* infection, the sample should be referred to a specialized reference laboratory for further analysis (3). Fig. 2 demonstrates the principles and key features of some important routine and reference serological methods for *T. gondii* detection.

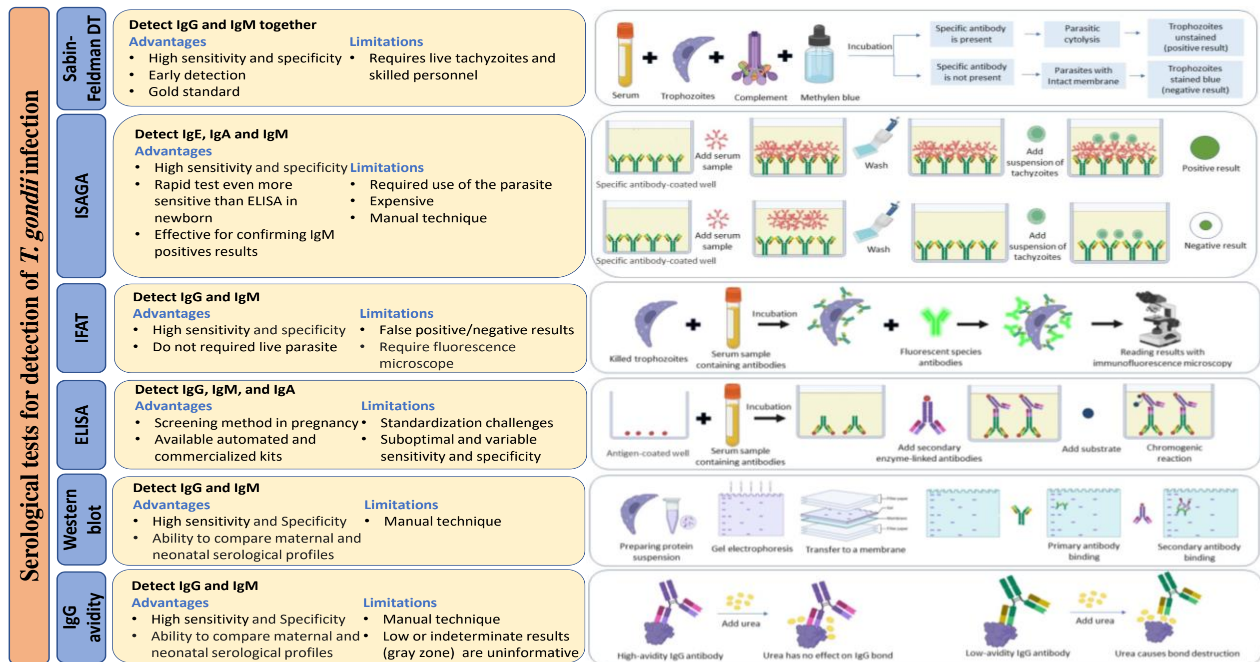

Fig. 2: Key features and principles of serological techniques for detecting *T. gondii* infection
Abbreviation: DT: dye test, ISAGA: immunosorbent agglutination assay, IFAT: immunofluorescence antibody test, ELISA: enzyme-linked immunosorbent assay (Original figure)

IgG detection

T. gondii-specific IgG antibodies typically develop within one to two weeks of the acute infection, reach their peak levels around 2-3 months, and then gradually decline, although they persist for life (11). The gold standard method for diagnosis of primary *T. gondii* infection is by confirming seroconversion, which is the detection of specific IgG in a previously seronegative pregnant woman (11). However, routine serological testing is not commonly performed before pregnancies, and a single sample with positive IgG results cannot differentiate between a chronic and primary infection.

IgM detection

T. gondii-specific IgM antibodies typically appear within one week of infection and peak around 4-12 weeks. These antibodies generally decline to undetectable levels within about three months (12), making them a useful marker for acute infection. However, approximately 9 to 27% of patients may exhibit high

IgM levels for two years or more, which can complicate interpretation (11, 12). This phenomenon, in addition to some possible technical and performance shortcomings of the IgM-specific assays, may lead to a relatively high frequency of IgM false-positive results, especially in populations with a low prevalence of acute *T. gondii* infection (13). Consequently, any positive IgM result should be confirmed with additional tests and interpreted alongside a comprehensive serological panel, including IgM, IgG, and IgG avidity assays. On the other hand, some patients with acute infections might not produce detectable IgM, resulting in false-negative outcomes. This could be due to a very recent infection or a rapid decline in IgM levels (11). Therefore, continuation of maternal and fetal monitoring in suspected clinical situations is essential, even if IgM results are negative.

IgA detection

A few days after the acute infection, they rise to reach a peak around one month and then decline to undetectable within weeks or months (14). IgA may appear earlier than IgM and is less likely to persist beyond the acute phase, potentially making IgA detection more specific than IgM for acute infection (11, 13). However, the rapid decline of IgA can lead to lower sensitivity and higher rates of false-negative results. Adding an IgA ELISA panel alongside IgM testing has improved the accuracy of distinguishing between acute and chronic infections in pregnant women (14).

IgE detection

The presence of *T. gondii* IgE antibodies in pregnant women suggests that these antibodies develop early in the infection, much like IgM, and usually persist for 3–5 months (15). An IgE immunocapture assay, which utilizes monoclonal antibodies against human IgE and formalinized *T. gondii* tachyzoites, detected specific IgE antibodies in 86% (25 out of 29 cases) involving pregnant women with documented IgG seroconversion or increased IgG titers alongside positive IgM or IgA results (16). Another study evaluated the IgE ISAGA and found it to have a specificity of 82.7% and a sensitivity of 94.4% in pregnant women categorized as having acute or chronic infections based on elevated or rising IgG titers and the presence of IgM and IgA antibodies (17). When evaluating *T. gondii*-specific IgE using both ISAGA and ELISA methods, both tests were highly accurate in identifying true negative cases, with a 100% specificity rate (18).

IgG avidity

T. gondii-specific IgG avidity tests, measuring the functional affinity of antibodies, are frequently used for serological screening of acute toxoplasmosis (9, 13). Various methods like ELISA, immunoblot, or fluorescence/chemiluminescence assays have been used for avidity assessment (8, 9). These techniques measure the concentration of high-affinity IgG by disrupting weak, low-affinity interactions through the use of denaturing agents such as hypermolar urea or potassium thiocyanate (9). The maturation of IgG antibodies following *T. gondii* infection can be delayed in some individuals, possibly due to individual differences or the effects of treatment regimens (19, 20). Hormonal and immunological changes (e.g., Th2 shift) during pregnancy may also influence antibody maturation (21). In other words, the positive predictive value (PPV) of high-avidity IgG results is close to 100%, while the negative predictive value (NPV) of low-avidity IgG results ranges between 61.1 and 77.7% (20, 22, 23).

A high *T. gondii*-specific IgG avidity result strongly suggests that an infection occurred more than 3 to 5 months ago, while a low or intermediate avidity result does not rule out a chronic infection. Although the avidity test is particularly useful during the first trimester of pregnancy, high avidity results obtained 3 to 4 months after conception do not exclude the possibility of a primary infection during preg-

nancy. In such cases, further evaluations such as reevaluation of IgG and IgM titers after 2-3 weeks, the *Toxoplasma* Serological Profile (TSP), and amniocentesis are recommended (9, 11). Some experts believe that a very low avidity index may indicate an acute infection. For instance, it has been demonstrated that IgG avidity results below 15% with the Elecsys assay, 17% with the Architect assay, and 0.05 with the Liaison XL Toxo test have been shown to accurately identify recent infections within the last three or two months (22, 23).

Molecular methods for T. gondii diagnosis

Several molecular techniques for the detection of *T. gondii* genetic material, such as loop-mediated isothermal amplification (LAMP) and PCR-based methods, are available (24). Nucleic acid sequence-based amplification (NASBA) is a novel isothermal method used for *T. gondii* RNA amplification (25); however, further studies on human samples are needed to validate its effectiveness. As depicted in Fig. 3, pregnant women who are strongly suspected or confirmed to have an acute *Toxoplasma* infection, immunocompromised patients with a chronic infection that may reactivate during pregnancy, or those with US findings indicative of CT should undergo amniocentesis and PCR-AF. Real-time PCR (RT-PCR) methods have enabled the quantification of parasite load even in samples with relatively low DNA loads (26). Romand et al. reported that severe prenatal and postnatal abnormalities were associated with higher parasite loads in AF (27). Similarly, other studies have shown that fetal US abnormalities (26) and postnatal symptoms (28) were associated with *T. gondii* DNA loads in AF.

Imaging techniques

Imaging techniques play a crucial role in the diagnosis and management of toxoplasmosis, particularly in assessing congenital infections and complications in pregnant women and their fetuses. US is the most commonly used imaging modality for detecting fetal abnormalities.

Ultrasound

US is the modality of choice for screening for fetal anomalies during pregnancy. Screening US is recommended routinely in the second trimester of all pregnancies (29). Suspicious US findings require a comprehensive evaluation of potential infectious causes, including toxoplasmosis (30). Infections during the first or second trimester are associated with more severe abnormalities, but they may not manifest until several months after the maternal infection. Therefore, in cases of suspected or confirmed CT, follow-up US should be performed to determine the severity of the fetal infection and guide the therapeutic approach. Common US findings in infected fetuses may consist of ventriculomegaly, hydrocephalus, microcephaly, cerebral calcifications, liver calcifications, ascites, hydrops, placentomegaly, hydropic placenta, and intrauterine growth retardation (30). However, these findings are only suggestive of fetal infection, and further serological and molecular assessments are required for a definitive diagnosis.

MRI

Although the imaging modality of choice for prenatal CT screening is US, a fetal MRI may be employed for better visualization of soft tissue lesions and identification of subtle CNS or other organ anomalies (31). MRI findings of fetuses with confirmed CT are comparable to US findings (31, 32). Nonetheless, MRI results are more difficult to interpret and need specialized neuroradiology consultation. US usually suffices for screening and diagnostic needs, and the usage of a relatively expensive and detailed imaging modality, such as MRI, is usually limited to research purposes.

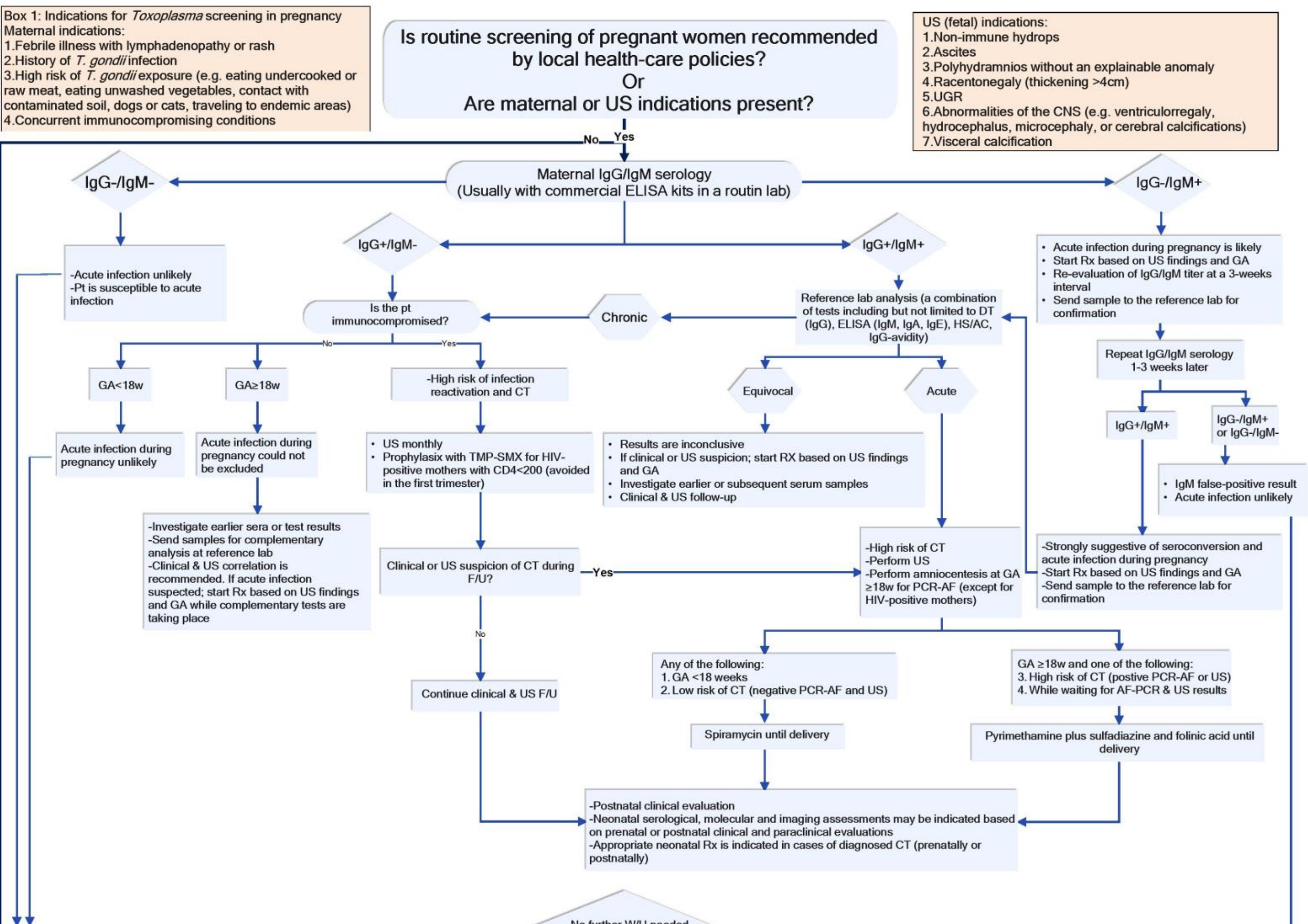

Fig. 3: The proposed algorithm for prenatal screening and diagnosis of congenital toxoplasmosis. Abbreviation: US: ultrasound, pt: patient, GA: gestational age, Rx: treatment, CT: congenital toxoplasmosis, TMP-SMX: Trimethoprim-sulfamethoxazole, HIV: human immunodeficiency virus, CD4: CD4+ T lymphocyte, F/U: follow-up, DT: dye test, ELISA: enzyme-linked immunoassay, HS/AC: differential agglutination, PCR-AF: polymerase chain reaction on amniotic fluid, W/U: work-up (Original figure)

Confirmation of CT diagnosis: Amniocentesis

A prenatal diagnosis of CT can be confirmed by detecting *T. gondii* DNA in an AF specimen. US-guided amniocentesis is performed preferably after 16–18 weeks of gestation and at least 4 weeks after confirmation of maternal primary infection to minimize the risk of false-negative results (33). The sensitivity of PCR-AF varies widely, reported between 64% and 98.3% (34, 35). A meta-analysis estimated the overall sensitivity and specificity of PCR-AF at 83% and 98.3%, respectively (35). Some studies have claimed that the sensitivity of PCR-AF is highest between 17 and 21 gestational weeks (36) and is increased in late pregnancy compared to the first trimester (37).

Recombinant antigens and epitope-specific assays

Advances in recombinant DNA technology and protein cloning have paved the way for the development of novel diagnostic techniques for toxoplasmosis. These recombinant antigens and epitopes allow for the identification and differentiation of antibodies produced during various stages of infection (38, 39). For instance, specific antigens like the microneme 8 (pMIC8) peptide are more reactive during the acute phase of infection, while soluble antigens of *T. gondii* (STAg), are more prominent in the chronic phase (38). These findings enable the development of immunoassays that can distinguish between early and late phases of *T. gondii* infection. Additionally, $CD8^+$ T cells targeting an epitope of GRA4 antigen were most prevalent two weeks after infection, whereas reactivity against an ROP7 epitope peaked at 6–8 weeks post-infection (39).Two antigens, P35 (GRA8) and P22 (SAG2), have been identified as early inducers of specific IgG antibodies (40). Indirect IgG- and IgM-ELISAs using a recombinant epitope of P22 (rP22a) have shown good performance in discriminating between infected and non-infected pregnant women (98.2% specificity and 94.4% sensitivity). Additionally, the recombinant P35a (rP35a) was more effective than rP22a in distinguishing between pregnant women with suspected acute infection (IgG+, IgM+, low IgG avidity) and those with chronic infection and persistent positive IgM (IgG+, IgM+, high IgG avidity), achieving 83.3% specificity and sensitivity. IgG avidity ELISA kits using GRA8 and SAG2 proteins have also shown promising results in discriminating between serum samples with low and high IgG avidities (41). Recently, Teimouri et al. developed in-house ELISA kits using a combination of recombinant SAG1 and GRA7 proteins. The tests had the best performance for detection of *T. gondii* IgG, IgM, and IgG avidity compared to those using either SAG1 or GRA7 alone, *Toxoplasma* lysate antigens (TLAs), or STAg (42, 43).

A recombinant multi-epitope fusion peptide (rMEP) composed of three epitopes from SAG1, SAG2, and SAG3 antigens showed good performance in distinguishing acute and chronic infection in pregnant women (44). The IgG-ELISA using rMEP showed sensitivity and specificity rates of 96.4% and 98.7%, respectively, while the IgM-ELISA exhibited a sensitivity of 96.6% and a specificity of 100%. Additionally, the rMEP-ELISA exhibited 93.2% and 95.7% concordance with 2 commercial ELISA kits (SERION ELISA classic *T. gondii* IgG/IgM, Germany) for the detection of IgG and IgM antibodies, respectively (45).

Rapid point-of-care-based methods

Point-of-care (POC)-based methods, including immunochromatography tests (ICT), biosensors, and novel molecular techniques such as NASBA, have obtained great attention in recent years for the diagnosis of *T. gondii* infection (25, 46, 47). Although studies on different biological samples may have shown promising results, studies on large populations of pregnant women are still required to determine the actual utility of these techniques. Primary studies have shown that ICT-based tests provide rapid and simple IgG and IgM detection with good sensitivity and specificity (46, 47).

Biosensors are novel classes of diagnostic tools that convert a biological reaction into a quantifiable signal (48). Some optical biosensors, known as plasmonic biosensors, are based on the interaction between antigen and antibody on a metallic surface that modifies the refractive index and causes a shift in the resonance curve of the reflected light (49). Laser-induced fluorescence (LIF)-based microfluidic immunosensors are other types of optical biosensors that use an anti-IgG antibody tagged with alkaline phosphatase to produce a fluorescent signal proportional to serum antibody concentration (48). Transforming biochemical processes into electrical signals, electrochemical biosensors are a sensitive and specific tool (50) for the detection of *T. gondii*-specific antibodies (51, 52) and DNA (53).

MicroRNAs as emerging biomarkers for acute T. gondii infection

Animal and cellular studies have addressed the fact that *T. gondii* infection produces a differential "miRNA signature" that is both specific to the infection phase (acute or chronic) (54, 55) and the parasite strain (56). miRNAs are small, non-coding RNAs that regulate various intra- and intercellular biological processes. Derangements in the "miRNA profile" and their diagnostic potential in many human diseases have emerged as a novel and promising field of research (57, 58). As an example, during the chronic phase of toxoplasmosis in mice, miR-146a is upregulated, while miR-204 is mainly downregulated during the acute phase (54). Another study showed that plasma levels of miR-712-3p, miR-511-5p, and miR-217-5p were *Toxoplasma*-specific and significantly elevated in mice infected by both the RH and ME49 strains of *T. gondii* (55). However, studies on pregnant populations are still lacking, and further investigations are required to evaluate the potential of plasma miRNAs as novel biomarkers for the diagnosis of acute toxoplasmosis.

Non-primary infections: reinfection or reactivation?

The majority of CT cases are caused by a primary infection after conception, however, reactivation of latent toxoplasmosis in immunocompromised pregnant women may also contribute to fetal infection (3). Nonetheless, there have been several reports of reinfection in immunocompetent mothers with evidence of prior immunization (59-63). In a newborn with CT, the isolated strain from blood was an atypical genotype of IPP-2002-URB, even though the mother was a native of and lived in France, where types I, II, and III are responsible for the majority of *Toxoplasma* infections (59). This finding is supported by other case reports in which the geographic distribution of the parasite strain was different from the region where the patient was currently living (60-62), suggesting that some women may have been immune to one strain of *T. gondii* but reinfected with another. Available serological assays cannot differentiate between typical and atypical *T. gondii* genotypes. Although it rarely occurs, clinicians should consider potential exposures to atypical genotypes during pregnancy and the possibility of reinfection in chronic cases. Developing screening methods that can differentiate parasite genotypes could be useful in assessing a patient's susceptibility to different *T. gondii* strains.

Maternal and congenital T. gondii-specific T-cell immunity; uncovering potential targets

Interferon-gamma release assays (IGRA) have been recently used to detect cellular immunity against *T. gondii*. Enzyme-linked immunosorbent spot (ELISPOT) and QuantiFERON (QFT) assays are the most standardized and employed platforms to evaluate *T. gondii*-specific T cell function (64). IGRA was a promising tool for differentiating chronic toxoplasmosis from non-infected pregnant women by producing large quantities of IFN-γ in response to STAg in infected patients (65). In addition, IFN-γ secretion increased from 8-

to 20-fold in the presence of fusion BAG1 with glutathione S-transferase (GST-BAG1) by peripheral blood monocytes (PBMCs) from 26 chronically infected women (contracted primary infection in gestation but samples collected after delivery) compared to *Toxoplasma* IgG-negative controls (66). Although few studies have shown that IGRA could potentially aid in the diagnosis of toxoplasmosis among pregnant women (66, 67), further studies are required to address its ability to differentiate acute and chronic infections, as well as its sensitivity and specificity.

Immunological and genetic studies have found that maternal or fetal genetic polymorphisms related to an uncontrolled T helper 1 (Th1) response, followed by inappropriate immunoregulation, may be involved in the pathogenesis of CT. These findings, although still scarce, may suggest a role for the immune system in pathogenesis of CT (68). Although studies on CT are lacking, molecular studies have revealed polymorphisms related to placenta damage, abortion, or fetal sequelae (69). Although these genetic polymorphisms have not been well studied, those related to the Th1 response, such as CCR5, CCR2, IFN-γ, or IL-12, and regulatory cytokines, such as transforming growth factor-β (TGF-β) or IL-10, are candidates for future research and potential targets (70).

To screen or not: balancing benefits and costs

Routine screening programs for prenatal diagnosis of CT have been implemented in some countries with a high seroprevalence of toxoplasmosis, such as France (71) and Austria (72). The serious neurological and other sequelae of the infection in infants, the development of simple screening methods, and the efficacy of anti-*Toxoplasma* medications in minimizing the risk of vertical transmission have encouraged countries to implement routine screening programs (3). In contrast, a consistent decline in toxoplasmosis prevalence and incidence rates, improvements in food hygiene, and the high expense of screenings argue against the routine CT screening of all pregnant women (73). Consequently, numerous countries like many European nations, the United States, Canada, the United Kingdom, and many other countries, carry out prenatal screening programs when certain maternal and fetal indications are present (3, 74).

Although prenatal CT screening may be less cost-effective in countries with low or decreasing seroprevalence, the majority of our knowledge about toxoplasmosis seroprevalence originates from high-income countries (75). In addition, the genetic diversity and common genotypes in each country should also be considered in health policy decisions. There is more than a twofold increase in the likelihood of clinical sequelae after infection with *T. gondii* type I and atypical strains, compared to types II and III. Moreover, the virulence and mother-to-child transmission ratio vary between different genotypes (74). Accordingly, it is essential for each country to establish its own screening and preventive guidelines, tailored to the local epidemiological and genetic features of *T. gondii* infection as well as the available diagnostic and therapeutic resources.

Conclusion

Acute toxoplasmosis poses significant teratogenic and fatal risks to developing fetuses, making effective prenatal screening and diagnosis for CT crucial. Despite advancements in screening methods, important challenges remain. Current serological assays are prone to false positive and false negative results, limiting their reliability. Emerging screening techniques, such as biosensors and noninvasive biomarkers, show promise in enhancing the accuracy of CT diagnosis, predicting fetal outcomes, and optimizing the choice of therapeutic strategies. Epidemiological data from low- and middle-income countries are limited and tailoring screening strategies to the specific

resources and virulence characteristics of *T. gondii* in each country is crucial.

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Conflict of interests

The authors declare that there is no conflict of interest.

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