Toxoplasmosis in the Fetus and Newborn

106

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Contents

Abstract

Globally, primary toxoplasmosis on gestation generates annually 190,100 new cases of congenital toxoplasmosis with a global burden of 1.20 million DALYs. Although *Toxoplasma gondii* infection is easily diagnosable and effectively treatable on the mother, out of prenatal screening setting, newborn diagnosis and early treatment might be problematic. In fact, the large majority of infected newborn display normal on clinical examination, with positive IgG of maternal origin and possibly negative IgA and IgM, with the consequence of late treatment on subclinical cases who are the ideal target of long-term pharmacological treatment. Moreover, toxoplasmosis is on the list of neglected disease of poverty. As consequence, the interest of manufacturers shows low, and standard of treatment continues to rely on a not curative and toxic medicine combination. Fortunately, research in progress on *Toxoplasma gondii* and host genetics and epigenetic machinery, including unusual histone variants and plantlike transcriptional and

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G. Buonocore et al. (eds.), *Neonatology*,
https://doi.org/10.1007/978-3-319-29489-6_253

posttranscriptional motifs, could pave the way to potential new drugs and/or to channel the choice to treat or not to treat (and how long) subclinical onset forms. On the chapter, practical sustain on management at birth and on the long term of infant exposed to maternal *Toxoplasma gondii* infection or definitely congenitally infected (text, tables, and figure) is updated on the state of the art.

106.1 Salient Points

- Congenital toxoplasmosis is widespread worldwide and a global burden of 1.20 million DALYs (Disability-adjusted life year) is estimated.
- Consequences on human infection vary according to parasite and host genetic and epigenetic factors, immune status of the host, and route of acquisition.
- Early gestation maternal infection, high parasite load on amniotic fluid, the presence of seizures, and/or neurologic findings and/or brain neuroimaging abnormalities and/or ocular toxoplasmosis near birth are predictors of negative outcomes.
- The large majority of infected newborn are normal on clinical examination, with positive IgG of maternal origin and possibly negative IgA and IgM, making diagnosis and early treatment difficult.
- Pharmacological treatment relies on a few toxic drug combinations (pyrimethamine and sulfonamide) on association with folinic acid to lower the side effect on bone marrow.
- Prompt ventriculoperitoneal shunt placement with frequent assessments of adequate drainage may be necessary. Seizures are treated effectively with levetiracetam, which does not interfere with pyrimethamine and sulfadiazine.
- Compensatory strategies help children compensate for the impact of the disease on cognitive function and quality of life.
- Antibodies against VEGF or focal laser photocoagulation and pars plana vitrectomy, in conjunction with standard antimicrobial therapy, are emerging as effective therapies for complications such as choroidal neovascular membranes.

106.2 Introduction

Toxoplasma gondii infection (TgI) in humans has been considered a benign anthropo-zoonosis harmful for immunocompromised person ("killer no. 3") and unborn child, on which a devastating disease might occur, even when primary maternal TgI is only unveiled by antibody presence (Saadatnia and Golkar 2012).

On the 1980s, focus has concentrated on congenital TgI (CTgI) *See* Toxoplasma gondi infection (TgI) sequelae and prevention and early treatment opportunities and claims for screening. On the EU, monthly or trimestral prenatal testing on antibody-negative pregnant women and fetal damage prophylaxis on seroconverters has been practiced, compulsively on France and Austria and on demand charge-free on Belgium, Germany, and Italy. On Austria and France, case treatment was further facilitated by mother-newborn pair postdelivery testing. On New England and New Hampshire, IgM testing on newborn dried blood spots and sequelae prophylaxis in confirmed positive infant had been launched, since 1986; feasibility of newborn screening had been confirmed in Denmark, Poland, Sweden, Brazil, and Colombia. Furthermore, it had closed on EU countries because of low sensitivity in a low prevalence setting.

Weak proofs of prophylaxis benefits, concerns about infrastructure costs and adverse treatment effects, and differences in test and drug local availability are still contributing to variability in clinical practices. Equipoise considerations in screening countries, lack of funding, and disinterest of manufacturers in developing countries where CTgI has the major impact are yet blocking randomized placebo-controlled trial realization. As consequence, promising new and/or multiplexed assays with potential diagnostic improvement remain not validated and commercialized. Treatment too relies on a few outdated and poorly understood pharmacokinetic/dynamic drugs unable to eradicate tissue cysts and thus to cure

toxoplasmosis. TgI has been included in the list of neglected tropical parasitic disease of poverty (Jones et al. 2014).

106.3 Etiology and Pathogenesis

T. gondii is an obligate intracellular protist (*Apicomplexa* phylum). Tachyzoite is the rapidly dividing parasite stage, segregated from host cell apparatus into a parasitophorous vacuole. The *apicoplast* is a complex structure at the apical end, including club-shaped secretory organelles with kinase or pseudokinase signature modulating innate immunity and parasite survival, namely, rhoptries (ROPs) and dense granule bodies (GRA) contributing to the development of microtubules and intra-vacuolar network (IVN), and polar rings sometimes surrounded by rodlike structures, namely, microneme (MIC), which participate on host cell invasion. Certain rhoptry proteins were found to be critical virulence factors that resist innate immunity, while other rhoptry proteins were found to influence chronic infection without affecting virulence (Fox et al. 2016).

Low-level genetic divergence among three major multilocus genotypes (I, II, III), stable in time and space, has been demonstrated on EU (mainly type II strain) isolates, whereas South America, Africa, and Asia isolates display more complex population structure with greater genetic diversity and virulence (atypical strain). On South America, where type II strain is very rare, 88 atypical strains have defined with severe acute course. Genetic diversity reaches the maximum on Amazonian area, where interpenetration of anthropized and wild forest leads to strain hybridization. In French Guiana, high rate fever, systemic disease, and ocular findings have reported on immunocompetent persons with such strains (Carme et al. 2009). In the USA, a fourth clonal type has been recently isolated (type 12) which account for 46.7% of wildlife isolates (Dubey et al. 2011). Genotype I–III crosses identified ROP 18 and ROP16 as key molecules for eye damage on humans (Torres-Morales et al. 2014).

Correlates between host genetic (and epigenetic) markers controlling immune response and outcomes, particularly on ocular toxoplasmosis (OT), are emerging. Linkage disequilibrium on single nucleotide polymorphisms (SNPs) has been shown on NOD2, IFN-γ point mutation (+874 T/A), and parental COL2A1 (Dutra et al. 2013; de Souza et al. 2012; Jamieson et al. 2008). Polymorphism on P2RX7 receptor has been shown in association with eye-brain involvement (Jamieson et al. 2010). More severe ocular lesions have been shown on Brazilian cohorts compared to the EU cohort (Gilbert et al. 2008), and atypical genotypes described in South America have been isolated on severe onset CTgI patient, on France and Tunisia (Delhaes et al. 2010; Boughattas et al. 2011). Type 1 and atypical parasites were associated with clinical problem, regardless of pregnancy period at infection (Rico-Torres et al. 2016).

The wealth of *T. gondii* epigenetic machinery, including unusual histone variants and plantlike transcriptional and posttranscriptional motifs, could open to new chemotherapy approach.

Chemokine release on infected cells leads to an amplification loop activated through direct interaction between dendritic and natural killer (NK) cells, mutually activating and amplifying IL-12 and IFN-γ synthesis and thus tumor necrosis factor alpha (TNF-a) synthesis. The further recognition of parasite antigen by pattern recognition receptors leads to an exacerbation of phagocytic activity with enhanced production of reactive oxygen and nitric oxide species and tryptophan starvation. A strong Th-1 immune response might overwhelm its goal and be responsible for severe inflammation, resulting in tissue damage or even death. Such a deleterious effect of an acute Th-1 immune response in the setting of primary acquired TgI on gestation can result in fetal loss, since IFN-γ destabilizes the Th-2 microenvironment necessary for eye, CNS, and maternal-fetal tolerance.

Transplacental transmission occurs on overall 29% of gestation complicated by primary TGI, conventionally during the temporary parasitemia accompanying early infection. Gestational week of maternal infection (AW), regardless of settings, including parasite genotype, study design, and methods, is the main factor for transmission rate

and CTgI severity. More newborns with clinical problems were born from mothers infected during the first half of gestation. Placenta plays a main role in the process as both natural barrier for the fetus protection and target tissue for parasite multiplication. Acute disease acquired ≥6 months before gestation shows a transmission rate approaching zero, and CTgI cases reported on maternal infection in the 2 months before conception are rare. On a recent meta-analysis study, the vertical transmission pooled rate was 20% (95% CI, 15–26%), and the rate by trimesters was 5% (95% CI, 2–16%), 13% (95% CI, 7–23%), and 32% (95% CI, 24–41%), respectively (Li et al. 2014). These values show lower than on EU prospective cohort meta-analysis, on which the odds of transmission increased by 12% per AW after seroconversion time, and the risk of giving birth to a child with clinical signs is greatest (10%) for women acquiring toxoplasmosis between 24 and 30 AW (Systematic Review on Congenital Toxoplasmosis Study Group (SYROCOT) 2007). In fact, the odds of intracranial lesion markedly decrease with older AW at maternal infection, whereas the risk of ocular lesions declines less significantly (Fig. 115.2). Besides pregnancy time at infection, clinical outcome in congenitally infected products depends on parasite burden. A concentration larger than 100 parasites/mL in amniotic fluid was strongly associated with a severe outcome (Romand et al. 2004).

In the fetus, the tachyzoite ability to cross non-permissive biological barriers includes eyes and CNS. Neuron chronic infection underlies *Toxoplasma's* ability to cause devastating neurologic disease and death. Although both astrocytes and neurons might be infected, only IFN-γ-stimulated astrocytes might clear intracellular parasite (Nguyen et al. 2016). Brain microcalcification and ventricular dilation on neuroimaging (Figs. 115.1 and 115.2), retinochoroiditis foci or scars on fundoscopic examination (Fig. 115.3), and/or optical coherence tomography (OCT) reveal tissue damages generated by local Th-1-mediated immune responses. Vasculitis with necrosis gradually sloughing into the ventricles might cause obstructive hydrocephalus (Fig. 115.2).

Four anatomical patterns secondary to CNS involvement have recently categorized (Hutson et al. 2015). The commonest is the aqueduct obstruction with enlargement of the lateral and third ventricles and abnormal fourth ventricle (43% of cases); the second one was Monro foramina obstruction with asymmetric lateral ventricle dilatation and normal third and fourth ventricles (25% of cases). The third pattern shows mixed aqueduct and foramen obstruction (11% of cases). The fourth pattern shows bilateral lateral ventricular dilatation, with normal third and fourth ventricle size, and Monro foramina without peri-foramen calcifications or intraventricular obstructive lesions (21% of cases). This peculiar pattern seems associated with no type II strains. Recurrent and/or progressive ocular damage on the long term has considered the consequence of bradyzoite to tachyzoite reconversion with at times invasion of adjacent cells (recrudescence) and secondary autoimmunity for retinal soluble antigen.

106.4 Epidemiology

T. gondii causes an extremely successful opportunistic infection, and approximately one-third of the human population display TGI serological markers. It is an emerging serious foodborne disease causing millions of disability-adjusted life years (Gangneux and Dardè 2012), depending on CTgI complication, and visual damage, psychosis, change in personality profile, and seizures complicating acquired TgI (Torgerson et al. 2015). OT is the fifth cause of acquired visual loss, with overall 24% of affected eyes becoming legally blind (Bodaghi et al. 2012).

Prevalence increases with age, but the rate of acquisition varies according to climate and anthropogenic factors (hygiene, dietetic habits, sanitation, and quality of water). Transmission occurs by ingestion of oocyst defecated by acutely infected feline on water or soil or of cyst on meat animal with chronic TgI. Sporadically, a Tg-IgG-negative recipient has been infected by organ transplantation or blood transfusion from latent infected subject or laboratory accident. On EU,

journey abroad or eating imported exotic food had sporadically reported on severe cases.

TgI is prevalent in socioeconomically disadvantaged groups. On Brazil, most person in the lower socioeconomic level are infected by age 15 years, whereas in the upper are infected after age 20 years, thus having an increased risk for primary TGI on reproductive age (Bahia-Oliveira et al. 2003). On the two last decades, progressive decrease in age-specific prevalence has been reported on economically privileged EU countries as consequence of farming, meat animal breeding, food cleaning and storage improved conditions, and prenatal standards of care, including prenatal screening and health education. In the EU, an incidence updating is in need as an increasing number of women are facing gestation while unprotected.

The global annual CTgI prevalence is estimated to be 190,100 cases, with a global burden of 1.20 million DALYs (Torgerson and Mastroiacovo 2013). In the Northern Hemisphere, gestational toxoplasmosis and CTgI prevalence range from 0.5 to 8 per 1000 susceptible pregnancies and 1 to 10 per 10,000 live newborns, respectively. On Italy, CTgI prevalence had shown 1.38 per 10,000 live newborn (Stagni et al. 2009).

106.5 Laboratory Diagnosis

TgI diagnosis mostly relies on antibody response kinetics (IgG, IgM, IgA isotypes) on blood, instead of direct detection by PCR or culture on placenta (60%–79.5% sensitivity, 92%–97% specificity), cord blood (21.2% sensitivity, 100% specificity), CSF (46% sensitivity), and urine (50% sensitivity) (Gangneux and Dardè 2012). As having a combination of IgM and IgA test results, in addition to IgG, has greater sensitivity than either test alone, IgG, IgM, and IgA should always be tested.

Nowadays, most clinical laboratories use an enzyme immunoassay (EIA) on whole soluble antigen for the routine, whereas further techniques have mostly reserved for pitfall exclusion on expert laboratories.

Maternal Diagnosis

Conversion from a negative postconceptional test to an IgM- and IgG-positive result marks a primary toxoplasmosis on gestation, whereas a negative postdelivery or a positive preconceptional IgG test reasonably rules out offspring risk. On prenatal screening setting, a *short therapeutic window* had shown that a delay of more than 3 weeks on starting treatment increased transmission odds, thus pointing out efficacy of monthly screening (Wallon et al. 2013).

Routinely, a multi-test strategy based on combination of highly sensitive IgM and IgG avidity (or stage specificity) assays has been considered appropriate in confirming or excluding maternal acute TGI (and thus fetal risk) when positive IgG and IgM were found at the first prenatal test ($\leq$12 weeks AW). High IgG avidity results point out residual IgM (Villard et al. 2016). Drug-induced delay on IgG avidity maturation has been reported on spiramycin-treated mother. High-performance low-cost multiplexed assays for IgG, IgM, and IgA on ultra-small blood volume are paving the way to universal prenatal screening implementation (Li et al. 2016).

Fetal diagnosis might carry out at $\geq$18 AW, at least 4 weeks after the estimated infection time, by PCR assay on amniotic fluid (AF) according to laboratory and/or ultrasonographic indications. On France, despite performance variation among systems, PCR has become standard of practice to channel prenatal management. The AF146527 RT-PCR is far more sensitive than B1 gene RT-PCR (Belaz et al. 2015). RT-PCR assay is highly specific, but a negative result does not rule out fetal infection as up to 46% of infected AF contain $<$10 tachyzoites/mL (Filisetti et al. 2015).

Newborn Diagnosis

Postnatal diagnosis is essential because of prenatal diagnosis failure on approximately 15% of cases and late on pregnancy maternal acquisition. Out of prenatal screening, early definition of infant infection status might show problematic. In fact, as most cases show normal on routine clinical examination, cranial findings are not pathognomonic and might

go undetected, funduscopic examination could be negative or imprecise even on expert examiner, and sensitivity of serological test is lower when mother acquired their infection early in gestation and/or received treatment during gestation. According to maternal infection AW, IgM sensitivity increases by 29% in the first to 71% in the third trimester, and IgA sensitivity increases by 40% in the first to 64% in the third trimester (EMSCOT- European Multicentre Study on Congenital Toxoplasmosis 2007). The most informative result is a positive IgM result on pairs with second trimester maternal seroconversion (with a pretest probability of 27% and a posttest probability of 79%) and a negative IgM result on pairs with third trimester maternal TgI (with a pretest probability of 59% and a posttest probability of 32%). Better scores have been achieved when the tests have been performed on age 2 weeks.

At birth, high IgG level might reflect transplacental transportation. Sequential specific IgG level monitoring (in parallel with the previous sample) compared to the expected decay on transported IgG (one on half per month) shows between ages 2 and 6 months the infant has been infected according to stabilized or rising IgG level. Beyond age 12 months, IgG persistence or disappearance definitely confirms or rules out CTgI, respectively; unfortunately, the gold standard for diagnosis is useless fore decision about treatment. In some infected infants, treatment can lead to IgG negativization during follow-up, creating the false sense that the infant is not infected. However, in infected infants, treatment discontinuation is followed by a *Toxoplasma* IgG rebound, whereas in uninfected, IgG remains permanently negative.

Demonstration of positive IgM and/or IgA on newborn whole blood or sera sample is highly specific when maternal antibody leak might be ruled out, because neither crosses the placenta. Overall, IgM and IgA sensitivities have shown 52% and 55%, respectively. Immunosorbent agglutination assay (ISAGA) is still preferred, because of better sensitivity (e.g., 81.1% versus 64.8%) and specificity, long-term persistence on infant blood, and few false-positive result (Pinon et al. 2001; Murat et al. 2015). Comparative immunoblot test (IB) for specific IgG and IgM analysis on paired mother-newborn sera (at birth and <3 months follow-up) can confirm CTgI diagnosis and rule out maternal serum contamination on cord blood. In fact, different band patterns on mother-newborn pair indicate antibody neosynthesis. Combination of IB with conventional serological methods has shown to be superior to IB or conventional methods used alone. The IB sensitivity at birth is 48–50% for IgG and 65–79% when combined with IgM detection. A multi-test strategy, including two tests (IgM and IgA) or three tests [IgM, IgA, and immunoblotting (IB)], increases sensitivity up to 73% and 78%, respectively (Rilling et al. 2003). Three-IgM band association at 75, 90, and 100 kDa, called the "IgM triplet," might increase sensitivity to 95.8%, when combined with prenatal and postnatal serological tests (L'Ollivier et al. 2012). The interpretation of IB may be difficult and should not have been performed after age 3 months. On cases with doubtful diagnosis, IFN-ɣ release after antigen stimulation of fresh resuspended blood pellet might increase diagnostic precision (94% sensitivity and 98% specificity, respectively). Unfortunately, the test is currently not commercially available (Chapey et al. 2015). PCR has more commonly utilized for neonatal diagnosis in regions without screening where higher sensitivity in PCR assays is expected according to the absence of maternal treatment.

On patient with missed diagnosis at birth and suggestive sequelae, retrospective diagnosis might be tempted on cards collected for newborn screening (Marangoni et al. 2014). Since suboptimal storage conditions can seriously affect sensitivity, negative results cannot rule out the diagnosis.

Protein microarray displaying *Toxoplasma* polypeptide products has identified several antigens for IgM antibody detection. Recently, high potential in serological diagnosis has been shown on rROP2186–533 ISAGA assay [100% (48/48) positivity on Toxo-IgM+-IgG– sera and 0% positivity on Toxo-IgG+ sera) (Liu et al. 2012). In the next future, multi-epitope-based antigen approach using software-based prediction tools and molecular techniques may provide a novel and alternative means of acquiring less expensive and more accurate diagnostic kits.

106.6 Clinical Aspects

106.6.1 Acquired Infection

After an incubation period of about 2–3 weeks, lymph node swelling (mostly posterior cervical) and fatigue associated with low fever are the commonest TGI symptoms. Adenopathy can involve other node groups. Hepatosplenomegaly and rash are rarer. Clinical course is usually self-limited, and symptoms resolve on weeks or months. OT might occur in the acute and latent phase and recur by time. Diagnosis is based on clinical feature but may be confirmed by biological tools applied to ocular fluids (Bodaghi et al. 2012). OT has reported on 2% of US population and on 7.7% of 130 mothers delivering a child with CTgI (Noble et al. 2010). In the context of French screening program, clinical signs have been reported only on 5% of mothers and without remarkable prenatal ultrasound (US) findings (Dunn et al. 1999). On HIV-infected patient, on direct parasite inoculation (laboratory accidents), where NE-II strains predominate, and/or small outbreak caused by contaminated water, the incidence of retinochoroiditis seems higher (Bowie et al. 1997). In a no screening setting (US), awareness of TgI compatible clinical signs seems to offer a chance of prompt treatment as 48% of mothers who gave birth to an affected child could recall cervical lymphadenopathy and/or flu-like illness (Boyer et al. 2005).

Although reactivation of chronic TgI in individuals with weakened immune systems causes severe toxoplasmosis, with rare exceptions, reactivation of latent toxoplasmosis poses no threat to the fetus. The potential for overt disease and fetal transmission during the chronic phase has been reported in severely immunocompromised pregnant women (CD4 count <200 cells/mm3) and on a few anecdotic cases (Elbez-Rubinstein et al. 2009; Silveira et al. 2003).

106.6.2 Congenital Infection

The clinical spectrum of fetuses, newborns, and children with CTgI could range widely from complete apparent normality on as much as 80% of cases to severe neurological and ocular disease and even death. Depending on maternal AW of infection and prenatal treatment, parasite genotype, genetic and immunologic status of the mother, and route of acquisition, a huge amount of complications have been reported at a different rate, including miscarriage, stillbirths, prematurity, intrauterine growth restriction (IUGR), purpura, blueberry muffin rash, splenomegaly, hepatomegaly, anemia, leukopenia, thrombocytopenia, abnormal cerebrospinal fluid cells and protein, ocular disease with or without visual loss, strabismus, nystagmus, cataracts, microphthalmus, intracranial calcification, hydrocephalus, microcephalus, electroencephalographic and/or cerebrospinal fluid abnormalities, seizures, intractable epilepsy, cerebral palsy, and neurodevelopmental delay (McLeod et al. 2014). Milder disease has been shown more prevalent in the EU, where overt signs were reported in a minority of infant enrolled in center performing prenatal or neonatal screening, and attributed to differences on *T. gondii* genetics and prenatal care (Gilbert et al. 2008).

Definition of onset severity at birth includes exclusion of systemic involvement by careful physical examination and general laboratory tests and of target organ (eye and CNS) damage.

Eye

The clinical diagnosis of OT on indirect funduscopic examination is usually straightforward. Typically, one or more fluffy white retinal lesion (less than half to four optical disk in diameter, macular, juxta-macular, or peripheral in location) might coexist with pigmented chorioretinal scar and prominent vitreous and anterior chamber cellular reaction. Papillitis, neuroretinitis, and retrobulbar neuritis might be unusual presentations and cataracts, glaucoma, optic atrophy, and microphthalmia late complications.

CNS

Hydrocephalus (approximately 4% of infected infants) is primarily obstructive with third ventricular dilation and associated with high CSF protein levels (≥1 g/dL). Not obstructive hydrocephalus might complicate fibrotic process (loss of brain

parenchyma or poor reabsorption). Cerebrospinal fluid (CSF) cells, protein, glucose, and *T. gondii* DNA presence have to investigate on the presence of neurological involvement on expert neurological examination or neuroimaging.

Neuroimaging has aimed to detect primarily ventricular dilation and cranial microcalcification. Ultrasound (US) examination, via the bregmatic fontanelle before age 6 months, is first-level imaging with high sensitivity (current probes) for ventricular dilation, low cost, large availability, and the absence of irradiation. Scanning (at 3-mm collimation with 5-mm interval and at 10-mm collimation) is second level, with high accuracy on calcification detection, but potential side effect secondary to irradiation. Magnetic resonance imaging (MRI) (1.6 to 4 mm slice thickness, sequence T1-W conventional spin echo or T2-W fast spin echo) is third level to search for parenchymal or cortical anomalies. A 45-s two-sequence MRI study, collectively referred to as "brain shunt hydrocephalus screen" in some US hospitals, which do not require sedation or contrast administration, can be used to follow the progress of ventricular dilatation or the correction of hydrocephalus in a manner that is easy and comfortable for both parent and child. These two sequences are T2 axial and coronal single shots with images at 3-mm intervals.

Ideally, a suspected CTgI should be confirmed on an experienced reference laboratory, and management carried out on (or with the formal support of) a specialized center, where a multidisciplinary expert team working in high-quality standardized conditions and performing valid and reliable measures, reporting in a large database standard format, including retinal photographs and neuroimaging, might minimize potentials (Blankenberg et al. 2000; Hintz et al. 2007). The more clinical onset definition is appropriate based on correct estimation of maternal infection time and interpretation of fetal diagnosis results, and posttest probability measures, the less the misdiagnosis rate, i.e., the number of treated among healthy infants and untreated among congenitally infected ones.

106.7 Differential Diagnosis

A severe TORCH syndrome, including *Cytomegalovirus*, herpes simplex virus, rubella, syphilis, varicella, B19, and West Nile, dengue, chikungunya, malaria, Zika, and Ebola on particular epidemiological conditions, has to rule out in symptomatic newborn infants by a combination of diagnostic tools. Systematic exclusion of congenital cytomegalovirus (cCMV) coinfection within age 2 weeks should channel appropriate onset severity definition/management and outcome measures. A negative postdelivery CMV-IgG test rules out cCMV, whereas a positive CMV-PCR on Guthrie card should allow differentiation between congenital and perinatal infection on case with testing delay. Exclusion of pseudo-TORCH syndrome might be indicated in multisystemic disease simulating TORCH syndrome upon which extensive screening for congenital infectious disease is negative (Knoblauch et al. 2003).

106.8 Therapy and Treatments

Guidelines for prenatal and postnatal treatment have been summarized in Table 115.2.

No safe and curative therapeutic strategy exists against TGI; pharmacological treatment of acquired infection has indicated only on complicated forms, including OT with visual damage, tropical toxoplasmosis (fever, myocarditis, and pneumonitis), and neurotoxoplasmosis. On gestational and periconceptional TGI (or periconceptional symptomatic TGI occurring <6 months before last menstrual data), prompt antibiotic secondary prophylaxis continued until delivery has advocated to reduce transmission rate and short- and long-term severity of fetal infection. Standard regimens rely on spiramycin and on synergistic combination of folate inhibitors, namely, pyrimethamine and sulfonamides, given with folinic acid (PSF) and regular monitoring of complete blood cell counts. The first is the only administrable before 16 AW, absorbed efficiently, well tolerated, and with little side effects to the fetus (Rajapakse et al. 2013). Change to PSF regimen has carried out on confirmed late maternal infection ($\geq$30 AW), positive fetal diagnosis, and

the presence of suggestive abnormalities on ultrasonography. On unproven maternal infection, an expert and exhaustive discussion with parents could channel appropriate treatment choices based on presumed AW of infection and offer relief to parental anxiety. Induction of labor or C-section delivery does not prevent CTgI (Wallon et al. 2015). Spiramycin-cotrimoxazole combination displayed comparable result with PSF combination on a single comparative study (Valentini et al. 2015), and should be regarded as an alternative in case of PSF regimen side effect and positive fetal diagnosis.

Although evidences of efficacy of postnatal treatment rely only on observational studies, tertiary prophylaxis has been applied in infected infants on the aim to reduce duration and severity of critical symptoms at birth, neurologic impairment, and new eye lesion. The lack of diffusion into the brain parenchyma and the potential for life-threatening arrhythmias excluded spiramycin from the use in newborns, and PSF combination has yet the standard of treatment (Stramba-Badiale et al. 1997).

Differences in treatment regimens exist among centers, including type of PSF regimen and duration of postnatal treatment. One-year long continuous regimen is the standard, whereas 3-month high-dose continuous and 2-year low-dose discontinuous were the shortest and longest regimens, respectively. On low fixed-dose discontinuous regimen, the potential for early and more severe sulfonamide side effects such as Lyell syndrome has been counterbalanced by potential improvement on adherence to treatment. Dosage adjustment, according to weekly complete blood count, might be necessary on as much as 14–58% of cases because of neutropenia. Precaution: glucose-6-phosphate dehydrogenase (G6PDH) deficiency screening should have been performed before sulfonamide administration. Signs of active infection appear to resolve early (in weeks) during treatment. In case of PSF withholding, depending on severe side effects, alternative treatment includes pyrimethamine combination with clindamycin or azithromycin, with standard dosages according to weight. Steroids (1 mg/kg daily) might have been given for a short time, after loading PSF doses, to shorten the course of vision-threatening retinochoroiditis such as that localized in the proximity of the optic nerve or the macula and/or encephalitis [suggested by protein concentration $\geq$ 1 g/dL on cerebrospinal fluid (CSF)]. When inflammation signs subsid, they are tapered and finally discontinued. Treatment reinstitution after a serological rebound has not been indicated.

OT is characterized by recurrent episodes with potential loss of vision when the macula and optic disk are involved and/or complications such as retinal detachment or neovascularization occur. The timing of recurrences varies between individuals and is unpredictable. If new eye lesions have been detected, standard treatment should have been given for 1–2 weeks after the resolution of acute phase in active lesions though on peripheral location and healed lesions. Four-month intermittent treatment with trimethoprim/sulfamethoxazole (once every 3 days) might have been considered as alternative regimen in children with recurrent eye lesion over age 1 year (Silveira et al. 2002). Treatment with antibiotics probably reduces the risk of recurrent toxoplasma retinochoroiditis, but there is currently no good evidence that this leads to better visual outcomes.

Prompt ventriculoperitoneal shunt placement might be necessary with frequent controls of adequate drainage suitability, as patients usually require repeated shunting procedures. As it is unclear from initial neuroimaging which children will benefit from CSF drainage, shunt placement has to consider in all patterns. Brain lesions can cause seizure secondarily (both the multifocal encephalomalacia and multifocal cerebral calcification). Seizures have been treated effectively with levetiracetam (Keppra) (7 mg/Kg twice a day), which has less sedative hypnotic type effects, does not induce hepatic degradation of pyrimethamine such as phenobarbital, and does not displace sulfadiazine albumin binding such as phenytoin nor does it trigger the bone marrow toxicity associated with carbamazepine.

Compensatory strategies such as large print and "talking" books, camera magnification of materials, and spectacles to maintain best-corrected visual acuity help children compensate for the impact of the disease on cognitive function and quality of life. Plate peripheral

neovascularization associated with plaques of cicatricial retinochoroiditis has sometimes been visualized by fluorescein angiography or ocular coherence tomography. The process might have been abrogated by antibodies against VEGF, ranibizumab, in conjunction with standard antiparasitic therapy or by focal laser photocoagulation and pars plana vitrectomy.

Breastfeeding has no contraindication as, even if *T. gondii* has been isolated in many animal species, there is no evidence of transmission on humans (Capobiango et al. 2015).

As neurological or ophthalmological disease might manifest later in life even on subclinical onset patient, psychological and developmental tests, visual evoked potential tests, and audiology tests have to be performed regularly in all cases, probably up to adolescent age.

In the next future, correlations between severity and type of disease and genotype of the infecting strain might be critical to determine appropriate disease treatment and outcome in human cases.

106.9 Prognosis

Maternal AW of infection and parasite load in amniotic fluid are early markers for CT onset severity. Evidences on parasite genotype effect on clinical outcomes are emerging, and an association between serotype II infection and better clinical outcome as compared to non-type II has shown in a large US cohort (Bodaghi et al. 2012).

Long-term evolution of CTgI was not fully documented. In outdated studies, only 11% of asymptomatic untreated patients remained sequelae-free. On the contrary, favorable cognitive, neurological, and auditory outcomes had been found in the group without substantial neurological disease near birth and in over 72% of moderate or severe neurological disease groups after 1-year standard postnatal treatment in the National Collaborative Chicago-based Congenital Toxoplasmosis Study (NCCCTS), recruiting referrals all over the USA (McLeod et al. 2006). New eye lesions remained undetectable in 91% of the children without neurological disease and in 64% of those with moderate or severe neurological disease. Reactivations have reported in as many as 34% of cases with early ocular involvement. More than 70% of patients in the untreated group developed new eye lesion after the first decade of life, whereas new central lesions were uncommon in treated children (Phan et al. 2008a; Phan et al. 2008b) (Table 1).

In the European Multicentre Study on Congenital Toxoplasmosis (EMSCOT), cohorts recruited through screening and treated displayed outcomes milder than anticipated based on historical data. Serious neurological sequelae had been found on 5% of patients on the first 2 years of life, and comparable development and behavior had been reported at 4 years age in treated cases compared to uninfected controls (Freeman et al. 2005). Moreover, 18% of 281 infected children had ≥ 1 retinochoroidal lesions, and 6% had recurrent retinochoroiditis during a median follow-up of 4.1 years. Half of the children who developed OT had already had their first lesion detected before age 4 months. The main determinants were the presence of clinical findings near birth and/or intracranial lesions. The highest risk (80%) had been found in cases with serious neurologic sequelae and the lower (12%) in subclinical cases (European Multicentre Study on Congenital Toxoplasmosis- EMSCOT 2008). On Lyon cohort, only one-fourth of the patients had bilateral ocular lesions, and less than one-sixth had foveal lesions, which probably explains their good performance on visual function assessments and quality-of-life score close to the expected normal range for the general population (74.7 ± 14.2 vs 73.7 ± 15.3) (Peyron et al. 2011; Wallon et al. 2014). Although at age 12 years, 29.8% of followed cases manifested at least one ocular lesion, and recurrences or new ocular lesions were shown on 33.8% of patient after the appearance of the first lesion, only 2.7% of infected children developed bilateral visual impairment severe enough to affect eligibility for a driving license. Lesions remained unilateral in 69.0% of cases and caused no vision loss in 80.6% (Table 2).

Table 1 Diagnosis of congenital toxoplasmosis

Criteria for diagnostic suspicion		
Toxoplasmosis on gestation	Maternal diagnosis precision	Proven Unproven Unlikely
	Gestational time of infection (weeks)	<13 GW 13–35 ≥36
	PCR on amniotic fluid	Negative Positive
The presence of clinical findings (isolate or on combination) in the first age months	Ocular	Nystagmus or strabismus Posterior segment abnormalities (retinochoroiditis, optic nerve atrophy) External eye abnormalities (microphthalmus, cataract)
	Intracranial	Microcalcifications (particularly spotty distributed) Ventricular dilatation or obstructive hydrocephalus
	Neurological	Seizures, EEG, and/or CSF abnormalities☼ Microcephaly, macrocephaly tone, or motor dysfunction
	Systemic (TORCH syndrome)	rash Hepatosplenomegaly Anemia, thrombocytopenia
Newborn screening	Toxo-IgM and/or IgA positivity	
Onset severity definition		Careful pediatric examination Neurologic evaluation* Expert ophthalmologic examination*°╦ Brain computed tomography scan╧ Brain US or 45-s two-sequence MRI (follow-up) ♣ Auditory brain stem response#
Infection status definition	Indirect criteria	Toxo-IgM♪ and IgA♫ Toxo-IgG increase or stabilization Toxo-IgG persistence beyond age 12 months Comparative analysis mother/newborn Toxo-IgG/IgM/IgA IB Toxo-IgM triplet IB (75, 90,100 kDa) IFN-ɣ release after 24-h stimulation with crude Toxo-antigen
	Direct criteria (not routinely)	Parasite isolation or positive inoculation on mice or tissue culture (placenta) Parasite DNA demonstration (PCR on amniotic or cerebrospinal fluid)

Legend: ♪ EIA and ISAGA-IgM; ♫ ELISA-IgA; * data sheets and narrative evaluations for each child at each examination; ° appropriate examination includes cycloplegia and under-sedation indirect fundoscopy to detect both central and peripheral lesions; ╦ fundus photographs and optical coherence tomography (OCT), whenever the patient cooperation allowed; ╧ without contrast medium enhancement. Brain computed tomography scan sensitivity has reported five times higher than ultrasonography (US) as interobserver agreement. ♣ US is widely practiced to monitor short-term treatment effect; # only on infected infant

Table 2 Treatment of Tg infection in pregnant women and congenital toxoplasmosis (CT)

Mother	Treatment	Dosage	Indication	Comment
	Spiramycin	1 g (3 million U) every 8 h (for a total of 3 g or 9 million U per day)	a. Pregnant women suspected of having acquired the infection <16 AW; b. unproven maternal diagnosis (any AW); c. negative amniotic fluid PCR and negative US at follow-up	Until delivery, not teratogenic
	Pyrimethamine (P), sulfadiazine, and folinic acid	(P) Loading dose: 50 mg each 12 h for 2 days; then 50 mg daily; *sulfadiazine* loading dose: 75 mg/ kg, followed by 50 mg/kg every 12 h (maximum, 4 g daily); *folinic acid*: 10–20 mg daily (during and 1 week after (P) therapy completion)	a. Women with proven infection acquired ≥17 AW; b. women with suspected infection acquired ≥30 AW; c. documented fetal infection (positive result of amniotic fluid PCR or abnormal US)	(P) is teratogenic; half-life is 100 h. Complete blood count twice a week (reversible neutropenia) (a) (b) (d)
	Pyrimethamine (P), sulfadoxine, and folinic acid	2 tablets (50 mg (P) 500 mg *sulfadoxine each tablet*) each 10 days; *folinic acid*: 50 mg weekly	a. Women with proven infection acquired ≥17 AW; b. women with suspected infection acquired ≥30 AW	Sulfadoxine half-life is 200 h. Potential for lethal hepatotoxicity (a) (b) (d)
CT▼	*Gold standard* pyrimethamine (P), sulfadiazine, and folinic acid	(P) Loading dose: 2 mg/kg every 12 h for 2 days; then 1 mg/kg daily for 2 or 6 months; then same dose each alternate day or half dose daily; *sulfadiazine*, 50 mg/kg every 12 h; *folinic acid*, 25 mg twice a week (during and 1 week after completion of (P) therapy)	2-month high-dose regimen on subclinical onset; 6-month high-dose regimen on symptomatic onset	1-year treatment; (P) half-life on infant is 60 h. CSF levels are 10–20% of concomitant serum levels (a) (b) (c)
	Alternative protocol Pyrimethamine (P), sulfadoxine, and folinic acid	(P) 1,25 mg /kg – sulfadoxine 25 mg / kg each 10 days; folinic acid, 50 mg weekly	Pre-regimen with (P) and sulfadiazine for 2 months. Loading dose: 1 mg/kg every 12 h for 2 days; then 1 mg/kg daily; *sulfadiazine*, 50 mg/kg every 12 h for 2 months; then P-sulfadoxine	10–24 months; sulfadoxine half-life on infant is 60 h (a) (b) (c)
	Prednisone	0.5 mg/kg each 12 h	a. CSF protein ≥1 g/dL; b. active chorioretinitis vision threatening; c. systemic findings	Until inflammatory marker subsides (1–2 weeks apart)

Footnotes Before starting sulfonamides, check for G6PDH deficit. Infants have weighed weekly and medications, made fresh each week, and are dosed based on increasing weight each week. Neutrophil counts should have been measured via heel prick once a week while taking (P), and in the week after, this regimen has discontinued. Care is taken to make certain that the baby's teeth are cleaned after medicine administration because of the sugar-suspending agents leading to the development of dental carries. Most children have an absolute neutrophil count 900–1200/mm3 during treatment. When neutrophil count is less than 1000 neutrophils/mm^3, a manual differential counting 500 white blood cells has performed to improve accuracy. (a) Sulfonamide discontinuation if hypersensitivity sign occurs, including rash, Stevens-Johnson, asthma, and micro-hematuria with urolithiasis; continue with (P) alone, (b) urine alkalinization, and diuresis maintenance; (c) on absolute neutrophil count (N) <1500/mm^3, double folinate dosage and repeat count; on N count <1000/mm^3, triple folinate (20 mg/day) and half (P) until N >1000/mm^3; and on N count <500/mm, stop P and S until N >1000/mmc. ▼None is manufactured as pediatric formulation. Seizure can occur on (P) regimen as overdosage consequence. (P) half-life is reduced while phenobarbital is administrated.

More severe OT (frequency, size, and multiplicity of retinochoroidal lesions) was shown in CTgI children in Brazil compared with EU, which might be a consequence of more virulent genotypes predominating there, but rare in Europe (Gilbert et al. 2008).

Sensorineural hearing defect is still associated with CT and neurological impairment.

In conclusion, on countries, performing screening CTgI displays mostly a sight-threatening eye disease in which occurrence of lesions during postnatal treatment contrasts with the regression or even disappearance of cranial lesions and in which long-term follow-up to adolescence is necessary. Ophthalmologic surveillance should be more active in cases with cranial findings. Little has known about the consequence of visual impairment on life quality and comprehension subscale upon which impaired vision might have impact. Further follow-up in recruited cohorts could allow better knowledge of long-term learning disabilities and behavioral problems in congenitally infected patients with and without intracranial lesions, namely, on speech development.

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