

Current Approaches to Prevent and Diagnose Congenital Toxoplasmosis: the Italian Experience Savremeni pristup prevenciji i dijagnozi kongenitalne toksoplazmoze: italijansko iskustvo

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Summary Congenital toxoplasmosis is an important cause of morbidity and mortality in congenitally infected newborns: even if the classic triad of hydrocephalus, intracranial calcifications and chorioretinitis is almost a remnant from the past, the infection can still cause retinal disease during childhood or adolescence. Preventive strategies and a correct diagnosis in pregnant women and in newborns are very important. The infection usually runs asymptomatic and the diagnosis relies primarily on serological tests. In pregnant women the differential diagnosis between early seroconversion and false positive IgM is always puzzling. The diagnosis of congenital toxoplasmosis at birth is usually a complicated task; the decision to treat with potentially harmful compounds relies exclusively on laboratory findings (either the detection of anti-Toxoplasma IgM and IgA, or the detection of persistent production of specific IgG during the first year of life) and their interpretation can be challenging when they give heterogeneous results. Researchers are trying to develop new tests that exploit the cellular immunity involved during the course of the infection like an interferon-gamma release assay similar to the Quantiferon for *Mycobacterium tuberculosis*.

Key words: Toxoplasmosis, diagnosis, IGRA test, newborns, pregnancy

Sažetak Kongenitalna toksoplazmoza je ozbiljan uzrok morbiditeta i mortaliteta inficirane novorođenčadi, i čak i kada klasičan trijas nije prisutan: hidrocefalus, intrakranijalne kalcifikacije i hiororetinitis, moguće je da se razvije retinalna bolest tokom djetinjstva ili adolescencije. Preventivne strategije i pravilna dijagnoza kod trudnica i novorođenčadi je od velike važnosti. Infekcija najčešće protiče asimptomatski i dijagnoza se postavlja serološki. Kod trudnica diferencijalna dijagnoza između rane serokonverzije i lažno pozitivnog IgM je uvek diskutabilna. Postavljanje dijagnoze na rođenju je komplikovano; odluka da se započne lečenje potencijalno štetnim agensima zasniva se prvenstveno na laboratorijskim nalazima (detekcija anti-Toxoplasma IgM i IgA, ili detekcijaperzistentne produkcije specifičnog IgG tokom prve godine života). Tumačenje nalaza je vrlo problematično, pošto su rezultati često heterogeni. U toku su istraživanja da se razvije test koji će ispitivati celularni imunitet uključen u infekciju kao što je interferon-gamma povezan sa Quantiferon testom za *Mycobacterium tuberculosis*.

Ključne reči: Toxoplasmosis, dijagnoza, IGRA test, novorođenčad, trudnoća

Introduction

Toxoplasma gondii (*T. gondii*) is a worldwide prevalent parasite: it is believed that approximately 25% to 30% of the global human population is infected by *Toxoplasma*. Prevalence varies widely between countries, and often within a given country or even among different communities in the same region (1). Average IgG seroprevalence in women of childbearing age ranges between 19.4 and 43.8% in Europe (2). Knowledge of the epidemiology of the infection in a given area is very important in order to introduce adequate preventive measures. Seroprevalence in Italy was found to be 23.6% among women of reproductive age, according the last study of this type conducted in Italy (3).

Despite the absence of a national register of congenital infections, 1-2 congenital toxoplasmosis cases per 10000 births per year are currently estimated in Italy (4). Therefore, Italy provides a free voluntary serological screening for toxoplasmosis in pregnancy supported by the public health system.

The majority of human infections are caused either by the consumption of raw meat contaminated with tissue cysts or by ingestion of soil, water or food contaminated by sporulated oocysts. Less frequently the infection is acquired directly from feline faeces.

Any meat from warm-blooded animals and birds has been traditionally considered a major source of infection, the risk of infection however is very different and depends on the type of meat (susceptibility of each species), how the animal is raised (indoor or outdoor, animal friendly facilities), age of the animal, parts of the animal that are eaten, post slaughter treatments (heating or freezing) (5): any cooking will anyway kill the parasite.

An adequate dietary education of the woman who is susceptible to *T.gondii* infection is a mandatory step in the prenatal follow-up of the pregnant woman and should be particularly focused on the less frequent routes of infection, given the general knowledge of women about traditional ways of acquiring the infection (6).

Maternal infection

Typically congenital infection results when primary infection is acquired during pregnancy. Frequency of vertical transmission and severity of the disease depend on the gestational age at maternal infection: the placental barrier is more efficient at the beginning of gestation, leading to transmission of the parasite in 10% of cases during the first trimester, but becomes more permeable as pregnancy progresses, with transmission rates of 30% during the second trimester and 60-70% during the last trimester.

The severity of foetal infection is instead inversely correlated with gestational age. When transplacental transmission occurs during the first trimester, the consequences are heavy, often leading to severe abnormalities or abortion (7). Neonates are instead asymptomatic in 80% of cases when infected during the third trimester of gestation (8).

Retinochoroiditis is the most particular clinical manifestation of congenital toxoplasmosis, as it can be observed whatever the time of maternal infection and it is frequently characterized by a delayed clinical expression after birth. If infection is suspected to have occurred during the first trimester of pregnancy, spiramycin is the drug of choice.

The usual dose in adults is 9 million IU divided in three times per day. The drug can clear the parasite from the blood and reduces the frequency of placental infection: if the treatment has been introduced early during the first trimester, it reduces the incidence of placental infection from 89%-90% to 75% (9). It is considered to be efficient when given within 4 weeks after maternal infection (10).

Due to the fact that it is not parasitocidal and to its limited ability to cross the placenta, its use is not recommended if foetal infection is confirmed and a switch to pyrimethamine/sulfadiazine regimen is recommended (11).

Materials and methods

Strategy for Literature Search

The available literature on the diagnosis of *T. gondii* infection was obtained by search on Pubmed. The literature was restricted to English and Italian language, and searched from their inception until December 2017 with the key words "Toxoplasma gondii" or "toxoplasmosis" or "*T. gondii*" combined with "diagnosis".

Discussion

Diagnosis in the mother

First level tests include measurement of IgG and IgM in the blood ideally before conception, otherwise as soon as the woman realizes to be pregnant.

The interpretation of positive anti-Toxoplasma IgM is challenging: IgM can persist in the blood for months or years after infection, and isolated positive IgM may be an unspecific reactivity against low molecular weight antigens of the parasite, referred to as "natural IgM production" (12). Isolated IgM positivity is also believed to result from cross reactions due to other viral infections, such as cytomegalovirus (CMV) or Epstein-Barr virus (EBV). As a general rule they should never be used alone as a sign of acute infection but must be tested in parallel to IgG (13).

Anti-Toxoplasma IgG antibodies are detectable 2 to 4 weeks after infection, they reach a peak in 2 or 3 months, then they stabilize at a plateau for several months before starting a slow decline until reaching very low levels that persist throughout life. Positivity of IgG and negativity of IgM is a marker of proven immunity and women with these findings do not need to be treated or to be tested for antibodies during pregnancy.

Avidity is a second level test that was developed in order to give a better temporal estimation of the infection. This test measures the strength of the antigen-antibody binding complex, which increases during the course of the immune response: a high avidity index allows to exclude an infection acquired in the last 12- 16 weeks. If high avidity is detected in the first trimester of pregnancy, it can be excluded that the infection was acquired after conception. Low avidity values instead have no diagnostic significance, as low IgG titres may persist for long time. Furthermore late gestational period at the time of infection and treatment initiation may contribute to delay avidity maturation (14, 15).

The prenatal diagnosis of foetal infection is based on ultrasound scans and PCR testing of amniotic fluid. Amniotic fluid PCR testing should be offered to all those women who seroconvert during pregnancy or who display abnormal findings on ultrasounds. Amniocentesis should be carried out after 18 weeks of gestation and at least 4 weeks after the estimated date of maternal infection: in order to detect

parasitic DNA in the amniotic fluid the parasite must be able to reach the foetus (16).

Infection in newborns

Congenital toxoplasmosis is considered a chronic ophthalmologic and neurologic disease because recurrences and relapses of ocular lesions can occur throughout life. Ocular lesions may develop in treated children at any age: they are more frequent before 5 years of age, but may be detected even more than 12 years after birth (17). Therefore all affected individuals should undergo regular ophthalmologic follow-up in early childhood and, when being able to report visual symptoms, at least until the age of 10-12 years of life.

Diagnosis in newborns

Laboratory evaluation of the newborn infant should be undertaken when either maternal infection during pregnancy has been suspected or documented or when the newborn presents clinical manifestations suggestive for congenital toxoplasmosis. Anti-toxoplasma IgG antibodies are not a reliable marker of congenital infection in newborns, as they only reflect the placental transfer of maternal IgG. Given the fact that IgA and IgM cannot cross the placenta, their presence in newborn blood could demonstrate congenital infection (18).

Some newborns with congenital infection may be negative for IgA and IgM: in these patients it is essential to determine if IgG present in their blood are due either to maternal transfer or to the infant's own immune response. They undergo monthly measurements of IgG titres up to 1 year of age, as maternal antibodies in non-infected offspring decrease by approximately half each month to finally become negative (14).

Western blots of paired maternal and baby sera have been developed to determine if antibodies recovered in the infant derive from maternal transfer or not: The specificity of IgG or IgM present in the serum of infected infants could be different from the mother's and demonstrates that antibodies formed by the foetus in response to the congenital infection may recognize different *T.gondii* antigens than do antibodies formed by the mother and therefore are confirmatory of congenital infection (19).

When congenital infection is confirmed, the newborn must be treated immediately and must then be followed with regular serologic and clinical assessments at 1 month of age and then every three months. Its serologic status should reflect a progressive decline of maternally derived IgG and the active production of its own IgG. A rebound of IgG titres has been frequently observed in infants after treatment discontinuation (20): serologic rebound is not associated to

the risk of intracranial calcifications, or to unfavourable ocular outcomes.

We must always take into account that a negative workup at birth cannot exclude congenital toxoplasmosis: disease presentation is subclinical in most of the cases, and results of serology are often unreliable in early infancy. The widely accepted criterion to rule out congenital infection in untreated infants is the persistent decline in specific IgG up to their negativization in the first year of life, demonstrating that the foetus has eliminated maternally transmitted IgG and has not synthesized his or her own (6).

Toxoplasma gondii can be detected at birth in blood, CSF, urine or placenta by using a PCR assay: the most used method is the detection of parasite in the placenta with sensitivities and specificities of 79.5% and 92%, respectively (21).

The diagnosis of congenital toxoplasmosis at birth is usually a complicated task; the decision to treat with potentially harmful compounds relies mostly on laboratory findings, and their interpretation can be challenging when they give heterogeneous results. There is therefore the need to develop more reliable and precise tests to confirm or exclude this serious condition in the newborn.

Discussion: IGRA test, a promising tool

The demonstration of the importance of host cellular immunity in the control of *T.gondii* infection, with the production of IFN- γ mainly by sensitized T-cells mirrors the immune response in the course of the infection with *Mycobacterium tuberculosis*. From the similarities between these two intracellular pathogens emerged the idea of exploiting IGRA tests (Interferon Gamma Release Assays) routinely used for the diagnosis of latent tuberculosis infection for a more precise diagnosis of toxoplasmosis. (22,23).

Therefore an in vitro miniaturized assay has been developed to measure IFN- γ responses of T-cells that had been stimulated by *T.gondii* antigens. The test requires only 1 ml of whole blood and therefore it is suitable for all patients, including newborns in whom it is usually difficult to collect big amounts of blood. Moreover, in addition to the IFN- γ release assay, serological tests can be performed using the same blood sample.

Chapey and Peyron investigated the validity of IGRA test for Toxoplasmosis applying it to a newborn population. They were able to demonstrate that infected infants displayed a significantly higher IFN-gamma production when their blood samples were stimulated by crude toxoplasmic antigens, with respect to uninfected newborns. They reported a sensitivity and specificity of 94 and 98%, respectively (24,25).

This test is a simple and easy-to-perform tool for the diagnosis of congenital toxoplasmosis in newborns: it could help to rule out a potential infection and avoid unnecessary drugs and follow up in infants. However, it is still an experimental test: new data are needed to set up standardized cut-offs. Furthermore, an application to pregnant women would be extremely useful in order to manage all those puzzling serological profiles. According to previous studies, which demonstrated that the specific T cell response to parasitic antigens is reduced in young children (26), a different cut-off should be applied for those 2 population of patients given the weaker immune system of infants, with respect to adult women.

Conclusion

The assessment of IFN- γ may help to interpret an ambiguous serological status which may be encountered during pregnancy. Furthermore it allows to treat truly seropositive patients and to stop treatment in those who are not infected, thus avoiding the potential side effects associated to therapy. This is especially important for newborns, who, in case of infection, should take pyrimethamine-sulfadiazine-folinic acid for at least one year after birth, with the risk of developing neutropenia. Being able to treat only those pregnant women who are for sure infected allows us to avoid, when not needed, the discomforts associated to the spiramycin or pyrimethamine-sulfadiazine-folinic acid regimens, which must be taken by the mother three times a week until birth in most instances. Further studies are needed in order to make IGRA tests a part of routine laboratory work-up for the diagnosis of *T. gondii* infection.

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Priljeno/Received: 07.03.2018

Prihvaćeno/Accepted: 20.03.2018.

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