

NEWS IN THE DIAGNOSIS OF THE OCULAR TOXOPLASMOSIS

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Abstract: Biological, serologic, histological or molecular assays are used for the diagnosis of *Toxoplasma gondii*. There are a lot of serologic tests for the detection of *Toxoplasma gondii*, but they are still not enough. New research continues to be done. We consider the IgG avidity test as a new valuable method for the diagnosis of a recent infection. A rapid, sensitive, DNA quantitative reaction is the polymerase chain reaction (PCR) with fluids or tissues, including the amniotic liquid. Real-time PCR has recently been introduced for the diagnosis of toxoplasmosis. It combines the steps of amplification and PCR product detection in a single phase, thereby shortening the turnaround time from 24 to 48 h to less than 4 h. DNA extraction methods have also recently been automated. Using magnetic-bead technology, DNA can be purified from a variety of clinical samples, while the DNA *toxoplasma gondii* fragments are analysed with the help of the polymerase chain reaction.

Keywords: *toxoplasma gondii*, retinochoroiditis, PCR.

Rezumat: Diagnosticul toxoplasmozei oculare se practică prin metode biologice, serologice, histologice, ori moleculare. Există numeroase proceduri serologice pentru detecția umorală a anticorpilor, cu limitele lor. Se cercetează în continuare și se caută noi teste. Considerăm ca metodă nouă de mare valoare testul de aviditate IgG care a fost descoperit pentru a ajuta la discriminarea infecției recente. O reacție rapidă, sensibilă, cantitativă este reacția de polimerizare în lanț cu fluide ale corpului sau țesuturi, inclusiv lichid amniotic. PCR (polimerizarea în lanț) în timp real, introdusă de curând, pentru diagnosticul toxoplasmozei, combină pașii amplificării și detecției într-o singură fază, reducând astfel timpul de la 24- 48 ore la 4 ore. Extragerea de ADN a fost automatizată. Utilizând tehnologia magnetică, ADN-ul poate fi purificat dintr-o varietate de probe, fragmentele de ADN *toxoplasma gondii* fiind analizate prin PCR.

Cuvinte cheie: *toxoplasma gondii*, corioretinita, polimerizare în lanț

INTRODUCTION

The tests of the ocular toxoplasmosis are: biological, serological, histological or molecular.

There are serological procedures for the humoral detection of the antibodies: the colour test, the dye-test or the Sabin-Feldman reaction; the indirect fluorescence

reaction which measures the specific IgG occurring 1-2 weeks after the infection, Remington test for IgM, ELISA-double sandwich, which isolates IgM from IgG and the rheumatoid factor, IDR with toxoplasmin.

A rapid and sensitive test in order to detect the infection with *Toxoplasma gondii* through PCR for a better prognostication with 2 DNA fragments, for example, gene B1 and the repetitive element 529-bp, which exist in 200-300 copies/genome.

Through the use of PCR, the genes TGR1E, respectively TGR1E-1, TGR1E-2 (with standard PCR) and genes B1, respectively TM1, TM2, TM3 (PCR with internal control) have been detected in the biological assays of *toxoplasma gondii* DNA.

The PCR positive results are confirmed through ELISA and RFC tests. The rapid real-time PCR examination, for the confirmation of an acute parasitemia with *toxoplasma gondii* is important especially for the patients to whom this infection may cause severe consequences, for example to fetuses or to the patients with immunodeficiency.

The excretion-secretion antigens of the tachyzoites which are also expressed by the bradyzoites have been emphasized: P24 and P25. They belong to a class of molecules stored in dense granules in *toxoplasma* and are discharged in the parasitophore vacuoles during the invasion of the host cell.

The testing of the ocular fluids must be taken into consideration for the patients with atypical features of chorioretinitis, for those with extended injuries or for those with resistance to the usual medicines.

These conditions are often encountered in the immunodepressed patients and in the old patients, as well.

Anterior chamber paracentesis is much more convenient than the vitreal puncture. However, the analysis of the aqueous humor may be suggested 2-3 weeks after the occurrence of chorioretinitis, when the specific antibodies have been produced and are PCR detectable.

During the last years, the IgG avidity test with serum, the PCR with fluids selected from the body or tissues, the immunoelectrophoresis of the umbilical cord serum are considered new methods of great value.

These methods are also useful for all categories of patients with *toxoplasma gondii*.

CLINICAL ASPECTS

The IgG avidity test was developed to help discriminate between past and recently acquired infection. Results are based on the measurement of the avidity (functional affinity) of toxoplasma-specific IgG antibodies. Following an antigenic challenge, the antibodies produced usually have a low average affinity. During the course of the immune response, there is a maturation of antibody affinity that increases progressively over weeks or months. The increase in IgG affinity results from an antigen-driven B-cell selection process, resulting in an increase in the complementarity of the antigen-antibody-binding site. Depending on the method used, the avidity tests, currently available, are helpful primarily to detect whether a patient's infection occurred in the last 4 or 5 months. This is most useful in pregnant women in their first months of gestation who have a positive test for both IgG and IgM toxoplasma antibodies. For example, a woman who has a high avidity test result in her first trimester did not acquire the acute infection in the last 3 months. Therefore, since her infection was acquired prior to gestation, her fetus is essentially not at risk (the likelihood of congenital transmission as a result of an infection acquired in the weeks before or near the time of conception is extremely low, approaching zero). This test reduces the number of pregnant women treated with spiramycin.

The antenatal diagnosis of congenital toxoplasmosis is set after the scheme: testing anti-toxoplasma gondii IgM of the fetal blood sampled under ultrasonic guidance, from the umbilical cord, at the insertion place of the placenta; the PCR of the amniotic fluid obtained through amniocentesis. If initially, it was observed that the immunoelectrophoresis of the mother and of the new-born were identical, the recent studies confirmed that the antibodies produced by the fetus, as an answer to the toxoplasma gondii antigens can be different from the mother's.

Serological follow-up 1 year after the congenital toxoplasmosis, through the standard serological tests and special tests on the first and fifth day of life and afterwards, 1 and 3 months later, the IgG and IgM studies revealed that through the ISAGA test, the specificity and the sensitivity of the results were significantly higher. The sensitivity of the precocious diagnosis reached 91,3% if both types of tests were used.

The PCR has been successfully used to diagnose congenital and ocular toxoplasmosis and toxoplasmosis in immunocompromised patients. For this purpose, PCR with amniotic fluid, placental and brain tissues, whole blood, cerebrospinal fluid, urine, vitreous fluid, aqueous humor, bronchoalveolar lavage fluid, and pleural and peritoneal fluids have proved their value.

The most common use of PCR is for the prenatal diagnosis of the congenital infection, using amniotic fluid. Most laboratories use the 35-fold-repeated B1 gene. Some laboratories in Europe are switching to the AF146527 sequence, a DNA fragment that is repeated 200- to 300-fold in the *T. gondii* genome (11, 18). An evaluation of three targets (18S ribosomal DNA, B1, and [AF146527](#)) in

parallel has recently been performed. There is a large variety of protocols used by different laboratories which use the PCR conventional procedure (extraction, amplification, detection). Different procedural steps were reported by different groups of researchers, including the DNA extraction, gene selection, the use of the uracil-DNA-glucosylase enzyme in order to prevent the contamination of the carrier, the size of the obtained PCR, the use of PCR with internal control.

Prenatal diagnosis of congenital toxoplasmosis is primarily based on ultrasonography and PCR with amniotic fluid. PCR with amniotic fluid for the detection of *T. gondii*-specific DNA performed in the 18th week of pregnancy is more sensitive, more rapid, and safer than conventional diagnostic procedures involving fetal-blood sampling.

Real-time PCR has recently been introduced for the diagnosis of toxoplasmosis. It combines the steps of amplification and PCR product detection in a single phase, thereby shortening the turnaround time from 24 to 48 h to less than 4 h. Real-time PCR uses a fluorescence-labeled oligonucleotide probe, which eliminates the need for post-PCR processing. DNA extraction methods have also recently been automated.

Using magnetic-bead technology, DNA can be purified from a variety of clinical samples, thereby eliminating the need for centrifugation, vacuum pumps, and other steps with high risk for contamination. It is likely that the use of real-time PCR and automated methods for DNA extraction will result in a decrease in the interlaboratory variability observed with the conventional three-stage PCR.

In addition, real-time PCR can be used to estimate the concentration of parasites in the amniotic fluid, which may be helpful for physicians to assess the neonatal outcome.

The diagnosis of toxoplasma gondii can also be set on tissues obtained through biopsy or necropsy. A rapid diagnosis can be set through the microscopic examination of the existing impression by spreading the injury on the blade. Toxoplasma gondii is detected through its half-moon form.

The infection spread in the body through blood may be transmitted from brain to eye through the optic nerve.

In conclusion, current laboratory methods for confirmation of toxoplasmosis include (i) direct detection of the parasites in tissues or body fluids, by using histological, Giemsa, or immunofluorescence stains or nucleic acid amplification techniques; (ii) isolation of the protozoan in mice or tissue culture; and (iii) investigation of *T. gondii* anti-immunoglobulin M (IgM), -IgG, -IgA, and -IgE antibodies in serum and intraocular fluids. Sensitivity and specificity may vary greatly between laboratories and applications.

The detection of *Toxoplasma gondii* DNA is under research in all well equipped laboratories in the world, in order to establish a future full diagnosis of this disease.

BIBLIOGRAPHY

1. Chai JY, Lin A, Shin EH et al. Laboratory passage and characterization of an isolate of *Toxoplasma gondii* from an ocular patient in Korea. *The Korean Journal of Parasitology* 2003;41(3):147-154.
2. Costa J M, Ernault P, Gautier E et al. Prenatal diagnosis of congenital toxoplasmosis by duplex real-time PCR using fluorescence resonance energy transfer hybridization probes. *Prenat Diagn* 2001; (21):85-88.
3. Edvinsson B, Lappalainen M, Evengard B. Real-time PCR targeting a 529-bp repeat element for diagnosis of toxoplasmosis. *Clinical Microbiology and Infection* 2006;(12):131.
4. Filisetti, D, Gorcii M, Pernot-Marino M and al. Diagnosis of congenital toxoplasmosis: comparison of targets for detection of *Toxoplasma gondii* by PCR. *J Clin Microbiol* 2003;(41):4826-4828.
5. Hofflin JM, Remington JS. Tissue culture isolation of *Toxoplasma* from the blood of a patient with AIDS. *ArchIntern Med* 1985;(145):925-926.
6. Howe DK, Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of a parasite genotype with human disease. *J Infect Dis* 1995;(172):1561-1566.
7. Kaul R, Chen P, Binder SR. Detection of Immunoglobulin M Antibodies Specific for *Toxoplasma gondii* with Increased Selectivity for Recently Acquired Infections. *Journal of Clinical Microbiology*, December 2004;42(12):5705-5709.
8. Miller Darlenne, Davis Janet, Rosa R et al. Utility of Tissue Culture for Detection of *Toxoplasma gondii* in Vitreous Humor of Patients Diagnosed with Toxoplasmic Retinochoroiditis *Journal of Clinical Microbiology* 2000;38(10):3840-3842.
9. Montoya JG. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *J Infect Dis* 185:S73-82.
10. Nielsen HV, Lauemoller Sanne Lise, Christiansen L et al. Complete Protection against Lethal *Toxoplasma gondii* Infection in Mice Immunized with a Plasmid Encoding the SAG1 Gene Infection and Immunity, December 1999;67(12):6358-6363.
11. Remington JS, Thulliez P, Montoya JG. Recent Developments for Diagnosis of Toxoplasmosis *Journal of Clinical Microbiology* 2004;42(3):941-945.