Screening for toxoplasmosis during pregnancy: One-year experience in an Italian reference laboratory

Triagem para toxoplasmose na gestação: um ano de experiência em um laboratório de referência italiano

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ABSTRACT

Aims: To describe the experience of the Toxoplasmosis Laboratory of Infectious Disease Department University of Pavia, IRCCS Foundation, San Matteo Polyclinic Pavia, a reference laboratory for diagnosis of toxoplasmosis, in the investigation of women with suspected acute toxoplasmosis. **Methods:** All sera were tested with LIAISON® Toxo IgM and IgG II, Toxo IgG Avidity II kits (DiaSorin, Saluggia, Italy), VIDAS Toxo IgG II and Toxo IgG Avidity (bioMérieux, Marcy l'Etoile, France), IgM ISAGA (bioMérieux, Marcy l'Etoile, France) and ETI-TOXOK-A reverse PLUS (DiaSorin, Saluggia, Italy). When required (IgG negative/IgM positive women), IgG/IgM Western Blot II (LDBio, Lyon, France) was also performed. Prenatal diagnosis on amniotic fluid was done by nested PCR. All newborns were followed up to one year of age in order to exclude or confirm the diagnosis of congenital toxoplasmosis. All pregnant women with acute or undetermined stages of infection were treated. **Results:** In the course of 2007, 236 women with suspected acute (IgM-positive) *Toxoplasma* infection were followed up. In the reference laboratory, 91 women had test results indicating acute toxoplasmosis, and 10 had undetermined status of infection. These 101 patients represented 42.8% of the 236 women referred. Acute toxoplasmosis could be excluded in the remaining 135 patients, of whom 53 were non-immune. Three infected newborns were observed, all from mothers tested for the first time during the third trimester of pregnancy. **Conclusions:** The role of a reference laboratory in suspected toxoplasmosis acquired during pregnancy is crucial to date the infection and discriminate between seroconversion and false positive anti-*Toxoplasma* IgM antibodies. This avoids unnecessary anxiety in immune women, provides correct counseling about primary prevention and periodic testing for seronegative ones, and allows early treatment and follow-up of pregnant women with acute infection and their newborns.

Keywords: TOXOPLASMOSIS, CONGENITAL; TOXOPLASMOSIS/diagnosis; *Toxoplasma gondii*; PRENATAL DIAGNOSIS; PRENATAL CARE; PREGNANCY COMPLICATIONS, INFECTIOUS; CROSS-SECTIONAL STUDIES; REFERENCE CENTERS; FEMALE; PREGNANCY; SCREENING.

INTRODUCTION

Screening for toxoplasmosis is not mandatory in Italy, but National Health Service reimbursement is provided for one test before pregnancy to assess immune status and a monthly follow-up for seronegative women, as well as after confirmed acute infection (DPR 245 10/09/98). Seroprevalence for *Toxoplasma gondii* antibodies has dramatically decreased in the last decades in Italy as well as in many other European Countries.[1] In our region it was 48% in 1981,[2] and now has dropped to 22% for Italian women and 33% for immigrants.[3]

Though screening is not mandatory, risk awareness causes almost 85% of pregnant women to be tested for the first time during the first trimester of pregnancy. Conversely, only few women are tested for *Toxoplasma* antibodies before becoming pregnant, usually only in cases of in vitro fertilization. In addition, most seronegative women undergo serological tests about three times during pregnancy, with a significant

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difference in number of sampling between Italian and immigrant women.[3] At the start of the screening program, 1.7% of women were positive for IgM, and were therefore referred as outpatients to the Infectious Disease Clinic, IRCCS San Matteo Hospital Foundation, Pavia, Italy, for further investigation. Our experience as a reference laboratory from January to December 2007 is described here.

METHODS

The study included all pregnant women with suspected toxoplasmosis acquired during pregnancy referred to the Infectious Disease Clinic, IRCCS San Matteo Hospital Foundation, Pavia, Italy, for further investigation.

At the reference laboratory, all sera were tested with LIAISON® Toxo IgM and IgG II kits (DiaSorin, Saluggia, Italy), VIDAS Toxo IgG II (bioMérieux, Marcy l'Etoile, France), and with the following confirmatory tests: LIAISON® Toxo IgG Avidity II (DiaSorin, Saluggia, Italy); VIDAS Toxo IgG II Avidity (bioMérieux, Marcy l'Etoile, France); IgG/IgM Western Blot II (LDBio, Lyon, France); IgM ISAGA (bioMérieux, Marcy l'Etoile, France); and ETI-TOXOK-A reverse PLUS (DiaSorin, Saluggia Italy). A nested polymerase chain reaction (PCR) (Clonit, Milan, Italy) was performed with target gene AF146527 on amniotic fluid in patients with confirmed acute infections who underwent prenatal diagnosis.

True seroconversion was defined by positive IgM antibodies confirmed with IgM ISAGA test and with type II Western Blot. Patients were managed according to our protocol illustrated in Figures 1 and 2.

All newborns were followed up to one year of age in order to exclude or confirm the diagnosis of congenital toxoplasmosis.[4] All pregnant women with acute and undetermined stages of infection in the second trimester were treated with pyrimethamine, sulfadiazine and folinic acid.

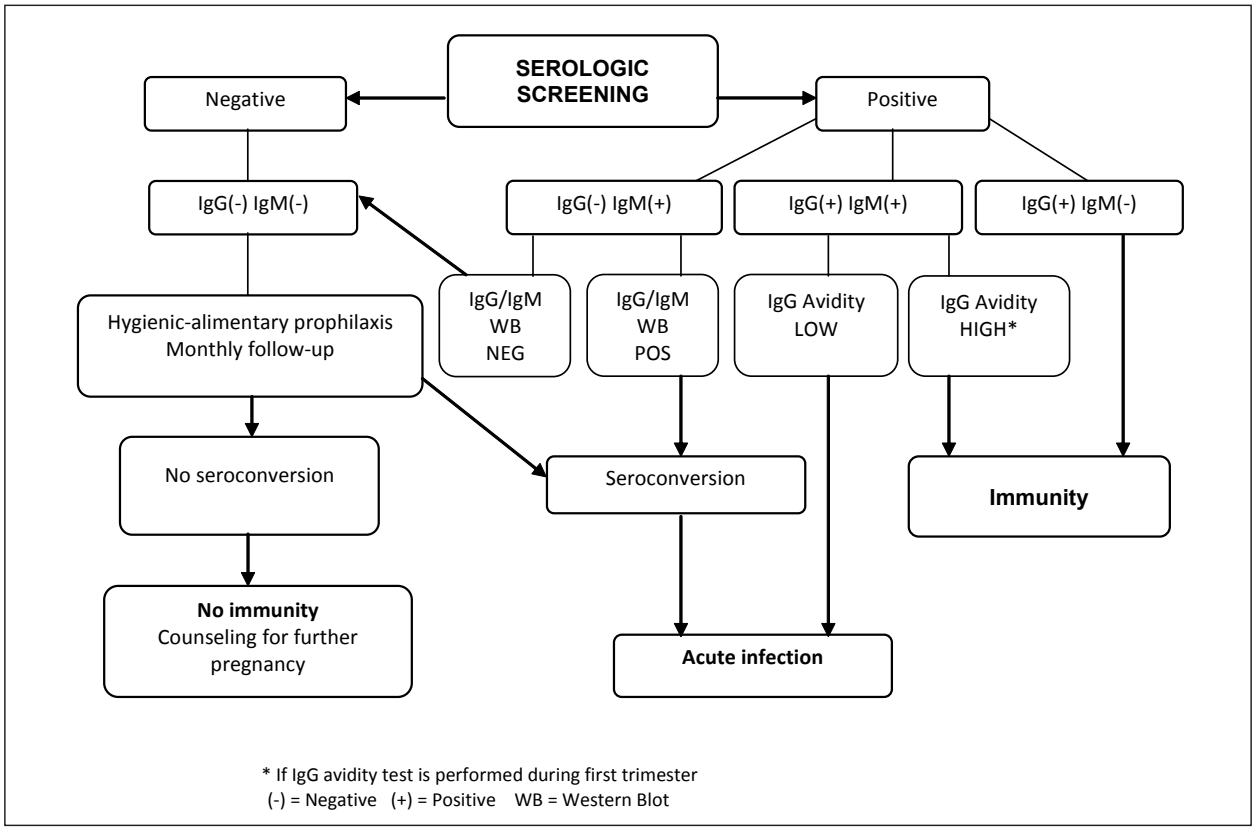

Figure 1. Prenatal serological screening for toxoplasmosis. Protocol of the Infectious Disease Clinic, IRCCS San Matteo Hospital Foundation, Pavia, Italy.

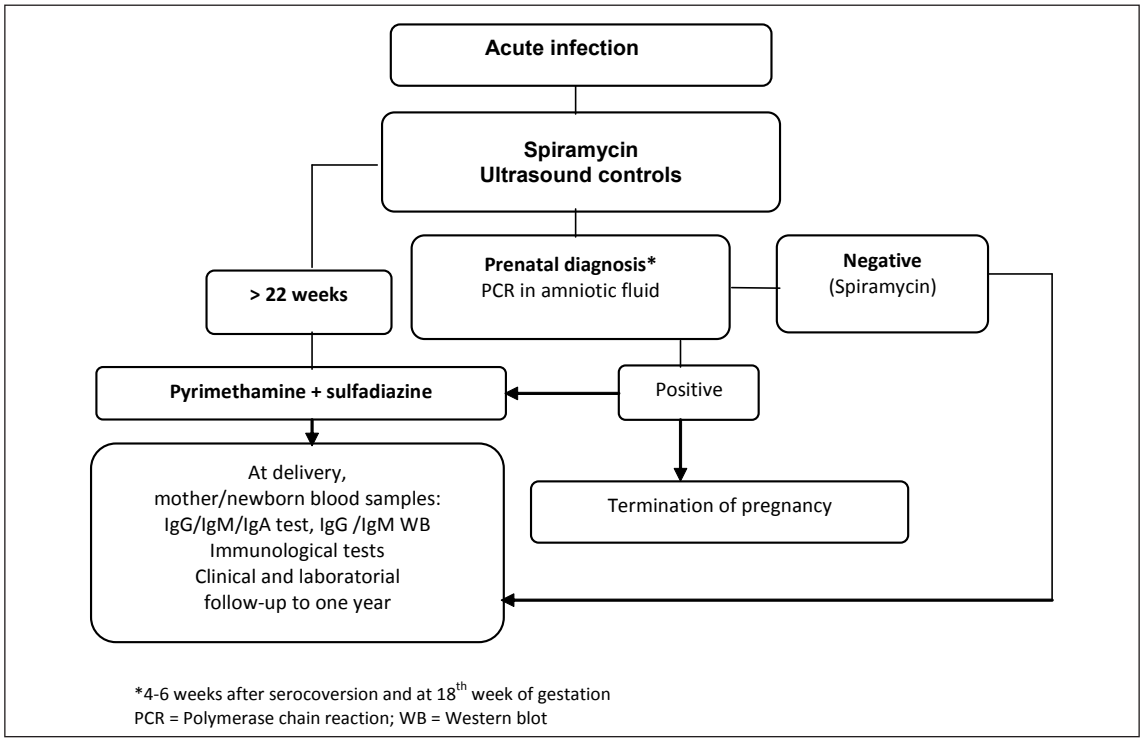

Figure 2. Diagnostic flow-chart for *Toxoplasma gondii* acute infection in pregnancy. Protocol of the Infectious Disease Clinic, IRCCS San Matteo Hospital Foundation, Pavia, Italy.

RESULTS

In the course of 2007, 236 women with suspected acute (IgM positive) *Toxoplasma* infection were followed up in the reference laboratory. Thirty five (15%) were tested before pregnancy, 134 (57%) during the first trimester of pregnancy, 45 (19%) during the second trimester, and 22 (9%) during the third trimester.

In the reference laboratory, 91 women had test results indicating acute toxoplasmosis, and 10 had undetermined status of infection. These 101 patients represented 42.8% of the 236 women referred. Acute toxoplasmosis could be excluded in the remaining 135 patients, of whom 53 were non-immune (Table 1).

Table 1. Results of one year experience on 236 women with suspected acute toxoplasmosis (positive anti-*Toxoplasma* IgM), referred to the Infectious Disease Clinic, IRCCS San Matteo Hospital Foundation, Pavia, Italy (reference laboratory), for further investigation.

Results in the reference laboratory	First test with positive anti-Toxoplasma IgM				Total
	Before pregnancy 35 (15%)	First trimester 134 (57%)	Second trimester 45 (19%)	Third trimester 22 (9%)	
Non Immune *	14	19	11	9	53
Immune †	11	71	0	0	82
Acute Infection ‡	10	44	25	12	91
(cases of confirmed seroconversion in pregnancy ‡)	(0)	(10)	(12)	(11)	(33)
Undetermined status of infection §	0	0	9	1	10
Congenital infection	0	0	0	3	3

* Non immune: IgG/IgM negative.
† Immune: IgG positive/IgM negative or IgG/IgM positive and high IgG avidity index.
‡ Acute infection and confirmed seroconversion in pregnancy as defined in Methods.
§ Undetermined status of infection: patients came too late to the reference laboratory.

Of the 35 women tested before pregnancy, 14 resulted non-immune, 11 immune, and 10 with acute infection (Table 1).

Of 134 sera collected from women in the first trimester of pregnancy, 19 resulted non-immune, 71 with past infection, and 44 with acute infection (Table 1). Twenty nine sera were negative for IgG and positive for IgM, but of these, only 10 patients had true seroconversion (positive IgM antibodies confirmed with IgM ISAGA test and/or with type II Western Blot, as defined in Methods). In these 10 cases IgG appeared, even if its production was delayed and lowered by therapy, as described elsewhere.[5] In 105 sera positive for IgG and IgM, acute infection was ruled out on the basis of high IgG avidity index in 71 women. All women with acute infection were treated with spiramycin and 31 underwent prenatal diagnosis with negative results. No infected newborns were observed in this group.

Forty-five women were tested for the first time in the second trimester of pregnancy, of whom 11 resulted non-immune, 25 with acute infection, and 9 with undetermined status (Table 1). Twenty-three scored negative for IgG and positive for IgM, of whom 12 had confirmed seroconversion (as defined above), and in 11 patients the infection was excluded because of a negative IgM ISAGA test and a negative type II IgG/IgM Western Blot. In all these 11 cases, after therapy discontinuation, no specific IgG appeared. In 13 women, sera scored positive for IgG and IgM with low IgG avidity index. Although a low avidity index is not always related to an acute infection (sometimes the avidity index remains persistently low), we could not exclude acute infection during pregnancy, so we treated and followed up these patients like we did with acute infections.[6] Hence, the possibility of infection occurred during pregnancy was considered in 25 cases, and infection status was undetermined in nine cases that came to our laboratory too late during pregnancy. Nine women underwent prenatal diagnosis and all scored negative. No infected newborns were observed.

Twenty-two women with suspected acute infection were tested for the first time during the third trimester of pregnancy. In the reference laboratory, acute infection was considered in 12 patients, of whom 11 patients were defined as having seroconversion during pregnancy and one woman with low avidity index was treated and followed up as an acute case. In this group we observed one case of undetermined stage of infection (IgG and IgM positive, high IgG index) and 10 negative women. All acute and undetermined stages of infection were treated with pyrimethamine and sulfadiazine. Three infected newborns were observed In this group. (Table 1)

The only newborn that showed severe signs of disease was born from an immigrant mother never tested before the 28th week of pregnancy. This woman was addressed to our laboratory when ultrasound abnormalities (hydrocephalus, cerebral calcifications) suggested *Toxoplasma* infection. High IgG titres and IgM and IgA positive results confirmed the diagnosis of maternal toxoplasmosis, even if IgG avidity index was already high. We could not define the gestational age at which seroconversion occurred and classified this infection as undetermined. The woman had not received therapy until diagnosis was established in our laboratory.

DISCUSSION

Seroprevalence data in pregnant women of our region showed a decrease during the last 30 years like in many other European Countries.[1] This fact may be due to a change in dietary habits, although seroprevalence in transplant recipients is higher (about 50%, Meroni V, unpublished data). Nevertheless, infection may still occur.

All women should be tested before pregnancy to evaluate their immunological status regarding toxoplasmosis, and should receive counselling on toxoplasmosis in pregnancy. Screening system in Italy is efficient in recruiting women in the first trimester of pregnancy, but we need to increase the use of preconceptional screening. Women should be encouraged to perform tests for toxoplasmosis before and during early pregnancy. This screening must be consistently based on specific IgG and IgM detection, and cases suspected of acute toxoplasmosis should be referred to a reference laboratory. This allows to reassure immune women and to avoid unnecessary treatment and follow up of pregnant women and their newborns.

All women were referred to our laboratory for the presence of anti-*Toxoplasma* IgM antibodies. The presence of anti-*Toxoplasma* IgM, however, is not synonymous with acute infection. The use of different tests, that only a reference laboratory can perform, allowed us to exclude acute infection in many cases. These tests have different specificity and sensitivity and were useful to date the infection. For instance, Liaison Toxo IgG is more sensitive and could detect serocoversion earlier than VIDAS Toxo IgG II. IgM ISAGA is more specific than Liaison IgM but could detect anti-*Toxoplasma* IgM too long. IgG/IgM Western Blot II is very specific because employs only purified antigens recognized by the specific antibodies at the beginning of infection.

No infected newborn was observed when infection occurred during the first and second trimesters, or from mothers whose infection in pregnancy was excluded. Unfortunately, for 11 women it was impossible to define the time of infection, because they arrived to the reference laboratory in the last weeks of pregnancy. They were not correctly treated, and among them we recorded the only case of severe congenital toxoplasmosis.

All patients who were referred in an appropriate time received the proper therapy, and in many cases prenatal diagnosis was performed. All PCR negative pregnant women gave birth to uninfected newborns.

Within this scenario, in which acute infection was confirmed or indeterminate in only 42.8% of cases, the role of a reference laboratory in suspected toxoplasmosis acquired during pregnancy is really crucial to date the infection and discriminate between seroconversion and false positive anti-*Toxoplasma* IgM antibodies. This avoids unnecessary anxiety in immune women, provides correct counseling about primary prevention and periodic testing for seronegative ones, and allows early treatment and follow up of pregnant women with acute infection and their newborns.

REFERENCES

1. Elsheikha HM. Congenital Toxoplasmosis: priorities for further health promotion action. Public Health. 2008;122:335-53.
2. Carosi G, Filice G, Meroni V, et al. Congenital toxoplasmosis: a serologic screening of 963 mothers and their children at birth. Int J Biol Res Pregnancy. 1981;2:117-22.
3. De Paschale M, Agrappi C, Belvisi L, et al. Management of *Toxoplasma gondii* screening in pregnancy: light and shade. Microbiol Med. 2008;23:178-83.
4. Remington JS, McLeod R, Thulliez P, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, Wilson CB, et al, editors. Infectious diseases of the fetus and newborn infant. 6th ed. Philadelphia: Elsevier-Saunders; 2006. p. 947-1091.
5. Meroni V, Genco F, Tinelli C, et al. Spiramycin treatment of *Toxoplasma gondii* infection in pregnant women impairs the production and the avidity maturation of *T. gondii*-specific immunoglobulin G antibodies. Clin Vaccine Immunol. 2009;16:1517-20.
6. Sensini A. *Toxoplasma gondii* infection in pregnancy: opportunities and pitfalls of serological diagnosis. Clin Microbiol Infect. 2006;12:504-12.