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
difference in number of sampling between Italian and immigrant women. 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. 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.
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.

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

* 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.

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.
**DISCUSSION**

Seroprevalence data in pregnant women of our region showed a decrease during the last 30 years like in many other European Countries. 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 seroconversion 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