PLACE OF INTERFERON-γ ASSAY FOR DIAGNOSIS OF CONGENITAL TOXOPLASMOSIS

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Abstract: The diagnosis of congenital toxoplasmosis relies mainly on serology. When results are doubtful, pediatricians have difficulties with respect to treatment. We report interferon-γ responses after the stimulation of blood by Toxoplasma gondii antigens in 17 infected infants and 80 infants free of infection. Sensitivity and specificity were 93.75% (95% confidence interval: 67%–99%) and 98.75% (95% confidence interval: 92%–99%), respectively.

Key Words: congenital toxoplasmosis, IFN-γ assay, perinatal diagnosis

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TOXOPLASMA GONDII infects one third of the world population. Infection is usually symptomless except in immunocompromised patients or fetuses. Depending on the stage of pregnancy at the time of maternal infection and also on strain virulence, clinical signs of fetal infection range from fetal loss, severe neurologic or ocular lesions to subclinical infection. Symptom-free newborns, who represent 75% of infants born to mothers who become infected during pregnancy in our cohort, are at risk of developing retinal diseases. Diagnosis at birth comprises mainly cerebral imaging and detection of Toxoplasma-specific immunoglobulin (Ig)M or IgA antibodies, but the sensitivity of the 2 tests combined did not exceed 71.4%. Additional tests, such as mother/child comparative western blot, yield a sensitivity of 78.8% for IgM and IgG. A negative perinatal workup cannot completely exclude a congenital infection. Uninfected infants at risk of congenital toxoplasmosis must undergo regular serologic testing for 1 year until maternally transmitted specific IgG have completely disappeared, ruling out congenital toxoplasmosis. Pediatricians are seeking reliable markers of congenital infection to effectively start the treatment immediately after birth or reassure parents. However, primary infection with T. gondii is known to stimulate production of high levels of interferon (IFN)-γ, a cytokine central to resistance to T. gondii, but few studies have investigated the significance of this cytokine for diagnosing congenital toxoplasmosis.

We previously reported the performance of an IFN-γ assay performed on pellets of blood samples after plasma was removed for serologic tests. This simple, easily performed test yielded a sensitivity and specificity of 94% and 98%, respectively. Here, we present the performances of this test in a prospective cohort of newborns and infants referred to our clinic because their mothers seroconverted for toxoplasmosis during pregnancy.

METHODS

Infants younger than 6 months, born to women who seroconverted during pregnancy and attending our outpatient department at Hôpital de la Croix Rousse, Lyon, France, between January 2010 and September 2014, were tested for toxoplasmosis serology, and an IFN-γ test was performed at their first visit. Maternal seroconversions were detected through monthly serologic testing, which is mandatory in France. In cases of infection, antenatal diagnosis was performed and treatment delivered. Termination was considered only in cases of fetal abnormalities. Congenital workup based on the presence of specific IgM and IgA in the newborn or of nonsynthesized IgG or IgM in mother/infant comparative western blot was performed between 3 and 5 days of life. Congenital infection was ruled out when specific IgG turned negative before month 12.

T. gondii-specific IgG antibodies were detected using AxSYM Toxo IgG microparticle enzyme immunoassays (Abbott Laboratories, IL). Specific IgM were detected using Platelia Toxo IgM enzyme immunoassays (BioRad Laboratories, WA). Anti-T. gondii IgA were detected using an immunosorbent agglutination assay, IgA Toxo-ISAGA (Biomerieux, Marcy-l’Etoile, France) or Platelia Toxo IgA enzyme immunoassay (BioRad Laboratories). Mother/child comparative western blots for specific IgG and IgM were performed with the IgG–IgM western blot kit (LDBIO Diagnostics, Lyon, France).

T-cell Stimulation

(i) T. gondii antigens were prepared by the Laboratory of Parasitology at the Hôpital de la Timone, Marseille. Briefly, T. gondii tachyzoites (RH strain) obtained from ascites of infected OF1 mice were disrupted by 4 freeze–thaw cycles and ultrasonic extraction. The final suspension was filtered through 0.2-μm pore size membranes.

(ii) Samples of 1 mL of peripheral blood were drawn into Vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ) containing lithium heparin anticoagulant. Tubes were centrifuged at 1600g for 15 minutes at room temperature. Plasma was collected for serologic testing and replaced by the same volume of Roswell Park Memorial Institute medium (Sigma-Aldrich, St. Louis, MO). Aliquots of 300 μL of diluted blood were cultured in sterile propylene tubes in the presence of T. gondii antigens at a final concentration of 3 μg/mL. Positive controls comprised a combination of lipopolysaccharide (LPS) and phytohemagglutinin (PHA) at a final concentration of 25 and 5 μg/mL, respectively. Negative controls comprised phosphate buffer solution. All cultures were incubated for 24 hours at 37°C in 5% CO2 in a humidified atmosphere. Culture supernatants were collected from each tube after centrifugation at 1600g for 15 minutes at room temperature and stored at −40°C until IFN-γ assays were carried out.

IFN-γ was assayed using 2 commercial enzyme-linked immunosorbent assay kits (AbCys, Paris, France and Life Technologies, Carlsbad, CA) successively. The optical density of phosphate buffer solution controls was subtracted from that of LPS–PHA-stimulated or antigen-stimulated samples. Results of Toxoferon tests were validated when the optical density of negative controls was below 0.75 and that of positive controls was higher than that of negative controls.

Statistical Analysis

Estimates for sensitivity, specificity and positive and negative likelihood ratios of IFN-γ tests were calculated using VassarStats website (Lowry R. VassarStats: Website for Statistical Computation, http://faculty.vassar.edu/lowry/VassarStats.html).
TABLE 1. Interferon-γ Results in Infants Less Than 1 Month of Age

<table>
<thead>
<tr>
<th>Age of Children When Test Was Performed (d)</th>
<th>Gestational Age at Maternal Infection (wk of Pregnancy)</th>
<th>Gestational Age at Time of Amniocentesis (wk of Pregnancy)</th>
<th>Antenatal Treatment</th>
<th>Congenital Toxoplasmosis Workup at Birth</th>
<th>Treatment of Children at Time of IFN-γ Assay</th>
<th>IFN-γ Assay Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>28</td>
<td>Positive at 32 wk</td>
<td>Spiramycin at 29 wk then sulfadiazine–pyrimethamine at 32 wk</td>
<td>IgM and IgA+, WB not done</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>None</td>
<td>Spiramycin at 37 wk then sulfadiazine–pyrimethamine 3 d after</td>
<td>IgM and IgA−, WB discrepant</td>
<td>None</td>
<td>Noninterpretable</td>
</tr>
<tr>
<td>9</td>
<td>33</td>
<td>None</td>
<td>Sulfadiazine–pyrimethamine at 36 wk then pyrimethamine–sulfadoxine on day later</td>
<td>IgM+, IgA−, WN not done</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>10</td>
<td>35</td>
<td>None</td>
<td>Spiramycin at 36 wk</td>
<td>IgM+, IgA−, WB discrepant</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>None</td>
<td>Spiramycin at 31 wk</td>
<td>IgM and IgA+, WB not done</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>12</td>
<td>19</td>
<td>Positive at 22 wk</td>
<td>Spiramycin at 21 wk then sulfadiazine–pyrimethamine at 24 wk</td>
<td>IgM−, IgA+, WB neg</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>Positive at 28 wk</td>
<td>Spiramycin at 23 wk then sulfadiazine–pyrimethamine at 29 wk</td>
<td>IgM and IgA+, WB not done</td>
<td>None</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>None</td>
<td>Spiramycin at 30 wk</td>
<td>IgM and IgA+, WB discrepant</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>14</td>
<td>32</td>
<td>None</td>
<td>None</td>
<td>IgM and IgA+, WB discrepant</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>21</td>
<td>21</td>
<td>Positive at 29 wk</td>
<td>Spiramycin at 26 wk</td>
<td>IgM and IgA+, WB not done</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>21</td>
<td>31</td>
<td>None</td>
<td>Spiramycin at 34 wk</td>
<td>IgM+, IgA−, WB not done</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td>26</td>
<td>32</td>
<td>Negative at 35 wk</td>
<td>Spiramycin at 34 wk</td>
<td>IgM−, IgA−, WB discrepant</td>
<td>None</td>
<td>Positive</td>
</tr>
</tbody>
</table>

IgM, IgA+ indicates presence or absence of specific IgM and IgA in the infant serum; WB discrepant, presence of neosynthesized IgG or IgM by the infant, which are not observed in the serum of the mother, demonstrating active secretion of antibodies by the infant; WB neg, identical IgG profile in both mother and child or lack of IgM in the infant sample, not in favor of congenital toxoplasmosis.

Ethical Aspect

Since our publication on the IFN-γ assay, it is routinely used for the diagnosis of congenital toxoplasmosis. Parents or legal guardians are informed that results of biological investigations could be used for publication and that they have the right to oppose this.

RESULTS

One hundred and seven infants, aged 4 days to 5 months, born to women who seroconverted during pregnancy, participated in this study. Congenital toxoplasmosis was diagnosed in 17 cases (15.8%) (Table 1); none of them were presented with clinical manifestations at the time of the test.

Eleven children (10%) displayed an invalid test result; no positive response to LPS–PHA as a positive control with a rate of invalidated tests of 5%. We are currently investigating other mitogenic agents. One infected patient displayed spontaneous IFN-γ secretion with no clinical signs of infection or inflammatory disease. Among the 80 interpretable tests performed in uninfected infants, only 1 scored positive then turned negative 1 month later. The infant had a normal clinical examination, maternal infection occurred at the end of the first trimester, and amniocentesis was negative. The only false-negative case was sampled erroneously on an EDTA-coated tube; whether this additive explained the wrong result remains unexplained.

In the workup of newborns and infants at risk of congenital toxoplasmosis, the performances of the test in this prospective study were comparable with those we reported previously. Initially, we only used PHA as a positive control with a rate of invalidated tests of 5%. We are currently investigating other mitogenic agents. One infected patient displayed spontaneous IFN-γ secretion with no clinical signs of infection or inflammatory disease. Among the 80 interpretable tests performed in uninfected infants, only 1 scored positive then turned negative 1 month later. The infant had a normal clinical examination, maternal infection occurred at the end of the first trimester, and amniocentesis was negative. The only false-negative case was sampled erroneously on an EDTA-coated tube; whether this additive explained the wrong result remains unexplained.

DISCUSSION

Congenital toxoplasmosis workup at birth comprises a constellation of serologic tests. Tests can be falsely negative, discordant or doubtful when scoring within the grey zone, and clinicians are in an uncomfortable situation with respect to treatment. We reported our experience of the IFN-γ assay routinely performed in the workup of newborns and infants at risk of congenital toxoplasmosis. The performances of the test in this prospective study were comparable with those we reported previously. One patient was under treatment at the time the test was performed and scored positive.
on the absence of congenital infection. Further control displayed specific IgM, confirming congenital infection.

In a subset of 12 patients tested during the first month of life (Table 1), an antenatal diagnosis was not performed in 7 cases, and diagnosis of congenital infection only relied on tests performed at birth. Except for 1 invalidated test (see above), the IFN-γ assay correlated with serologic workup and was not affected by maternal treatment. These data show that even at 4 days of life cellular-specific responses can be investigated successfully. For infections occurring in the third trimester, the pretest probability is 56%, using the positive and negative likelihood ratio; the posttest probability of infection will reach 98.5% in cases of positive IFN-γ assays; conversely a negative test will reduce the probability from 56% to 5%. The rate of nonresponders to mitogens and the delay between sampling and processing the blood (after 10 hours the sensitivity declines) are limitations to this test.

Nevertheless, IFN-γ tests run on an otherwise discarded pellets of blood are easy to perform and well adapted to infants. As they explore cellular immune responses, they appear complementary to serologic tests.

REFERENCES


CASE REPORT

A 6-year-old male Caucasian, born in and living in Santa Luzia do Paruá, Maranhão, Northeast Brazil, was admitted to the Infectious and Parasitic Diseases Unit in Pediatrics of the University Hospital of the Federal University of Maranhão with abdominal pain and intermittent diarrhea for 2 months, which improved gradually. Fifteen days before being admitted, holocranial headache, intermittent fever and pain and paresthesia of the lower limbs started, followed by paraparesis and urinary incontinence. Upon physical examination, pallor, weight loss, decreased motor sensitivity and strength of the lower limbs and difficulty in sitting were observed. Additionally, there were absent tendon reflexes in the legs and decreased tendon reflexes in the arms.