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Late diagnosis of congenital toxoplasmosis based on serological follow-up: A case report

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ABSTRACT

Toxoplasma gondii is a protozoan parasite infecting up to one third of the world's population. *T. gondii* infection is usually benign in immunocompetent patients but can be life-threatening when congenitally transmitted. Congenital toxoplasmosis presentation ranges from severe central nervous system and ocular features, to a well appearing newborn with onset of complications late in childhood. The diagnosis of subclinical form remains important since early treatment reduces later complications such as chorioretinitis.

We report an atypical case of congenital toxoplasmosis with a delayed diagnosis, based on *Toxoplasma*-specific serological follow-up. The infant was born to a mother who became infected during pregnancy, thus inducing infant biological and clinical follow-up. Neither biological nor clinical arguments favored a diagnosis of congenital toxoplasmosis until ten months of life. Congenital toxoplasmosis was then suspected because of an unusual increase of specific IgG levels. Diagnosis was confirmed by detection of newly synthesized newborn Ig isotypes using complementary comparative mother-to-child immunological profile techniques and specific treatment therefore administered.

This report highlights the importance to follow up newborns at risk of congenital toxoplasmosis with specific and newborn-appropriate techniques until *Toxoplasma*-IgG titers are completely negative. This allows not only the exclusion of congenital toxoplasmosis when serology becomes negative, but also the diagnosis and treatment of congenital toxoplasmosis when infection is detected later in development.

1. Case report

Toxoplasmosis is a disease caused by the intracellular parasite *Toxoplasma gondii*. This disease is usually benign in immunocompetent individuals, but can be serious in immunocompromised patients and when congenitally transmitted. In congenital transmission, the risk of vertical transmission increases during the term whereas the gravity of fetal defects decreases. Clinical manifestations in infected infants range from asymptomatic disease to immediate or progressive retinochoroiditis and cerebral malformations. Miscarriage and stillbirth can occur in cases of severe infection [1].

In France, primary infection during pregnancy is detected through monthly serological monitoring [2,3]. When a primary infection is diagnosed, a thorough medical follow-up is provided in reference centers to detect vertical transmission to the fetus. Prenatal diagnosis based on amniocentesis is generally performed to detect fetal infection through the detection of *T. gondii* by PCR and mouse inoculation. Fetal ultrasound examination is also regularly performed to detect symptomatic clinical toxoplasmosis with malformations. Treatment with spiramycin or a pyrimethamine-sulfonamide combination is generally provided to the mother to prevent the risk of vertical transmission and malformations, respectively [2]. If no prenatal diagnosis is established or if the data cannot confirm congenital toxoplasmosis (CT), biological and clinical studies are performed at birth and a serological follow-up is initiated during the first year of life [3]. The interest of early diagnosis is to provide early

Abbreviations: CIP, Compared Immunological Profiles; ELIFA, Enzyme-Linked Immuno-Filtration Assay; Ig, Immunoglobulin; *Toxoplasma gondii*, *T. gondii*; PCR, Polymerase Chain Reaction; CT, Congenital Toxoplasmosis; IB, Immuno-Blotting; AW, Weeks of Amenorrhea; ELISA, Enzyme-Linked Immunosorbent Assay; ISAGA, Immuno-Sorbent Agglutination Assay; IC, Immuno-Capture; D, Day; M, Month.

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treatment to limit clinical adverse outcomes [2]. Neonatal diagnosis consists of serological testing for IgG, IgM, and IgA antibodies in cord blood and a neonatal serum sample, with comparative mother-to-child immunological profiles (CIP) if necessary [4–6]. *T. gondii* detection in placenta and/or cord blood by PCR and mouse inoculation can also be performed [3,7,8]. Moreover, transfontanellar ultrasonography and fundus oculi allow a primary evaluation of cerebral and ocular damages.

Serological follow-up – mainly IgG, IgM, and IgA detection – occupies a major place in the diagnosis of CT but requires a high level of expertise as serological profiles vary considerably depending on the infants and the techniques [4–6]. Indeed, synthesis of anti-*T. gondii* Ig by the newborn itself is proof of CT. However, the detection of Ig can be masked by maternal antibodies and missed due to low Ig production, below the sensitivity thresholds of the techniques used. IgM and/or IgA detection in newborns is consistent with CT as they cannot cross the placental barrier. On the contrary, detection of IgG in newborns is not sufficient to prove a CT as they can be transplacentally transmitted by the mother. The disappearance of IgG within 9 months from the infant's serum demonstrates the absence of infection whereas the persistence of IgG beyond one year is consistent with a diagnosis of CT [5]. The CIP method is required for the earliest detection of newly synthesized newborn Ig isotypes. Two analytical techniques are used by reference centers to perform IgG and IgM CIP: immunoblot (CIP-IB) and Enzyme-Linked Immunofiltration Assay (CIP-ELIFA) [5,6,9]. CIP-IB analysis is based on the detection and characterization of antibodies by exploiting antigen-antibody recognition after electrophoresis and transfer of antigens to nitrocellulose strips. CIP-IB on sera from infants with CT displays new molecular-mass bands [8]. CIP-ELIFA is based on a co-immunoelectrodiffusion procedure to simultaneously determine the antibody specificity and isotype by immunoprecipitation and immunofiltration, respectively. CIP-ELIFA establishes the maternal, fetal or mixed (maternal-fetal) origin of specific IgG detected in an infant [5].

A 29-year-old pregnant woman was referred to the Grenoble-Alpes University Hospital (France) after a toxoplasmic seroconversion diagnosed in a local laboratory and confirmed by the laboratory of Parasitology-Mycology in Grenoble-Alpes University Hospital. The beginning of pregnancy was estimated to be 08/26/2013. She was known to be "seronegative" for *T. gondii* and was consequently serologically monitored monthly according to French recommendations [3]. Seroconversion was observed during the second trimester of pregnancy by the apparition of specific IgM and IgG, with a fivefold increase of specific IgG titers (Vidas, bioMérieux® and Architect, Abbott®) in two serum samples taken one month apart (Table 1). The primary infection of the mother was estimated to have occurred at 20 weeks of amenorrhea (WA) mid-January 2014. Fetal ultrasound examinations at 21, 28, 32 and 37 WA were strictly normal. An amniocentesis performed 6 weeks after toxoplasmic contamination at 26 WA did not reveal the presence of *T. gondii* in amniotic fluid by PCR and mouse inoculation. Spiramycin treatment was administered from the diagnosis of the primary infection until delivery.

The baby was born by normal vaginal delivery at 38 WA + 6 days (05/18/2014). PCR on the placenta was negative. Clinical evaluation, transfontanellar ultrasonography to detect cerebral abnormalities, and fundus oculi in the first days after birth were both normal. Serology at day 3 (D3) revealed the presence of high IgG levels (Vidas, bioMérieux® and Architect, Abbott®) (classical hemoconcentration pattern of maternal IgG antibodies) and the absence of IgM (Vidas and ISAGA, bioMérieux®) and IgA (ISAGA, bioMérieux®) specific antibodies. Serological follow-up of the infant showed normal kinetics of IgG decrease between D3 and month 7 (M7) (Table 1). CIP-IB IgG and IgM (LD Bio Diagnostics®, France) analyses at D3, M1, and M3 did not reveal specific neonate Ig isotypes (see Table 2, Supplementary material). No biological or clinical arguments favored a diagnosis of CT, through the seventh month of life.

Surprisingly, serological analysis at M9 revealed a slight increase of IgG levels (by ELFA-ELISA Vidas, bioMérieux® only) and IgM levels (by ISAGA, bioMérieux® only and below the threshold of positivity) (Table 1). Asymptomatic CT with delayed detection was therefore suspected. The serological control three weeks later confirmed the abnormal Ig kinetics showing stable IgG levels using both ELISA techniques and an increase of IgM by the ISAGA (bioMérieux®) technique beyond the threshold of positivity. Treatment with pyrimethamine-sulfadoxine and folinic acid was initiated at 10 months of life for a duration of 12 months. The treatment was well tolerated and clinical examinations with fundus oculi every two months were all normal during two years.

We pursued further biological investigations in light of these atypical results. The remaining sera stored in a biobank at −20 °C (biobank declared to the French Ministry of Health, number DC-2008-582) were sent to the laboratory of Parasitology-Mycology at Reims University Hospital to confirm these results with others serological techniques. IgG antibodies were titrated using High Sensitivity Direct Agglutination (HSDA) (cutoff value = 6 U/mL) and IgM and IgA were assayed using lab-developed Immuno-Capture (IC) tests (cutoff value = 1 in neonates) [5]. IgG CIP between mother and infant were performed using CIP-ELIFA [5,6] (see Table 3, Supplementary material). IgM CIP-ELIFA was not performed because of small sample volume. CIP-ELIFA on the M5 serum showed an increase of the preexisting IgG with neo-antibody synthesis by the infant, confirming with certainty the suspected diagnosis of CT. In parallel, IgGs appeared to decrease by HSDA until five months – as they would normally in the absence of neonatal infection – and were stable between five and eight months with no apparition of specific IgM or IgA.

The main feature of this case report is the late diagnosis of CT, despite a strict biological and clinical follow-up. The main issue of delayed diagnosis is delayed therapy which could lead to ophthalmological problems, even in adolescence. This case highlights that some CT diagnoses are late, but also supports that these cases are generally associated with asymptomatic or less serious clinical outcomes [10]. There are several possible reasons for the long delay of CT diagnosis. The most likely is weak parasitic load transmission from mother to infant generating a weak infection with a slight and late immune response. Another is primary-infection of the infant but this is unlikely (no cat at home and no risk of contamination through food) and the CIP-ELIFA patterns confirmed the congenital infection. The fetal transmission of CT probably occurred at the end of pregnancy in the absence of clinical and ophthalmological signs in the infant. Symptomatic clinical toxoplasmosis is often associated with early fetal infection during pregnancy [10].

Here, the diagnosis was suspected using classical serological methods only at M9, because of an increase of IgG detected using the ELFA-ELISA (Vidas, bioMérieux®) technique. In this case, CIP-ELIFA would have led to an earlier diagnosis with the detection of newly synthesized IgG antibodies from the infant at M5. In a single case, the superiority of CIP-ELIFA relative to CIP-IB cannot be established in a general way [6]. In the age of quality assurance, CIP-ELIFA remains a lab-developed, non-commercialized, non-standardized, manual technique and is only used in very few laboratories. However, this case demonstrates the complementarity between classical quantitative methods and qualitative analyses of specific immunoglobulins using CIP to perform an early diagnosis of congenital toxoplasmosis, when IgM and IgA antibodies are not detected at birth [5,6]. A cumulative sensitivity of 98% during a one-year follow-up was described in a previous publication using a combination of ELIFA/IB and IC [6]. Another study showed that the combination of CIP-ELIFA, IC-IgM, and IC-IgA tests provided a positive diagnosis in 90% of infants within one month of life and 94% at three months of life [5].

To conclude, this case highlights (a) the importance of a strict serological follow-up of infants suspected of CT during the first year of life until *Toxoplasma*-IgG titers are completely negative to exclude or detect late expression of CT whatever the perinatal work-up and (b) the

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