Western blotting for the diagnosis of congenital toxoplasmosis

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SUMMARY

Toxoplasmosis is a common congenital infection. It does not usually produce recognizable signs of infection at birth so most infected newborns are not detected by routine clinical examination and remain untreated. Infected children without clinical symptoms should nonetheless be identified and treated as early as possible.
Serological diagnosis of congenital toxoplasmosis is quite difficult. The aim of this study was to evaluate the utility of Western blot for the diagnosis of congenital toxoplasmosis.
We compared the immunological profiles of mothers and children to differentiate between passively transmitted maternal antibodies and antibodies synthesized by the infants in the first three months of life.
The method enabled us to diagnose congenital toxoplasmosis in cases in which the infection had not been detected by classical serology techniques.

KEY WORDS: Congenital toxoplasmosis, Western blotting

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Congenital toxoplasmosis is a disease that can be prevented by education and providing information to mothers about risk factors. It can be diagnosed using appropriate serological and molecular assays and treated with therapeutic protocols that reduce the risk of vertical transmission and sequelae in newborns (Foulon *et al.*, 1999).

Although there are several accurate analytical methods for the diagnosis of toxoplasmosis during pregnancy, early diagnosis of congenital toxoplasmosis is more difficult, because the production of antibodies against *Toxoplasma* in the fetus or newborn baby is often inhibited and/or masked by maternal antibodies (Robert-Gangneux *et al*., 1999a). Postnatal serological diagnosis is usually performed by detecting specific IgM or IgA antibodies, but in a large percentage of congenitally infected children, these antibodies may be absent or produced in concentrations below the sensitivity thresholds of the methods available. Thus, in the absence of clinical signs, the diagnosis may be delayed until the persistence or increase in Toxoplasma specific IgG is observable.

The aim of the present study was to test the utility of the Western blot technique in diagnosing congenital toxoplasmosis in the first months of life, by revealing specific neosynthesized antibodies in neonatal serum.

Fifty-six mother-child pairs were enrolled in the study, in the period 2001-2008. All pregnancies had a regular course, without clinical signs of pathology due to *Toxoplasma* infection. None of the babies had clinical manifestations suggesting congenital toxoplasmosis.

All mothers were tested during pregnancy for specific IgG and IgM and for IgG avidity and, on the basis of the results, they were considered at risk for transmission. Seroconversion was observed during the course of pregnancy or strongly suggested by the presence of specific IgM and/or a threefold elevation of specific IgG titers in two serum samples taken at a distance of three weeks and analysed in parallel. In a few cases the pres-

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ence of specific IgM and a significant and stable IgG titer with low/average avidity made it impossible to exclude a primary infection during the pregnancy or in the periconceptional period. Indeed a high IgM anti-toxoplasma titer has a low positive predictive value in pregnant women (De Paschale *et al.*, 2008), low avidity results may persist for as long as 1 year and some patients can have borderline or equivocal results (Remington *et al.*, 2004). The women were treated with spiramicin until 18 weeks of gestation and with pyrimethamine, sulfadiazine and folinic acid from 19 week until the end of gestation (Montoya and Remington, 2008), independently of the prenatal diagnosis by PCR on amniotic fluid, which was only possibile in 32% of the cases analysed.
At birth the classical serological tests for IgG and IgM as well as IgM-IgG Western blot were performed in all mother-child pairs. The presence of IgG was investigated by direct agglutination (screening test) and ELISA (quantitative test). Direct agglutination was performed using the Toxo-Screen DA kit (BioMerieux). Quantitative determination and the avidity test for IgG were carried out using the Platelia Toxo IgG TMB kit (Bio-Rad). The anti-*Toxoplasma gondii* IgM test was carried out with the ISAGA technique (Immuno Sorbent Agglutination Assay) using the Toxo ISAGA kit (Biomerieux). For the Western blot assay we used the *Toxoplasma* Western blot IgG/IgM kit (LDBIO Diagnostics), Rilling *et al.*, (2003). This technique consists of a qualitative analysis by comparison at the birth of immunological profiles of neonatal blood taken 48 hours after birth and maternal blood, and in the follow-up, of neonatal blood taken 48 hours after birth and at later times (1, 2, 3 months). The test was performed as described by the manufacturer. The IgG and IgM patterns are compared with the Western blot technique independently. Any well-defined band, with a molecular weight of less than 120 kDa in infant serum, but not in maternal serum (or in follow up serum, but not in the previous sample), indicated specific antibodies synthesized by the baby (Figure 1).
A different immunologic profile from that of the mother was found in five newborn babies, indicating immunoglobulin production by the baby and therefore *Toxoplasma* infection. Specific IgM were found by ISAGA in four babies, but were not detectable in the fifth. A further baby, which

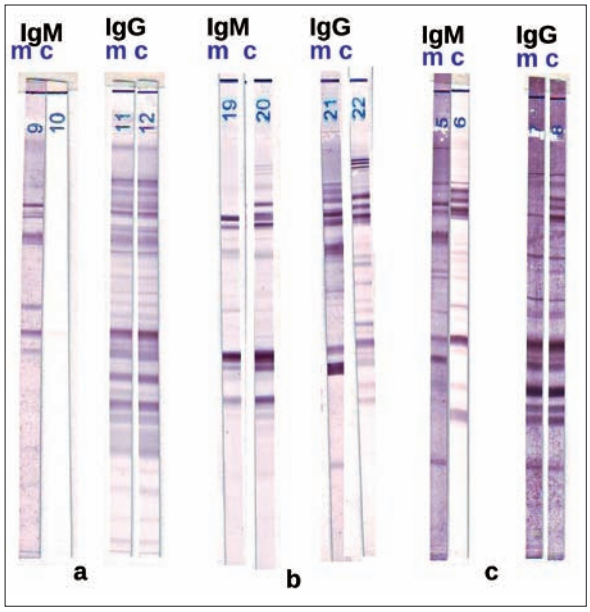

FIGURE 1 - ***a)*** *Example of a negative Western blot pattern. Presence of transmitted IgG without IgM in a non-infected newborn.* ***b-c)*** *Examples of two positive Western blot patterns. Presence of neosynthesized IgM and IgG in two infected newborns (m = mother; c = child).*

TABLE 1 - *Summary of Western blot results. Seven infections were diagnosed by Western blot and ISAGA, but three cases by Western blot only.*

	ISAGA +	*ISAGA -*
Western blot -	0	49
Western blot +	4	3

was negative for IgM at birth by ISAGA and Western blot, was positive to Western blot at the first and second months of life. In a seventh baby, Western blot for IgG and IgM became positive in the third month, while ISAGA remained negative (Table 1). IgG measured by ELISA were also negative in the third month. Western blot enabled us to detect infection in three babies at birth or in their first months of life. Diagnosis of these cases would normally have been delayed using classic serological tests. In fact without a Western blot assay, only the increase or persistence of IgG antibodies within the first 12 months of life can demonstrate congenital infection. Otherwise a delayed diagnosis and consequently delayed therapy can lead to vision problems even in adolescence. Western blot also proved useful

for demonstrating the active synthesis of IgG in one case, whereas ELISA was negative in the same sample.

We can therefore conclude that of the several serological tests used to detect congenital toxoplasmosis Western blot was the most sensitive, demonstrating IgM with a greater sensitivity than the ISAGA technique and being the only technique to differentiate IgG of maternal origin from that of fetal and neonatal origin. In one case Western blot also proved useful for demonstrating active synthesis of IgG, as it showed bands of IgG directed against Toxoplasma antigens that were not present in the previous sample. IgG ELISA was negative in the same serum sample.

Our experience with Western blot enabled us to confirm four cases of congenital toxoplasmosis diagnosed by the classic method and to diagnose three other cases (one at birth, one at 1 month and the other at 3 months of age) that classic serological tests identified as negative. In accordance with the observations of other authors (Robert-Gangneux *et al.,* 1999b; Rilling *et al*., 2003; Tissot Dupont, 2003; Gallego-Marin *et. al.,* 2006, Tridapalli *et al.,* 2008), our result confirm that the comparison of immune profiles is a valid aid for the early diagnosis of connatal toxoplasmosis.

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