Early diagnosis of acute toxoplasmosis in IgG negative IgM positive pregnant women

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OBJECTIVES

During maternal primary infection *Toxoplasma gondii* can be trasmitted to the fetus in a time dependent way. Congenital toxoplasmosis could be more severe and less frequent if the mother became infected at the beginning of gestation and mainly asymptomatic but more frequent for third trimester infections.

As previously reported early treatment (during the first 4 weeks after contamination) can decrease the severity of congenital infection and sequelae in newborns (Wallon M et al. 2013).

In pregnant women who are anti-toxoplasma IgG negative and IgM positive, the differential diagnosis between early seroconversion and false positive IgM result is always puzzling as a result of the high sensitivity of new automatized tests. Furthermore therapy with spyramicin, usually given when positive IgM are found, can delay IgG production and mask seroconversion (Tab. 1).

The objective of our study is to evaluate a diagnostic flow chart aimed to identify acute *Toxoplasma* infection in pregnant women who are IgG negative IgM positive at the first sampling.

Tab. 1 Antibodies kinetics in treated pregnant woman

TEST	02/11/2010	10/11/2010	30/11/2010	23/12/2010	04/01/2011
IgG CLIA	NEG	NEG	Gray-zone	Gray-zone	25
IgG ELFA	NEG	NEG	ND	4	24
IgM CLIA	POS	POS	POS	POS	POS
IgM ISAGA	12+	12+	12+	12+	12+
IgA ELISA	200	250	200	150	150
WB IgG/IgM	NEG/POS		POS/POS	POS/POS	
IgG AVIDITY					0,114

METHODS

Patients: Sixty eight pregnant women referred to the Infectious Diseases Department outpatient for a suspected seroconversion for toxoplasmosis (IgG negative - IgM positive) were enrolled. Every woman was treated with spyramicin at the first visit and therapy discontinued if infection excluded. All these patients underwent weekly serological follow up. All infected women received the adequate therapy (spiramycin and/or pyrimethamine + sulfadiazine + folinic acid) counseling, prenatal diagnosis and serological and clinical follow up of the newborn was recommended.

Tests: The serological results were confirmed with LIAISON® Toxo IgG II / IgM CLIA (DiaSorin - Saluggia- Italy), ETI-ToxoA (DiaSorin Saluggia Italy) VIDAS TOXO IgG ELFA, ISAGA Toxo IgM (Biomerieux Marcy l'Etoile France). In most of them a miniaturized in house IGRA test was also done and the IFN production was evaluated by IGRA - Quantiferon – ELISA (Cellestis Australia) after stimulation of peripheral whole blood with sonicated *Toxoplasma* antigen kindly provided by DiaSorin. Immunoblot Toxo IgG / IgM Type II (LD-Bio Lyon France) was always performed. The test CE mark only for IgG diagnosis but we used it also for early detection of IgM using as control IgM sera from previous diagnosed seroconvesion. Briefly IgG WB is considered positive when are present at least 3 band to antigens with molecular weight of 30 - 40 KD and IgM when are present at least 2 band including band to 30 Kd antigens (Fig 1). All the tests were performed accordingly to manufacturers instructions.

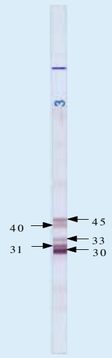


Fig.1 LDBIOTOXO II

- (immunoblot)
- ready to use
- standardized (90'- 60'- 30')
- positive control For IgG included

5 specific band:
30 – 31 – 33 – 40 – 45 kDa

IgG positive: 3 specific bands
IgM positive: 2 specific bands including 30 kDa band

RESULTS

All the women had at least one positive test for IgM (CLIA / IgM ISAGA) they are equally distributed in all the pregnancy trimester (Tab. 2).

Thirtyone women were IgM positive on Immunoblot at the first sample and 22 of them also for IgG undetectable with traditional tests (Fig. 2). All but 5 showed seroconversion 1-2 months after the beginning of therapy. In 16 of them IGRA was performed and was always positive. Newborns follow-up was completed in 20 newborns and two infected babies were recorded. Thirtyseven women were Immunoblot IgM and IgG negative (Fig. 3); 22 had also a negative IGRA test. For all of them therapy was discontinued and serology monitored weekly. No seroconversion was recorded and 9 became completely negative at the end of pregnancy. Follow up was completed in 14 newborn without any congenital infection (Tab. 3).

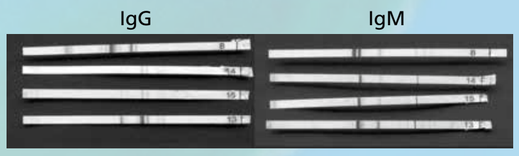
Tab. 2

	I TRIM	II TRIM	III TRIM	Unknown	Tot.
Maternal Infection	11	12	8	0	31
No inFection	9	7	14	7	37

Tab. 3

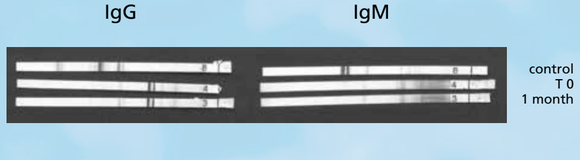
WB IgMpos	WB IgGpos	ISAGA neg	ISAGA pos	IGRA pos	IGRA neg	SEROCONVERSION YES	SEROCONVERSION NO	SERONEGATIVATION	Followed newborns	New borns POS	New borns NEG
31	22	1	30	16	0	26	5	0	21	2	19
0	0	33	4	0	19	0	37	14	14	0	14

Fig. 2 IgG-IgM WB II Seroconversion



Serological test	T 0	7 days	15 days	1 m
IgG ELFA	Neg	Neg	Neg	9
IgM CLIA	Pos	Pos	Pos	Pos
IgM ISAGA	12+	12+	12+	12+
IgA ELISA	Neg	Neg	100	100

Fig. 3 IgG-IgM WB II aspecificity



Serological test	T 0	1 m	2 m
IgG ELFA	Neg	Neg	Neg
IgM CLIA	Pos	Pos	Neg
IgM ISAGA	12+	12+	Neg
IgA ELISA	100	Neg	Neg

CONCLUSION

Screening for toxoplasmosis in pregnancy requires very sensitive tests in order to find out as early as possible all the primary infections. All suspected cases must be sent to reference laboratory in order to perform more specific tests. Type II Immunoblot and IGRA when correctely performed can discriminate in in uninfected women. Most of cases between real seroconvesion and false positive results thus avoiding anxiety, unnecessary therapy, and prenatal diagnosis