Multidisciplinary approach to congenital Toxoplasma infection: an Italian nationwide survey.

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Multidisciplinary approach to congenital Toxoplasma infection: an Italian nationwide survey

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SUMMARY

Italy provides a free voluntary serological screening for toxoplasmosis in pregnancy supported by public health system, as there is an estimated congenital toxoplasmosis rate of 1-2/10,000. The aim of this study was to make an inventory of diagnostic and therapeutic protocols in use in Italy in the absence of a national guideline. A semi-structured questionnaire was distributed to AMCLI (Italian Association of Clinical Microbiologists) members who were asked to involve other specialists to fill in the form. Data from 26 centers show:

a) a general use of the IgG avidity test to solve diagnosis in IgG/IgM positive, pregnant women;
b) a widespread attitude to spyramicin antenatal treatment in suspected, unconfirmed maternal infection;
c) avoidance of invasive antenatal diagnosis only in suspected early or late (>24 weeks), even confirmed, maternal infection
d) fetal diagnosis performed by PCR assays on amniotic fluid;
e) variability of both indications and dosage of pyrimethamine-sulfadiazine (P-S) as fetal treatment;
f) use of comparative mother and newborn IgG/IgM Immuneblot in most centers;
g) no diagnostic tests performed on placenta and cord blood;
h) spyramicin is no longer used in congenital infections;
i) no P-S-based treatment for children at high risk of congenital infection (late maternal infection) in the absence of diagnosis.

As there is the opportunity to test pregnant women for *Toxoplasma gondii* infection in Italy free of charge, standardized diagnostic and therapeutic national guidelines would focus on a more uniform approach.

KEY WORDS: Toxoplasma, pregnancy, diagnosis, treatment.

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INTRODUCTION AND RATIONAL

Despite a substantial decrease in *Toxoplasma gondii* seroprevalence (from 40 to 20-30% in the adult population in the last 20 years) (Castelli *et al.*, 1995; Buffolano *et al.*, 1996; Tomasoni *et al.*, 2010; De Paschale *et al.*, 2010) and although no national register of congenital infections is available, 1-2 congenital Toxoplasma cases per 10,000 births are currently estimated in Italy (Stagni *et al.*, 2009); 1-4% of them are at risk of death or serious neurological sequelae (Gilbert and Peckham, 2002).

A recent paper by Wallon *et al.* demonstrated that monthly prenatal screening and improvements in antenatal diagnosis may lead to a de-

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crease in the congenital infection rate and a better outcome of infected children (Wallon M. *et al.*, 2013).

In Italy free serological screening before and during pregnancy is considered cost-effective and supported by the public health system (Ministero della Salute- DPM 10 settembre 1998; Ministero della Salute, 2011). The screening is rarely performed during the preconception period (Tomasoni *et al.*, 2010), and is often carried out late in pregnancy (after 1st trimester) in 15% of Italian women and in 30% of immigrants (De Paschale *et al.*, 2010; Tomasoni *et al.*, 2010).

As a consequence, both doctors and pregnant women often have to deal with diagnostic and psychological problems linked to the poor specificity of serological tests for primary infections: about 1-5% of screened women have specific anti-Toxoplasma IgG and IgM antibodies during pregnancy (De Paschale *et al.*, 2008; Thaller *et al.*, 2011).

IgG avidity test can exclude a recent primary infection and fetal risk in 50% of them only when it is performed during the first trimester (Flori *et al.*, 2009; Iqbal and Khalid, 2007). In all the other cases uncertainty about maternal diagnosis complicates counseling as well as subsequent diagnostic procedures on the fetus and therapeutic decision-making.

Despite the fact that Italian legislation supports free non-mandatory *Toxoplasma gondii* screening in pregnancy, no official diagnostic and therapeutic national guideline is currently available.

Only recently, Italian scientific Societies with different skills in this field, AMCLI (Italian Association of Clinical Microbiologists), SIMIT (Italian Society of Infectious and Tropical Diseases), SIGO (Italian Society of Gynecology and Obstetrics), SIMaST (Interdisciplinary Society of Sexually Transmissible Diseases), SIN (Italian Society of Neonatology) and SIP (Italian Society of Pediatrics), set up a multidisciplinary consensus on diagnosis, therapy and follow-up of toxoplasmosis in pregnancy and in newborns (www.amcli.it).

The aim of this study was to make an inventory of the management protocols for congenital Toxoplasma infection in use in Italy, with particular emphasis on the multidisciplinary approach.

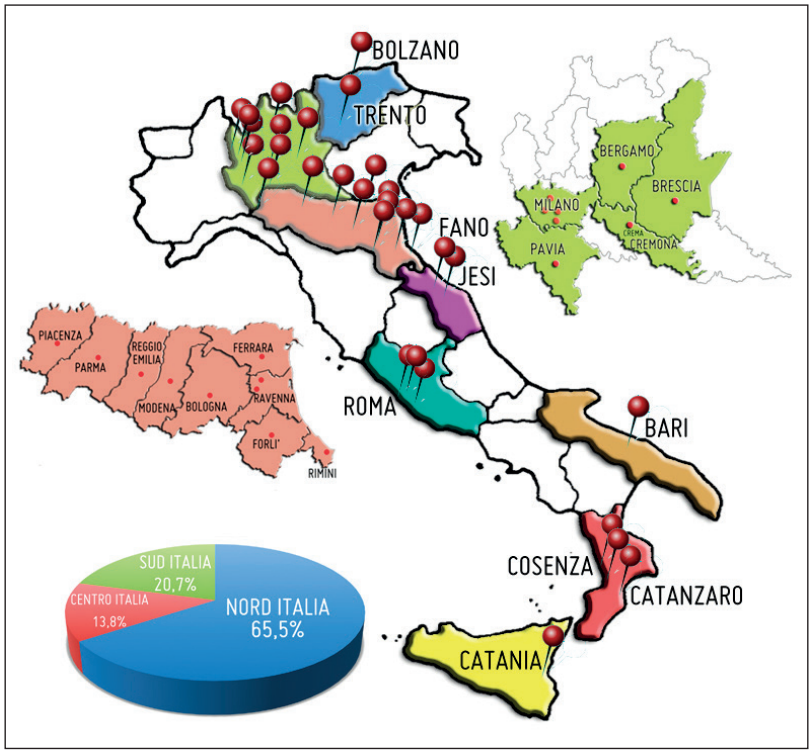

FIGURE 1 - *Geographical distribution of the centers involved in the survey.*

METHODS

A semi-structured questionnaire was designed by a pool of microbiologists, infectious diseases specialists, gynecologists and neonatologists with working experience in the field of congenital toxoplasmosis, and was distributed to AMCLI members. As diagnosis is always crucial for subsequent interventions, microbiologists can be considered the main bridge between different specialists. For this reason they were asked to involve others specialists in filling in the questionnaire. The survey included sections on the diagnosis of pregnant women, fetus and newborn, and ante and post-natal therapeutic decisions.

RESULTS

From June to August 2012, 29 completed questionnaires from 26 cities (66% from north Italy, 21% from the center and 13% from the south) were returned (Figure 1). Not all the questionnaire sections were completed by all the centers, thus justifying different denominators (Table 1).

Twenty centers reported a close collaboration among different specialists but only 10 teams included the infectious diseases specialist. In 18 Centers different specialists work separately sharing operational protocols, while only two (7%) provide multidisciplinary joint consultations for parents.

The temporal definition of pre-conception period at risk of congenital toxoplasmosis resulting from maternal primary infection varies among Centers: 7 centers out of 26 (27%) consider a risk period of 1 month, 10 (38.5%) 2 months and 9 (34.5%) a period up to 6 months before conception.

In the absence of documented seroconversion, all Centers use the IgG avidity test and 14/26 (54%) add IgG kinetics in the subsequent 3-4 weeks (to differentiate a probable from a possible recent primary infection) to date the infection in IgG and IgM positive women at their first screening in pregnancy.

Antibiotic prenatal treatment is always offered to seroconverted cases. At least half of the Centers (16/26, 61.5%) also recommend it to IgG and IgM positive women without a high IgG avidity certified in the first 12-14 weeks of gestation (recent primary infection not ruled out). The presence of an additional criterion (evocative clinical manifestations or variation of IgG titer) is required by 7/26 (27%) centers to prescribe antenatal treatment. Most centers (66%) start spiramycin immediately after an IgM positive test while waiting to complete maternal diagnosis.

TABLE 1 - *Main results.*

Topics (N. of answering Centers)		
Pre-conceptional risk timing (26)	6 months	9
	2 months	10
	1 month	7
Maternal diagnosis (26)	IgG avidity test	26
	IgG title	14
Fetal diagnosis (21)	cordocentesis	0
	amniocentesis	21
	after 18th week	15
	after 15th week	6
PCR assay on Amniotic Fluid(17)	Real time	15
	Nested	2
	Performed in case of late (>24weeks) infection? (18)	10
	Performed in case of very early infection? (21)	18
	Performed in case of suspected maternal infection? (21)	9
Neonatal diagnosis (27)	Immunoblot	16
	IgM ISAGA	8
	IgA ELISA	16
Ante-natal treatment (26)	**Spiramicine**	26
	While waiting confirmed dignosis	17
	If primary infection is not rulled out	16
	Pyrimetamine-sulphamidic	17
	Only for ascertained fetal infection	5
	Always for late (>24 w) infection	12
Post-natal treatment (21)	Pyrimetamine-sulphamidic	21
	While waiting definite diagnosis if III trimenster infection	4
	Only after confirmed diagnosis	17
	Different schedule in asymptomatic infected baby	12

Among 17 centers reporting criteria for antenatal pyrimethamine(P)-sulfonamide treatment, 5 (27.7%) exclusively reserve it to cases with confirmed fetal infection by prenatal diagnosis; all the others also use it for women with a primary infection occurring after 24 weeks of gestation regardless of proof of fetal infection. Two centers consider its use also for women with earlier (II trimester) confirmed infection who do not agree to undergo an invasive procedure for fetal diagnosis. Sulfadiazine (S) is the sulfonamide of choice in all but one center. When prescribed, the pyrimethamine-sulfonamide treatment is continuous in 50% of centers. Variable periods of suspension of these drugs replaced with better tolerated spiramycin are considered in the remaining centers.

Twenty-one centers filled in the questionnaire section about fetal diagnosis. Cordocentesis is no longer performed. Amniocentesis is performed only after the 18th week of gestation in 15/21 (71.4%) centers, after the 15th in the remaining centers, but in any case at least 4-6 weeks after maternal infection. While 12/21 (66%) consider it also for women with suspect primary infection, three centers do not perform this invasive procedure when maternal infection occurred early in pregnancy (before 4th, 8th, and 12th weeks of gestation, respectively). Amniocentesis is not considered when maternal infection occurs after 24 weeks in 8/18 (44.4%) centers.

No center uses *in vitro* or *in vivo* culture of amniotic fluid, while molecular diagnostic test, based on PCR assay (real-time in 15/17 centers; nested in 2/17), is the test used for fetal diagnosis. Commercial kits (mainly Elitech Group Nanogen®) for PCR assay are employed in 80% of the centers, with an automated extraction system in 90%. The number of PCR repetitions is however variable from 1 to 6 in different centers.

All centers perform periodical ultrasound examination of the fetus. This monitoring is stopped in case of negative antenatal diagnosis in 2 centers.

Neonatal diagnostic procedures are performed in newborns of mothers with confirmed or possible primary infection in pregnancy or in periconception period. The placenta is never tested, serological assays on cord blood are performed rarely (2/28 centers). On newborn peripheral blood IgG, by automated assay (Diasorin in 11, Biomerieux in 5, Siemens in 4 centers), and IgM, by automated assay (Diasorin in 12, Biomerieux in 5, Siemens in 4 centers), are always performed. IgM ISAGA and IgA ELISA assays are available in 8/27 (22%) and in 16/27 (59%) centers, respectively. Comparative mother and newborn IgG/IgM Immuneblot is routinely used in 16/27 centers. All centers continue serological follow-up until at least 12 months, every month in the first trimester of life in 14/23 (60%).

Cerebral ultrasound is always performed at birth. Only 4 centers consider it conclusive if normal; the others repeat it periodically until congenital infection is serologically excluded. During the same period, an ophthalmoscopic evaluation of the baby is repeated periodically.

Child treatment is almost always started only after confirmation of congenital infection. Only 4/21 centers treat the newborn even before confirmation of its infection, when maternal toxoplasmosis occurred in the III trimester. Pyrimethamine and sulfadiazine (P-S) are used in all centers. About 60% of centers treat symptomatic and asymptomatic babies with a different schedule of P-S. The treatment lasts one year. Two centers report the use of spiramycin alternating with P-S in the second semester of treatment. Azithromycin is used as an alternative drug in intolerant cases in 11 centers.

DISCUSSION

Our survey aimed to define diagnostic and therapeutic approaches to congenital toxoplasmosis in different centers in Italy. However, the picture it offers could be biased by the concentration of participating centers mainly in the north of the country. Another selection bias may have been linked to questionnaire distribution modality: as AMCLI is one of the two national microbiology societies, not all Italian centers dealing with congenital toxoplasmosis could be necessarily reached by the study. Even with these limits, some data can be emphasized. The widescale diffusion of the IgG avidity assay is justified by its high positive predictive value for old infection (100%) (Villard *et al.*, 2013) and by recent simplification of the original Hedman method (Hedman *et al.*, 1989) thanks to the in-

troduction of cheaper standardized automated assays producing an IgG avidity index by only two measurements with or without urea. So it is used to exclude the risk of congenital infection in IgG and IgM positive pregnant women, even with an unknown previous serostatus (Flori *et al.*, 2009), when performed early in pregnancy and with the caveat that different commercial kits have different cut-offs for high avidity index and are validated to exclude a primary infection in a precise time interval. On the contrary, a low avidity index is not an accepted predictor of recent primary infection (Lefevre-Pettazzoni *et al.*, 2006). About half of the women undergoing IgG avidity test do not solve their diagnostic dilemma. In our survey, only half of the centers employ further tests (monitoring of IgG titer) to confirm or exclude the infection. In any case, 60% of centers suggest treatment if the infection is not ruled out.

Congenital infection after periconception (2 months) maternal infection is rarely reported (Dollfus *et al.*, 1998; Chemla *et al.*, 2002) and likely due to an unusual persistent maternal parasitemia. No available study has determined a precise risk for such a situation. If a 6-9 months delay of conception is then advisable after a diagnosis of primary infection in non-pregnant women, no study has evaluated the cost/benefit ratio to treat or even to expose pregnant women to invasive procedures when infection occurred just before conception (Villena *et al.*, 1998).

In contrast to this, three centers of the survey avoid invasive procedures. The same is reported by some French centers (Binquet *et al.*, 2004) and was recently considered in the literature (Mandelbrot, 2012), due to the very low risk of congenital infection at the onset of pregnancy (1-2%).

As cordocentesis has been abandoned since the 1990s due to its higher invasiveness and lower performance (Grover *et al.*, 1990), amniocentesis is at present the only fetal diagnostic procedure. It is however performed too early by 6 centers as insufficient data are available to certify the sensitivity of the procedure before the 18th week of gestation (Montoya and Remington, 2008).

A high proportion (30%) of specialists avoid amniocentesis when infection is estimated to have occurred in the III trimester. This may depend on different factors: the need to delay the procedure 4 weeks after maternal infection; the high (50-80%) rate of congenital infection in this period; the risk of false negative results with an assay, PCR, whose sensitivity ranges from 85% (Sterkers *et al.*, 2012) to 92% (Wallon *et al.*, 2010), and the risk of premature delivery. Real-time PCR employing AF146527 as a target gene has a 100% positive predictive value and a 99% negative predictive value and is the most widespread assay to test amniotic fluid (Sterkers, *et al.* 2010; Wallon *et al.*, 2010; Calderaro *et al.*, 2006). Commercial kits and automated extraction methods are predominant in our survey. However, some authors still claim their in-house PCR performs better (Morelle *et al.*, 2012).

Even if PCR has a high specificity and positive predictive value for congenital infection (99% and 93% respectively) when applied to placental samples (Filisetti *et al.*, 2010), this technique is not used probably because of its low (25%) sensitivity. So neonatal diagnosis in Italy is entrusted to serological assays. Next to ELISA and automated assays for IgM and IgA, about 60% of the centers use immunoblot assay to detect IgM and to compare maternal and neonatal IgG patterns. This combination can offer a sensitivity of 78% at birth and of 85%-95% at three months of life (Rilling *et al.*, 2003; L'Ollivier *et al.*, 2012).

While at international level the debate is focused around the real effectiveness of an antimicrobial treatment of toxoplasmosis in pregnancy and in congenital cases (Petersen, 2007; Elsheikha, 2008; McLeod *et al.*, 2009), in Italy all centers adopt spiramycin even if maternal infection is only suspected. Furthermore, interruption of treatment after a negative antenatal diagnosis is never considered. Elsewhere this is at least discussed (Mandelbrot, 2012) as long as an assay with a high negative predictive value is available. This is not exactly what we have now (Rabilloud *et al.*, 2010; Sterkers *et al.*, 2012). It should also be evaluated whether anecdotal cases of congenital infection with negative antenatal diagnosis (Robert-Gangneux *et al.*, 2009) are due to low sensitivity or to a vertical infection transmission after amniocentesis. The inverse relationship between transmission

rate and gestational age at maternal infection, with less probable infected fetus even if exposed for a longer time to an infected mother, and experimental models of congenital toxoplasmosis in animals would support fetal infection temporally coinciding with maternal parasitemia (Gilbert and Peckham, 2002). Practice of short (but lasting at least 4 weeks) pyrimethamine-sulfonamide treatment is reported in Germany after a negative antenatal diagnosis at the 16th week of gestation (Hotopo *et al.*, 2012).

As regards postnatal therapy, spiramycin treatment is no longer used. It was proposed in the past while waiting for a conclusive diagnosis, but no benefit has ever been demonstrated and it can cause cardiac toxicity (QT elongation) (Stramba-Badiale *et al.*, 1997). Some centers start pyrimethamine-sulphadiazine (pyrimethamine is not registered in Italy) immediately after birth even in the absence of confirmed congenital infection when maternal toxoplasmosis was acquired late in pregnancy. This practice, however, interferes with the diagnostic procedure because it can lead to a confusing serological pattern with declining IgG titer and negativization even in infected children.

Postnatal treatment lasts 12 months in accordance with the National Collaborative Chicago-Based, Congenital Toxoplasmosis Study (McLeod *et al.*, 2006). No definitive international agreement exists on postnatal treatment, with some European Countries practising a shorter (3 months) schedule (Roser *et al.*, 2010). No pediatric formulation is available for the two drugs and this can cause an overdose (Genuini *et al.*, 2011). The well-known bone marrow toxicity of the drugs makes them unsafe and requires a strict monitoring of babies by blood sampling.

CONCLUSIONS

The survey, even with an uneven geographical distributions of the centers, offers information on diagnostic and therapeutic procedures for congenital toxoplasmosis in a country funding specific screening but without national guidelines to address the problem and without a systematic surveillance. One of the most common attitudes is to suspect a maternal diagnosis in need of prenatal treatment. The multidisciplinary consensus on the diagnosis, therapy and follow-up of toxoplasmosis in pregnancy and in newborns (www.amcli.it) set up by different Italian scientific societies could help to standardize these different approaches.

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