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Contribution of Neonatal Amniotic Fluid Testing to Diagnosis of Congenital Toxoplasmosis

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We evaluated the molecular diagnosis of congenital toxoplasmosis (CT) on neonatal amniotic fluid samples from 488 mother-child pairs. Maternal infection during pregnancy was diagnosed and dated or could not be ruled out. Forty-six cases of CT were defined according to the European Research Network on CT classification system and case definitions. Neonatal amniotic fluid testing had an overall sensitivity of 54% (95% confidence interval [95% CI], 39 to 69%) and a specificity of 100% (95% CI, 99 to 100%). Its sensitivity was 33% (95% CI, 13 to 59%) when antenatal diagnosis was positive and 68% (95% CI, 48 to 84%) when antenatal diagnosis was negative or lacking. This difference in sensitivity may have been due to treatment of antenatally diagnosed cases. Relative to postnatal serology, neonatal amniotic fluid testing allowed an earlier diagnosis to be made in 26% of the cases (95% CI, 9 to 51%).

The clinical severity of congenital toxoplasmosis (CT) ranges from lethal to subclinical, and there is a risk of late severe ocular lesions (1). CT may be diagnosed in the antenatal, neonatal, or postnatal period. Antenatal diagnosis by PCR on amniotic fluid (AF) currently has a sensitivity of 80 to 90% (2–4) and therefore fails to detect 10 to 20% of cases. Moreover, monitoring during pregnancy is often erratic or not done, and some mothers at risk may decline amniocentesis or be infected very late. In these situations, CT can be diagnosed at birth by molecular testing of AF, placental tissue, cord blood, or peripheral blood specimens, as well as by comparative maternal-newborn serologic tests. Placental tissue and blood testing is useful for CT diagnosis at birth (3, 5–9), but little data are available for amniotic fluid (4, 10). We therefore evaluated PCR on neonatal AF samples for the diagnosis of CT.

Neonatal AF samples were collected between 2006 and 2013 from 488 mother-child pairs monitored for CT at Strasbourg University Hospital (Strasbourg, France), Lille 2 University Hospital (Lille, France), or Paris Centre University Hospital group (Paris, France). Maternal infection during pregnancy was diagnosed and dated by monthly IgG and IgM testing, as recommended by the French health authorities, after serologic conversion, or by studying the kinetics of IgG titers and IgG avidity testing (6, 11, 12). Maternal infection during pregnancy could not be ruled out when the first serologic tests were done after the third month and showed IgM and IgG with low avidity and stable titers after 3 weeks. Antenatal diagnosis was performed by PCR on amniotic fluid specimens obtained by amniocentesis and by fetal ultrasonography to detect CT signs (dilatation of the lateral ventricles, intracranial calcification, hepatosplenomegaly, or ascites). Neonatal and postnatal diagnoses were made by PCR on AF specimens collected at birth, serologic testing, as previously described (4, 6, 11), transfontanellar ultrasonography, and clinical and ophthalmological examination of the infants for neurological or ocular abnormalities, such as retinochoroiditis. Postnatal follow-up of the apparently uninfected infants was recommended until the disappearance of maternal IgG during the first year of life. CT was defined according to the classification system and case definitions developed by the European Research Network on Congenital Toxoplasmosis (13). An antenatal AF sample was drawn during amniocentesis from the 18th week of amenorrhea and 4 weeks after the date of maternal infection, as recommended by the French National Reference Center for Toxoplasmosis (http://cnrtoxoplasmose.chu-reims.fr). A neonatal AF sample was obtained during delivery at the time of amniotic sac rupture. The antenatal and neonatal AF samples (10 ml) were centrifuged at 1,300 × *g* for 10 min. DNA was extracted from the pellet with the QIAamp DNA minikit (Qiagen) and amplified by real-time PCR targeting the 529-bp repetitive DNA element of *Toxoplasma gondii* (14), either with hybridization probes on a LightCycler I device (15, 16) or with a hydrolysis probe on an ABI Prism7000 (16, 17). As previously reported, the performances of the two assays for detecting the DNA target were similar (4). The absence of PCR inhibitors was checked in each DNA extract by amplifying an autologous internal control or a noncompetitive internal control (16, 17). A negative extraction control and a negative PCR control

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were included in each assay. The 95% exact confidence interval (95% CI) was calculated using the binomial distribution.

Most of the mothers received specific therapy (spiramycin) during their pregnancy. Forty-six cases of CT were diagnosed by ultrasonography and antenatal molecular diagnosis, followed by serological, radiological, and clinical neonatal or postnatal monitoring. Eighteen cases (39%) were diagnosed in the antenatal period and 28 (61%) in the postnatal period only (Table 1). Maternal infection occurred during the first (5%), second (41%), and third (54%) trimesters. When CT was diagnosed during the pregnancy, the women were systematically treated with a combination of sulfadiazine and pyrimethamine.

The overall diagnostic sensitivity of PCR on neonatal AF specimens was 54% (95% CI, 39 to 69%) (Table 2), and its specificity was 100% (95% CI, 99 to 100%). Its sensitivity was 33% (95% CI, 13 to 59%) in the 18 cases in which the infection was diagnosed antenatally and 68% (95% CI, 48 to 84%) in the 28 cases in which antenatal diagnosis was negative or not done. This difference in sensitivity may have been due to antenatal treatment, which is recommended in France when antenatal AF is positive for *T. gondii* and fetal ultrasonography is normal. This treatment, combining sulfadiazine and pyrimethamine, is parasiticidal for tachyzoite stages and may lead to a reduction in or the disappearance of the parasite load in body fluids. PCR on neonatal AF samples had a sensitivity of 86% (95% CI, 42 to 100%) in the 7 cases with a negative antenatal diagnosis and only 62% (95% CI, 38 to 82%) in the 21 cases with no antenatal diagnosis. The detection of parasite DNA at birth but not before birth might be due to late parasite transmission from the mother to the fetus. The sensitivity of the PCR on neonatal AF was 0% in the two cases diagnosed during the first trimester, 33% (95% CI, 12 to 62%) in those diagnosed in the second trimester, and 85% (95% CI, 62 to 97%) in those diagnosed during the third trimester. A similar trend was found for cases with negative or absent antenatal diagnosis, with a sensitivity of 44% (95% CI, 14 to 79%) in the second trimester and 87% (95% CI, 60 to 98%) in the third trimester. This increase in PCR sensitivity with later maternal infection suggests that parasite circulation in AF may be transient.

In comparison with serology, PCR on neonatal AF would have allowed earlier diagnosis in 5/19 cases (26% [95% CI, 9 to 51%]) (Table 1). In these cases (22, 29, 30, 35, and 37), the infection remained undiagnosed at birth, and serology became positive only at two, four (2 infants), eight, or 24 weeks of life, leading to a delay in postnatal treatment. In practice, PCR on neonatal AF might prove useful when the diagnosis has not been made at birth, which corresponds to about 10% of the CT cases in France (French National Reference Center for Toxoplasmosis, unpublished data) but probably more in countries without a national prevention policy. In these cases, postnatal serologic follow-up during the first year of life may be hindered by poor compliance. Earlier diagnosis would allow earlier treatment of the child, which is known to reduce the frequency and severity of CT lesions (18, 19).

The reported sensitivity and specificity of PCR on placental tissue are 51.9 to 79.5% and 90.5 to 99%, respectively (3, 5–8). However, preanalytical sample processing is cumbersome, and its sensitivity varies widely across studies (8). Neonatal AF samples are much easier to transport and require simpler preanalytical treatment (centrifugation only). Moreover, a positive PCR result on a neonatal AF sample confirms CT at birth, while a positive PCR result on a placental tissue sample has to be confirmed by

TABLE 1 Data from the cohort of infants with congenital toxoplasmosis in this study

Case no.	Time of maternal infection (WA)[a]	PCR result using AF at[b]:		Serology result, delay (days)[c]	Clinical feature(s)
		PND	NND		
1	7	P	N	P, 1	Ventriculomegaly
2	14	P	N	N, 1	
3	16	N	P	P, 1	
4	21	N	P	P, 1	
5	22	P	N	P, 28	
6	22	ND	N	P, 1	
7	24	P	N	P, 168	
8	19	N	P	ND	Ventriculomegaly, TTP[d]
9	19	ND	N	P, 2	
10	19	P	N	N, 21	
11	26	ND	N	P, 112	
12	26	P	P	N, 1	
13	26	P	N	N, 28	
14	26	ND	N	P, 14	
15	28	P	N	P, 224	
16	28	ND	P	P, 1	
17	28	ND	N	P, 1	
18	29	P	P	ND	TTP
19	30	N	P	P, 1	
20	30	ND	N	P, 1	
21	30	P	P	ND	Cerebral anomalies, TTP
22	30	ND	P	P, 168	
23	24–31	ND	P	P, 1	
24	32	P	P	P, 70	
25	32	N	P	P, 1	
26	33	P	P	P, 1	
27	34	ND	P	P, 1	
28	35	ND	P	P, 4	
29	36	ND	P	P, 14	
30	36	ND	P	P, 28	
31	36	ND	P	P, 1	
32	17–34	P	N	P, 1	
33	38	N	P	P, 1	
34	38	ND	N	P, 112	
35	38	ND	P	P, 56	
36	38	ND	P	P, 1	
37	38	ND	P	P, 28	
38	33	P	N	P, 2	
39	33	ND	P	P, 1	
40	U	N	N	P, 210	
41	U	ND	P	P, 1	
42	U	P	N	P, 1	
43	U	P	P	N, 1	
44	U	ND	N	P, 28	
45	U	P	N	N, 252	
46	U	P	N	N, 112	

[a] WA, weeks of amenorrhea; U, unknown.
[b] AF, amniotic fluid; PND, prenatal diagnosis; NND, neonatal diagnosis; P, positive; N, negative; ND, not done.
[c] Delay from birth to the first positive serology (i.e., presence of IgM or IgA or synthesis of IgG) or to the last negative serology (i.e., absence of arguments supporting specific Ig production).
[d] TTP, therapeutic termination of pregnancy.

TABLE 2 Results of the molecular diagnosis on neonatal amniotic fluid samples

Neonatal AF sample from[a]:	No. with PCR result for fetuses: with CT[b] Negative	with CT[b] Positive	without CT Negative	without CT Positive	Total no.
All fetuses	21	25	442	0	488
Fetuses with positive PND	12	6	0	0	18
Fetuses with negative PND	1	6	287	0	294
Fetuses without PND	8	13	155	0	176

[a] AF, amniotic fluid; PND, prenatal diagnosis.
[b] CT, congenital toxoplasmosis.

serologic follow-up (3, 5–8). The sensitivity and specificity of PCR on neonatal cord blood samples are reported to be 21.2% and 100%, respectively (3), while in one study, PCR on neonatal peripheral blood samples revealed CT in 5 of the 6 children infected during the third trimester of pregnancy (9). However, if PCR on neonatal blood samples shows good specificity, its sensitivity is variable (3, 9). Consequently, in view of its good sensitivity (68%) and specificity (100%), PCR on neonatal AF samples might be useful when antenatal diagnosis is negative or not done. In addition, it may allow earlier diagnosis than that with postnatal serology, allowing prompt patient management (20). The main drawback is the difficulty of collecting AF at birth, as the amniotic sac may rupture before arrival in the labor room.

To our knowledge, this is the first study of the contribution of PCR on neonatal AF to the diagnosis of congenital toxoplasmosis at birth. More studies are needed to determine the sensitivity of this method according to the date of maternal infection and to compare it directly with neonatal PCR on placental tissue and infant peripheral blood.

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