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Prognostic factors for the long-term development of ocular lesions in 327 children with congenital toxoplasmosis

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SUMMARY

The aim of this study was to identify the high-risk factors associated with the development of ocular lesions in a large cohort of children with congenital toxoplasmosis (CT), irrespective of their gestational age at the time of maternal infection. Children were managed according to a standardized protocol and monitored for up to 14 years at the Croix-Rousse Hospital, Lyon, France. Cox model and a flexible regression, spline-based method were used for the analysis. During a median follow-up time of 6 years, 79 of the 327 children (24%) had at least one retinochoroidal lesion. No bilateral impairment of visual acuity was observed. The risk of a child developing ocular disease was higher not only when mothers were infected early during pregnancy, which was expected, but also when CT was diagnosed prior to or at the time of birth, when non-ocular manifestations were present at baseline and when birth was premature.

INTRODUCTION

Congenital toxoplasmosis (CT) results from a vertical transmission of the *Toxoplasma* parasite from the mother to the foetus, usually after acute maternal infection during pregnancy. With the exception of severely affected infants, who, having contracted CT very early during gestation, suffer from widespread inflammation of the central nervous system which often leads to hydrocephalus, most infected babies are asymptomatic at birth [1]. Although any organ can become affected by congenital infection, ocular disease is the most common manifestation [2–4]. The hallmark of congenital ocular toxoplasmosis is focal necrotizing retinochoroiditis, which may appear or reappear at any time during childhood or adolescence and threaten vision due to macula involvement [5, 6].

There is a general consensus that CT should be treated upon diagnosis, since an estimated 80% of untreated children develop ocular sequelae [4, 7, 8]. In 1994, the Chicago Collaborative Treatment Trial [9] proposed a specific treatment programme for children with an initially severe form of CT. Although protocols for treatment and follow-up may need to be supplemented in children at high risk of developing ocular lesions, we believe that no one has yet developed a strategy adapted to the real risks incurred by particular individuals. One reason for this may be that

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little is known about the characteristics of children with the highest risk of developing such lesions. An earlier work [10] aimed at estimating the risk of developing clinical signs, in addition to the risk of mother-to-child transmission of toxoplasmosis, and included children born between 1987 and 1995 identified through antenatal screening. However, clinical outcome was measured at 3 years of age and this study did not specifically address the question of ocular prognosis or prognostic factors associated with retinochoroiditis development. We believe that the factors prognostic for long-term ocular disease have not been investigated. The aim of the present study was to identify the high-risk factors associated with the development of ocular lesions in a large cohort of children with CT; children were identified through systematic prenatal screening and included irrespective of their gestational age at the time of maternal infection, managed according to a standardized protocol and monitored for up to 14 years.

SUBJECTS AND METHODS

Study design and population

The study was designed as an observational prospective cohort study. The study population comprised all children with confirmed CT who had been followed up for at least 6 months after birth to mothers monitored for primary *Toxoplasma* infection through antenatal screening between 1988 and March 2002 at the Croix-Rousse Hospital, Lyon, France. Monthly testing was commonly performed before it became required by law in 1992.

Definition and monitoring of maternal infection

Maternal infections were identified through the monthly testing of susceptible women which was recommended in 1985 and became mandatory in 1992. Samples that suggested seroconversion were received for confirmation from private and public laboratories of the Rhône department, France. Maternal *Toxoplasma* infection was defined as the appearance of specific serum IgG antibodies in previously seronegative women or as a significant rise in specific IgG in women with specific IgM [10]. Up to 1994, IgG was tested by indirect haemagglutination (Cellognost, Behring, Marburg, Germany) and subsequently by indirect immunofluorescence ELISA [11] (Behring). Similarly, detection of IgM was based on indirect haemagglutination until 1994 and then on ISAGA (bioMérieux, Marcy l'Etoile, France), indirect immunofluorescence. Avidity of IgG was determined from 1995 onwards. Gestational age at maternal infection was estimated prospectively and was therefore blind to the outcome. Standard maternal treatment consisted of the daily administration of spiramycin (3 g/day) until delivery. If the infection had occurred after the 32nd week of gestation or in case of positive antenatal diagnosis, 3-week courses of spiramycin (3 g/day) were alternated with 3-week courses of pyrimethamine (50 mg/day) and sulphadiazine (3 g/day) (PS).

Definition and monitoring of congenital infection

At 1 year of age, children were considered to be infected if at least one of the following criteria [12] were satisfied: (1) positive mouse inoculation of foetal blood (performed until 1994) or amniotic fluid, positive PCR on amniotic fluid and positive specific IgM and IgA in foetal blood; (2) positive IgM (index $\geqslant 3$) after birth, positive IgA ($\geqslant 0{\cdot}70$) after birth [detected by ELISA (SFRI, Bordeaux, France)]; (3) a rise in specific IgG during the first year of life; (4) persistent specific IgG (> 5 IU/ml) after the first year of life; or (5) clinical signs of toxoplasmosis. The antibody load at birth was also calculated as the ratio of specific IgG to total IgG; it was considered to be positive if the ratio was $\geqslant 10{\cdot}7$.

Mouse inoculation was used as the standard test until 1994 when it was superseded by PCR of amniotic fluid samples. Amniocentesis was performed not less than 4 weeks after the date of maternal infection. Children were managed according to a standard protocol. Assessments at birth included cerebral ultrasonography, X-ray of the skull and examination of the fundus oculi, in addition to tests for specific IgM, IgA and IgG on cord and neonatal blood samples. Paediatric check-ups, assessment of neurological development and IgG and IgM determination were scheduled every 3 months for at least 1 year for all children. Those with proven infection had additional 3-monthly eye examinations and their follow-up (neurological, ophthalmological and serological) was repeated every trimester for the second year, every semester for the third year and yearly thereafter without age limit.

Children whose infection was diagnosed before 2 months of age (weighing < 5 kg bodyweight) received a 3-week course of pyrimethamine (3 mg/kg every 3 days), sulphadiazine (25 mg/kg every 8 h) and

folinic acid (50 mg orally every 7 days) with weekly haematological and renal monitoring. Fansidar® [pyrimethamine (1·25 mg/kg every 10 days) and sulphadoxine (25 mg/kg every 10 days)] and folinic acid (50 mg every 7 days) was started when bodyweight reached 5 kg, or at any time for children whose infection had been diagnosed later in the first year. Treatment was given for 12 months with monthly haematological surveillance. In the event of an active retinal lesion being detected upon treatment termination, Fansidar® was resumed for a further 3 months.

Data collection

All eye examinations were performed by experienced ophthalmologists and reported on a standard form. Their findings were retrospectively reviewed by an independent, external adjudication committee. After pupillary dilatation, the anterior and posterior segments of each eye were usually examined by direct ophthalmoscopy. The retina was alternatively visualized either directly, using a three-mirror-lens system, or indirectly using wide-field lenses, depending on the child's age and compliance, and on the ophthalmologist's preference. For children up to 3 years of age, Parinaud charts were used to assess visual acuity, which was age-matched according to standard norms [13]. Far vision was assessed using Snellen charts. Irrespective of the cause, blindness and low vision were defined as in ICD-10 [14]. Blindness was defined as visual acuity of less than 1/20 or corresponding visual field loss in the better eye with best possible correction and low vision corresponded to visual acuity of less than 3/10, but equal to or better than 1/20 in the better eye with best possible correction.

For each child information had been collected prospectively during routine visits on mother's date of infection and her treatment during pregnancy, as well as that of the child after birth (date, type and dosage), and the results of antenatal (ultrasonography; foetal blood and amniotic fluid analyses) and neonatal (umbilical cord and peripheral blood analyses; neurological, radiological and ophthalmological) evaluations.

Statistical analyses

In all time-to-event analyses, time zero (baseline) was defined either as the date of birth, if CT had been diagnosed before or at birth, or as the date of diagnosis if the disease had been identified thereafter. The time to the first occurrence of retinochoroiditis after the diagnosis of CT was defined as the interval between the baseline date and the detection of retinochoroiditis. As negative delay could not be taken into account, the study was limited to children for whom no ocular lesion was identified before CT had been confirmed biologically. Subjects who manifested no retinochoroiditis during the follow-up course were censored at the endpoint, i.e. 31 March 2002, if they were not lost to follow-up, or at their last ophthalmological examination otherwise. Children were considered as lost to follow-up when they had no diagnosis of retinochoroiditis and no follow-up after October 2000 (1·5 years before endpoint).

In the first step of the analysis, a univariate Cox proportional hazards (PH) model [15] was used to select the factors associated with the occurrence of ocular lesions at a P value less than 0·25. The following maternal characteristics were tested: mother's age at delivery, gestational age (in weeks) at the estimated time of maternal infection, clinical signs of infection and the duration of PS treatment (in days) during pregnancy. Offspring factors tested included: gender, gestational age at birth, age at CT diagnosis (with 0 assigned to children diagnosed at or before birth), non-ocular clinical signs of CT at baseline, serum levels of specific IgM and IgA and the antibody load (IgG) at birth (positive *vs.* negative). The effect of treatment received after the diagnosis of CT was modelled as a time-dependent covariate [15]. A calendar-year effect was also tested to take into account the routine use of PCR for the detection of *Toxoplasma gondii* DNA in amniotic fluid samples from 1994 onwards. A multivariable Cox PH model which included previously selected factors was then estimated using a backward strategy to identify independent risk factors for the occurrence of retinochoroiditis. During the backward elimination process, variables were excluded from the final model if the corresponding P value for the Wald test was higher than 0·05. First-order interactions between prognostic factors were also explored by forward selection into the model, which included the main effects of all variables selected by the backward procedure. We applied the same strategy while restricting the analyses to the cohort of children diagnosed with CT at or following birth, since in many countries, this disease is not usually diagnosed before birth.

In order to avoid biased estimates of the effects of prognostic factors in the Cox model, and to minimize the risk of type-II error, it is important to test the underlying PH assumption [16]. If the PH hypothesis

is rejected, the effect of a given prognostic factor changes during the follow-up and, in such cases, flexible methods should be applied to ensure accurate modelling of time-dependent effects [17–19]. In addition, an accurate representation of the effects of quantitative variables requires their log-linearity to be tested [20]. Indeed, in any survival analysis, it is important to test the PH and log-linearity hypotheses for each continuous predictor simultaneously [18]. We therefore used a flexible regression, spline-based method for the simultaneous modelling of time-dependent and nonlinear effects, which represents an extension of the time-dependent model proposed by Abrahamowicz et al. [18]. This method permits the joint testing and estimation of the time-dependence and linearity of continuous covariates, as well as the time-dependency of binary ones. The best trade-off between the number of parameters to be estimated and the optimal representation of each covariate effect (in terms of fit to data that minimize the model's variance), was chosen according to the Akaike Information Criterion (AIC) [21]. We also checked this model for a potential hidden impact of the other covariates that were found to be associated with the occurrence of ocular lesions at a P value below 0·25 in the univariate analysis.

For all the analyses, a P value below 0·05 was considered to be significant. The statistical analyses were performed using STATA v. 7.0 (Stata Corp., College Station, TX, USA) and a customized C program provided by Abrahamowicz and McKenzie.

RESULTS

Study population

A total of 1506 consecutive pregnant women were monitored for primary *Toxoplasma* infection between September 1987 and March 2002 at the Croix-Rousse Hospital, Lyon, France. Fifty-three pregnancies (4%) were interrupted either due to spontaneous abortion ($n=22$), to stillbirth ($n=4$) or to induced termination ($n=27$) for suspected or proven foetal infection. The other 1453 pregnant women gave birth to 1466 live-born children (26 twins) of whom 1384 (94%) could be followed until infection was either ruled out ($n=1026$; 74%) or confirmed ($n=358$; 26%). Thirty-one infected newborns were excluded from the survey because a retinal lesion was detected before the infection was confirmed serologically ($n=3$) and/or because the follow-up was shorter than 6 months at the end of the study ($n=29$). Five of these 29 children (17%) experienced morbidity ($n=4$) and/or mortality ($n=1$). One child developed hepato-splenomegaly and died within the first week. Among the four remaining children, intracranial calcifications were discovered at birth in three including one with mild hydrocephalia and normal development. A retinochoroiditis was discovered at 4 months of age for the last child. The three children with retinochoroiditis detected before toxoplasmosis had no other clinical sign of CT.

The remaining 327 infected children were included in the study. These children (sex ratio female/male: 0·96) were born to 324 mothers, who had a mean ($\pm$S.D.) of 28 ± 5 years at delivery. Three sets of twins were included among which two were monozygotic. Most mothers become infected during the second ($n=89$; 28%) or third trimester ($n=219$; 68%). Most mothers ($n=272$; 84%) were treated during pregnancy; in the other 52 (16%), infection was diagnosed at delivery. A total of 149 mothers (46%) were treated with spiramycin alone, 104 (32%) with spiramycin followed by pyrimethamine + sulphadiazine, and 19 (6%) with pyrimethamine + sulphadiazine alone. The distribution of gestational age at birth coincided ($P=0·371$) with a French population standard (unpublished data from the Perinatal Network of Burgundy in the year 2000; $n=17\,092$). Congenital infection was diagnosed during pregnancy in 27% of the children ($n=87$) and at birth in 50% ($n=163$). In the other 23% ($n=77$), the diagnosis was based on an increase in specific IgG between 1 and 12 months of life (median, 6 months). In children who where diagnosed with CT at birth, maternal infection was detected at a later time (median gestational age, 30 weeks; interquartile range (IQR), 27–33 weeks) than in those whose CT was diagnosed before birth (median gestational age, 22 weeks; IQR, 18–22 weeks; $P<10^{-4}$) or after parturition (median gestational age, 28 weeks; IQR, 23–32 weeks; $P=0·002$). Pyrimethamine and sulphadiazine were administered *in utero* to 38% of the foetuses, and all but two children were treated with Fansidar for an average duration ($\pm$S.D.) of 337 ± 23 days.

Development of retinochoroidal lesions

During a median follow-up time of 6 years (IQR, 3–10 years) after birth or after the post-birth diagnosis of CT, 79 of the 327 children (24%) manifested at least one retinochoroidal lesion. All but 13 lesions were inactive at the time of detection. The median age of

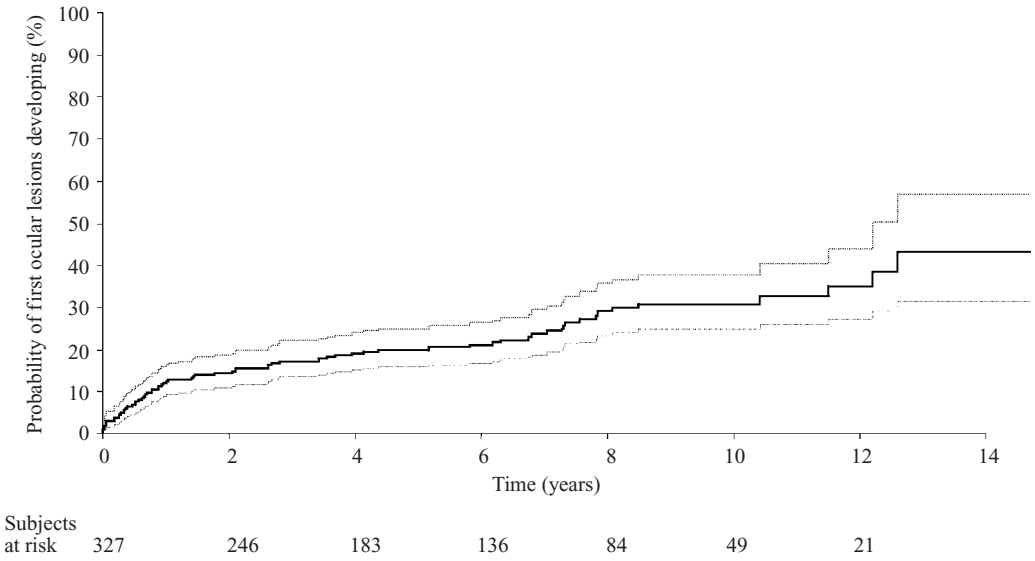

Fig. 1. Kaplan–Meier curve of the cumulative probability [and its 95% confidence interval (CI)] of retinochoroidal lesions developing (Lyon cohort: 1987–2002).

children with a first ocular lesion identified as active was 1·2 years (IQR, 5 months to 6·7 years) and for children with inactive lesions it was 1·0 year (IQR, 6 months to 4·1 years; $P=0{\cdot}953$). Thirty lesions were diagnosed in children who were treated with Fansidar®. Thirty-eight children (12%) manifested at least one lesion by the age of 1 year. Half of the initial lesions were diagnosed before 2 years of age, 76% before 5 years and 95% before 10 years. Figure 1 represents the Kaplan–Meier curve for the cumulative probability of a retinochoroidal lesion occurring.

The estimated probability increases from 12·6% (95% CI 9·0–16·1) at 1 year to 19·9% (95% CI 15·8–25·0) at 5 years, to 29% (95% CI 23·3–35·7) at 8 years and to 35% (95% CI 27·3–44·1) at 12 years after birth or after the post-birth diagnosis of CT. Ophthalmological findings at the final examination are summarized in Table 1.

Nearly 76% (248/327) of children had no ocular involvement. Among the 79 children with at least an ocular lesion, 24 had both eyes affected. Their characteristics did not statistically differ from those of children with unilateral lesions (Table 2).

Final visual acuity, available for 66 of the 79 children with retinochoroiditis, was below 20/20 in 24 cases (36%), as a consequence of a toxoplasmic macular lesion in 15 instances and to additional factors in the other 9 (strabismus, $n=9$; microphthalmia, $n=4$; retinal detachment, $n=3$; cataract, $n=1$; hyalitis, $n=1$). When the WHO's categories of visual impairment were applied, no children could be considered as having a low vision or being blind as their second eye had a normal or subnormal visual acuity. A moderate unilateral alteration of visual acuity was observed in 9 children, 7 children had a low

Table 1. *Ophthalmological findings at the final examination* (*Lyon cohort: 1987–2002*)

	n	%
No ocular involvement	248	76
Ocular lesions	79	24
Peripheral	38	48
Macular	31	39
Peripapillary	8	10
Peripapillary + macular	2	3
Eyes involved		
Right eye	32	41
Left eye	23	29
Both eyes	24	30
Visual impairment		
Unilateral lesion*	55	100
Low vision ($>1/20$ and $\leqslant 3/10$)	5	9
Blindness ($\leqslant 1/20$)	4	7
Unknown	10	18
Bilateral lesion†	24	100
Low vision ($>1/20$ and $\leqslant 3/10$)	2	8
Blindness ($\leqslant 1/20$)	4	16
Unknown	3	13

* No retinochoroiditis in the other eye.

† No bilateral visual impairment was observed but the visual acuity could be reduced in one of the two eyes involved.

Table 2. *Description of mothers and children characteristics according to the presence or absence of ocular lesions* (*Lyon cohort: 1987–2002*)

	No ocular lesion ($n=248$)		Ocular lesion ($n=79$)						
			Unilateral (55)		Bilateral (24)			Total	
Covariates	Mean	s.d.	Mean	s.d.	Mean	s.d.	P*	Mean	s.d.
Maternal age	29	5	29	6	28	5	0·60	29	6
	Median	IQR†	Median	IQR	Median	IQR		Median	IQR
Gestational age at contamination (WP)‡	29	24–33	25	19–30	23·5	19–29	0·61	25	19–30
Gestational age at birth (WP)	38	37–39	37	36–39	37·5	36–38	0·97	37	36–38
Maternal treatment by pyrimethamine/sulphonamides duration (days)	0	0–21	0	0–21	0	0–21	0·66	0	0–21
	n	%	*n*	%	*n*	%		*n*	%
Clinical signs during infection							0·97		
No	220	89	50	91	22	92		72	91
Yes	17	7	3	5	1	4		4	5
Unknown	11	4	2	4	1	4		3	4
Sex							0·37		
Male	127	51	26	47	14	58		40	51
Female	121	49	29	53	10	42		39	49
Time of diagnosis							0·41		
Before birth	59	24	17	31	11	46		28	35
At birth	126	51	27	49	10	42		37	47
After birth	63	25	11	20	3	12		14	18
Presence of at least one other symptom of CT§ at baseline							0·90		
Yes	16	7	13	24	6	25		19	24
No	232	93	42	76	18	75		60	76
IgM at birth							0·65		
Positive	133	54	33	60	11	46		41	58
Negative	98	40	20	36	13	54		36	39
Unknown	17	6	2	4	0	0		2	3
IgA at birth							0·22		
Positive	75	31	18	33	5	21		23	29
Negative	83	33	12	22	3	12		15	19
Unknown	90	36	25	45	16	67		41	52
Antibody load at birth							0·86		
Positive	142	26	43	78	19	79		62	78
Negative	65	57	8	15	4	17		12	15
Unknown	41	17	4	7	1	4		5	6

* P value for the comparison of unilateral *vs.* bilateral involvement.
† IQR, Interquartile range.
‡ WP, weeks of pregnancy.
§ CT, congenital toxoplasmosis.

vision and 8 were blind in one eye. In seven instances, low vision or blindness was due to toxoplasmosis.

Other manifestations of CT

At baseline, 35 children (11%) exhibited at least one of the following signs: hydrocephalus ($n=4$), isolated splenomegaly ($n=2$), hepato-splenomegaly ($n=2$), microcephaly ($n=1$), intracranial calcifications ($n=31$). Such manifestations were associated with an early diagnosis of CT ($P=0·008$). At the final paediatric check-up, two more children were found to have developed hydrocephalus. Three children

Table 3. *Predictive factors for the occurrence of retinochoroidal lesions* (*Lyon cohort: 1987–2002*). *Results of Cox model analyses*

	Univariate analysis		Cox's multivariate analysis				
			Complete model		Final model		
Covariates	HR*	*P*†	HR‡	*P*†	HR‡	95% CI§	*P*†
Maternal covariates							
Maternal age	1·01	0·592					
Duration of the maternal treatment with pyrimethamine/sulphamides	1·00	0·500					
Clinical signs during infection							
Yes *vs.* no	0·77	0·599					
Unknown *vs.* no	1·14	0·824					
Gestational age at contamination (weeks)	0·96	0·010	0·98	0·134	0·96	0·94–0·99	0·017
Offspring covariates							
Gender (male *vs.* female)	0·88	0·560					
Gestational age at birth	0·88	0·090	0·95	0·135			
Age at baseline	0·99	0·034	1·00	0·104	1·00	0·99–1·00	0·044
Presence of at least one non-ocular sign of CT‖ at baseline (yes *vs.* no)	3·43	$<10^{-4}$	2·44	0·002	2·61	1·49–4·59	0·001
IgM at birth							
Positive *vs.* negative	1·02	0·924					
Unknown *vs.* negative	0·58	0·457					
IgA at birth							
Positive *vs.* negative	1·44	0·269					
Unknown *vs.* negative	1·32	0·372					
Antibody load at birth							
Positive *vs.* negative	2·11	0·018	1·57	0·170			
Unknown *vs.* negative	0·68	0·470	0·67	0·452			
Treatment (time-dependent covariate)							
Spiramycine alone *vs.* no treatment	1·51	0·469					
Pyrimethamine + sulphadiazine *vs.* no treatment	0·81	0·801					
Pyrimethamine + sulphadoxine *vs.* no treatment	1·25	0·639					
Spiramycine + pyrimethamine + sulphamide *vs.* no treatment	1·05	0·966					

* Unadjusted hazards ratio (HR).
† *P* value for the Wald test of no association.
‡ HR adjusted for all other variables in the column.
§ CI, confidence interval.
‖ CT, congenital toxoplasmosis.

with hydrocephalus had moderate psychomotor retardation, but the other three had normal development and school progression. Two of the children with calcifications had only an isolated episode of seizures despite the lack of long-term therapy.

Predictive factors for the existence or development of retinochoroiditis

The results of the univariate and multivariate Cox's regression analyses are summarized in Table 3. In the univariate analysis, a younger gestational age at the time of maternal infection, the diagnosis of CT either before or at birth and the manifestation at baseline of at least one non-ocular sign of CT were all associated with a statistically significant increase in the risk of developing an ocular lesion. Since the age of a child at baseline and the timing of CT diagnosis were closely correlated in the multivariable analysis, we chose to include only the age at baseline which was more predictive according to AIC and was not significantly

correlated ($P=0{\cdot}396$) to gestational age at the time of maternal infection. Variables that were not associated with the risk of developing ocular lesions included the following: gender, serum levels of IgM and IgA at birth, maternal treatment strategy during pregnancy and offspring treatment strategy during childhood (Table 3). Similarly, no calendar-year effect (before *vs.* after 1994) was identified ($P=0{\cdot}496$). The results of the multivariable Cox model are likewise presented in Table 3. The risk of a child developing retinochoroiditis was reduced by 14·8% (95% CI 2·8–25·3) with each advancing month of pregnancy before the mother became infected. Similarly, older age at baseline was also associated with a lower risk: the risk of a child developing retinochoroiditis was reduced by 10·5% (95% CI 0·3–19·6) for each additional month of the child's age. The presence at baseline of at least one non-ocular clinical sign of CT increased the risk of developing retinochoroiditis by a factor of 2·6 (95% CI 1·5–4·6). When children who where diagnosed with CT before birth were excluded, the final model achieved was similar: the gestational age at the time of maternal infection and the age of a child at baseline were still associated with a higher risk of occurrence of ocular lesions. However, the manifestation at baseline of at least one non-ocular clinical sign of CT lost statistical significance ($P=0{\cdot}079$; data not presented).

The three covariates identified as independent prognostic factors in the multivariable Cox model were introduced into the flexible regression, spline-based model in order to test and model time-dependence and/or non-loglinear effects. This analysis revealed the effects of gestational age at the time of maternal infection, a child's age at baseline and the presence at baseline of at least one non-ocular clinical sign of CT to be constant over time, i.e. to be consistent with the PH hypothesis. However, the relationship between a child's age at baseline and the risk of developing ocular lesions was not log-linear ($P=0{\cdot}028$ for the log-linearity test): the age of a child at baseline had a significantly increasing impact on the development of ocular lesions if the time elapsing between birth and the diagnosis of CT exceeded 9 months than if the interval was 1 month.

Finally, we tested for the existence of nonlinear and/or time-dependent effects of the other covariates that were found to be associated with the occurrence of ocular lesions at a P value below 0·25 in the univariate analysis. These were the gestational age of a child at birth and antibody load (IgG) at birth after adjusting for gestational age at the time of maternal infection, for the age of child at baseline and for the presence at baseline of at least one non-ocular clinical sign of CT. The impact of antibody load remained statistically insignificant in the flexible analyses ($P>0{\cdot}081$ in testing the PH hypothesis). However, the gestational age of a child at birth had a significant impact when non-proportionality ($P=0{\cdot}0004$) and nonlinearity ($P=0{\cdot}0006$) were simultaneously taken into account. The estimated pattern of time-dependence indicated that the impact of gestational age at birth on the development of ocular lesions was limited to the period between 2·5 and 5 years after baseline. Overall, the risk of retinochoroiditis occurring decreased as the gestational age of a child at birth increased. Premature infants (with a gestational age at birth <32 weeks) were at considerably higher risk of developing an ocular lesion than were infants born at term (37 weeks), although this tendency did not attain statistical significance.

DISCUSSION

Many studies have reported on the long-term ocular prognosis of children with CT and several authors [10, 22, 23] have recognized that a relationship exists between the severity of CT and gestational age at the time of maternal infection. However, to the best of our knowledge no study has specifically addressed the factors prognostic for long-term ocular disease, as we have done. Such information is important for the counselling of parents and the planning of a child's follow-up.

This study was conducted in a large cohort of unselected children with CT confirmed using a standardized protocol and born to mothers monitored for primary *Toxoplasma* infection which was identified through systematic antenatal screening. Early postnatal acute infections are unlikely for epidemiological reasons and would have been recognized by their very distinctive serological pattern (kinetics of IgA and IgM, low avidity of IgG). Serological follow-up was extended up to the date of the last ophthalmological examination and allowed for clear biological confirmation in all cases. None of the children included in this study was referred to our centre for complementary investigations of signs or symptoms detected before the diagnosis of CT was suspected. Moreover, among the 327 children included, only 49 could be considered as lost to follow-up (15%) and for those children the median follow-up was 4·6 years

(IQR, 2·2–8·2 years) after baseline. Considering that 75% of retinochoroiditis occurred in our study before 5 years, and the rather low percentage of losses to follow-up, we believe that such a bias only had very little impact on our results. As ocular outcome was assessed based on the last examination, only the techniques used for this latest assessment were taken into account. Indirect ophthalmoscopy may lack sensitivity, especially for the detection of lesions in the peripheral retina, but the risk of underdiagnosis was reduced by the repetition of eye examination at close intervals (10 examinations on average in the first 3 years). Furthermore, there was no statistical difference between the age of children at the discovery of active and inactive lesions, suggesting that the technique used for examining retina had a little impact on our results. Moreover, the 24% cumulative incidence of ocular disease found in our cohort corresponds closely to that documented by other French authors whose subjects were identified and monitored according to protocols that were similar to ours [24–27]. Although almost half of the children with retinochoroiditis were identified as having this condition during the first 2 years following CT diagnosis, the first occurrence of ocular disease could nevertheless occur at any time during the follow-up period, even during adolescence, which confirms earlier findings [5, 7, 28]. This implies that long-term ophthalmological follow-up should be recommended for children with CT.

Our findings reveal that the risk of developing an ocular disease is higher not only when mothers are infected early during pregnancy, which was expected [10, 22] but also when CT is diagnosed prior to or at the time of birth and when children have non-ocular clinical signs of CT at baseline. Most of the non-ocular symptoms were of a neurological nature, with intracranial calcifications predominating. Nevertheless, neurological development was normal in most of these treated children [29], even in those with hydrocephalus, and episodes of seizures remained isolated despite the lack of long-term therapy. Previous studies have shown neurological damage to be associated with both the occurrence and severity of ocular disease [5, 9, 12]. This finding may reflect the limited availability of antibody in the central nervous system and the eye, which could account for the parasite continuing to proliferate at these sites whilst disappearing from extraneural sites [30]. However, even though the periodicity of ophthalmological examination was not conditioned by the presence of non-ocular signs, we cannot exclude the fact that the ophthalmologist may have observed children with extra-ocular signs more closely.

As far as we are aware, no previous study has suggested that an early diagnosis of CT could increase the risk of ocular lesions developing. An early diagnosis could reflect early infection, which would result in a more severe form of CT. In our study, we failed to identify a relationship between a child's age at the time of CT diagnosis and gestational age at the time of maternal infection, but the existence of non-ocular signs of CT at baseline was associated with an earlier CT diagnosis. This latter finding implies that if an antenatal PCR result (when available) and/or serological findings at birth are positive, then the risk of ocular lesions occurring is increased. This result may be understood in terms of the severity of infant infection, which, when great and extensive, may lead to positive results at an earlier stage.

Premature birth also appears to increase the risk of ocular disease developing within 2·5–5 years after CT diagnosis, irrespective of gestational age at the time of maternal infection. This finding may be partially explained by the circumstance that premature children have a less-well-developed immune system in which antibody synthesis does not begin until the 22nd week of gestation [31], and are thus less able to fight infection. Some authors argue that CT could be a reason for premature birth and/or intrauterine growth retardation [9, 32, 33] in untreated children. However, the distribution of gestational age at birth in our cohort of individuals with confirmed CT did not differ from that of the standard French population, which may reflect the fact that children born to treated mothers manifest signs of generalized disease less frequently than do those born to untreated ones [34].

Some authors [35, 36] have suggested that females are more liable to develop ocular toxoplasmosis or that female foetuses may be more susceptible to congenital infection. We did not find gender to be a high-risk factor in the development of retinochoroidal lesions and the sex ratio lay close to 1. There may be several reasons for this discrepancy between our findings and those reported by other authors. The study of Chapman et al. [35] included much older individuals (mean age 40 years) than did ours. Furthermore in the report by Gomez-Marin et al. [36] puberty may have interfered with the natural history of CT, in that a higher frequency of retinochoroiditis was observed in adolescent girls (13–16 years) than in boys of the same age.

Some of our results were unexpected. However, the present study was designed to identify the high-risk factors associated with the development of ocular lesions in a cohort of unselected children with confirmed CT. Moreover, we used flexible statistical methods allowing for the simultaneous modelling of time-dependent and nonlinear effects. This method not only reduced the risk of making incorrect inferences but also permitted a more accurate assessment of the effect of each identified prognostic factor. Although such statistical analysis only allowed us to include children who did not already have retinochoroiditis discovered before CT diagnosis, these are the children for whom therapy to prevent vision loss will be most important. We considered the six twins as single individuals as a previous work [37] showed that marked divergence of the clinical course could be observed even in monozygotic twins.

We are aware that our cohort of children were identified as having CT by a systematic antenatal screening programme. When the *P* values relating to the associations between particular characteristics and the risk of developing ocular lesions were examined, the most significant risk factors appeared to be gestational age at the time of maternal infection and the existence of non-ocular clinical signs of CT at baseline. However, in many countries, no systematic antenatal screening is undertaken, and gestational age at the time of maternal infection cannot therefore be established except by sporadic screening or when toxoplasmosis is suspected on the basis of ultrasonographic or clinical signs during pregnancy. Hence, in order to assess the relative prognostic utility of gestational age *vs.* other clinical and biological characteristics, we compared the goodness of fit of different versions of the Cox model. Specifically, the loss of predictive value was much less when gestational age was excluded from the model (negative log-likelihood with and without this parameter being 405·4 and 408·1, respectively) than when other characteristics were omitted (negative log-likelihood = 419·3). This finding suggests that the model's ability to identify children who are at high risk of developing ocular lesions relates mostly to clinical and biological information collected after birth. Moreover, the general results were confirmed when the analyses were restricted to children whose CT was diagnosed at the time of or after birth. This being the case, our results could hold true for other screening contexts. It must be also emphasized that, except for gestational age at birth, the effects of prognostic factors were constant over time, which suggests that their value in predicting the risk of ocular lesions developing is as valid at 1 year as it is, for example at 10 years of age.

We did not identify any association between the serum levels of IgM or IgA at birth and the occurrence of ocular lesions. The synthesis of specific IgM and IgA is correlated to the gestational age of a child at the time of maternal infection [38], which also has a bearing on the time at which CT is diagnosed. These correlations could mask a prognostic impact of IgA and IgM levels, even if the child's age at the time of CT diagnosis and not the actual time at which the CT diagnosis was made is taken into account. However, it seemed more appropriate to predict the risk of ocular disease on the basis of readily obtained clinical information rather than on that of more exacting biological data.

The standardization of treatment did not permit us to assess the real impact of this parameter, owing to the inherent correlation between gestational age at the time of maternal infection, the juncture at which CT was diagnosed, and the treatment received by individual children. However, this study did not aim at assessing treatment efficacy, rather at estimating the effects of other plausible prognostic factors, with a view to identifying those children at high risk of developing ocular disease. Although numerous attempts have been made to assess treatment efficacy, published results are still the subject of controversy [39, 40]. Despite the overriding impression that treatment does benefit a child [5, 9, 26, 34, 41], randomized controlled trials are required to confirm this belief [23, 39, 42].

Our results constitute a first step in delineating treatment and follow-up protocols that are better suited to the characteristics of individual children. Ongoing studies that are part of the European Multicentre Study on Congenital Toxoplasmosis may go one step further [34]. At this juncture, parents should be informed that despite the risk of late-onset of ocular lesions, the overall ocular prognosis is satisfactory in treated children. Nevertheless, annual life-long ophthalmological follow-up is to be recommended for children with a high risk of developing ocular lesions as treatment has been reported to result in the rapid resolution of active lesions, usually without visual loss [5]. Our study suggests that children who contracted CT early during pregnancy, who had non-ocular clinical signs of CT at baseline, and who where born prematurely should be followed closely.

Further studies should now be undertaken to refine these results, in particular, a prognostic score should be derived and confirmed for children with CT in various screening contexts.

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