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Original Article

Postnatal ocular toxoplasmosis in immunocompetent patients

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Abstract

Introduction: Ocular toxoplasmosis is the most common cause of infectious posterior uveitis worldwide. It can be prenatal or postnatal in origin. Despite estimations that postnatal ocular toxoplasmosis is more prevalent, only several cases of proven postnatal ocular toxoplasmosis have been reported in non-epidemic settings. Here, the clinical evolution of ocular toxoplasmosis of conclusively proven postnatal origin in immunocompetent patients is reported.

Methodology: Postnatal ocular toxoplasmosis was diagnosed based on clinical diagnosis supported by the longitudinal detection of *Toxoplasma gondii*-specific IgG, IgM and IgA antibodies in the serum as well as by direct detection of the parasite (bioassay) and/or its DNA (real-time PCR) in aqueous humor.

Results: Three cases involved adults in whom ocular toxoplasmosis developed during primary *T. gondii* infection, as part of the clinical presentation in two and as the sole manifestation in one patient. The fourth patient was a case of inactive ocular toxoplasmosis in a 14-year-old boy, where postnatal infection was confirmed by exclusion of maternal infection. The causative parasite strain was genotyped in only one case and it belonged to genotype II, the dominant type in Europe. One patient acquired the infection in Africa, suggesting an atypical strain.

Conclusions: The distinction between prenatal and postnatal ocular toxoplasmosis is only possible in particular clinical situations, and requires extensive laboratory investigation. Genotyping of the parasite strain involved may be important, particularly if atypical strains are suspected, requiring tailored treatment approaches.

Key words: *Toxoplasma gondii*; ocular toxoplasmosis; postnatal infection; immunocompetent patients; strain genotype.

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Introduction

Toxoplasma gondii is a ubiquitous parasite capable of infecting a wide range of hosts. The sexual cycle of *T. gondii* occurs only in the feline hosts and results in the formation of highly infectious oocysts. In all other hosts, including humans, only the asexual cycle occurs, characterized by fast replicating tachyzoites, and slowly dividing bradyzoites, localized within tissue cysts. Tachyzoites are responsible for the acute stage of infection. The crucial event in the pathogenesis of toxoplasmosis is the conversion of tachyzoites into bradyzoites, which tend to encyst in the brain tissue, retina, and muscles. *T. gondii* cysts perpetuate latent infection in the host. Since stage conversion is a process triggered and controlled by the host immune response, life-threatening *T. gondii* infection can occur in the fetus and in immunocompromised individuals [1].

The *T. gondii* population consists of three global clonal lineages, referred to as genotype I, II and III, of endemic ones such as haplotype 12 found in the US [2], and of atypical strains, with a mixture of alleles or entirely new alleles. Some of the latter have been grouped into Africa 1-4, Caribbean 1-3, and Brazil 1-4 [2,3]. In Europe, a vast majority of human toxoplasmosis cases have been associated with the mildly virulent genotype II [4]. In contrast, atypical strains are highly pathogenic, more frequently affecting the eyes and resulting in severe outcomes regardless of the host's immune status [5,6].

Ocular toxoplasmosis (OT) is the most common complication of *T. gondii* infection. However, the prevalence and incidence of OT are difficult to establish as they depend on the overall prevalence of the infection in a given population and on the genotypes of the local strains of *T. gondii*. An estimate based on US data

where *T. gondii* genotype II predominates indicates that 2% of all *T. gondii*-infected individuals may develop ocular symptoms [7]. OT is the most common cause of infectious posterior uveitis worldwide, and can result in serious loss of vision or blindness. Furthermore, due to tissue cysts persisting in the retina, patients with OT have a lifelong risk of recurrence [8]. Since no drug is able to eradicate tissue cysts, treatment focuses on reducing inflammation and subsequent retinal scarring [9], and antiparasitic drugs are needed for the control of released tachyzoites, pushing them to re-encystation. OT can be acquired postnatally or be a consequence of congenital infection [10]. Contrary to the formerly widespread opinion that congenital OT is the more prevalent one, postnatal OT is today considered to be more frequent than congenital OT [11,12]. Gilbert and Stanford [13] calculated that at least two-thirds of OT were caused by postnatal infection.

The diagnosis of OT relies most heavily on typical clinical findings on the retina; laboratory examination can support a presumptive clinical diagnosis in case of positive *T. gondii* serology, or rule it out in case of negative serology. PCR detection of parasite DNA in aqueous humor (AH) can provide a rapid diagnosis in patients with active lesions, but requires invasive anterior chamber puncture and is thus seldom carried out. Although some specific clinical features, such as bilateral involvement or delay in onset, have been more frequently associated with OT resulting from congenital infection, there are no unique clinical characteristics associated with either prenatal or postnatal OT, and they cannot be distinguished clinically [10]. Thus, distinction between prenatal and postnatal origin of OT relies entirely on laboratory findings.

Many reports regarding postnatal OT were published during epidemic outbreaks in South America, mostly attributed to atypical strains [14,15]. In Europe, however, cases of confirmed postnatal etiology of OT have been rarely reported [16,17], although description and analysis of such cases can help to understand the frequency, clinical course, and outcome of postnatally acquired OT. We here report four cases of postnatally acquired OT in immunocompetent patients, conclusively proven using a combination of immunodiagnostic and molecular tests.

Methodology

Suspected OT patients were referred by their ophthalmologists to the Serbian National Reference Laboratory for Toxoplasmosis (NRLT) for diagnostic workup. Peripheral blood samples were drawn at the NRLT, while AH samples were taken at the Clinical Centre of Serbia (CCS) Clinic for Eye Diseases, and immediately transported to NRLT. The study has followed the tenets of the Declaration of Helsinki. All patients have given informed consent for participation in the study. The study was approved by a local Ethics Committee.

Serology

T. gondii specific IgM and IgG antibodies, and specific IgG avidity, were detected using commercial assays based on an enzyme-linked fluorescence technique on the fully automated VIDAS system (VIDAS TOXO IgM – TXM, VIDAS TOXO IgG II – TXG, and VIDAS TOXO IgG Avidity – TXGA, bioMérieux, Marcy l'Etoile, France). Results were interpreted according to the manufacturer's recommendations. For TXM the cut-off value is 0.55, results between 0.55 and 0.65 are considered borderline, and results above or equal to 0.65 are considered positive. Results for TXG are expressed in IU/ml and were interpreted as follows: < 4 IU/mL, negative; 4–8 IU/mL, borderline; ≥ 8 IU/mL, positive. TXGA results are expressed as indices that correspond to the avidity percentages (< 20%, 20-30%, ≥ 30%, respectively); the cut-off value is 0.2; results below the cut-off are considered low avidity; 0.2-0.3 borderline, and results above or equal to 0.3 indicate high avidity. Specific IgA antibodies were detected by Platelia TOXO IgA assay (Bio-Rad, California, USA). Results are expressed as indices and were interpreted according to the manufacturer's recommendation, as follows: < 0.8, negative; 0.8–1, borderline; ≥ 1, positive.

Parasite detection

DNA detection

Complete DNA from the blood samples and AH was extracted using the QIAmp DNA mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. Real-time PCR (qPCR) was performed according to a protocol described in Štajner *et al*. [18]. Briefly, the *T. gondii* 529-bp repetitive element (GenBank accession number AF146527.1) was detected with Taqman probe (10 pmol/μL) 6FAM-ACG CTT TCC TCG TGG TGA TGG CGTAMRA [19,20]. Amplification was performed in an Eppendorf Mastercycler realplex 1.5 device (Eppendorf, Hamburg, Germany), using the following cycling conditions: 2 minutes at 50 °C for UDG pre-treatment and 10 minutes at 94 °C for initial denaturation followed by 40 cycles of 15 seconds at 95 °C for denaturation and 60 seconds at 60 °C for annealing/extension.

Bioassay

Isolation of the parasite was attempted by intraperitoneal (i.p.) inoculation of two female Swiss Webster mice (Medical Military Academy Animal Research Facility, Belgrade, Serbia), each with 500 µL of blood sample as described previously [21]. After six weeks, mice were euthanized, and brains harvested; brain homogenates were examined microscopically for *T. gondii* tissue cysts. A bioassay was considered positive if at least one *T. gondii* cyst was detected in either mouse.

Results

In the past 12 years, since a medical information system for the collection, maintenance and archiving of medical records has been implemented in the NRLT, out of a total of 95 patients with retinochoroiditis of confirmed toxoplasmic etiology, only four patients were conclusively diagnosed with postnatal OT. To describe in detail the evolution of OT, cases are presented individually, with a focus on laboratory assays and steps leading to the final diagnosis.

Case 1

A 40-year-old female developed fever, generalized lymphadenopathy and splenomegaly, simultaneously with loss of vision in the left eye two weeks after returning from a vacation in Mauritius, for the New Year 2008 holidays. The patient was diagnosed with focal necrotizing retinochoroiditis and was immediately referred to the NRLT.

Serological analysis of the initial sample showed absence of specific IgG antibodies but high levels of specific IgM and specific IgA antibodies (Table 1). In a follow-up sample drawn three weeks later seroconversion was demonstrated, by the detection of specific IgG antibodies of extremely low avidity, along with a still high level of specific IgM antibodies. This blood sample was also bioassayed, and six weeks later *T. gondii* cysts were visualized on microscopic slides of brain homogenates, thus confirming acute infection.

The patient was treated with systemic clindamycin and trimethoprim/sulfamethoxazole, in alternation with pyrimethamine, and with subconjunctival injections of dexamethasone.

Table 1. *Toxoplasma gondii* diagnostic assay results in cases 1-3.

		Case 1	Case 2	Case 3
Initial and first follow-up serology*	VIDAS TOXO IgM	9.04; 8.36	8.28; 7.43	
	Platelia TOXO IgA	11; 10.90	4.36; 1.32	
	VIDAS TOXO IgG II	1 IU/mL; 82 IU/mL	512 IU/mL; 308 IU/mL	
	VIDAS TOXO IgG Avidity	NA; 0.052	0.055; 0.066	
	Bioassay	Positive	Negative	
	qPCR	ND	Positive	
Aqueous humor	qPCR	ND**	Positive, Ct = 29	
Serology at time of conversion to HA	Time since first sampling	10 months	9 months	
	VIDAS TOXO IgM	1.06	2.03	
	Platelia TOXO IgA	2.06	0.38	
	VIDAS TOXO IgG II	2,030 IU/mL	76 IU/mL	
	VIDAS TOXO IgG Avidity	0.549	0.356	
Serology at time of IgM negativization	Time since first sampling	6 years	8 years 9 months	
	VIDAS TOXO IgM	0.55	0.44	
	Platelia TOXO IgA	0.89	0.54	
	VIDAS TOXO IgG II	212 IU/mL	248 IU/mL	
	VIDAS TOXO IgG Avidity	0.531	0.553	
First available serum, 1997	VIDAS TOXO IgM			0.06
	VIDAS TOXO IgG II			0 IU/mL
February 2020, onset of symptoms	VIDAS TOXO IgM			5.03
	Platelia TOXO IgA			1.74
	VIDAS TOXO IgG II			990 IU/mL
	VIDAS TOXO IgG Avidity			0.048
November 2020	VIDAS TOXO IgM			0.37
	Platelia TOXO IgA			0.57
	VIDAS TOXO IgG II			72 IU/mL
	VIDAS TOXO IgG Avidity			0.228

* For case 1 first follow-up sample was taken three weeks later, and for case 2 five weeks later; ** Ophthalmologist advised against sampling due to severe symptoms. HA: high avidity; NA: not applicable; ND: not done; qPCR: real-time PCR.

Almost a year was needed for the conversion of acute infection to chronicity, which was marked by a rise in the avidity of specific IgG antibodies and a low but detectable level of specific IgM antibodies (Table 1). After the acute stage, the patient developed a large macular scar which caused loss of central vision in the affected eye. Six years after the initial presentation, the levels of specific IgM antibodies still lingered at borderline levels.

Case 2

A 47-year-old male presented at the CCS Clinic for Eye Diseases with painless but sudden loss of vision in the left eye five days after the onset of symptoms in late January 2011, and was diagnosed with retinochoroiditis. Physical examination revealed painless cervical lymphadenopathy and sub-febrile temperature, and the patient was referred to the NRLT.

Initial serology showed a high concentration of specific IgG antibodies of low avidity, a high level of specific IgM antibodies, and detectable specific IgA antibodies (Table 1). *T. gondii* DNA was detected by qPCR both in this blood sample and in AH (Table 1). These immunological and molecular findings supported toxoplasmic etiology of retinochoroiditis and the patient was immediately started on systemic treatment with clindamycin, trimethoprim / sulfamethoxazole in alternation with pyrimethamine, and prednisone, and subconjunctival injections of dexamethasone. In March 2011, mild improvement in visual acuity was registered, but concentric narrowing of the visual field was still present. Three months after the onset of symptoms, cervical lymph nodes were no longer enlarged, but the formation of a retinal scar was noted. A decrease in the level of specific IgM antibodies and a rise in the avidity of specific IgG antibodies were first detected nine months after the first presentation (Table 1). However, 12 months after disease onset, there was a sudden 24-fold increase in the concentration of specific IgG antibodies (from 76 IU/mL to 1,840 IU/mL). This systemic serological reactivation did not result in the appearance of new ocular symptoms. The patient has been followed up ever since, every six months for five years, then once a year for the last three years. Residual specific IgM lingered at borderline levels until the eighth year of follow-up, when ultimately they could not be detected. Initial cataract and epiretinal membranes in the posterior pole (interpapillomacular region) of the affected eye developed as long-term consequences of OT.

Case 3

A 47-year-old female reported sudden loss of central vision in the right eye in early February 2020. The first ophthalmological examination revealed a macular edema in the affected eye. The patient was hospitalized at the CCS Clinic for Eye Diseases, and was empirically started on systemic trimethoprim/sulfamethoxazole and subconjunctival injections of dexamethasone. After serology done at the CCS showed the presence of *T. gondii*-specific IgM and IgG antibodies, a final diagnosis of macular punctate outer retinal toxoplasmosis (PORT) was made, and systemic clindamycin was added to the treatment. Three months after the initial presentation, a macular scar started to form. On follow-up, visual acuity of the affected eye was found to be permanently reduced by 95% (unilateral legal blindness) due to the formation of an atrophic scar in the macular region.

After a follow-up ophthalmological examination in November 2020, the patient was referred to the NRLT. Interestingly, our database search showed that the patient's serological status had already been evaluated in the NRLT in 1997, in association with a recent miscarriage, when she was found to be non-immunized. Since the 1997 serum sample was stored in the NRLT biobank, analysis was repeated in 2020 with currently used assays, and negative serology was confirmed (Table 1). These findings confirmed postnatal etiology of the current OT. The February 2020 sample stored at the CCS was re-evaluated in the NRLT, and the results showed specific IgG antibodies of extremely low avidity along with high levels of specific IgM and specific IgA antibodies, all indicative of acute infection (Table 1). In a follow-up sample collected in November 2020, nine months after the onset of ocular symptoms, a markedly lower concentration of specific IgG of (still) borderline avidity was detected, while specific IgM and specific IgA antibodies were no longer detectable (Table 1).

Case 4

During a school-related ophthalmological examination, a retinal scar in the left eye was noted in a nine-year old boy in 2014. The patient had no visual field defects or any other ocular symptoms, and no further etiologic diagnostic examinations were carried out at the time. In October 2019, now aged 14, the patient had a routine annual ophthalmological check-up for myopia, when a whitish lesion of a 1.5-disc diameter, with pigmented fractions near the macula of the left eye, was noted. After this examination, the patient was referred to the NRLT.

Table 2. Case 4 – comparative serology of patient and mother.

	Patient (14 years old)	Mother
VIDAS TOXO IgM	0.07	0.08
Platelia TOXO IgA	0.30	0.27
VIDAS TOXO IgG II	52 IU/mL	0 IU/mL
VIDAS TOXO IgG Avidity	0.516	NA

NA: not applicable.

Serological analysis showed a high concentration of specific IgG antibodies of high avidity, negative specific IgA, and negative specific IgM antibodies (Table 2). As five years had elapsed since the initial ophthalmological examination, it was impossible to determine whether OT had developed because of postnatal or congenital infection based solely on the patient's serological status. Hence, the patient's mother was tested, and the results were negative for specific IgM, specific IgA, and specific IgG antibodies (Table 2). This ruled out congenital infection and afforded a definitive diagnosis of postnatally acquired OT. After establishing the etiology of the lesion, optical coherence tomography showed an atrophic scar on the posterior pole of the left eye that did not affect the fovea.

Discussion

Identifying whether the origin of a diagnosed OT is pre- or postnatal is difficult. Our results reflect this difficulty, since only four cases out of 95 patients with retinochoroidal lesions and positive *T. gondii* serology could be confirmed as postnatal. In the remaining 91 cases the origin of infection could not be definitively determined. OT can be definitively established as postnatally acquired only if it occurs during the course of acute infection. If serology confirms chronic infection, congenital OT cannot be ruled out in the absence of a previous seronegative sample. In line with this, among the four patients with proven postnatal origin of OT presented here, three adult immunocompetent patients had an initial OT episode during primary toxoplasmosis. The fourth, a 14-year-old boy, had a chronic infection and an inactive lesion at the time of initial serological evaluation but postnatal origin was confirmed by negative maternal serology.

The prevalence of OT varies considerably around the world and generally depends on the prevalence of *T. gondii* infection in an area. Such statistics are generally underestimates as they do not include cases with smaller or peripheral lesions that do not cause symptoms, and patients never seek medical attention. In the United States, it has been estimated that out of 1,075,242 persons infected with *T. gondii*, 21,505 will have ocular lesions, and 4,839 will develop symptomatic OT each year [22]. Among uveitis cases, the OT prevalence ranges from 1.3% reported in Japan to approximately 40% in South America [23,24], in Europe from 2.85% in Italy to 14% in France [25-27]. Fitting within this range, a recent study performed at a Serbian referral center established OT in 12.9% of all uveitis cases [28]. In contrast, a household survey of the general population in Erechim, Brazil reported retinal lesions in even 17.7% of individuals [29]. Such a high prevalence in the general population could be attributed to highly divergent and more virulent *T. gondii* strains in South America [30].

The presence of atypical strains has also been reported in isolates from Africa, including those from the Reunion Island [31]. This is relevant for patient 1 in the presented series, since symptoms of acute OT developed after her return from Mauritius, an island 226 km away from Reunion. Despite the initial isolation of the parasite by bioassay, we were not able to further maintain the strain nor was it genotyped. However, it may be assumed that it was an atypical strain of *T. gondii*, since the clinical presentation included severe systemic manifestations and severe ocular inflammation. A recently published case series by Leroy *et al*. [32] presented four cases of severe acute toxoplasmosis imported from Africa in which symptoms occurred after return to France, with two patients developing ocular sequelae. This parallels our case, which further reinforces toxoplasmosis as a travel risk and the necessity for screening symptomatic patients after their return home from countries with a higher prevalence of atypical strains [33]. In Europe, strains of genotype II have been shown to be the dominant causative agent of OT in France [34]. Indeed, in our patient 2, who had a milder clinical presentation and no history of travel overseas, the *T. gondii* strain belonged to genotype II as shown by microsatellite (MS) marker analysis of AH DNA (kindly performed by Daniel Ajzenberg, Limoges, France). In both cases IgM antibodies were detectable for long periods of time, years after the diagnosis of acute OT. Persistence of residual IgM antibodies, as detected by diagnostic tests of high sensitivity, has been described [35,36], and is more frequently associated with clinically manifest infections (unpublished observation).

Except for the parasite genotype, patient age can be a risk factor for ocular involvement and severity of the disease, as older age at the time of the first active lesion has been associated with a higher risk of recurrences [8]. Indeed, Bosch-Driessen *et al*. [37] showed that patients with OT and recent seroconversion were of a mean age of 50.6. During an outbreak in British Columbia, the mean age of patients with ocular symptoms was 54 [38]. In line with this, the three adult patients in this series developed primary OT at the ages of 40 (case 1) and 47 (cases 2 and 3). Importantly, patients 1 and 2 did not develop new retinal lesions during a respective follow-up of six and eight years, while patient 3 is a recent patient. Moreover, serological reactivation which occurred one year after the diagnosis of postnatal OT in patient 2, probably reflecting a boosted immune response following reinfection i.e., by ingestion of new cysts, was not accompanied by clinical exacerbation of OT. Of note is also that the patient in case 3 presented atypical OT – PORT – which has been described to most often occur in the first two decades of life, whereas our patient was 47 at disease onset [10].

Patient 4 is also interesting in the context of age, as the patient's young age could misdirect the diagnosis to congenital OT. However, since the patient's mother was seronegative, congenital infection was excluded, and consequently, postnatal OT was confirmed. Given the downward trend in the prevalence of *T. gondii* infection in women of reproductive age [39], exclusion of congenital origin of OT in a young child by a negative maternal serological profile may be expected to increase in frequency.

The presented cases also illustrate the disease burden of acquired toxoplasmosis, for which retinochoroiditis was shown to be the major contributor in the Netherlands, accounting for 1,350 DALYs, one-third of the total annual disease burden of toxoplasmosis [12]. In cases 1 and 3, OT led to complete loss of central vision and legal blindness in the affected eye, which enormously reduced the patients' quality of life. Moreover, a diagnosis of PORT (patient 3) bears a high risk of further complications including involvement of the fellow eye, and secondary optic neuropathy [10]. On the other hand, in patient 2, treatment restored visual acuity to a 20/30 vision in the affected eye (legal eyesight for driving), despite progressing sequelae and a risky localization of the scar near the macula; evolving sequelae may potentially require surgery in the future. Patient 4 had a perfect visual acuity with prescription glasses when last seen. Nevertheless, the life-long risk of developing new recurrences and exacerbating sequelae, warrants regular ophthalmological check-ups of all OT patients.

Given the available therapeutic options, it may be argued that the distinction between prenatal and postnatal OT, only possible through extensive laboratory investigation, is of purely academic relevance at this time. However, genotyping of the parasite strain involved may be an important addition to the diagnosis, particularly if atypical strains are suspected, as infection with different strains / strain types may require different therapeutic approaches. Treatment of acute symptomatic toxoplasmosis and/or different dosing regimens have been suggested for infection with atypical strains [33,40], and it is to be hoped that in the near future more options will become available to tailor treatment according to the infecting strain.

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