Clinical and Biological Factors Associated With Recurrences of Severe Toxoplasmic Retinochoroiditis Confirmed by Aqueous Humor Analysis

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• PURPOSE: To investigate clinical and biological factors influencing recurrences of severe toxoplasmic retinochoroiditis (TRC) confirmed by aqueous humor analysis.
• DESIGN: Retrospective case series.
• METHODS: Retrospective analysis of 87 subjects with severe TRC, proven by positive Goldmann-Witmer coefficient (GWC), *Toxoplasma gondii* (*T. gondii*) immunoblot, or *T. gondii*–specific polymerase chain reaction (PCR) in aqueous humor. Cases with immunosuppression or retinal scars without previous recorded episode were excluded. Time-dependent, clinical, treatment-related, and biological factors were explored by univariate and multivariate shared frailty survival analyses.
• RESULTS: Among 44 included subjects (age, 40.4 ± 17.6 years; follow-up, 8.3 ± 2.7 years), 22 presented recurrences. There was 0.11 recurrence/patient/year and mean disease-free interval was 5.0 ± 2.9 years. The risk of recurrence was higher immediately after an episode ($P < .0001$). Among recurrent cases, the risk of multiple recurrences was higher when the first recurrence occurred after longer disease-free intervals ($P = .046$). In univariate analysis, the recurrence risk declined with higher number of intense bands on aqueous *T. gondii* immunoblot ($P = .006$), and increased when venous vasculitis was present initially ($P = .019$). Multivariate analysis confirmed that eyes with more intense bands on immunoblot had fewer recurrences ($P = .041$). There was a near-significant risk elevation after pyrimethamine/azithromycin treatment ($P = .078$ and $P = .054$, univariate and multivariate). Intravenous corticosteroid administration, oral corticosteroid administration, aqueous GWC, and *T. gondii* PCR did not influence recurrences ($P = .12$, $P = .10$, $P = .39$, and $P = .96$, respectively).
• CONCLUSIONS: Recurrences of severe TRC are not random and may be influenced by clinical and biological factors possibly related to blood-retinal barrier alterations. These results may contribute to identifying biomarkers for TRC reactivation. (Am J Ophthalmol 2019;199:82–93. © 2018 Elsevier Inc. All rights reserved.)

AJO.com Supplemental Material available at AJO.com.

Accepted for publication Nov 21, 2018.

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TOXOPLASMIC RETINOCHOROIDITIS, CAUSED BY THE obligate intracellular parasite *Toxoplasma gondii* (*T. gondii*), is the most frequent infectious posterior uveitis, and may lead to irreversible visual impairment. Following congenital or postnatal infection, it manifests as foci of retinochoroiditis associated with intraocular inflammation of varying severity. After episode resolution, parasites convert to the bradyzoite stage of their life cycle and remain quiescent within intraretinal cysts, for an unpredictable duration. At some point, nonelucidated factors precipitate the conversion back to the active, tachyzoite form, producing 1 or several new foci of retinal necrosis, most often adjacent to an old toxoplasmic scar, triggering a new intraocular inflammatory episode.

Over time, new recurrences lead to an increased risk of ocular complications, contributing to a worse visual outcome,[1] and an increased risk of treatment-related adverse events. The time pattern of recurrences is variable,[1,2] resulting in an apparent random occurrence over the lifetime of infected individuals. However, large case series have demonstrated that the timing of recurrences is governed by nonstochastic processes, with a tendency for episode clustering and a decreased risk of recurrence over years since the first episode.[3,4]

Factors associated with parasite reactivation after disease-free intervals are still debated. Among others, younger age at diagnosis,[3,5] older age at an active episode,[3] absence of antibiotic treatment for an active episode,[6] or treatment by steroids alone,[7] pregnancy,[8] and cataract extraction[9] may favor reactivations, but several of these factors remain controversial.[10,11]

Although the diagnosis of toxoplasmic retinochoroiditis relies on clinical criteria in routine practice, recent aqueous humor analysis techniques are helpful for diagnostic

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2018.11.013

confirmation in severe, vision-threatening cases that frequently share features with other causes of posterior or panuveitis, and require immediate effective therapy.[12–15] Moreover, these tools provide unique biological information regarding intraocular host/pathogen interaction and the local immune response.

No study has yet focused on factors influencing the risk of recurrence in severe, biologically proven cases of toxoplasmic retinochoroiditis. In addition, standardized laboratory techniques used for aqueous humor analysis provide molecular information that can be included among investigated parameters, thus opening perspectives on biomarkers linked to the prognosis of toxoplasmic retinochoroiditis.

In this study, we investigated the influence of clinical and biological factors on the risk of disease recurrence in severe toxoplasmic retinochoroiditis confirmed by aqueous humor analysis.

METHODS

• **STUDY DESIGN:** For this retrospective case series, consecutive patients presenting from January 1, 2005 to December 31, 2008 with a clinical suspicion of severe toxoplasmic retinochoroiditis, as defined below, at a tertiary uveitis center, the Ophthalmology Department of Pitié-Salpêtrière Hospital (Paris, France), and for whom biological confirmation by aqueous humor analysis was obtained, were identified from a clinical database. Charts were reviewed for clinical and biological parameters, treatments received, and the precise timing of previous and subsequent recurrences. Episodes not seen at our institution were considered when confirmed by a referring ophthalmologist.

This study adhered to the tenets of the Declaration of Helsinki, and approval by the Ethics Committee of the French Society of Ophthalmology was obtained. The need for written consent was waived owing to the retrospective design.

• **STUDY DEFINITIONS:** *Severe toxoplasmic retinochoroiditis* was defined for the purpose of the present study by at least 1 of the following clinical features: vitritis grade ≥3, hypertensive panuveitis (intraocular pressure >25 mm Hg with vitritis grade ≥2 and anterior chamber cells), extended retinal necrosis covering an area of 5 disc diameters or more, multifocal lesions, arterial or venous vasculitis (vascular sheathing visible on fundus examination, or vascular leakage detected on fluorescein angiography), and involvement of the macula or optic nerve head.

Total disease duration was defined as time elapsed since the initial episode of toxoplasmic retinochoroiditis.

Time since previous episode was defined as time elapsed since the last-recorded episode (corresponding to "disease-free duration").

Time from initial episode to first recurrence was defined as disease-free interval immediately after the initial episode.

• **CLINICAL AND BIOLOGICAL INCLUSION CRITERIA:** Consecutive cases of severe toxoplasmic retinochoroiditis, as defined above, were retrospectively included.

In these severe cases, the aim of aqueous humor sampling was to confirm the clinical suspicion of toxoplasmic retinochoroiditis, and to discriminate from potential differential diagnoses of posterior infectious uveitis, such as viral retinitis caused by herpes simplex virus (HSV1-2), varicella-zoster virus (VZV), or cytomegalovirus (CMV).

The diagnosis of toxoplasmic retinochoroiditis was biologically confirmed when aqueous humor analysis showed Goldmann-Witmer coefficient (GWC) ≥2; and/or immunoblot with at least 1 additional or more intense band, as compared to plasma; and/or positive polymerase chain reaction (PCR) specific for *Toxoplasma gondii*.

• **EXCLUSION CRITERIA:** To reduce potential bias in the analysis of recurrence patterns, patients with follow-up shorter than 3 years, human immunodeficiency virus (HIV) infection, or immunodeficiency/immunosuppression were excluded. Episodes occurring in female patients during pregnancy were not considered and their follow-up was censored from that time point.

To avoid misclassification of recurrent cases as nonrecurrent, we excluded patients harboring scars on funduscopy at the first observed episode (index episode) without knowledge of previous episodes recorded by an ophthalmologist.

• **BIOLOGICAL ANALYSIS OF AQUEOUS HUMOR:** Aqueous humor samples were obtained during an active inflammatory episode (either the initial episode or the first recurrence). Paracentesis was performed under topical anesthesia with tetracaine chlorhydrate 1% drops, using a 30 gauge needle and a 1 mL syringe, and retrieved ~0.1 mL of aqueous. A blood sample was taken simultaneously for analysis.

Aqueous samples were centrifuged for 10 minutes at 10 000*g*. The supernatant was used for GWC determination and immunoblot, and the pellet for PCR. GWC determination, immunoblot, and PCR were performed as previously described.[14]

Briefly, specific anti–*T. gondii* and total immunoglobulin G in aqueous humor and serum samples were measured using enzyme-linked immunosorbent assay (ELISA, Platelia Toxo IgG; Bio-Rad, Marnes-la-Coquette, France) and immunodiffusion (Human IgG NL BINDARID Kit; Binding Site, Birmingham, UK), respectively. The GWC was calculated as (anti–*T. gondii* IgG in aqueous humor/total IgG in aqueous humor)/(anti–*T. gondii* IgG in serum/total IgG in serum). A value of 2 or more was considered evidence of intraocular anti–*T. gondii* antibody synthesis.

The immunoblot technique (Toxoplasma Western Blot IgG; LDBIO Diagnostics, Lyon, France) consists in

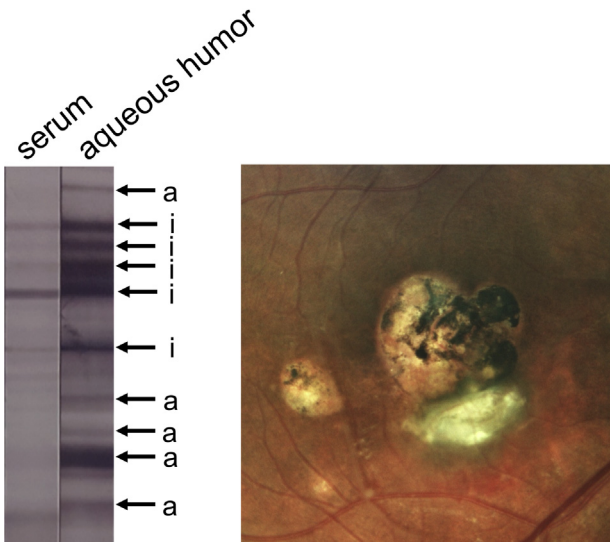

FIGURE 1. Immunoblot for anti–*Toxoplasma gondii* antibodies and clinical example of recurrent ocular toxoplasmosis. (Left) Magnified area from specific immunoblots detecting antibodies directed against *Toxoplasma gondii* antigens, showing differential bands in serum (left blot) and aqueous humor (right blot). This section harbors 5 additional bands (a) and 5 more intense bands (i) in the aqueous sample, as compared to serum. (Right) Color fundus photograph in a 21-year-old woman with multirecurrent ocular toxoplasmosis showing an active retinochoroiditis lesion adjacent to a large pigmented macular scar and a smaller atrophic scar. The patient had 3 documented previous episodes that occurred over a 5-year period, resulting in these scars. Note the discrete retinal hemorrhages adjacent to the active foci, and to an arteriovenous crossing, suggestive of venous vasculitis.

detecting antibodies binding *T. gondii* antigens (from 20 to 120 kDa) on a Western blot gel, and in comparing bands corresponding to these antibodies in the aqueous sample with those of the serum sample. Additional or more intense bands indicate specific intraocular synthesis of *T. gondii* antibody (Figure 1). The number of bands was assessed by a senior parasitology specialist (L.P.).

The presence of *T. gondii* DNA was investigated by real-time quantitative PCR targeting the repetitive B1 gene and the 529-base-pair repeat element (REP; 200–300 copies/genome), as reported elsewhere.[16]

In cases presenting a clinical suspicion of viral retinitis, specific PCR for viruses of the Herpesviridae family was also performed on the aqueous sample, as previously described.[17]

• **PATIENT EXAMINATION AND FOLLOW-UP:** At presentation, patients underwent comprehensive ophthalmologic examination, consisting of best-corrected visual acuity, biomicroscopy, tonometry, and funduscopy. Anterior chamber cells were graded on a 0–4 scale according to the Standardization of Uveitis Nomenclature classification,[18] and the degree of vitritis was determined from 0 to 4 (0, no vitritis; 1, vitritis without impairment of fundus visualization; 2, optic disc and major vessels visible; 3 optic disc visible; 4, fundus not visualized). Presence of retinal scars and papillitis, location, size (in disc diameter), number, and pigmentation of retinochoroiditis foci were recorded. Fluorescein angiography was performed at presentation or as soon as the degree of vitritis allowed appropriate visualization of major vessels, searching for signs of arterial of venous vasculitis, fluorescence pattern at the retinochoroiditis foci, papillitis, and neovascularization.

Lesions were considered central (vs peripheral) when located posterior (vs anterior) to the temporal vascular arcades. Retinal necrosis was defined as extended when spanning an area ≥5 disc diameters. The presence of arterial, venous, or combined vasculitis was determined on funduscopy, when visible, and otherwise on fluorescein angiography.

After episode resolution, patients were told to return immediately to the emergency outpatient clinic of our tertiary center or to their referring ophthalmologist in the case of symptom recurrence.

• **TREATMENT SCHEME:** All patients with severe toxoplasmic retinochoroiditis received specific anti–*T. gondii* treatment for at least 6 weeks, consisting of oral pyrimethamine (100 mg the first day, then 50 mg daily), combined with either oral sulfadiazine (1g 4 times daily), oral azithromycin (500 mg the first day, then 250 mg daily), or, more rarely, clindamycin (300 mg 4 times daily), and oral supplementation by folinic acid (5 mg daily). Two days after antiparasite treatment initiation, systemic corticosteroid treatment was initiated when foci were located in or adjacent to the macula or optic disc, or when vision was impaired by dense vitritis. Patients received intravenous methylprednisolone (~15 mg/kg daily for 3 days) and/or oral prednisone (~0.5 mg/kg daily for ~2 weeks, followed by progressive dose tapering over 6–8 weeks). Topical or oral treatments to lower the intraocular pressure were prescribed when appropriate. Treatment decisions were based on current practice guidelines at our department and the clinical expertise of uveitis consultants (B.B., P.L.H., V.T., C.F., N.C., E.C., C.T.).

When clinical presentation was also suggestive of viral retinitis, patients received intravenous acyclovir (10 mg/kg every 8 hours) in addition to anti–*T. gondii* treatment, until the results of the aqueous humor analysis were known.

Based on clinical decisions by uveitis consultants at our institution, certain patients received a 3-month prophylactic course of oral trimethoprim/sulfamethoxazole (800/160 mg daily) after episode resolution.

• **STATISTICAL ANALYSES:** Descriptive and comparative statistics were performed on GraphPad Prism (version 5.0f; GraphPad Software, La Jolla, California, USA).

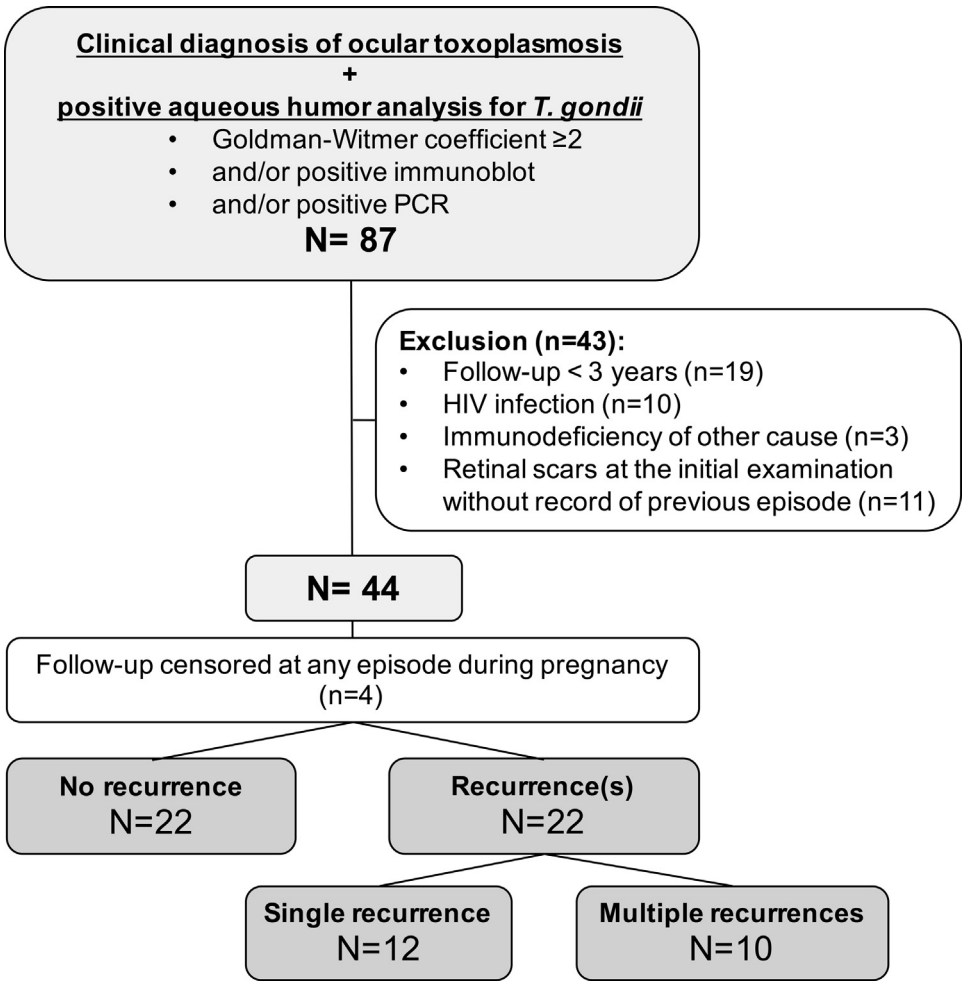

FIGURE 2. Chart of retrospective inclusion and exclusion criteria for the study of recurrences in severe toxoplasmic retinochoroiditis with biologic confirmation.

The risk of recurrence was estimated as a function of clinical and biological characteristics at the index episode. Recurrences occurring after this episode were considered. The pattern of recurrences over time and the effect of potential predictors on the risk of recurrence were analyzed separately with a shared frailty survival model, using the "frailtypack" package[19,20] and R Software (Version 3.3.0; R Foundation for Statistical Computing, R Core Team, 2016, Vienna, Austria; http://www.R-project.org/). This model consists of a survival analysis of recurrence times, based on a Cox proportional hazard regression with an additional patient-related parameter accounting for the fact that recurrences occur within single individuals. A series of univariate regressions, and a multivariate regression with those covariates that reached P values ≤ .25 in the univariate analysis, were performed to investigate disease recurrence, as previously reported.[21]

The strategy for statistical modeling consisted of analyzing in 2 separate models: (1) time-related factors (total disease duration, time since previous episode, time from initial episode to first recurrence), providing a descriptive overview of the time pattern of recurrences; and (2) clinical and biological factors potentially associated with recurrences, potentially providing predictive factors for recurrences.

RESULTS

FROM A DATABASE OF 313 AQUEOUS HUMOR SAMPLES tested for *T. gondii* during the study period, 87 samples from patients with a clinical suspicion of toxoplasmic retinochoroiditis were positive for *T. gondii* (positive GWC, and/or positive immunoblot, and/or positive PCR). Forty-three cases were excluded owing to follow-up <3 years (n = 19), HIV infection (n = 10), immunodeficiency/immunosuppression (n = 3), or presence of retinal scars at the first examination without record of previous episodes (n = 11). Of the remaining 44 cases, 4 were censored at the

TABLE 1. Indication for Aqueous Humor Tap in 44 Patients With Severe Toxoplasmic Retinochoroiditis

Clinical Features	Subjects, N (%)
Dense vitritis	16 (34%)
+ nonvisible fundus	4 (9%)
+ retinal necrosis	4 (9%)
+ retinal tears	3 (6%)
+ chronic or recurrent pattern	3 (6%)
+ encephalitis	1 (2%)
+ vasculitis	1 (2%)
Hypertensive panuveitis	12 (25%)
+ nonvisible fundus	6 (13%)
+ retinal vasculitis	3 (6%)
+ papillitis	2 (4%)
+ multifocal lesions	1 (2%)
Retinal vasculitis	7 (15%)
Isolated vasculitis	4 (9%)
+ retinal necrosis	2 (4%)
+ papillitis	1 (2%)
Other findings	
Multifocal lesions	4 (9%)
Papillitis	4 (9%)
Atypical macular lesion	3 (6%)
Exudative retinal detachment	1 (2%)

Dense vitritis was defined as vitritis of grade ≥3 (scale, 0–4). The cumulative count is more than 44 cases since a fraction of patients presented several indications for aqueous humor tap.

time of an episode occurring during pregnancy, and 22 presented recurrences, with single recurrences in 12 subjects and multiple recurrences in 10 subjects (Figure 2). There were 22 men and 22 women, with a mean age of 40.4 ± 17.6 years (range, 13–74 years).

Indications for aqueous humor tap are detailed in Table 1. The most frequent indications were dense vitritis (34%) and hypertensive panuveitis (25%) impeding fundus visualization or associated with retinal lesions suggestive of severe posterior uveitis. Less frequently, retinal vasculitis (15%), multifocal fundus lesions (9%), or papillitis (9%) prompted aqueous humor tap. Biological confirmation helped to discriminate toxoplasmic retinochoroiditis from possible differential diagnoses, the most frequently suspected being viral retinitis (68%), Behçet disease (21%), ocular lymphoma (13%), and ocular sarcoidosis (13%) (Supplementary Table 1; Supplemental Material available at AJO.com).

Among the 44 cases with biological confirmation, 10 samples were positive for *T. gondii* in 1 of the 3 laboratory tests, 26 samples in 2 tests, and 8 samples in 3 tests. The GWC was found positive in 17 patients with recurrence and 14 patients without recurrence, the immunoblot in 20 (with) and 20 (without) patients, and the *T. gondii* PCR in 7 (with) and 7 (without) patients (Table 2). Aqueous humor was sampled at the time of the first episode in 32 patients and at the time of the second episode in 12 patients.

A total of 82 episodes were observed (44 initial episodes and 38 recurrences) over a mean follow-up of 8.3 ± 2.7 years and a cumulative follow-up of 329.4 patient-years. During follow-up, 1 subject experienced 4 recurrences, 3 subjects had 3 recurrences, 6 subjects had 2 recurrences, and 12 subjects had 1 recurrence. The chronology of recurrences among recurring patients is displayed in Figure 3, and the clinical and biological characteristics of subjects who experienced single, multiple, or no recurrences are summarized in Table 2. Overall, the observed recurrence rate after the initial episode was 0.11 recurrence/patient/year and the mean disease-free interval following the first episode was 5.0 ± 2.9 years. The follow-up duration between nonrecurring and recurring patients did not differ significantly (7.9 ± 2.5 years vs 8.7 ± 2.8 years, $P = .17$). Treatments received at each recorded episode are reported in Table 2. The antibiotic regimen was pyrimethamine/sulfadiazine in 46 episodes, pyrimethamine/azithromycin in 29 episodes, and a 3-month prophylactic course of trimethoprim/sulfamethoxazole administered following resolution of 9 episodes (of which 7 were recurrences and 5 were followed by further recurrences). This treatment was occasionally prescribed, most frequently when there was knowledge of previous episodes and based on the physician decision. Intravenous methylprednisolone and oral prednisone were administered in 23 and 51 episodes, respectively.

A shared frailty survival model analyzing the chronology of recurrences and their distribution (Table 3) revealed that the risk of recurrence was higher immediately after an episode and decreased over time as patients remained recurrence-free ($P < .0001$). The risk of recurrence also declined with longer total disease duration since the initial episode ($P = .0001$). A multivariate analysis indicated that the only time-related variable independently associated with a decreased risk of recurrence was longer recurrence-free duration since the previous episode ($P < .0001$). An additional multivariate analysis was restricted to recurrent cases in order to investigate the influence of time to first recurrence on the risk of subsequent recurrences (Table 4). For this model, the first recurrence was excluded because it was directly related to this variable, and time to subsequent recurrences was considered. The risk of recurrence was higher when patients had presented a first recurrence after longer disease-free intervals ($P = .046$). Moreover, this analysis confirmed that longer duration since previous episode ($P < .0001$), but not total disease duration ($P = .18$), was independently associated with lower recurrence risk in this subgroup.

The influence of clinical and biological parameters at the index episode on the risk of subsequent recurrences was analyzed using a shared frailty survival model, reported in Table 5. A series of univariate analyses revealed that this risk declined with the number of intense bands observed on aqueous humor immunoblot ($P = .006$) and increased when venous vasculitis was present ($P = .019$). A

TABLE 2. Clinical and Biological Characteristics of 44 Patients With Severe Toxoplasmic Retinochoroiditis Confirmed by Aqueous Humor Analysis, According to the Number of Recurrences

	No Recurrence	Single Recurrence	Multiple Recurrences
Patients, n	22	12	10
Episodes, n	22	24	36
Age at first episode, years	38.4 ± 17.0	37.8 ± 16.3	40.6 ± 18.5
Sex (male/female), n	10/12	7/5	5/5
African subjects, n	9	3	3
Follow-up, years	7.1 ± 1.1	7.2 ± 2.2	8.7 ± 2.8
Interval from first episode to first recurrence, years	NA	1.8 ± 1.7	4.3 ± 3.1
Clinical parameters at baseline			
Anterior chamber cell grade, 0–4	1.7 ± 0.9	1.7 ± 1.0	2.0 ± 0.7
Vitritis grade ≥2, n	11	6	7
Intraocular pressure ≥25 mm Hg, n	7	3	2
Arterial vasculitis, n	7	6	1
Venous vasculitis, n	0	3	1
Active lesions, n/eye	1.5 ± 1.5	1.0 ± 0.7	1.9 ± 1.4
Peripheral active lesions, n	12	7	9
Size of active lesions, DD	1.7 ± 0.9	3.0 ± 1.5	2.1 ± 1.0
Presence of scars at index episode, n	0	3	6
Extended retinal necrosis (≥5 DD), n	1	4	2
Biological parameters at baseline			
GWC	30.5 ± 74	12.2 ± 9.5	9.3 ± 8.7
GWC ≥2, n	14	9	8
Positive *T. gondii* immunoblot, n	20	11	9
Intense bands on immunoblot, n/blot	4.9 ± 2.9	4.6 ± 2.2	2.1 ± 2.5
Additional bands on immunoblot, n/blot	2.3 ± 2.1	1.5 ± 1.7	2.1 ± 1.7
Positive *T. gondii* PCR, n	7	4	3
Treatments (treated episodes, n)			
Pyrimethamine/sulfadiazine	17	15	14
Pyrimethamine/azithromycin	5	4	20
Pyrimethamine/clindamycin	0	4	0
Trimethoprim/sulfamethoxazole (3-month prophylactic course after episode resolution)	2	2	5
Intravenous methylprednisolone	11	7	5
Oral prednisone	13	14	24

CI = confidence interval; DD = disc diameter; GWC = Goldmann-Witmer coefficient; PCR = polymerase chain reaction; *T. gondii* = *Toxoplasma gondii*.

Quantitative variables are expressed as mean ± standard deviation. Clinical parameters were assessed during the first episode evaluated at our institution, and corresponded to the time of aqueous humor tap. Biological parameters were obtained by analyzing the aqueous humor sample. Anterior chamber cells and vitritis intensity were graded from 0 to 4. Lesions were classified as peripheral when located anteriorly to the temporal vascular arcades. Retinal necrosis was classified as extended when covering ≥5 disc diameters.

multivariate analysis confirmed that patients with more intense bands on aqueous humor immunoblot experienced fewer recurrences ($P = .041$) and did not identify other contributing parameters. Treatments did not influence the risk of recurrence, although there was a near-significant risk elevation after episodes treated with pyrimethamine/azithromycin, in both univariate and multivariate models ($P = .078$ and $P = .054$, respectively). Owing to the low number of episodes treated with pyrimethamine/clindamycin, this treatment was not included as a possible risk factor in the recurrent event analysis. Noticeably, prophylactic trimethoprim/sulfamethoxazole did not reduce the risk of subsequent recurrences ($P = .44$). The administration of corticosteroids, either intravenous methylprednisolone or oral prednisone, did not influence the risk of later recurrences in the univariate or multivariate models ($P > .10$). No significant differences in terms of clinical features were found between episodes treated with different regimens, as summarized in Supplementary Table 2 (Supplemental Material available at AJO.com).

Given the clinical relevance of patient age, we forced age at first episode and age at active episode into the

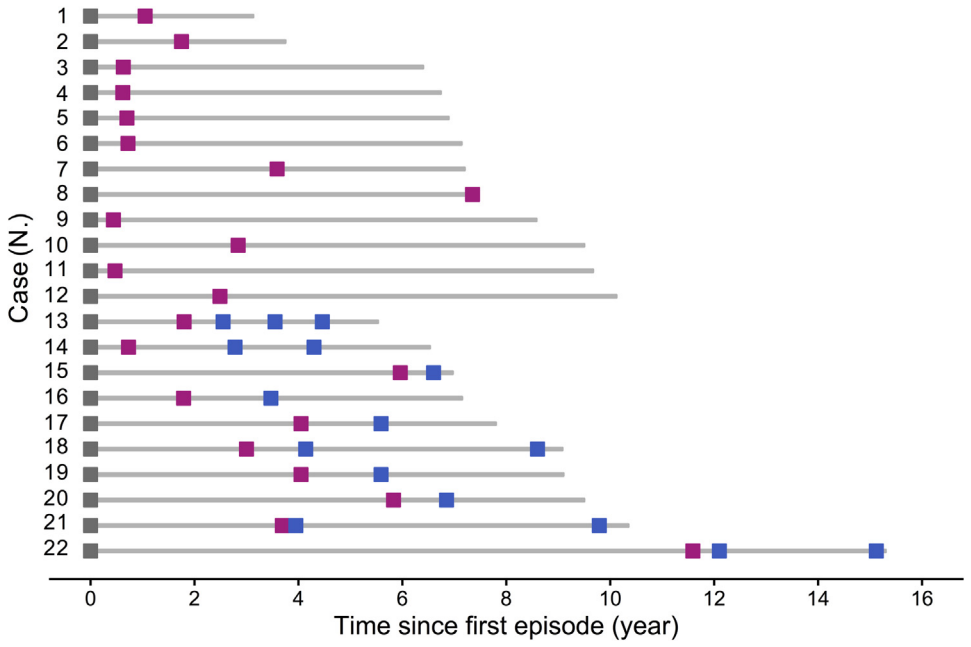

FIGURE 3. Chronology of recurrences among 22 patients with severe recurrent toxoplasmic retinochoroiditis confirmed by aqueous humor analysis. For graphical clarity, 22 patients who did not recur during follow-up were not displayed. Gray squares represent initial episodes, purple squares first recurrences, and blue squares additional recurrences. The higher number of additional recurrences among patients who experienced a delayed first recurrence is visible, and was confirmed in a shared frailty survival model (univariate, $P < .0001$ and multivariate, $P = .046$).

TABLE 3. Time-related Factors Indicating the Nonstochastic Distribution of Episodes in 44 Cases of Toxoplasmic Retinochoroiditis

	Univariate		Multivariate	
	Hazard Ratio (95% CI)	*P* Value	Adjusted Hazard Ratio (95% CI)	*P* Value
Time since previous episode (per year)	0.23 (0.18–0.28)	<.0001	0.23 (0.19–0.29)	<.0001
Total disease duration since first episode (per year)	0.65 (0.52–0.81)	.0001	1.0 (0.87–1.15)	.96

Univariate and multivariate analyses were based on a shared frailty survival model. All episodes, including past episodes observed elsewhere, were considered.

multivariate models computed over the entire cohort (Tables 3 and 5). Older age at first and at active episode were associated with a higher risk of recurrence when analyzed along with time-related parameters ($P = .0004$ and $P < .0001$, respectively), without altering the significance level of the other variables. When forced into the model of clinical/biological variables, age had no effect.

An attempt to compute into a combined model all time-related, clinical, and biological variables retained for multivariate analysis failed to provide a converging model.

DISCUSSION

IN THE PRESENT STUDY, WE INVESTIGATED THE time-dependent distribution of recurrences, and potential predictive factors, in severe toxoplasmic retinochoroiditis proven by aqueous humor analysis. The risk of recurrence decreased with prolonged disease duration since the first episode, and with longer disease-free intervals following active episodes. Surprisingly, patients with shorter intervals between the first and second episodes were at lower risk of subsequent recurrences than those with longer first disease-free interval. Among clinical and biological predictive factors, the only independent factor identified was the higher number of more intense bands on aqueous humor immunoblot, associated with a reduced risk of recurrence.

Regarding the chronology of episodes, our findings are consistent with previous studies evaluating recurrences of toxoplasmic retinochoroiditis. Holland and associates[3] and, more recently, Reich and associates[4] identified that the risk of recurrence is highest immediately after a given

TABLE 4. Time-related Factors Associated With the Distribution of Recurrences Among 22 Cases of Recurrent Toxoplasmic Retinochoroiditis

	Univariate		Multivariate	
	Hazard Ratio (95% CI)	*P* Value	Adjusted Hazard Ratio (95% CI)	*P* Value
Time since previous episode (per year)	0.15 (0.11–0.20)	<.0001	0.20 (0.09–0.44)	<.0001
Total disease duration since first episode (per year)	0.69 (0.67–1.01)	.059	0.71 (0.43–1.17)	.18
Time from initial episode to first recurrence (per year)	1.19 (1.03–1.36)	.015	1.64 (1.01–2.66)	.046

Univariate and multivariate analyses were based on a shared frailty survival model. This model analyzed the risk of subsequent recurrences following the first recurrence, in order to include time to first recurrence as variable.

TABLE 5. Clinical Factors Associated With Disease Recurrence in 44 Patients With Toxoplasmic Retinochoroiditis

	Univariate		Multivariate	
	Hazard Ratio (95% CI)	*P* Value	Adjusted Hazard Ratio (95% CI)	*P* Value
Age at first episode (≥40 years vs <40 years)	1.31 (0.55–3.10)	.54		
Age at time of active episode (per 10-year interval)	0.94 (0.73–1.21)	.62		
Sex (male vs female)	1.06 (0.45–2.49)	.89		
Ethnicity (black vs white)	0.90 (0.36–2.25)	.82		
Biological parameters				
Goldmann-Witmer coefficient (≥2 vs <2)	2.17 (0.64–7.35)	.21	1.80 (0.47–6.85)	.39
Additional bands on immunoblot (per band)	0.93 (0.73–1.19)	.58		
Intense bands on immunoblot (per band)	0.78 (0.65–0.93)	.006	0.80 (0.65–0.99)	.041
T. gondii PCR (positive vs negative)	1.02 (0.41–2.55)	.96		
Clinical parameters				
Vitritis grade (0–4)	1.25 (0.77–2.02)	.37		
Intraocular pressure (≥25 mm Hg vs <25 mm Hg)	0.39 (0.11–1.35)	.14	0.69 (0.17–2.88)	.61
Arterial vasculitis (presence vs absence)	0.61 (0.22–1.71)	.35		
Venous vasculitis (presence vs absence)	3.35 (1.22–9.19)	.019	2.41 (0.69–8.44)	.17
Number of active lesions (per lesion)	1.08 (0.83–1.40)	.58		
Localization of lesions (posterior pole vs periphery)	0.45 (0.15–1.35)	.16	0.47 (0.13–1.69)	.25
Extended retinal necrosis (presence vs absence)	1.54 (0.56–4.20)	.40		
Treatment of previous episode				
Pyrimethamine/sulfadiazine	0.56 (0.24–1.31)	.18	4.43 (0.60–32.67)	.14
Pyrimethamine/azithromycin	2.15 (0.92–5.01)	.076	6.90 (0.97–49.19)	.054
Trimethoprim/sulfamethoxazole (3-month prophylactic course after episode resolution)	1.55 (0.51–4.64)	.44		
Intravenous methylprednisolone	0.40 (0.13–1.19)	.10	0.33 (0.09–1.31)	.12
Oral prednisone	1.89 (0.70–5.12)	.21	2.52 (0.84–7.54)	.10

CI = confidence interval; PCR = polymerase chain reaction; *T. gondii* = *Toxoplasma gondii*.

Univariate and multivariate analyses were based on a shared frailty survival model. Age at time of active episode, disease duration since first episode, and treatments were time-dependent covariates. The other clinical and biological covariates were recorded at the index episode. Recurrences occurring after the index episode were considered.

episode and decreased as subjects remain recurrence-free. However, in the present study, longer duration since the first episode (total disease duration) was not retained as an independent factor associated with the risk of recurrence, as evidenced in Tables 3 and 4. We also observed that among recurring cases, those with earlier first recurrences carry a lower risk of additional recurrences, whereas more additional recurrences were observed in cases with late first recurrences (Table 4). This finding had not been previously reported. The same underlying phenomenon may explain the high proportion of "late clusters" reported by Holland and associates,[3] defined as 3 episodes occurring within a 5-year period, after a 5-year disease-free interval, indicating that patients recurring after a long disease-free period seem at higher risk for additional recurrences. It is also consistent with the chart of recurrence timing in Colombian patients with 2 or more recurrences of toxoplasmic retinochoroiditis provided by De la Torre and associates,[7] where 7 patients with a first recurrence less than 3 years after the initial episode experienced only 1 subsequent recurrence, while 10 patients with a first recurrence occurring 3 years or more after the initial episode experienced 3 ± 1.5 (range, 1–5) additional recurrences ($P = .008$, Mann-Whitney test, post hoc analysis performed on the data presented by authors). Taken together, our observations confirm the nonstochastic distribution of episodes over time, identified by Holland and associates,[3] in a population with severe, biologically proven toxoplasmic retinochoroiditis.

Several previous works pointed out an effect of patient age on the risk of recurrence. Garweg and associates concluded that individuals who experienced a first toxoplasmic retinochoroiditis episode during teenage years or early adulthood had a higher risk of recurrence than those experiencing a first episode at older age.[5] Holland and associates deduced from a multivariate survival analysis that older age at diagnosis had a near-significant protective effect, but that patients older than 40 years at any active episode were at higher risk of subsequent recurrences, whereas this parameter was protective in the univariate analysis.[3] The present study did not identify patient age as influencing the risk of recurrence when computed among clinical factors. Therefore, we forced age-related variables into the multivariate models, a step permitted for parameters of high clinical relevance, and identified that older age at first or at active episode were independently associated with a higher recurrence risk, when computed with time-related parameters. This finding confirms the complex interaction of patient age with other covariates, in particular total disease duration and duration since previous episode, and is consistent with observations by Holland and associates.[3] These interactions probably result from the strong risk reduction over life, related to longer duration since first episode (as discussed above), responsible for the apparent higher risk at younger age. Consistently, age older than 40 years was also an independent risk factor in the study by Holland and associates, after adjusting for total disease duration since the first episode.[3] The paradoxical findings from the present study may also result from potential selection bias induced by strict inclusion and exclusion criteria, restricting the population to severe cases in immunocompetent hosts with positive aqueous humor analysis, and no scar at presentation in the absence of previous episodes. Similar criteria were not applied in the 2 above-mentioned studies. Moreover, severe manifestations tend to occur more frequently in older patients,[22,23] which may modify the age distribution in our study as compared to other study populations. Finally, we opted for a different strategy than Holland and associates,[3] in that we analyzed separately chronological parameters related to the timing of recurrences and clinical parameters potentially predictive of subsequent recurrences, which explains why age and total disease duration, for instance, were not computed together in a survival model.

When assessing the effect of different antibiotic treatments on subsequent recurrences, we observed that none of the regimens seemed to influence this risk, even though a near-significant risk increase was noted after episodes treated by the combination of oral pyrimethamine and azythromycin. These findings are consistent with the observation by Bosch-Driessen and associates[2] that standard treatment regimens do not influence the risk of recurrence, and with the clinical notion that the antiparasitic action of azythromycin might be less potent than sulfadiazine in combination with pyrimethamine. However, the association of azythromycin and pyrimethamine is frequently prescribed in the clinical setting because it carries less systemic adverse effects.[24,25] Treatment by intravenous or oral corticosteroids during an active episode did not influence the risk of subsequent recurrences. De la Torre and associates[7] and Reich and associates[6] have reported an increased rate of recurrent episodes after treatment by corticosteroid alone, but in the present study no patient received corticosteroid monotherapy. In addition, we found that a 3-month course of trimethoprim/sulfamethoxazole prophylaxis did not influence the risk of subsequent recurrence, which may be explained by the short duration of prophylactic treatment, as compared to a recent prospective trial demonstrating the protective effect of a 311-day course of trimethoprim/sulfamethoxazole on the risk of recurrences, assessed over a 3-year follow-up.[26] In order to rule out confounding-by-indication, wherein patients with low-risk clinical findings are treated differently than those with high-risk clinical findings, we investigated whether treatment indications differed according to clinical features at each episode. No significant differences were observed regarding antiparasitic and anti-inflammatory treatment regimens, as reported in Supplementary Table 2.

To the best of our knowledge, molecular characteristics in the aqueous humor have not been previously explored with respect to the risk of subsequent recurrence. The

present results indicate that neither the level of local specific anti–*T. gondii* antibody synthesis (reflected by the Goldmann-Witmer ratio), nor the number of additional specific *T. gondii* proteins recognized by these local antibodies as compared to circulating antibodies (reflected by additional bands on immunoblot), nor the presence of *T. gondii* DNA in the aqueous humor influenced the risk of recurrence. However, they indicate an influence of a greater binding intensity of anti–*T. gondii* antibodies to parasite proteins (intense bands on immunoblot) in the aqueous humor as compared to serum. This observation suggests that the quality of antibody-antigen binding, rather than the magnitude of the local immune response, allows a longer control of parasite activity and delays pathogen reactivation. Future translational studies on biological samples from affected patients should further investigate the humoral immune reaction to *T. gondii* in relation to the risk of recurrences, since the intensity of bands on immunoblot does not provide an accurate quantitative measure of an antibody's affinity for its antigen. Moreover, it does not reflect other important aspects, such as the complement fixing ability of a given antibody, or its ability to stimulate secondary immune responses involved in the killing of the organism.

Retinal vasculitis is a clinical manifestation of inner blood-retinal barrier rupture, disrupting the ocular immune privilege by allowing contact between circulating immune mediators and intraocular media.[27] Among identified causes, venous vasculitis is most frequently associated with lymphocyte-mediated immune or infectious disorders, such as Behçet disease,[28] viral retinitis[29] including HIV retinitis,[30] leukemia,[31] and lymphoma.[32] One of the mechanisms of ocular immune privilege involves the limitation of cytotoxic responses, which may damage ocular tissues, while preserving the humoral response.[33] Since the eye is a nonlymphoid organ, the local humoral response relies on a specific population of immunoglobulin-producing B lymphocytes, with a wider epitope recognition profile than circulating B cells. These local B clones are derived from lymphoblasts paused in their development that migrated from lymph nodes before being exposed intraocularly to specific *T. gondii* antigens.[34] Therefore, in the context of vasculitis, the ocular compartment is not protected from the systemic circulation, and the process of selection of this specific anti–*T. gondii* local humoral response may be impeded. Here, venous vasculitis was not retained in the multivariate analysis as an independent risk factor of recurrences, suggesting a connection with the number of more intense bands on immunoblot, the only retained factor. This observation is consistent with an interplay between immune privilege, the quality of local humoral response, and the control of *T. gondii* reactivations.

The combined observations that fewer episodes are observed after an earlier first recurrence, and in cases with intense *T. gondii* bands on aqueous humor immunoblot, suggest that recurrent disease may be controlled by an immunization-related mechanism. The stronger the initial local immune reaction to the parasite, the fewer recurrences. Despite the limited data available from experimental models of ocular disease reactivation,[33–35] the parasite cycle and its modulation by host stimuli have been extensively studied, both in vitro and in vivo.[36–40] During the initial episode, the key pathophysiology event predisposing to future *T. gondii* reactivation is the conversion of active tachyzoites to quiescent, slow-replicating bradyzoites. At this stage, a weak local immune response may suppress tachyzoite replication but still allow parasites to reach a requisite premitotic cell-cycle checkpoint for entry into bradyzoite differentiation, thus contributing to bradyzoite conversion that would later induce recurrences. In contrast, a strong local immune response may destroy living tachyzoites, thereby preventing cell-cycle progression and bradyzoite conversion.[41] Moreover, in vivo histopathologic analyses of *T. gondii* latency in the central nervous system of rodents suggested that bradyzoite-surrounding cysts, which may persist for years within host tissue, tend to rupture unpredictably.[42,43] This slow phenomenon leads to a random release of bradyzoites that would be destroyed in an immune-competent environment[44] but may convert to tachyzoites and elicit reactivations in an immunocompromised host or environment, such as a weak anti–*T.gondii* local humoral reaction.

The main strength of the present study relies on the biological diagnosis confirmation. Also, we analyzed separately parameters reflecting the *timing* of recurrences, and clinical or biological parameters, in an attempt to identify clinically relevant *predictors* of recurrences. Although relying on statistical modeling, by essence different from real biological causative effects, the present results indicate time patterns describing the nonstochastic chronology of toxoplasmic recurrences, and clinical/biological factors potentially associated with a higher risk of recurrences. On the other hand, since anterior chamber tap was indicated in severe cases only, weaknesses of this study include the limited population, with a potential selection bias. Our study definition of severity was based on clinical criteria used to indicate aqueous humor tap when suspecting infectious posterior uveitis. We considered a case as severe when presenting a high risk of visual loss owing to the localization, extension of foci, the density of vitritis, the intensity of the blood-retinal barrier breakdown, and elevated intraocular pressure, often present in viral necrotizing retinitis. All parameters do not reflect parasite burden, but rather the intensity of host response to the parasite. A selection bias may also have been introduced by the strict exclusion criteria, such as patients with scars at presentation without knowledge of previous episodes, as well as those with immunodeficiency. Moreover, the intensity of clinical manifestations included in the statistical analyses are likely to reflect host factors in response to the parasite, but not the parasite burden. Both host factors and parasite burden could influence recurrences, but parasite burden cannot be directly assessed with

currently available clinical or biological investigation tools. Another limitation regarding the biological data is the fact that these data were obtained for diagnosis, not research purposes, and that many more molecular agents, including numerous cytokines,[33] are of interest in the aqueous humor of toxoplasmic retinochoroiditis patients.

To summarize, recurrences of toxoplasmic retinochoroiditis are not random processes. Shorter intervals before the first recurrence are associated with a reduced risk of subsequent episodes. More intense bands on *T. gondii*–specific immunoblot in aqueous humor compared to serum are also related to fewer recurrences. This latter finding likely reflects a more efficient humoral response against the parasite, either at the initial episode, limiting the conversion to the dormant bradyzoite stage, or at reactivations. A better understanding of processes driving reactivations, and further clinical evidence, are required to validate prognosis biomarkers of recurrences.

FUNDING/SUPPORT: NO FUNDING OR GRANT SUPPORT. FINANCIAL DISCLOSURES: THE FOLLOWING AUTHORS HAVE NO financial disclosures: Alexandre Matet, Luc Paris, Christine Fardeau, Céline Terrada, Emmanuelle Champion, Arnaud Fekkar, Nathalie Cassoux, Valérie Touitou, Phuc LeHoang, and Bahram Bodaghi. All authors attest that they meet the current ICMJE criteria for authorship.

Other Acknowledgments: The authors thank Virginie Rondeau, PhD, Université de Bordeaux, Bordeaux, France, for statistical counseling.

REFERENCES

1. Bosch-Driessen EH, Rothova A. Recurrent ocular disease in postnatally acquired toxoplasmosis. *Am J Ophthalmol* 1999; 128:421–425.
2. Bosch-Driessen L, Berendschot TJM, Ongkosuwito J, Rothova A. Ocular toxoplasmosis: clinical features and prognosis of 154 patients. *Ophthalmology* 2002;109:869–878.
3. Holland GN, Crespi CM, ten Dam-van Loon N, et al. Analysis of recurrence patterns associated with toxoplasmic retinochoroiditis. *Am J Ophthalmol* 2008;145:1007–1013.
4. Reich M, Ruppenstein M, Becker MD, Mackensen F. Time patterns of recurrences and factors predisposing for a higher risk of recurrence of ocular toxoplasmosis. *Retina* 2015; 35(4):809–819.
5. Garweg JG, Scherrer JN, Halberstadt M. Recurrence characteristics in European patients with ocular toxoplasmosis. *Br J Ophthalmol* 2008;92(9):1253–1256.
6. Reich M, Becker MD, Mackensen F. Influence of drug therapy on the risk of recurrence of ocular toxoplasmosis. *Br J Ophthalmol* 2016;100(2):195–199.
7. de la Torre A, Rios-Cadavid AC, Cardozo-García CM, Gomez-Marín JE. Frequency and factors associated with recurrences of ocular toxoplasmosis in a referral centre in Colombia. *Br J Ophthalmol* 2009;93:1001–1004.
8. Reich M, Ruppenstein M, Becker MD, Mackensen F. Risk of recurrence of preexisting ocular toxoplasmosis during pregnancy. *Ocul Immunol Inflamm* 2015;23(3):240–245.
9. Bosch-Driessen LH, Plaisier MB, Stilma JS, Van der Lelij A, Rothova A. Reactivations of ocular toxoplasmosis after cataract extraction. *Ophthalmology* 2002;109:41–45.
10. Braakenburg AMD, Crespi CM, Holland GN, Wu S, Yu F, Rothova A. Recurrence rates of ocular toxoplasmosis during pregnancy. *Am J Ophthalmol* 2014;157:767–773.
11. Heringer GC, Oueghlani E, Dell 'omo R, et al. Risk of reactivation of toxoplasmic retinitis following intraocular procedures without the use of prophylactic therapy. *Br J Ophthalmol* 2014;98:1218–1220.
12. De Groot-Mijnes JDF, Rothova A, Van Loon AM, et al. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. *Am J Ophthalmol* 2006;141(2):313–318.
13. Garweg JG, de Groot-Mijnes JDF, Montoya JG. Diagnostic approach to ocular toxoplasmosis. *Ocul Immunol Inflamm* 2011;19(4):255–261.
14. Fekkar A, Bodaghi B, Touafek F, Le Hoang P, Mazier D, Paris L. Comparison of immunoblotting, calculation of the Goldmann-Witmer coefficient, and real-time PCR using aqueous humor samples for diagnosis of ocular toxoplasmosis. *J Clin Microbiol* 2008;46(6):1965–1967.
15. Garweg JG, Garweg S-DDL, Flueckiger F, Jacquier P, Boehnke M. Aqueous humor and serum immunoblotting for immunoglobulin types G, A, M, and E in cases of human ocular toxoplasmosis. *J Clin Microbiol* 2004;42(10): 4593–4598.
16. Edvinsson B, Lappalainen M, Evengård B. Real-time PCR targeting a 529-bp repeat element for diagnosis of toxoplasmosis. *Clin Microbiol Infect* 2014;12:131–136.
17. Tran THC, Rozenberg F, Cassoux N, Rao NA, Lehoang P, Bodaghi B. Polymerase chain reaction analysis of aqueous humour samples in necrotising retinitis. *Br J Ophthalmol* 2003; 87:79–83.
18. Jabs DA, Nussenblatt RB, Rosenbaum JT. Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am J Ophthalmol* 2005;140(3):509–516.
19. Rondeau V, Gonzalez JR. frailtypack: a computer program for the analysis of correlated failure time data using penalized likelihood estimation. *Comput Methods Programs Biomed* 2005;80(2):154–164.
20. frailtypack: An R Package for the Analysis of Correlated Survival Data with Frailty Models Using Penalized Likelihood Estimation or Parametrical Estimation | Rondeau | Journal of Statistical Software. Available at https://www.jstatsoft.org/article/view/v047i04. Accessed October 9, 2016.
21. Matet A, Daruich A, Zola M, Behar-Cohen F. Risk factors for recurrences of central serous chorioretinopathy. *Retina* 2018; 38(7):1403–1414.
22. Holland GN. Ocular toxoplasmosis: the influence of patient age. *Mem Inst Oswaldo Cruz* 2009;104(2):351–357.
23. Fardeau C, Romand S, Rao NA, et al. Diagnosis of toxoplasmic retinochoroiditis with atypical clinical features. *Am J Ophthalmol* 2002;134(2):196–203.

24. Rothova A, Bosch-Driessen LE, van Loon NH, Treffers WF. Azithromycin for ocular toxoplasmosis. *Br J Ophthalmol* 1998;82(11):1306–1308.
25. Bosch-Driessen LH, Verbraak FD, Suttorp-Schulten MSA, et al. A prospective, randomized trial of pyrimethamine and azithromycin vs pyrimethamine and sulfadiazine for the treatment of ocular toxoplasmosis. *Am J Ophthalmol* 2002;134(1):34–40.
26. Paulo Fernandes Felix JO, Pessoa Cavalcanti Lira R, Brito Cosimo A, Lourenç Cardeal Da Costa RO, Abujamra Nascimento M, Eduardo Leite Arieta C. Trimethoprim-sulfamethoxazole versus placebo in reducing the risk of toxoplasmic retinochoroiditis recurrences: a three-year follow-up. *Am J Ophthalmol* 2016;170:176–182.
27. Rosenbaum JT, Sibley CH, Lin P. Retinal vasculitis. *Curr Opin Rheumatol* 2016;28(3):228–235.
28. Shulman S, Kramer M, Amer R, et al. Characteristics and long-term outcome of patients with noninfectious retinal vasculitis. *Retina* 2015;35(12):2633–2640.
29. Pathanapitoon K, Tesavibul N, Choopong P, et al. Clinical manifestations of cytomegalovirus-associated posterior uveitis and panuveitis in patients without human immunodeficiency virus infection. *JAMA Ophthalmol* 2013;131(5):638–645.
30. Furrer H, Barloggio A, Egger M, Garweg JG. Retinal microangiopathy in human immunodeficiency virus infection is related to higher human immunodeficiency virus-1 load in plasma. *Ophthalmology* 2003;110(2):432–436.
31. Merle H, Hage R, Meniane J-C, et al. Retinal manifestations in adult t-cell leukemia/lymphoma related to infection by the human t-cell lymphotropic virus type-1. *Retina* 2016;36(7):1364–1371.
32. Akpek EK, Ahmed I, Hochberg FH, et al. Intraocular-central nervous system lymphoma. *Ophthalmology* 1999;106(9):1805–1810.
33. Jones LA, Alexander J, Roberts CW. Ocular toxoplasmosis: in the storm of the eye. *Parasite Immunol* 2006;28:635–642.
34. Klaren VN, Peek R. Evidence for a compartmentalized B cell response as characterized by IgG epitope specificity in human ocular toxoplasmosis. *J Immunol* 2001;167(11):6263–6269.
35. Friedrich R, Simon HU, Müller WA, Sych FJ. Ocular toxoplasmosis: the role of cellular immune defense in the development of recurrences. Results from animal experiments. *Ger J Ophthalmol* 1992;1(1):54–57.
36. Hunter CA, Sibley LD. Modulation of innate immunity by Toxoplasma gondii virulence effectors. *Nat Rev Microbiol* 2012;10(11):766–778.
37. Dupont CD, Christian DA, Hunter CA. Immune response and immunopathology during toxoplasmosis. *Semin Immunopathol* 2012;34(6):793–813.
38. Carneiro ACAV, Machado AS, Béla SR, et al. Cytokine signatures associated with early onset, active lesions and late cicatricial events of retinochoroidal commitment in infants with congenital toxoplasmosis. *J Infect Dis* 2016;213(12):1962–1970.
39. Blader IJ, Saeij JP. Communication between Toxoplasma gondii and its host: impact on parasite growth, development, immune evasion, and virulence. *APMIS* 2009;117(5-6):458–476.
40. Maenz M, Schlüter D, Liesenfeld O, Schares G, Gross U, Pleyer U. Ocular toxoplasmosis past , present and new aspects of an old disease. *Prog Retin Eye Res* 2014;39:77–106.
41. Skariah S, McIntyre MK, Mordue DG. Toxoplasma gondii: determinants of tachyzoite to bradyzoite conversion. *Parasitol Res* 2010;107(2):253–260.
42. Frenkel JK, Escajadillo A. Cyst rupture as a pathogenic mechanism of toxoplasmic encephalitis. *Am J Trop Med Hyg* 1987;36(3):517–522.
43. Ferguson DJ, Hutchison WM, Pettersen E. Tissue cyst rupture in mice chronically infected with Toxoplasma gondii. An immunocytochemical and ultrastructural study. *Parasitol Res* 1989;75(8):599–603.
44. Dubey JP, Lindsay DS, Speer CA. Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin Microbiol Rev* 1998;11(2):267–299.