Diagnosis of Ocular Toxocariasis by Establishing Intraocular Antibody Production


- PURPOSE: To investigate the role of *Toxocara canis* in posterior uveitis of undetermined origin.
- DESIGN: Retrospective case-study.
- METHODS: Paired ocular fluid (47 aqueous humor [AH] and two vitreous fluids) and serum samples of 37 adults and 12 children with undetermined posterior uveitis were retrospectively analyzed for intraocular IgG antibody production against *Toxocara canis* by enzyme-linked immunosorbent assay and Goldmann-Witmer coefficient (GWC) determination. Previous diagnostic investigation by polymerase chain reaction and GWC for Herpes simplex virus, Varicella zoster virus, and Toxoplasma gondii had not provided a cause of the posterior uveitis.
- RESULTS: Three of 12 (25%) children showed intraocular IgG production against *Toxocara canis*. One child had vitritis, one presented with a low-grade uveitis and a peripheral retinal lesion, and the third had posterior uveitis and a chorioretinal scar. All three children had AH IgG titers exceeding those of the corresponding serum. In fact, two children had low *Toxocara* serum IgG titers (<1:32) and would have been considered seronegative upon routine serology screening. Intraocular antibody production against *Toxocara canis* was absent in all 37 adults, including five seropositive patients.
- CONCLUSIONS: Our results indicate that ocular toxocariasis is mainly a pediatric disease. Serological screening is not informative for the diagnosis of intraocular *Toxocara* infection. *Toxocara* GWC analysis, however, can be of value when diagnosing patients with posterior focal lesions or vitritis of unknown etiology. (Am J Ophthalmol 2008;145:369–374. © 2008 by Elsevier Inc. All rights reserved.)

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**OXOCARA CANIS IS A ROUNDWORM WITH THE DOG** as its natural host. Humans can become infected by ingestion of soil or contaminated meat containing *Toxocara* larvae. In particular, children eating dirt or in close contact to puppies are at risk of being infected. In humans, the *Toxocara* larvae can invade several organs, such as the lungs, liver, brain, and eye, where they are encysted by a granulomatous cellular reaction.\(^1\)\(^–\)\(^3\)

Not much is known about the prevalence of human toxocariasis, but the disease occurs worldwide. Seroepidemiological studies may vary widely depending on the population examined. Reported *Toxocara* seroprevalences range from 4% to 46% in adults and can be as high as 77.6% in schoolchildren.\(^2\)\(^–\)\(^12\) High percentages are associated with low hygienic standards and high exposure to infected dogs.

Ocular toxocariasis or ocular larva migrans (OLM) is a local complication of a *Toxocara* canis infection and is usually suspected in children.\(^1\)\(^–\)\(^3\),\(^13\) although it has been reported in adults.\(^14\),\(^15\) The clinical signs of ocular toxocariasis often include diminished vision, leukocoria, red eye, and strabismus. Lesions occur mostly unilaterally and might be falsely diagnosed as retinoblastoma or endophthalmitis of bacterial origin. The diagnosis is usually based on the presence of chorioretinal granuloma or focal lesions in the posterior eye segment in the presence of positive serology.\(^1\)\(^–\)\(^2\),\(^15\)

However, low or undetectable *Toxocara* serum immunoglobulin (Ig) G titers have been reported in patients with ocular toxocariasis.\(^16\) Therefore, the diagnosis of ocular toxocariasis is difficult and in the majority of cases remains only presumptive.

In this study, we examined the possibility of intraocular infection with *Toxocara canis* in 49 patients (37 adults and 12 children) with posterior uveitis of undetermined origin by means of serum antibody and Goldmann-Witmer coefficient (GWC) determination.

**METHODS**

FROM 2001 TO 2006, 49 PATIENTS WITH POSTERIOR AND panuveitis of unknown etiology were examined at the Department of Ophthalmology (n = 43) at the University Medical Center in Utrecht (UMCU), The Netherlands, or at Ophthalmology Clinics in other Dutch hospitals (n = 6).
From all patients the clinical characteristics were recorded. All patients had been subjected to extensive general screening, which included erythrocyte sedimentation rate, red and white blood cell counts, glucose levels, determination of serum angiotensin-converting enzyme levels and serological tests for syphilis, and chest radiography. Based on the general screening and clinical presentation, none of the patients were considered immunocompromised. Moreover, intraocular fluid analysis was performed at the UMCU for Herpes simplex virus (HSV), Varicella zoster virus (VZV), and Toxoplasma gondii by polymerase chain reaction (PCR) and GWC determination, with negative results. All patients had clinical characteristics compatible with ocular toxocariasis (granuloma, focal chorioretinitis, or multiple focal chorioretinal lesions) and Toxocara canis serology had been previously requested, but did not provide conclusive evidence about the cause of uveitis. Of 49 patients, 12 (24%) were children (under 17 years of age) and 37 (76%) were adults. The children included seven (58%) boys and five (42%) girls, with a mean age of 9.6 years at the time of sampling (range, two to 16 years). The adults included 20 (54%) men and 17 (46%) women, with a mean age of 35.9 years at the time of sampling (range, 17 to 65 years) (Table 1).

The simultaneously taken serum and ocular fluid samples (46 aqueous humors [AH] and three vitreous fluids), previously used for examination for HSV, VZV, and T. gondii, were retrospectively analyzed for intraocular antibody production against Toxocara canis by GWC determination ([specific IgG in AH/specific IgG in serum] / [total IgG in AH/total IgG in serum]). Toxocara canis–specific serum and AH IgG were determined by using the Toxocara canis IgG enzyme-linked immunosorbent assay (ELISA) kit (DRG Instruments, Marburg, Germany), which contains micro test wells coated with an excretory/secretory antigen derived from second-stage larvae of Toxocara canis. The assays were performed according to the instructions of the manufacturer. However, instead of a single 1:64 dilution, which is the manufacturer’s screening dilution for seropositivity, four two-fold dilutions ranging from 1:32 to 1:256 were used for both serum and AH. Serum and AH IgG titers were calculated using the Mikrowin software version 3.0 (Mikrotek Laborsysteme, Overath, Germany). In case of undetectable serum IgG (titer <32), the GWC value was calculated using a serum titer of 32 and referred to as larger than (>), the outcome of the GWC. Total IgG titers in serum and aqueous humor were determined by an in-house ELISA which has been previously described. Intraocular antibody production was considered positive when the GWC value exceeded three.

### RESULTS

GENERAL CLINICAL CHARACTERISTICS ARE GIVEN IN TABLE 1. Two of 37 adults were seropositive for Toxocara canis at the screening dilution of 1:64 and an additional three were positive at the 1:32 dilution (5/37; 14%). Of the children, one was seropositive at 1:64, one was just positive at the 1:32 dilution (2/12; 17%) and the remainder were negative. Intraocular antibody production against Toxocara canis (GWC > 3) was absent in all 37 adults, including the five seropositive adult patients. Moreover, in none of the 37 adults was Toxocara canis IgG detected in the aqueous humor. In contrast, three of 12 (25%) children demonstrated intraocular IgG production against Toxocara canis. Two of these three children were negative at dilution 1:32. The third child was positive at dilution 1:64. All three children had an intraocular Toxocara IgG titer which exceeded that of the serum (Table 2). In the remaining nine children no Toxocara canis IgG was detected in the aqueous humor. The three children with a positive Toxocara canis GWC are described.

**CASE 1:** A 7-year-old Turkish boy was referred to our clinic because of recently detected decrease in visual acuity of the left eye (LE). He had no ophthalmic history, except intermittent redness of the LE for several months. Ocular examination of this eye revealed the presence of keratic precipitates, cells in the anterior chamber, iris bombe with papillary seclusion, mature cataract, and dense vitreous membranes. Funduscopy was not possible attributable to mature cataract. Ultrasonography revealed vitreous opac-
ities with a funnel-shaped structure in the vitreous, adhesion to the optic disk, and disk edema (Figure 1). The right eye (RE) was unremarkable. He was referred to a pediatrician, but there were no indications of tuberculosis, juvenile idiopathic arthritis, and sarcoidosis by Purified Protein Derivative, anti-nuclear antibodies, and radiological examination of the chest. *Ascaris lumbricoides* serology was negative, *Toxocara canis* serology was positive, aqueous analysis revealed negative GWC results for HSV, VZV, *Rubella virus*, *T. gondii* and *Borrelia Burgdorferi*, and negative PCR results for HSV, VZV, and *T. gondii*. At that time the diagnosis remained inconclusive. Cataract extraction was performed in combination with vitrectomy with silicone oil, because of retrolental vitreous membranes and tractional retinal detachment. After removing the silicone oil, the patient developed proliferative vitreoretinopathy. Subsequently, the eye became atrophic and because of severe psychological and cosmetic problems, enucleation followed and the eye was investigated at the pathology laboratory. Microscopic examination revealed a hyperplastic cornea and a round nuclear inflammatory infiltrate in the underlying fibrous tissue. In addition to fibrosis, neovascularization, and papillary seclusion, the retina was completely detached and prolapsed anteriorly with adhesion to the fibrous tissue. This piece of the retina showed reactive gliosis. The angles of the anterior chamber were completely obstructed, partly with reactive choroid proliferation. Locally, macrophages with multinuclear giant cells were observed on the retinal pigment epithelium. Eosinophils were not observed. Stainings to detect microorganisms were all negative, but this does not exclude an infectious cause. Based on histopathological analysis, no specific diagnosis could be made, other than evidence for recurrent uveitis.

Retrospectively, serum and AH were analyzed for *Toxocara canis* immunoglobulin, yielding a very high AH titer (1,609), exceeding the serum titer (94). The resulting

<table>
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<th>Patient</th>
<th>Gender</th>
<th>Age</th>
<th>Immune Status</th>
<th>Location Involvement</th>
<th>Anterior Segment Involvement</th>
<th>Anterior Segment Activity</th>
<th>Aqueous IgG Titer</th>
<th>Serum IgG Titer</th>
<th>GWC</th>
<th>Ocular toxocariasis</th>
</tr>
</thead>
<tbody>
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<td>7</td>
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<td>Panuveitis</td>
<td>Unilateral</td>
<td>Yes</td>
<td>1609</td>
<td>94</td>
<td>&gt;243</td>
<td>Unknown</td>
</tr>
<tr>
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<td>Male</td>
<td>8</td>
<td>Normal</td>
<td>Posterior</td>
<td>Unilateral</td>
<td>No</td>
<td>nd</td>
<td>1085</td>
<td>&gt;1085</td>
<td>Ocular toxoplasmosis or OT</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>13</td>
<td>Normal</td>
<td>Posterior</td>
<td>Unilateral</td>
<td>No</td>
<td>nd</td>
<td>103</td>
<td>&lt;32</td>
<td>Ocular toxoplasmosis or OT</td>
</tr>
</tbody>
</table>

**TABLE 2**: Clinical and Laboratory Data of the Three Children with a Positive Goldmann-Witmer Coefficient for *Toxocara canis*

**FIGURE 1**: Ultrasonography revealing a funnel-shaped structure and adhesion to the optic disk in a child with ocular toxocariasis.
GWC was positive (144), and the diagnosis of ocular toxocariasis was made.

**CASE 2:** An 8-year-old boy was referred to our clinic because of recently detected uveitis of the LE with vitreous cells and a peripheral retinal scar. The RE was normal. The initial visual acuity of the left eye was 1.0. The diagnosis of ocular toxocariasis or toxoplasmosis was suspected. The general medical history was not remarkable, however the patient was born in Sri Lanka and visited it several times. On ocular examination, the visual acuity of the LE was 0.8, the anterior chamber revealed sporadic cells, the lens was clear, the vitreous exhibited cells and opacities, and in the inferior peripheral retina a white lesion was observed. The RE had full visual acuity and no abnormalities. Fluorescein angiography demonstrated a peripheral active lesion, possibly a granuloma with vitreous traction (Figure 2). The patient was referred to a pediatrician for examination for systemic diseases. The erythrocyte sedimentation rate was 5 mm/hour and blood counts and angiotensin converting enzyme were within normal range. Radiological chest examination was normal. Ascaris serology was negative and Toxocara titers were less than 1:32. Toxoplasma IgM was negative and IgG was positive. There was no evidence for an active infection with Coxiella burnetii, Rickettsia conorii, Rickettsia typhi, Strongyloides stercoralis, Filaria, cytomegalovirus (CMV), HSV, and VZV. Aqueous analysis was negative for HSV, VZV, T. gondii, and Rubella virus, both by PCR and by GWC. Despite undetectable serum IgG against Toxocara, the AH titer was clearly positive (109) and a GWC value of >243 was determined, establishing intraocular antibody production against Toxocara canis. The patient was not treated for toxocariasis because the lesion became quiet and atrophic over time. The uveitis, however, persisted and was treated with topical corticosteroids.

**CASE 3:** A 13-year-old boy was seen at the ophthalmology clinic because of a decrease in visual acuity of the LE existent for six months. He was in general good health and had no ophthalmic history. On ocular examination, the visual acuity of the LE was 0.1, the anterior chamber revealed no cells, and the vitreous exhibited some pigment cells, vitreous strands with retinal traction, and a macular scar with a pucker. Ultrascanography revealed a posterior vitreous detachment with adhesion to the optic disk. On fluorescein angiography, no vasculitis was seen. The RE was normal. Vitrectomy with removal of internal limiting membrane was performed. A vitreous sample was obtained and subsequent screening by Toxocara canis, Ascaris lumbricoides serology was negative. Serum IgG against Toxocara was undetectable, however the IgG titer in the vitreous was 103, resulting in a GWC value of at least 1,085, establishing ocular toxocariasis. Visual acuity did not improve after vitrectomy.

**DISCUSSION**

IN THIS STUDY WE FOUND THREE CHILDREN WITH LOCAL antibody production against Toxocara. Antibody detection in serum and in ocular fluid of patients suspected of ocular toxocariasis has been reported, but only one report included GWC determination to correct for passive leakage of antibodies from the serum in the aqueous attributable to blood–aqueous barrier breakdown.

The three children with a positive GWC had very low serum IgG titers. One child was positive at the screening dilution of 1:64. Two were negative even at dilution 1:32 and would have been designated seronegative. Very low serum titers or seronegativity in patients with ocular toxocariasis have been reported previously. Therefore, it has been suggested that sera should be tested at dilutions as low as 1:2 and that any positive result in combination with clinical correlation is relevant in ocular toxocariasis. Moreover, Hagler and associates found a positive result at a 1:8 serum dilution or highly accurate in association with typical clinical findings. By screening at lower dilutions, the seroprevalence in patients with ocular toxocariasis may be higher than reported thus far. Interestingly, the seroprevalence in patients with ocular toxocariasis was reported to be higher in children than in adults. This is most likely attributable to waning antibody titers, as was demonstrated in a follow-up study of 20 patients with ocular toxocariasis, where 85% showed a decrease in serum titers. Therefore, patients with a low or undetectable serum titer against Toxocara, including two of our GWC-positive children, may have had higher titers in the past.
Still, the presence of serum IgG against Toxocara does not unambiguously prove ocular involvement even in the presence of typical clinical findings, as is exemplified by six seropositive patients in our study who had no detectable intraocular antibody against Toxocara. Therefore, determination of intraocular antibody production can help to establish the diagnosis of ocular toxocariasis.

All three GWC-positive patients had low or undetectable serum IgG titers, but very high AH titers. Similar antibody distributions have been reported previously.14,25 This most likely is a reflection of the localized nature of an intraocular Toxocara infection, with extensive intraocular immunostimulation, but a systemic decrease in antibody titers.22

Although ocular toxocariasis has been described in adults,14,15,20 none in our study, including the five seropositive patients, had intraocular antibody production against Toxocara canis. The significantly higher incidence of GWC proven ocular toxocariasis cases in juveniles (P = .012), is in agreement with ocular toxocariasis being mainly a pediatric disease.1–3,13

It is difficult to establish the diagnosis of ocular toxocariasis based on clinical manifestations solely, because ocular symptoms may be diverse and inflammatory signs such as redness and pain are not always present. The diagnosis of ocular toxocariasis is often made coincidentally in eyes without inflammation, for instance, during an evaluation for strabismus, in cases of decreased vision, or while undergoing a routine examination.13 Our first GWC-positive patient presented with a decrease of visual acuity, intermittent redness, and cataract in combination with severe vitritis. The second patient had a low-grade uveitis and a peripheral retinal lesion and the third presented with posterior uveitis and a chorioretinal scar. Posterior focal lesions were found in two patients and led to the suspicion of ocular toxoplasmosis or toxocariasis. However, ocular toxocariasis can also cause severe vitreous inflammation mimicking endophthalmitis, which applies to our first case.13

Taking into account that establishing the diagnosis of ocular toxocariasis based on clinical features and serologic results is unreliable, we suggest the addition of Toxocara canis GWC determination to the diagnostic repertoire in patients with unexplained focal chorioretinitis or vitritis. Moreover, toxocaral granuloma might be mistaken for retinoblastoma, because both diseases can clinically present with leukocoria, strabismus, and loss of visual acuity.11 In 1950, Wilder reported 24 patients whose eyes were enucleated because of suspected retinoblastoma.26 The enucleated eyes were found to have nematodes, four of which later appeared to be Toxocara canis.27 Toxocara GWC determination might play a role in the differentiation between retinoblastoma and toxocaral posterior pole granuloma in children. However, the decision to perform paracentesis should be made reluctantly, attributable to the risk of spreading malignant cells in case of retinoblastoma.

Summarizing, intraocular IgG production against Toxocara canis was demonstrated by GWC determination in three children with posterior focal lesions or vitritis, despite negative or very low serum IgG titers. Toxocara GWC analysis might be of value when diagnosing patients with posterior focal lesions or vitritis of unknown etiology.

### REFERENCES