# Identification of New Pathogens in the Intraocular Fluid of Patients With Uveitis

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• PURPOSE: To determine infectious causes in patients with uveitis of unknown origin by intraocular fluids analysis.

• DESIGN: Case-control study.

• METHODS: Ocular fluids from 139 patients suspected of infectious uveitis, but negative for herpes simplex virus, varicella-zoster virus, cytomegalovirus, and Toxoplasma gondii by polymerase chain reaction and/or antibody analysis in intraocular fluids, were assessed for the presence of 18 viruses and 3 bacteria by real-time polymerase chain reaction (PCR). The ocular fluids from 48 patients with uveitis of known etiology or with cataract were included as controls.

• RESULTS: Positive PCR results were found for Epstein-Barr virus, for rubella virus, and for human herpesvirus 6 each in 1 patient and for human parechovirus in 4 patients. Of the human parechovirus–positive patients, 1 was immunocompromised and had panuveitis. The other 3 patients were immunocompetent and had anterior uveitis, all with corneal involvement.

• CONCLUSIONS: Human parechovirus might be associated with infectious (kerato)uveitis.   (Am J Ophthalmol 2010;150:628–636. © 2010 by Elsevier Inc. All rights reserved.)

U VEITIS CAN BE OF INFECTIOUS OR NONINFECTIOUS origin. Infections are thought to cause approximately 20% to 25% of cases; about 30% are associated with a noninfectious systemic disease. Although for patient management and the efficacy of treatment, the differential diagnosis is crucial, in more than half of the uveitis patients the underlying cause remains unknown.

The pathogens most commonly associated with infectious uveitis in immunocompetent patients are Toxoplasma gondii, herpes simplex virus (HSV), and varicella-zoster virus (VZV). In recent years, a few other infectious agents have been implicated in the etiology of uveitis, most notably rubella virus and cytomegalovirus (CMV).[1–7] CMV is currently recognized as the most common cause of uveitis in immunocompromised patients.

In this study we performed an extensive search for infectious agents that cause uveitis but so far have escaped attention. Aqueous humor samples from 139 uveitis patients were analyzed retrospectively in our laboratory by available real-time polymerase chain reaction (PCR) assays for a variety of viruses and bacteria. Included were pathogens previously associated with uveitis (enteroviruses, Epstein-Barr viruses [EBV], human herpesvirus 6 [HHV6], influenza virus, rubella virus, Mycoplasma pneumoniae) and those causing conjunctivitis and/or keratitis or encephalitis (adenoviruses, coronaviruses, enteroviruses, influenza virus, parainfluenzaviruses, human parechovirus [HPeV], respiratory syncytial virus, Chlamydia pneumoniae, Chlamydia trachomatis)[1,5,8–30] Our findings suggest that human parechovirus may be involved in the pathogenesis of infectious (kerato)uveitis.

## METHODS

• PATIENTS AND SAMPLES: Ocular fluid samples analyzed in this study were from 629 uveitis patients who visited the ophthalmology clinic of the University Medical Center Utrecht from October 1, 2001 until June 30, 2006 and were suspected of infectious uveitis. The patients were classified using the uveitis nomenclature according to the recommendations of the Standardization of Uveitis Nomenclature working group 2005.[31] All patients had undergone the uveitis screening consisting of erythrocyte sedimentation rate, red and white blood cell counts, determination of serum angiotensin–converting enzyme levels, serologic tests for syphilis, and chest radiography. Selected patients also underwent serologic testing for Borrelia burgdorferi. For all 629

**TABLE 1.** General Characteristics of Uveitis Patients and Controls for Which the Intraocular Fluids Were Analyzed for the Presence of New Pathogens

|  | N | Immunocompromised | Gender (M:F) | Mean Age ± SD |
|---|---|---|---|---|
| Patients |  |  |  |  |
| Anterior uveitis | 49 | 2 (4%) | 29:20 | 50.8 ± 16.7 |
| Panuveitis/posterior uveitis | 90 | 8 (9%) | 46:44 | 48.9 ± 18.3 |
| Controls |  |  |  |  |
| Ocular toxoplasmosis | 13 | 0 | 7:6 | 47.2 ± 15.4 |
| HSV anterior uveitis | 10 | 0 | 5:5 | 44.1 ± 22.1 |
| Rubella virus–associated Fuchs heterochromic uveitis syndrome | 14 | 0 | 10:4 | 42.3 ± 16.5 |
| Cataract | 11 | 0 | 6:5 | 71.3 ± 15.8 |

F = female; HSV = herpes simplex virus; M = male; SD = standard deviation.

patients aqueous sampling was performed for diagnostic purposes. The samples were stored at −80°C within 5 hours of collection before processing for laboratory analysis. Initial analysis was performed for HSV, VZV, and in the case of posterior uveitis also for *Toxoplasma* and CMV, by PCR and by Goldmann-Witmer coefficient (GWC), to determine intraocular antibody production. Of the 629 patients, 486 were negative for the above mentioned agents. A sufficient amount of ocular fluid remained for this study in 139 of these cases. Forty-nine patients had anterior uveitis (AU) and 90 had posterior uveitis (PU) or panuveitis (Table 1). Of the 49 AU patients, 2 were immunocompromised as a result of immunosuppressive medications (1 for lethal midline granuloma and the other after allogeneic stem cell transplantation for hematologic malignancy). Of the 90 patients with PU and panuveitis, 8 were immunocompromised, 5 of whom had acquired immunodeficiency syndrome and 3 of whom received immunosuppressive drugs (Table 1).

The remainders of ocular fluid samples from patients with PCR- and/or GWC-confirmed infectious uveitis (ocular toxoplasmosis, n = 13; HSV anterior uveitis, n = 10; rubella virus–associated Fuchs heterochromic uveitis syndrome (FHUS), n = 14) and from patients with cataract in the absence of intraocular inflammation (n = 11) served as controls.

• NUCLEIC ACID ISOLATION AND REAL-TIME PCR: The ocular fluid samples were analyzed for the presence of adenovirus, EBV, HHV6, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Chlamydia trachomatis* DNA and of coronaviruses 229E, OC43, and NL63, enteroviruses, human metapneumovirus, influenza A and B virus, parainfluenza virus 1 to 4, HPeV, respiratory syncytial virus A and B, and rubella virus RNA. If not done previously, samples from patients with anterior uveitis were also analyzed for CMV and *Toxoplasma*. DNA and RNA were extracted from 30 μL of ocular fluid using the MagNa Pure LC Total Nucleic Acid isolation kit (Roche, Mannheim, Germany). To monitor the quality of the extraction and the subsequent amplification procedure, a standard dose of phocine herpesvirus type 1 and encephalomyocarditis virus was added to each sample as an internal control prior to extraction.[32–34] Nucleic acid was collected in a volume of 240 μL. For detection of RNA viruses, copyDNA (cDNA) was produced by mixing 40 μL of extracted nucleic acid with 60 μL of reverse transcriptase mix (Taqman, reverse transcription reagents; Applied Biosystems, Foster City, California, USA) and incubating the mixture for 10 minutes at 25°C and 30 minutes at 48°C. The cDNA synthesis reaction was stopped by incubating for 5 minutes at 95°C. Per amplification reaction 10 μL of extracted nucleic acid (for DNA detection) or 10 μL of cDNA (for RNA detection) was used. Real-time PCR assays were performed on an ABI Prism 7700 sequence detection system (Applied Biosystems, Branchburg, New Jersey, USA). For *Chlamydia trachomatis*, 25 μL of extracted nucleic acid was analyzed using the Cobas Amplicor *Chlamydia trachomatis* detection kit according to the instructions of the manufacturer (Roche). All samples were examined once. In case of positive outcomes the real-time PCR reaction was repeated. Two human parechovirus–positive samples were confirmed by nucleic acid sequencing. Samples for which the internal control was inhibited were excluded. The primers and probes used are listed in Table 2.

• ANTIBODY DETECTION ASSAYS: Intraocular production of antibody against rubella virus (Goldmann-Witmer coefficient) was assessed as described previously.[1] Serum and intraocular immunoglobulin (Ig)G titers against HHV6 were determined using the Biotrin International Human Herpes Virus 6 IgG immunofluorescence assay (Dublin, Ireland). Serum and intraocular IgG against EBV was determined using the Panbio VCA IgG ELISA (Grenoble, France).

| Pathogen | Primers/Probes | Sequence 5′ to 3′ | Sensitivity | References |
|---|---|---|---|---|
| Adenoviruses | Forward | TTT GAG GTG GA(C/T) CC(A/C) ATG GA | 100 copies/mL | 51 |
| | | TTT GAG GTG GA(C/T) CC(A/C) ATG GA | | |
| | Reverse | AGA A(G/C)G G(G/C)G T(A/G)C GCA GGT A | | |
| | | AGA A(G/C)G GTG T(A/G)C GCA GAT A | | |
| | Probe | **FAM-**ACC ACG TCG AAA ACT TCG AA-**MGBNFQ** | | |
| | | **FAM-**ACC ACG TCG AAA ACT TCA AA-**MGBNFQ** | | |
| | | **FAM-**ACA CCG CGG CGT CA-**MGBNFQ** | | |
| Coronavirus 229E | Forward | CAG TCA AAT GGG CTG ATG CA | | |
| | Reverse | CAA AGG GCT ATA AAG AGA ATA AGG TAT TCT | ND | 53 |
| | Probe | **FAM-**CCC TGA CGA CCA CGT TGT GGT TCA -**TAMRA** | | |
| Coronavirus NL63 | Forward | AAG GGT TTT CCA CAG CTT GCT | ND | 53 |
| | | AAA GGT TTT CCA CAG CTT GCT | | |
| | Reverse | ATC ACC CAC TTC ATC AGT GCT AAC | | |
| | Probe | **FAM-**TCA CTA TCA AAG AAT AAC GCA GCC TGA TTA | | |
| | | GGA A -**TAMRA** | | |
| Coronavirus OC43 | Forward | CGA TGA GGC TAT TCC GAC TAG GT | | |
| | Reverse | CCT TCC TGA GCC TTC AAT ATA GTA ACC | ND | 53 |
| | Probe | **FAM-**TCC GCC TGG CAC GGT ACT CCC T -**TAMRA** | | |
| Enteroviruses | Forward | TCC TCC GGC CCC TGA | | |
| | Reverse | AAT TGT CAC CAT AAG CAG CCA | ND | 50 |
| | | GAT TGT CAC CAT AAG CAG CCA | | |
| | Probe | **FAM-**CGG AAC CGA CTA CTT TGG GTG ACC GT -**TAMRA** | | |
| | | **FAM-**CGG AAC CGA CTA CTT TGG GTG TCC GT -**TAMRA** | | |
| Epstein-Barr virus | Forward | GGA ACC TGG TCA TCC TTT GC | | |
| | Reverse | ACG TGC ATG GAC CGG TTA AT | 50 copies/mL | 42 |
| | Probe | **FAM-**CGC AGG CAC TCG TAC TGC TCG CT -**TAMRA** | | |
| Human herpesvirus 6 | Forward | GAA GCA GCA ATC GCA | 160 copies/mL | 42 |
| | Reverse | ACA CA ATG TAA CTC | | |
| | Probe | GGT GTA CGG TGT CTA | | |
| | | **FAM-**AAC CCG TGC GCC GCT -**TAMRA** | | |
| Human metapneumovirus | Forward | CAT ATA AGC ATG CTA TAT TAA AAG AGT CTC | ND | 49 |
| | Reverse | CCT ATT TCT GCA GCA TAT TTG TAA TCA G | | |
| | Probe | **FAM-**TG(C/T) AAT GAT GAG GGT GTC ACT GCG GTT | | |
| | | G -**TAMRA** | | |
| Influenza virus A | Forward | AAG ACC AAT CCT GTC ACC TCT GA | | |
| | Reverse | CAA AGC GTC TAC GCT GCA GTC C | ND | 52 |
| | Probe | **FAM-**TTT GTG TTC ACG CTC ACC GTG CC -**TAMRA** | | |
| Influenza virus B | Forward | AAA TAC GGT GGA TTA AAC AAA AGC AA | | |
| | Reverse | CCA GCA ATA GCT CCG AAG AAA | ND | 52 |
| | Probe | **FAM-** CAC CCA TAT TGG GCA ATT CCT AT | | |
| | | GGC -**TAMRA** | | |
| Parainfluenza virus 1 | Forward | TGA TTT AAA CCC GGT AAT TTC TCA T | | |
| | Reverse | CCT TGT TCC TGC AGC TAT TAC AGA | ND | 51 |
| | Probe | **FAM-** ACG ACA ACA GGA AAT C -**TAMRA** | | |
| Parainfluenza virus 2 | Forward | AGG ACT ATG AAA ACC ATT TAC CTA AGT GA | | |
| | Reverse | AAG CAA GTC TCA GTT CAG CTA GAT CA | ND | 51 |
| | Probe | **FAM-** ATC AAT CGC AAA AGC TGT TCA GTC ACT GCT | | |
| | | ATA C -**TAMRA** | | |
| Parainfluenza virus 3 | Forward | TGA TGA AAG ATC AGA TTA TGC AT | | |
| | Reverse | CCG GGA CAC CCA GTT GTG | ND | 51 |
| | Probe | **FAM-**TGG ACC AGG GAT ATA CTA CAA AGG CAA AAT | | |
| | | AAT ATT TCT C -**TAMRA** | | |

**TABLE 2.** Primers, Probes, and Sensitivities of the Real-Time Polymerase Chain Reaction Assays Used to Identify New Pathogens in the Intraocular Fluids of Patients With Unidentified Uveitis *(Continued)*

| Pathogen | Primers/Probes | Sequence 5' to 3' | Sensitivity | References |
|---|---|---|---|---|
| Parainfluenza virus 4 | Forward | CAA ATG ATC CAC AGC AAA GAT TC | | |
| | Reverse | ATG TGG CCT GTA AGG AAA GCA | ND | 51 |
| | Probe | **FAM**- GTA TCA TCA TCT GCC AAA TCG GCA ATT AAA CA -**TAMRA** | | |
| Human parechovirus | Forward | TGC AAA CAC TAG TGG TA(A/T) GGC CC | | |
| | Reverse 1 | TCA GAT CCA TAG TG(C/T) CAC TTG TTA CCT | | |
| | Reverse 2 | TCA GAT CCA CAG TGT CTC TTG TTA CCT | 1 TCID50/mL | Forthcoming |
| | Probe | **FAM**- CGA AGG ATG CCC AGA AGG TAC CCG -**TAMRA** | | |
| Respiratory syncytial virus A | Forward | AGA TCA ACT TCT GTC ATC CAG CAA | | |
| | Reverse | TTC TGA ACA TCA TAA TTA GGA GTA TCA AT | ND | 54 |
| | Probe | **FAM**- CAC CAT CCA ACG GAG CAC AGG AGA T -**TAMRA** | | |
| Respiratory syncytial virus B | Forward | AAG ATG CAA ATC ATA AAT TCA CAG GA | | |
| | Reverse | TGA TAT CCA GCA TCT TTA AGT ATC TTT ATA GTG | ND | 54 |
| | Probe | **FAM**- TCC CCT TCC TAA CCT GGA CAT AGC ATA TAA CAT ACC T -**TAMRA** | | |
| Rubella virus | Forward | CAC GCC GCA CGG ACA | | |
| | Reverse 1 | CAC CGG GAC TG(C/T) TG(A/G) TTG C | | |
| | Reverse 2 | CAC CGG GAC TGT TGG TTG C | 1.7 PFU/mL | Forthcoming |
| | Probe | **FAM**- AGG TCC CGC CCG AC-**MGBNFQ** | | |
| *Mycoplasma pneumoniae* | Forward | GGT CAA TCT GGC GTG CAT CT | | |
| | Reverse | TGG TAA CTG CCC CAC AAG C | 50 CCU/mL | 34 |
| | Probe | **FAM**- TCC CCC GTT GAA AAA GTG AGT GGG T -**TAMRA** | | |
| *Chlamydia pneumoniae* | Forward | TCC GCA TTG CTC AGC C | | |
| | Reverse | AAA CAA TTT GCA TGA AGT CTG AGA A | 4.9 IFU/mL | 34 |
| | Probe | **FAM**-TAA ACT TAA CTG CAT GGA ACC TTC TTT TAC TAG G -**TAMRA** | | |

CCU = color-changing units; FAM = 6-carboxyfluorescein fluorescent label; IFU = inclusion-forming units; MGBNFQ = minor groove binding nonfluorescent quencher; ND = not determined; PFU = plaque-forming units; TAMRA = tetramethylrhodamine quencher; TCID50 = tissue culture infectious dose 50.

# RESULTS

IN NONE OF THE OCULAR FLUIDS THE INTERNAL CONTROL was inhibited. In the patient samples positive PCR reactions were found for Epstein-Barr virus (n = 1), rubella virus (n = 1), human herpesvirus 6 (n = 1), and human parechovirus (n = 4). The PCR reactions for all other pathogens were negative. All control samples were negative except for 3; 2 toxoplasma chorioretinitis control samples were positive for EBV and 1 sample positive for rubella virus intraocular antibody production also tested PCR-positive for rubella virus RNA.

The patients with uveitis of unknown cause and a positive PCR result for rubella virus, HHV6, and human parechovirus are described below.

• RUBELLA VIRUS (CASE 1): A 40-year-old female patient complained of gradual decrease of visual acuity in the right eye (OD). Her medical history included pneumothorax and bilateral pneumonia many years ago, but she had no signs of systemic disease.

The visual acuity of the OD was 20/80. The anterior chamber and vitreous of the OD revealed cells, but no synechiae. There was a subcapsular posterior cataract, fine keratic precipitates (KPs) and vitreous opacities. The retina was normal. The left eye (OS) had full visual acuity and no abnormalities on examination. Uveitis screening results were within normal limits. The clinical diagnosis of Fuchs heterochromic uveitis syndrome was made and a cataract extraction with implantation of an intraocular lens was performed as well as pars plana vitrectomy for vitreous opacities. On examination of the vitreous, there was no evidence for systemic and/or intraocular infection using PCR and GWC for CMV, HSV, VZV, *Toxoplasma*, *Borrelia burgdorferi*, and *Bartonella henselae*. Microbiological cultures were negative and cytologic examination revealed no malignant cells. By PCR, rubella virus was detected in the vitreous fluid. Subsequent antibody analysis for rubella

**TABLE 3.** Clinical Data of Uveitis Patients With a Human Parechovirus–Positive Polymerase Chain Reaction on Intraocular Fluid

| Case | Gender | Age | Immune Status | Location | Unilateral or Bilateral | Cells Ant[a] | Cells Post[a] | Corneal Involvement | Retinitis | Vasculitis | Papillitis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | M | 54 | Normal | Anterior | Unilateral | 1+ | No | Corneal scar | No | No | No |
| 4 | M | 53 | Normal | Anterior | Unilateral | No | No | Corneal Infiltrate | No | No | No |
| 5 | F | 73 | Normal | Anterior | Unilateral | No | No | Corneal edema and keratic precipitates | No | No | No |
| 6 | M | 37 | HIV-positive | Panuveitis[b] | Unilateral | 3+ | 2+ | Corneal edema and keratic precipitates | Yes | Yes | Yes |

Ant = anterior; F = female; M = male; Post = posterior.

[a] Grading of cells was performed as recommended by Jabs and associates.[31]

[b] This patient was also diagnosed with neurosyphilis.

virus revealed the presence of intraocular IgG, but the GWC was negative (ratio 2.02). However, in comparison with HSV, VZV, CMV en *Toxoplasma*, intraocular antibody production against rubella virus was elevated.

● HUMAN HERPESVIRUS-6 (CASE 2): A 42-year-old man was referred because of decrease in visual acuity of his OD and floaters since 3 months prior. His medical history was not contributory and the patient used no medications. Uveitis screening results were within normal range. Remarkable was the heterochromia of his eyes, present since childhood. There was no serologic evidence for an active infection with CMV, HSV, VZV, *Toxoplasma*, or *Treponema pallidum*. *Borrelia burgdorferi* serum IgG and immunoblot were positive; however, a distinction between a past and an ongoing infection could not be made.

On ocular examination, the visual acuity of the OD was 20/25; the cornea revealed the presence of keratic precipitates, but the anterior chamber was clear. There were no synechiae, but several small noduli were present on the pupillary edge of the iris. Cataract was not observed. Funduscopy of the OD revealed vitreous cells and several peripheral snowballs. The fundoscopic findings were normal. The OS had full visual acuity; however, some peripheral vitreous opacities were observed.

Because of the possible (previous) infection with *Borrelia*, the patient was treated with intravenous ceftriaxone and additionally with periocular steroids, but with no effect. Diagnostic vitrectomy was performed and cytologic and microbiological examinations did not reveal a cause of his uveitis. Vitreous analysis was negative for CMV, HSV, VZV, and *Borrelia*, both by PCR and by GWC. The rubella virus GWC was negative (ratio 2.68), although intraocular IgG was detected and the GWC was elevated in comparison to CMV, HSV, and VZV. Therefore, rubella virus–associated FHUS could not be excluded. Three months after vitrectomy the patient regained full visual acuity, although the keratic precipitates in his OD remained. Retrospectively, the vitreous fluid appeared to be positive for HHV6 by PCR. Immunofluorescence assay demonstrated that the patient was seropositive for HHV6.

● HUMAN PARECHOVIRUS:CASE 3. A 54-year-old male patient was referred to our center with anterior uveitis of 2 years' duration in his pseudophakic OS. Twenty-nine years prior, the patient underwent cataract extraction and implantation of an iris-clip lens in his OS because of previous trauma. On ocular examination, the visual acuity of the OS was hand movements (Table 3). A central corneal scar was seen, cells were present in the anterior chamber, and the vitreous was clear. Funduscopy revealed no abnormalities. The OD had full visual acuity and no abnormalities. Both eyes had normal intraocular pressure. The patient had no systemic complaints and used no medications. Uveitis screening results were within normal limits.

Uveitis was clinically attributed to irritation caused by the iris-clip lens, which was therefore surgically removed. A vitreous sample was obtained during surgery. Analysis of the ocular fluid was negative for HSV and VZV, both by PCR and GWC, and for CMV and *Toxoplasma* by PCR, but was retrospectively positive for human parechovirus by PCR. Ocular examination 4 months after removal of the intraocular lens revealed a quiet OS.

*Case 4.* A 53-year-old male was referred because of persistent keratitis of his OS. It was thought to be caused by HSV, but the patient did not respond to systemic and topical treatments with acyclovir and valacyclovir. His previous medical and ophthalmic histories were unremarkable. Uveitis screening did not reveal any abnormalities.

On ocular examination, the visual acuity of the OS was 20/100. An infiltrate in the upper part of the cornea with epithelial defect and sporadic cells in the anterior chamber were observed (Table 3). Corneal sensitivity was normal. The vitreous was clear and the retinal findings were unremarkable. Intraocular pressure was normal. The OD had full visual acuity and no abnormalities. Aqueous analysis was negative for HSV, VZV, and *Toxoplasma* by both PCR and GWC and for CMV by PCR; however, retrospectively PCR was positive for human parechovirus. The patient was treated with antibiotic eye ointment and the corneal lesion and anterior uveitis slowly became quiet.

*Case 5.* A 73-year-old female patient with an ophthalmologic history of cataract extraction in both eyes at the age of 70 was referred to our institution because of secondary glaucoma in the OD. The patient had no systemic complaints and used no medications. The OD revealed pupillary seclusion with an intraocular pressure (IOP) of 50 mm Hg. On examination corneal edema and keratic precipitates were noted in the OD (Table 3). The iris revealed atrophic areas. Fluorescein angiography revealed slight optic disc leakage and cystoid macular edema in the OD. The IOP initially normalized with laser iridotomy and local treatment; however, intermittent periods with IOP elevations up to 50 mm Hg were regularly encountered and trabeculectomy was required. The presumed diagnosis included low-grade *Propionibacterium* endophthalmitis and other various causes of hypertensive uveitis (Table 3). Screening examinations were within normal limits as well as serology for *Borrelia* and *Bartonella*. Aqueous sampling was performed and PCR was negative for HSV, VZV, CMV, and *Toxoplasma* and GWC was negative for HSV and VZV. Cultures were negative for *Propionibacterium*. Retrospectively, the patient was found positive for human parechovirus by PCR.

*Case 6.* A 37-year-old male patient was referred because of panuveitis with a focal chorioretinitis lesion in his OS since 3 months prior. At that time visual acuity in his OS

decreased to finger counting. On examination KPs and cells in the anterior chamber and vitreous were noted with an active lesion located in the periphery of the retina (Table 3). Intraocular pressure was normal. Fluorescein angiography demonstrated optic disc leakage and vasculitis with changes of retinal pigment epithelium in the mid-periphery of the retina. The OD had full visual acuity and no abnormalities. The patient had no systemic complaints and used no medications. The presumed diagnosis of toxoplasma chorioretinitis was made. Extensive screening for panuveitis revealed positive human immunodeficiency virus (HIV) serology, an HIV RNA plasma load of 69 700 copies/mL, 619 CD4 cells/mL, and positive syphilis serology (*Treponema pallidum* hemaglutination [TPHA] >1: 2560 and a Veneral Diseases Research Laboratory [VDRL] test result of 1:256). Aqueous analysis was negative for *Treponema pallidum* by PCR and for HSV, VZV, CMV, and *Toxoplasma* both by PCR and GWC. The aqueous was, however, retrospectively positive for human parechovirus by PCR. Although both the aqueous and cerebrospinal fluid analyses were negative for syphilis, the tentative diagnosis of ocular syphilis was made and the patient was treated with systemic penicillin. Antiretroviral treatment was considered not necessary at that time. Ocular inflammation subsided slowly, visual acuity increased to 20/20, and the eye remained quiet during 2 years of follow-up.

## DISCUSSION

IN 139 OCULAR FLUID SAMPLES FROM PATIENTS WITH UVEitis that were analyzed with a panel of 21 real-time PCRs, we found positive results in 7 cases (5%): 1 case with EBV, 1 case with rubella virus, 1 case with human herpesvirus 6, and 4 cases with human parechovirus.

EBV, the causative agent of infectious mononucleosis and several malignancies, has been implicated as a possible cause of uveitis and in primary ocular non-Hodgkin lymphoma of the central nervous system.[16,26,35] In our study EBV was detected in the ocular fluid of 1 patient with anterior uveitis of unknown cause and in 2 patients with toxoplasma chorioretinitis. IgG analysis in these 3 patients did not show any evidence of intraocular antibody production against EBV. The presence of EBV genome in the eyes of patients with various causes of uveitis was demonstrated previously and was found independent of the clinical diagnosis. The clinical significance of this phenomenon has not yet been established.[18,36,37] Moreover, as in our study, Ongkosuwito and associates found EBV also in ocular fluids from patients with laboratory-confirmed toxoplasmosis and in ocular fluids of patients without ocular inflammation.[18] Apparently, PCR detection of EBV in ocular fluids should be interpreted with caution, and may in most cases be considered an epiphenomenon, mostly likely attributable to the presence of EBV in B cells present in the inflamed eye. Further studies combining PCR and

intraocular antibody production analysis are required to determine whether EBV is a true cause of intraocular inflammation.

One patient was PCR-positive for rubella virus, the causative agent of German measles and congenital rubella syndrome.[38] Rubella virus has been associated with FHUS and FHUS-like uveitis.[1,5,39,40] This patient was clinically diagnosed with incomplete FHUS, as she did not have iris heterochromia or atrophy.

HHV6 is a beta-herpesvirus and the causative agent of roseola infantum (or exanthema subitum), a childhood disease.[41] In addition, HHV6 is being recognized as an important opportunistic infection following bone marrow and/or stem cell transplantation.[42] Our HHV6 PCR-positive patient (Case 2) was neither a child nor immunosuppressed. Antibody analysis of the ocular fluid by immunofluorescence assay did not reveal the presence of intraocular IgG against HHV6. However, absence of intraocular antibody production does not necessarily exclude intraocular infection. Previously, we reported that by simultaneous use of PCR and GWC in immunocompetent patients a diagnosis by PCR only was established in 9% of cases.[2] Moreover, de Boer and associates found that in patients with presumed herpetic anterior uveitis, PCR was more frequently positive than GWC.[43] HHV6 has been implicated in ocular inflammation, most notably when the posterior part of the eye was affected.[14,15,17,19] Our patient had anterior uveitis with heterochromia that had been present since childhood. Heterochromia is classically associated with FHUS but can develop in other viral infections such as HSV or VZV.[44] The detection of HHV6 in the eye might not be a clinically relevant finding; however, like other (herpes)viruses, HHV6 can reside latently in cells of the lymphoid and myeloid lineage, and it may have entered the inflamed eye via immune cells, similar to HIV and possibly EBV.[6,18,19,36,45] The role of HHV6 as a cause of anterior uveitis is inconclusive and further studies are required.

Human parechoviruses belong to the genus parechovirus within the family of *Picornaviridae*. They may cause gastroenteritis, encephalitis, and flaccid paralysis in young children, but rarely in adults.[29,46] Ocular diseases attributable to other picornavirus infections, particularly enteroviruses such as echoviruses 11 and 19 and coxsackieviruses, have been published, but an association between parechoviruses and ocular disease has not been reported yet.[8–12] In this study the ocular fluids of 4 patients with undiagnosed unilateral uveitis were PCR-positive for HPeV. Unfortunately, intraocular antibody production could not be established as appropriate serologic assays are not available and there was not enough ocular fluid left to perform viral culture.

One patient (Case 6) with intraocular HPeV was immunocompromised (Table 3). He had been diagnosed with active syphilis, but there were no indications for neurosyphilis or ocular syphilis. However, upon treatment with penicillin his ocular condition improved. As this patient was HIV-positive, multiple uveitis entities may have contributed to ocular disease.

The other 3 patients all had unilateral anterior uveitis with corneal involvement and cells in the anterior chamber, which suggested an ocular viral infection (Table 3). Further research is required to determine if HPeV plays a role in the pathogenesis of infectious (anterior) uveitis. It is surprising to find a virus that is associated with disease in children in the eyes of adults. However, other infections of childhood are known to cause intraocular disease in adults, as is the case for rubella virus.[1,5,39]

The vast majority (132/139 [95%]) of ocular fluid samples were negative by PCR analysis for multiple viruses and bacteria. There are several explanations for this result. First, the uveitis might be of noninfectious origin. Second, the number of DNA or RNA copies present in the ocular samples may have been below the detection level. This may be attributable to the low amount of input nucleic acid, which is inherent to diagnostic assays with intraocular fluid. Alternatively, the time of sampling may have been such that the causative agent had already been cleared from the eye. In this case, other diagnostic approaches may be more useful, such as the detection of intraocular antibody production by Goldmann-Witmer coefficient calculation. It is known, also for systemic and neurologic viral infections, that a PCR assay is most sensitive early in infection, whereas antibody can be detected over a much longer period of time and thus provide a wider window of detection.[2,43,47,48] Finally, it may be that other pathogens not covered by our assays were involved in these cases.

Our study addressed multiple infectious causes in patients with undiagnosed uveitis and revealed a possible new cause of infectious uveitis. Further investigations are required to narrow the diagnostic gap in patients with presumed infectious uveitis.

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