CLINICAL INVESTIGATION

The 2009 prospective multi-center epidemiologic survey of uveitis in Japan

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Abstract

Purpose To investigate etiologic data on intraocular inflammation in Japan collected in the 2009 epidemiologic survey of uveitis in Japan and assess the current state of etiology compared with that reported in a previous survey.
Methods Thirty-six university hospitals participated in this prospective etiologic study. Patients who visited the outpatient uveitis clinic of each hospital for the first time between 1 June 2009 and 31 May 2010 were enrolled in the study. Uveitic diseases were diagnosed according to the guidelines when available or following commonly accepted diagnostic criteria.
Results A total of 3,830 patients were enrolled in the survey and 2,556 cases of uveitis were identified, of which 1,274 cases were described as unclassified intraocular inflammation. In the identified cases, the most frequent intraocular inflammatory disease was sarcoidosis (10.6 %), followed by Vogt–Koyanagi–Harada disease (7.0 %), acute anterior uveitis (6.5 %), scleritis (6.1 %), herpetic iridocyclitis (4.2 %), Behçet's disease (3.9 %), bacterial endophthalmitis (2.5 %), masquerade syndrome (2.5 %), Posner–Schlossman syndrome (1.8 %), and retinal vasculitis (1.6 %).
Conclusions The current etiology of uveitis in Japan was elucidated by means of a multi-center prospective survey. Conducting such surveys on a periodic basis may help clinicians in their management of uveitis.

Keywords Epidemiology · Survey · Intraocular inflammation · Uveitis

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Introduction

Genetic, geographic, social, and environmental factors affect the distribution of the types and etiology of uveitis. A significant correlation had been reported between acute anterior uveitis (AAU) and human leukocytic antigen (HLA)-B27 [1], birdshot retinochoroidopathy and HLA-A29 [2], and Vogt–Koyanagi–Harada disease (VKH) and HLA-DR4 [3]. Human T cell lymphotropic virus type-1 (HTLV-1)-associated uveitis is localized to southern Japan [4], and Behçet's disease is seen frequently in Asia and the Mediterranean basin [5]. However, the incidence of Behçet's disease in Japan has decreased in recent decades [6, 7], suggesting that the onset of this disease might be correlated with social and environmental factors. Therefore, studies of the distribution of the various types of uveitis and their etiology are important for establishing an appropriate diagnosis and management.

In 2002, the Japanese Ocular Inflammation Society (JOIS) conducted a multi-center retrospective survey to delineate the status of intraocular inflammation in university hospitals nationwide [8]. The survey found that sarcoidosis was the most frequent intraocular inflammatory disease identified, followed by VKH and Behçet's disease. However, since social and environmental factors can change over time, it is important to conduct this kind of research periodically. Consequently, a working group of the JOIS conducted a multi-center prospective survey to accumulate etiologic data on uveitis in Japan and assess the changes over time.

Materials and methods

Thirty-six university hospitals participated in this prospective etiologic study. The Institutional Review Board of each center approved the study protocol.

Patients who presented for the first time at the outpatient uveitis clinic of each hospital between 1 June 2009, and 31 May 2010 were enrolled. Once the cause of the uveitis was diagnosed in each patient, we recorded the disease. When possible, the diagnosis of the uveitic disease was based on the guidelines; when this was not possible, common diagnostic criteria reported in the literature were used [9–13].

Several changes in the classification criteria had been made between the 2002 [8] and 2009 survey. First, scleritis was not included in the previous survey, but was included in the 2009 survey. Second, the previous survey differentiated ankylosing spondylitis and HLA-B27 from other types of AAU and recorded them as either ankylosing spondylitis-associated uveitis or uveitis associated with HLA-B27. The 2002 survey recorded AAU from unknown etiologies as unclassified intraocular inflammation. In the 2009 survey, since our aim was to determine the precise relationship between HLA-B27 and AAU, we classified AAU as HLA-B27-positive, HLA-B27-negative, and unknown HLA. Third, we classified viral infectious diseases into groups based on the findings of viruses detected by PCR assays, the absence of viruses based on PCR assays, and clinical diagnosis only (without PCR assay). Fourth, we added a masquerade syndrome to the 2009 survey.

All data were collected at the end of December 2010. Patients with undiagnosed uveitis at that time were classified as having unclassified intraocular inflammation.

Results

A total of 3,830 patients were enrolled in the study, among whom 2,556 cases of uveitis were identified with a specific etiology and 1,274 cases were recorded with unclassified intraocular inflammation. Among the identified cases, 75.4 % were non-infectious diseases and 24.6 % were infectious diseases.

Table 1 shows the distribution of specific intraocular inflammatory diseases in this survey. The most frequent intraocular inflammatory disease in our Japanese patient population was sarcoidosis, followed by VKH. The third most frequent disease in the 2009 survey was AAU, of

Table 1 Diagnostic distribution in 2009 for new patients with intraocular inflammatory diseases

Disease	No. of patients (%)
Sarcoidosis	407 (10.6)
Vogt–Koyanagi–Harada disease	267 (7.0)
Acute anterior uveitis	250 (6.5)
Scleritis	235 (6.1)
Herpetic iritis	159 (4.2)
Behçet's disease	149 (3.9)
Bacterial endophthalmitis	95 (2.5)
Masquerade syndrome	95 (2.5)
Posner–Schlossman syndrome	69 (1.8)
Retinal vasculitis	61 (1.6)
Diabetic iritis	54 (1.4)
Ocular tuberculosis	54 (1.4)
Acute retinal necrosis	53 (1.4)
Ocular toxoplasmosis	48 (1.3)
Multiple evanescent white dot syndrome	40 (1.0)
Fungal endophthalmitis	39 (1.0)
Cytomegalovirus retinitis	37 (1.0)
Rheumatoid arthritis-associated uveitis	29 (0.8)
Human T cell lymphotropic virus type-1-associated uveitis	29 (0.8)
Inflammatory bowel disease-associated uveitis	28 (0.7)
Multifocal posterior pigment epitheliopathy	28 (0.7)
Uveitis associated with other systemic diseases	27 (0.7)
Peripheral uveitis	26 (0.7)
Multifocal choroiditis	23 (0.6)
Fuchs' heterochromic iridocyclitis	21 (0.5)
Acute posterior multifocal placoid pigment epitheliopathy	16 (0.4)
Tubulointerstitial nephritis and uveitis syndrome-associated uveitis	15 (0.4)
Syphilis-associated uveitis	15 (0.4)
Lens-induced uveitis	13 (0.3)
Punctate inner choroidopathy	13 (0.3)
Juvenile idiopathic arthritis-associated uveitis	11 (0.3)
Geographic chorioretinopathy	11 (0.3)
Sympathetic ophthalmia	10 (0.3)
Ocular toxocariasis	9 (0.2)
Others	112 (2.9)
Unclassified intraocular inflammation	1,282 (33.5)

Table 2 Cause of scleritis

Cause	No. of patients (% of total scleritis cases)
Rheumatic disease	59 (25.1)
Rheumatoid arthritis	22 (9.4)
Wegener's granulomatosis	10 (4.2)
Others	27 (11.5)
Viral infection	11 (4.7)
Tuberculosis	4 (1.7)
Others	23 (9.8)
Unclassified	138 (58.7)

Table 3 Methods for diagnosing herpetic iritis

Method	No. of patients (%)
Clinical diagnosis only	85 (53.5)
Herpes simplex virus detection	31 (19.5)
Varicella zoster virus detection	23 (14.5)
Cytomegalovirus detection	20 (12.6)

Table 4 Causes of viral retinitis

Cause	No. of patients (%)
Acute retinal necrosis	53 (58.9)
Herpes simplex virus	12
Varicella zoster virus	39
Cytomegalovirus	1
Other	1
Cytomegalovirus retinitis	37 (41.1)

Table 5 Background of masquerade syndrome

Background	No. of patients (%)
Primary intraocular malignant lymphoma	48 (50.5)
Primary central nervous system malignant lymphoma	12 (12.6)
Systemic malignant lymphoma	14 (14.7)
Primary intraocular tumor	15 (15.8)
Metastatic tumor	6 (6.3)

which 71 patients were HLA-B27 positive, 74 patients were HLA-B27-negative, and the remaining 105 patients had an unknown HLA type.

Table 2 summarizes the causes of scleritis, the fourth most frequent disease in the 2009 survey. Many cases were associated with rheumatoid arthritis; however, some cases were identified as infectious. In herpetic iridocyclitis, the PCR assays detected similar numbers of cases of herpes simplex virus, varicella-zoster virus (VZV), and cytomegalovirus (Table 3). VZV was the most frequent disease entity detected by PCR assays in acute retinal necrosis (Table 4), while about 50 % of the disease entity in masquerade syndrome was primary intraocular lymphoma (Table 5).

When the disease frequencies were analyzed by geographic area, the frequency of uveitis associated with HTLV-1 in the Kyushu area (3.1 %; average of five university hospitals) was significantly greater than the national average (0.8 %; $P < 0.05$, chi-square test). The other diseases did not differ significantly among the geographic areas.

Discussion

This was the first multi-center prospective nationwide etiological survey of uveitis in Japan. Using the data from this survey, we have elucidated the current etiology of uveitis in Japanese university hospitals and found that sarcoidosis, VKH, AAU, and scleritis occur at a high frequency, followed by a relatively large number of cases of Behçet's disease and masquerade syndrome.

The 2009 survey differs somewhat from the retrospective survey of intraocular inflammation conducted at 41 university hospitals in 2002 [8]. Different institutions participated in the two surveys, although there was an overlap of many institutions. In addition, the disease classifications differed between the two surveys. For example, in the 2009 survey, we included scleritis, which was not included in the 2002 survey. Scleritis comprised 6.1 % of all cases in the 2009 survey; one-fourth of these cases were associated with rheumatoid arthritis, while half had an unknown etiology. The masquerade syndrome had also been excluded from the 2002 survey, and only intraocular lymphoma was included. In the 2009 study, the masquerade syndrome comprised 2.5 % of all cases; about one-half of these (1.3 %) were cases of intraocular lymphoma. Intraocular lymphoma comprised 1.0 % of the diseases surveyed during 2002, indicating that the incidence of this disease remained virtually unchanged. Lymphoma is usually associated with a poor prognosis, and care should be taken not to misdiagnose patients with this disease entity. In the 2002 survey, of all the AAU cases, only HLA-B27-associated uveitis was included and the other types were classified in the unclassified category. HLA-B27-associated uveitis accounted for 1.5 % of the cases in the 2002 survey and 1.9 % in the 2009 survey; therefore, the incidence of HLA-B27-associated uveitis would appear to be largely unchanged.

The number of patients with Behçet's disease in Japan has been reported to decrease [6, 7], and the incidence in the 2009 study was 3.9 %. Compared to the previous

survey [8], the incidence of patients with newly diagnosed Behçet's disease in Japan has decreased from 6.2 to 3.9 %, although the two surveys cannot really be compared because of the different participating institutions. The decrease in the number of patients with Behçet's disease in Japan over nearly a decade suggests that the disease might be correlated with exogenous factors, such as climate, public health, and dietary habits, rather than endogenous factors, such as age, sex, ethnicity, and immunogenetic background.

Geographically, HTLV-1-associated uveitis was still more frequently diagnosed in the Kyushu area, a result similar to that of the 2002 survey. However, there was no marked variation in the geographical distribution of the other types of uveitis. This indicates that ophthalmologists can consider the results of this survey to be valid on a nationwide basis.

The limitation of the 2009 survey is that only university hospitals participated. It should be kept in mind that Sakai and associates report that diabetic iritis and herpetic iritis are seen significantly more frequently in general eye clinics, whereas VKH and Behçet's disease are seen significantly more often in university hospitals [14]. In addition, the 2009 survey did not consider age and sex. These factors should be considered in any future survey.

Investigations of epidemiologic changes over time require comparisons of periodically acquired etiologic data from the same diagnostic categories and from the same institutions. In addition, to standardize the diagnosis in all participating institutions in the survey, easily understandable diagnostic guidelines for intraocular inflammation are needed. Moreover, a national epidemiologic survey should include not only university hospitals but also clinics. In the next survey, these factors need to be considered in order to establish a well-designed format for a periodic epidemiologic national survey.

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Virological Analysis in Patients with Human Herpes Virus 6–Associated Ocular Inflammatory Disorders

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PURPOSE. To determine whether human herpes virus 6 (HHV-6) genomic DNA and mRNA can be detected in ocular samples from patients with inflammatory disorders, and whether viral replication is involved in the development of inflammation in the eye.

METHODS. After informed consent was obtained, ocular fluid samples (aqueous humor and vitreous fluids) were collected from 350 patients with uveitis or endophthalmitis. Corneal samples were also collected from 65 patients with corneal infections. Multiplex PCR was performed to screen ocular samples from the patients for HHV-1 to HHV-8. Samples were also assayed for HHV-6 DNA using quantitative real-time PCR. Primers for nested RT-PCR were designed to detect amplification of mRNA (HHV-6 A IE1 U90).

RESULTS. PCR results indicated a total of seven patients with uveitis or endophthalmitis (7/350, 2% +) and a single patient with corneal inflammatory disease were positive for HHV-6 DNA (1/65, 1.5% +). These eight patients had high copy numbers of HHV-6 DNA, with values ranging from 4.0×10^3 to 5.1×10^6 copies/mL. Real-time PCR analysis indicated that two of these cases were HHV-6 variant A and six cases were variant B. In addition, HHV-6 mRNA was clearly detected in vitreous cells collected from one of the patients, suggesting that viral replication may occur in the eye.

CONCLUSIONS. Our results indicate that HHV-6 infection/ reactivation is implicated in ocular inflammatory diseases. (www.umin.ac.jp/ctr/index/htm number, R000002708.) (*Invest Ophthalmol Vis Sci.* 2012;53:4692–4698) DOI:10.1167/ iovs.12-10095

H uman herpesvirus 6 (HHV-6) is the causative agent of exanthema subitum in children and has been associated with a number of inflammatory and neurological disorders worldwide. It has been implicated in hepatitis, pneumonitis, and severe infections of the central nervous system in both immunosuppressed and immunocompetent patients. HHV-6 can reactivate from its latent form after primary infection. In the case of eye diseases, it has been implicated in AIDS-associated retinitis,[1-3] uveitis,[4-8] corneal inflammation,[9] and optic neuropathy.[10-12] Two variants of HHV-6 have been identified. HHV-6A is less often associated with disease and has a greater predilection for neural cells than HHV-6B.[13] Although HHV-6A DNA is frequently found in the nervous system of infected adults, HHV-6B DNA is rarely present in ocular fluids, although it is found in most documented primary HHV-6 infections.

Diagnosis of clinically relevant HHV-6 can be challenging due to the high prevalence of infection and viral persistence. Detection of viral nucleic acids may indicate active or latent infections, depending on the clinical setting and specimens tested. Quantitative PCR methods have been established to detect active infections. Detection of HHV-6 DNA in plasma or serum is indicative of active replication and is therefore more directly interpretable.[14,15] Using these PCR techniques, several investigators previously reported that HHV-6 genomic DNA is found in ocular inflammatory diseases, including infectious uveitis and endophthalmitis[1-8]; however, involvement of HHV-6 in ocular infections has not yet been clearly demonstrated.

Therefore, we designed experiments to investigate whether ocular samples from patients with various ocular inflammatory disorders contain HHV-6 genomic DNA, whether ocular samples from noninflammatory patients also contain HHV-6 DNA, whether positive cases are either HHV-6 variant A or B, and whether HHV-6 mRNA as well as a high copy numbers of HHV-6 DNA can be detected in positive samples.

MATERIALS AND METHODS

Subjects

The first patient group was examined between 2006 and 2010 at the Tokyo Medical and Dental University Hospital, Kyoto Prefectural University Hospital, and Shinkawabashi Hospital in Japan. After informed consent was obtained, ocular fluid samples were collected from patients with uveitis (infectious and noninfectious) or endophthalmitis. This group included consecutive patients with uveitis or endophthalmitis ($n = 350$), including a previously HHV-6–positive severe panuveitis case.[7] Corneal tissues were also collected from patients with ocular surface diseases (e.g., keratitis, $n = 65$). At this time, we excluded ocular tumor diseases (e.g., intraocular lymphoma) from the patient group.

In addition to the patient group, we also analyzed samples from a control group. A total of 100 samples (50 aqueous humor and 50 vitreous fluids) were collected from patients who did not have any type of ocular inflammation (age-related cataract, macular edema, retinal
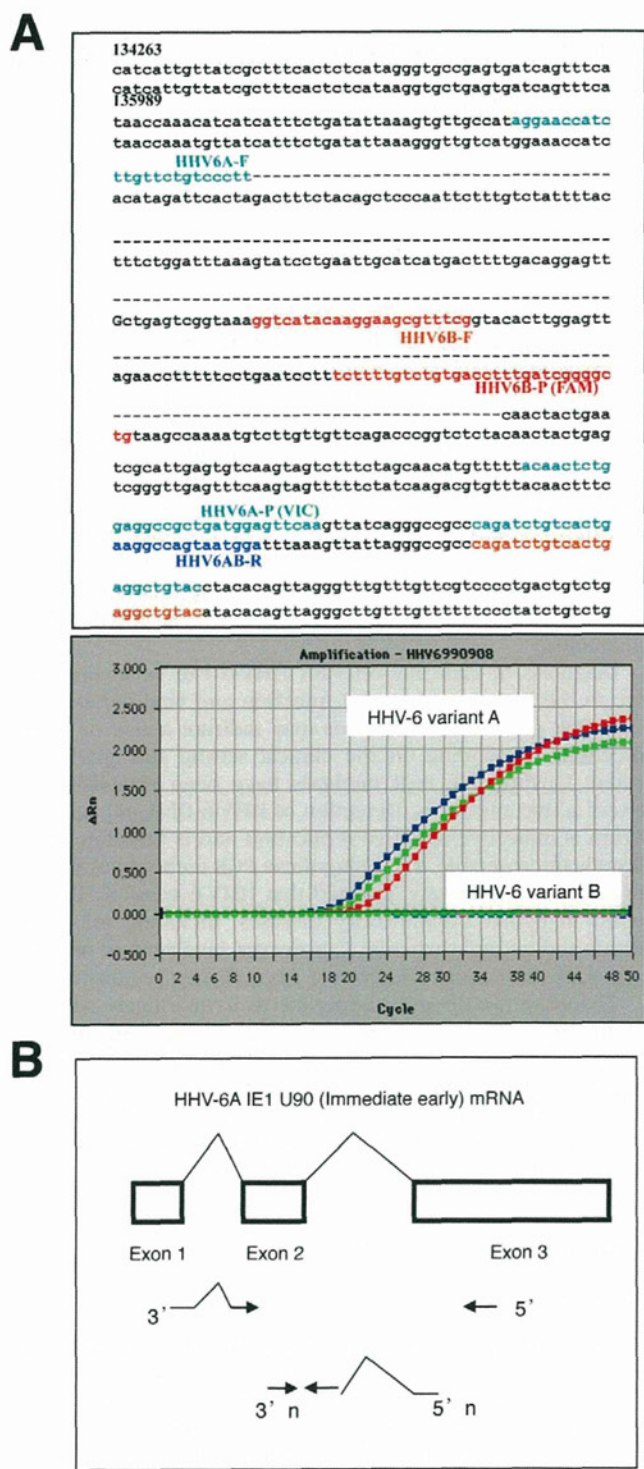
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FIGURE 1. Amplification of HHV-6–specific DNA and mRNA. (**A**) TaqMan probes and primers used to amplify HHV-6 DNA (HHV-6A and HHV-6B). HHV-6 subtypes were identified using PCR with variant-specific primers and probes (*lower graph*). (**B**) Nested RT-PCR primers were designed to amplify HHV-6A mRNA.

detachment, idiopathic macular hole, or idiopathic epiretinal membrane).

The research followed the tenets of the Declaration of Helsinki and all study protocols were approved by the Institutional Ethics Committee of Tokyo Medical and Dental University. A clinical trial registration was conducted and information is available at www.umin.

TABLE 1. Clinical Findings in Patients with HHV-6–Associated Ocular Inflammatory Disorders

Case	Age / Sex	Eye	Initial Diagnosis	VA	IOP	Cornea	AC	KPs	VO	Fundus	Bacterial Examination*	Final Diagnosis
1	75 / Male	R	Pan-uveitis	0.02	15	None	Hypopyon	Mutton fat	Grade III	Retinal exudates	Culture (–) / PCR (–)	Ocular toxocariasis
2	64 / Female	L	Corneal endotheliitis	0.5	33	Edema	Cell 2+	Mutton fat	None	None	PCR (–)	HSV-1 corneal endotheliitis
3	70 / Male	L	Bacterial endophthalmitis	sl-	35	None	Hypopyon	Fine	Grade III	Retinal necrosis	Culture (+) / PCR (+)	Endogenous endophthalmitis
4	74 / Female	R	Idiopathic uveitis	0.8	16	None	Cell1+	None	Grade II	None	PCR (+)	Late postoperative endophthalmitis
5	79 / Female	L	Bacterial endophthalmitis	mm	19	None	Hypopyon	Fine	Grade II	Retinal exudates, hemorrhage	Culture (+) / PCR (+)	Acute postoperative endophthalmitis
6	71 / Female	L	Necrotic retinitis	0.04	12	None	None	None	None	Retinal necrosis, hemorrhage	PCR (–)	Cytomegalovirus retinitis
7	24 / Female	L	Posner-Schlossman synd.	1.2	24	None	Cell 1+	Mutton fat	None	None	PCR (–)	Idiopathic uveitis
8	22 / Male	R	Karatitis	0.7	15	Infiltration	Cell1–	None	None	None	Culture (–) / PCR (+)	Bacterial karatitis

* Bacterial examination: Results for bacterial culture and/or PCR (bacterial 16S rDNA).
AC, anterior chamber; KPs, keratic precipitates; VA, visual acuity by Landolt Chart; VO, vitreous opacity.

TABLE 2. Virological Analysis and Treatment in Patients with HHV-6–Associated Ocular Inflammatory Disorders

Case	Ocular Sample	HHV Genome	Viral Copy No. by Real-Time PCR	HHV-6A or B	Treatment
1	Aqh	HHV-6	HHV-6: 2.4×10^6 copies/mL	HHV-6A	PSL, PPV, VCV, VGV
	VF	HHV-6, EBV	HHV-6: 2.0×10^4 copies/mL, EBV: <50 copies/mL		
2	Aqh	HHV-6, HSV-1	HHV-6: 7.5×10^3 copies/mL, HSV-1: 2.8×10^5 copies/mL	HHV-6B	VGV
3	VF	HHV-6	HHV-6: 5.1×10^6 copies/mL	HHV-6B	PPV, SA, IAI
4	VF	HHV-6	HHV-6: 1.1×10^4 copies/mL	HHV-6B	PPV, VGV
5	VF	HHV-6	HHV-6: 1.1×10^6 copies/mL	HHV-6B	PPV, SA, Betametasone
6	VF	HHV-6, CMV	HHV-6: 4.4×10^4 copies/mL, CMV: 1.6×10^6 copies/mL	HHV-6A	VGV
7	Aqh	HHV-6	HHV-6: 4.0×10^3 copies/mL	HHV-6B	None
8	Cornea	HHV-6	HHV-6: 3.9×10^6 copies/µg · DNA	HHV-6B	Antibiotics

Aqh, aqueous humor; IAI, intravitreal antibiotic injection; PPV, pars plana vitrectomy; PSL, prednisolone; SA, systemic antibitotics; VCV, valacyclovir; VF, vitreous fluids; VGV, valganciclovir.

PCR

DNA was extracted from samples using an E21 virus minikit (Qiagen, Valencia, CA) installed on a robotic workstation for automated purification of nucleic acids (BioRobot E21, Qiagen). HHV genomic DNA in ocular samples was detected using two independent PCR assays: a qualitative multiplex PCR and a quantitative real-time PCR.[16]

The multiplex PCR was designed to qualitatively measure genomic DNA of eight human herpes viruses as follows: herpes simplex virus type 1 (HSV-1), type 2 (HSV-2), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpes virus 6 (HHV-6), 7 (HHV-7), and 8 (HHV-8). PCR was performed using a LightCycler (Roche, Rotkrevz, Switzerland). Primers for HHV-6 were as follows: Forward – ACCCGAGAGATGATTTTGCG and Reverse – GCAGAAGA-CAGCAGCGAGAT. Probes were used as follows: 3′FITC-TAAG-TAACCGTTTTCGTCCCA and LcRed705-5′-GGGTCATTTATGTTATAGA. These primers and probes do not distinguish between HHV-6A and B. PCR conditions, primers, and probes specific for other HHV have been described previously.[17]

Real-time PCR was performed for detection of HHV only, following identification of genomic DNA by multiplex PCR. Real-time PCR was performed using Amplitaq Gold and the Real-Time PCR 7300 system (ABI, Foster City, CA). The sequence of the HHV-6 primers and probes are as follows: Forward – GACAATCACATGCCTGGATAATG and Reverse – TGTAAGCGTGTGGTAATGTACTAA. The probe was AG-CAGCTGGCGAAAAGTGCTGTGC. The primers and probes of other herpes viruses and the PCR conditions have been described previously.[16,17] These primers and probes do not distinguish between HHV-6A and B. TaqMan probes and primers used in the HHV-6 DNA amplifications, HHV-6 type A and HHV-6 type B, are shown in Figure 1A. The value of viral copy number in the sample was considered to be significant when more than 50 copies/mL were observed.

RT-PCR

The primers for nested RT-PCR were designed to detect mRNA (HHV-6 A IE1 U90 immediate early) as follows: first PCR Forward – GATGAACGTATGCAAGACTACC and ATGAACATGGATTGTTGCTG and Reverse – CAGCGGACTGAGCAGCTA; nested PCR Forward – CCGATCCAATGATGGAAGAA and Reverse – CAGCGGACTGAGCAGC-TA (Fig. 1B). A one-step RT-PCR was performed on 100 ng of total RNA with 0.5 µM of each primer and SuperScript III One-Step RT-PCR with platinum Taq (Life Technologies Co., Tokyo, Japan) in a final volume of 50 µL. Samples were reverse transcribed for 30 minutes at 54°C and amplified for 40 cycles consisting of denaturation for 15 seconds at 94°C, annealing for 30 seconds at 54°C, and polymerization for 20 seconds at 72°C. Following identification of a PCR product of 340 bp, nested PCR was performed on 1 µL of the first PCR solution using 0.5

µM of each primer and 200 mM deoxynucleotide triphosphates and 1.25 U of Taq DNA polymerase (Thermo Fisher Scientific, Tokyo, Japan). Monoclonal antibody (anti-taq high: Toyobo Life Science, Tokyo, Japan) was used at 0.25 µg in a buffer containing 75 mM Tris-HCl (pH = 8.8), 0.01% Tween-20, 20 mM $(NH_4)_2SO_4$, and 1.5 mM $MgCl_2$ in a final volume of 50 µL. Twenty cycles of amplification consisting of denaturation for 15 seconds at 94°C, annealing for 30 seconds at 55°C, and polymerization for 15 seconds at 72°C were performed to give a positive PCR product of 198 bp.

All ocular samples were tested for the presence of β-actin as an internal control. β-Actin mRNA RT-PCR was performed on 100 ng of total RNA with 0.5 µM each primer and SuperScript III One-Step RT-PCR with platinum Taq in a final volume of 50 µL (Forward-CTTCCTTCCTGGGCAT and Reverse-TCTTCATTGTGCTGGGT). Samples were reverse transcribed for 30 minutes at 55°C followed by 40 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 60°C, and polymerization for 1 minute at 72°C on a thermal cycler TP-400 instrument (Takara Bio Inc., Tokyo, Japan). Raji cell lines were used as a positive control, and MOLT-4 cells were used as a negative control. PCR products were analyzed using 2% agarose gel electrophoresis and ethidium bromide staining and the positive product was 215 bp.

RESULTS

Detection of HHV-6 Genomic DNA in Patients with Uveitis, Endophthalmitis, and Ocular Surface Diseases

We first performed multiplex PCR to screen for 8 HHVs after collecting intraocular samples from patients with various ocular inflammatory diseases. PCR results indicated that 7 (2%) of 350 patients with uveitis or endophthalmitis were positive for HHV-6 DNA. In addition, 1 (1.5%) of 65 patients tested positive for HHV-6 in a corneal tissue sample. These HHV-6–positive cases together with clinical findings are summarized in Tables 1 and 2. These eight HHV-6–positive patients were clinically suspected to have HHV-6–associated infectious diseases based on the detection of HHV-6 genome in ocular fluid or corneal tissue samples. HHV-6 DNA was not detected in any of the 100 control samples that were collected from patients without ocular inflammation.

The clinical features observed in HHV-6–positive cases at their initial presentation are summarized in Table 1. Almost all of the patients with uveitis and endophthalmitis had active ocular inflammation, that is, there were anterior chamber cells (except case 6), keratic precipitates (except cases 4 and 6), vitreous opacity (except cases 2 and 7), and fresh retinal exudates/necrosis (except cases 2, 4, and 7). In the single patient with HHV-6$^+$ keratitis (case 8 in Table 1), corneal
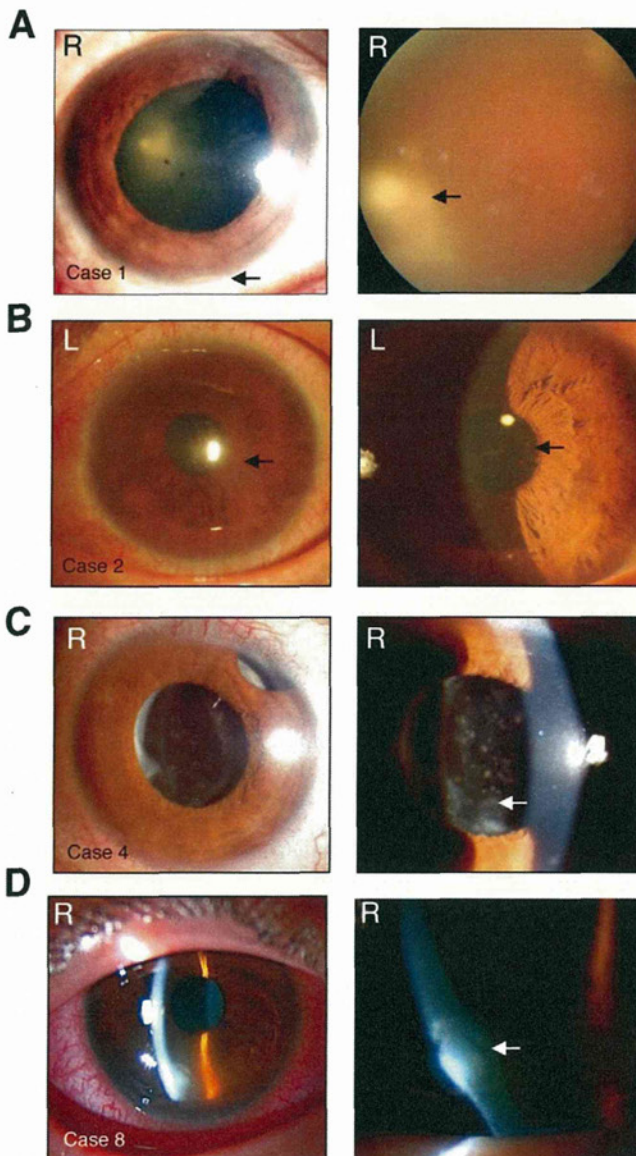
FIGURE 2. Slit-lamp and fundus photographs for HHV-6 infections. (A) Case 1: A case of ocular toxocariasis. Slit-lamp examination of right eye (RE) disclosed ciliary injection, moderate mutton-fat keratic precipitates (KPs), and severe anterior chamber cells with hypopyon (*arrow*). Funduscopic examination of the RE revealed dense vitreous opacities and yellowish white massive retinal lesions (*arrow*) in the peripheral fundus. HHV-6 DNA was detected in both aqueous humor and vitreous samples. (B) Case 2: A case of HSV-1–associated corneal endotheliitis. Slit-lamp examination of left eye (LE) disclosed pigmented mutton-fat-like KPs with high intraocular pressure, mild anterior chamber cells, and small-size corneal stromal edema (*arrow*). HSV-1 and HHV-6 DNA were detected in aqueous humor, but other HHV-DNA, such as VZV and CMV, was not detected. (C) Case 4: A case of late postoperative endophthalmitis. This patient with Vogt-Koyanagi-Harada disease had postcataract surgery 6 months earlier. Slit-lamp examination of RE disclosed ciliary injection and mild anterior chamber cells. White plaque (*arrow*) on the intraocular lens and mild inflammation were seen, and an aqueous humor sample was obtained. HHV-6 DNA and *Propionibacterium acnes* DNA were detected in the aqueous humor sample. The final diagnosis was *P. acnes*–associated late postoperative endophthalmitis. (D) Case 8: A case of bacterial keratitis. Slit-lamp examination of RE disclosed keratitis (*arrow*) with ciliary injection. A corneal infiltration with epithelial defect was observed and a high copy number of HHV-6 DNA was detected in corneal tissue samples.

infection, such as corneal epithelial ulcer and ciliary injection, was indicated. Representative findings including slit-lamp or fundus photographs for HHV-6–positive cases are shown in Figure 2. In addition, ocular samples from all patients were subjected to bacterial examinations, including conventional bacterial culture and bacterial broad-range PCR (bacterial 16S rDNA)[18] (Table 1). The final diagnoses were as follows: case 1, ocular toxocariasis; case 2, HSV-1 corneal endotheliitis; case 3, endogenous endophthalmitis; case 4, late postoperative endophthalmitis; case 5, acute postoperative endophthalmitis; case 6, CMV retinitis; case 7, idiopathic uveitis; case 8, bacterial keratitis (Table 1).

We next summarized the virological analysis of ocular samples from these eight HHV-6–positive patients (3 aqueous humor, 5 vitreous fluids, and 1 corneal tissue) in Table 2. Multiplex PCR was used to detect HHV infection (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, and HHV-8). HHV-6 was found together with EBV (only case 1), HSV-1 (only case 2), or CMV (only case 6). Figure 3 is representative of the results of the multiplex PCR where HHV-6 DNA was detected in aqueous and vitreous fluid from case 1. HHV DNA in nine ocular samples from eight cases was also measured by real-time PCR. These patients had high copy numbers of HHV-6 DNA, with values ranging from 4.0×10^3 to 5.1×10^6 copies/mL (Table 2), suggesting that viral replication may occur in the eye. Following diagnosis, 4 patients received antiviral treatment (i.e., valacyclovir or valganciclovir), which controlled their ocular inflammation (Table 2).

Detection of HHV-6 Variant A or B in Patients with HHV-6–Associated Ocular Inflammatory Disorders

HHV-6 can be classified into two groups: a variant A (HHV-6A) and a variant B (HHV-6B).[13] Distinguishing between HHV-6 subtypes is mainly accomplished using PCR techniques, including melting curve[19] or variant-specific primers.[20] Therefore, we next determined whether the HHV-6–positive cases were HHV-6A or B using real-time PCR. In this study, we designed a probe and primers for use in the HHV-6 DNA amplification. The paired primers and TaqMan probes used for detection of HHV-6A and HHV-6B are shown in Figure 1A. By using several different primers and probes, we were able to detect each of these HHV-6 types separately (Fig. 1A). The PCR results from case 1 showed that intraocular samples included HHV-6A but not HHV-6B DNA (Fig. 4). Final analysis with quantitative PCR indicated that two of the cases were positive for HHV-6A and six cases were positive for HHV-6B (Table 2).

Detection of HHV-6 mRNA in Intraocular Samples

RT-PCR has previously been used on mRNA from peripheral blood mononuclear cells to detect actively replicating virus.[21] We therefore tested ocular samples for the presence of HHV-6 mRNA. Various samples, such as aqueous humor, vitreous fluid, retinal membrane tissues, and collected vitreous cells from an HHV-6A–positive case (case 1), were available for the RT-PCR assay. We designed primers to amplify mRNA using a nested RT-PCR (HHV-6 A IE1 U90, Fig. 1B). As revealed in Figure 5, HHV-6A mRNA was clearly detected in vitreous cell samples, but other ocular samples from the same patient were all negative.

DISCUSSION

In this study, we demonstrate that seven patients with uveitis or endophthalmitis were positive for HHV-6 DNA. In addition, one patient with infectious keratitis was also found to be HHV-6–positive. These patients had high copy numbers of HHV-6
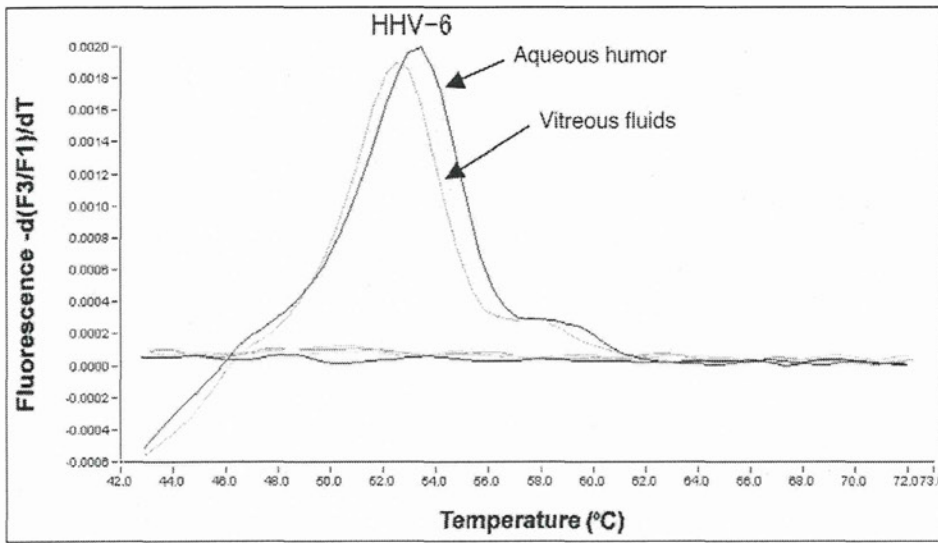
FIGURE 3. Results for multiplex PCR in a patient with HHV-6–positive uveitis. A significant positive curve was seen at 52°C, indicating detection of HHV-6 genomic DNA in the ocular fluids (case 1). DNA from other herpes viruses, such as HSV1, HSV2, VZV, EBV, CMV, HHV7, and HHV8, was not detected in this sample.

DNA, and two cases were found to be HHV-6 type A and six cases were type B. In addition, HHV-6 mRNA was detected in intraocular samples from HHV-6–positive patients, suggesting that viral replication or reactivation may occur in the eye.

Recently, Cohen et al.[5] reported that HHV-6A DNA could be detected by PCR in vitreous fluid from a patient with CMV-associated retinitis when vitreous fluids were assayed from 101 patients with ocular inflammation for HHV-6A, HHV-6B, and HHV-7. HHV-6B DNA was also detected in vitreous fluid from a patient with idiopathic uveitis in the absence of CMV DNA. This study suggests that HHV-6A and HHV-6B DNA are detectable in approximately 1% of vitreous samples from patients with ocular inflammation. In our study, we show that HHV-6 DNA was detectable in 2% of ocular samples from patients with intraocular inflammation following screening for HHV-1 to −8 infection using multiplex PCR.

In a previous study,[16] we found that intraocular HHV DNA was detectable in a wide range of herpes virus–associated uveitis cases when analysis was performed using multiplex PCR. PCR is a valuable tool for the diagnosis of herpetic uveitis and it is now possible to exclude nonherpetic uveitis patients using this method. Moreover, de Boer et al.[8] previously found that in patients with herpetic anterior uveitis, PCR was more frequently positive than the Goldmann-Witmer coefficient. HHV-6 has been implicated in ocular inflammation, most remarkably when the posterior segment of the eye was affected.[6,7,10-12] On the other hand, the role of HHV-6 as a cause of anterior uveitis is inconclusive and further studies are required. As revealed in this study, we found three cases of anterior inflammatory diseases including keratitis and five cases of pan- or posterior inflammatory diseases in the eye.
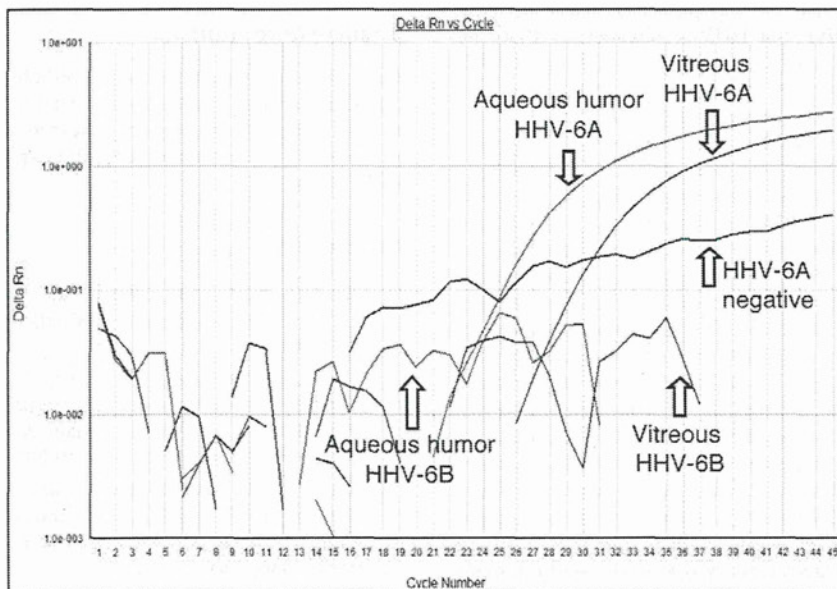

FIGURE 4. Detection of HHV-6 DNA by quantitative real-time PCR. The real-time PCR results for the samples from case 1 showed that intraocular samples, such as aqueous humor and vitreous fluids, contained a high copy number of HHV-6A DNA, but not HHV-6B DNA.
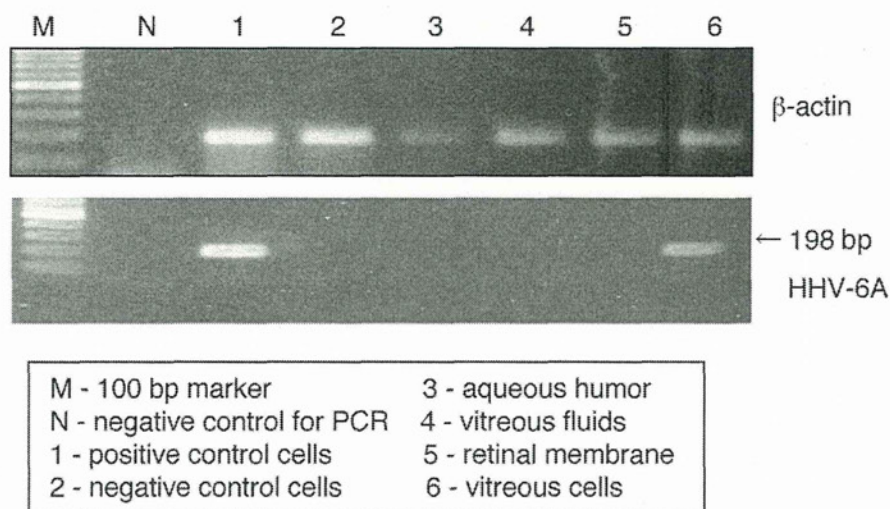
FIGURE 5.   Detection of HHV-6 mRNA in intraocular samples. HHV-6A mRNA was detected in samples from vitreous cells, but other ocular samples, such as aqueous humor, vitreous fluids, and retinal membrane tissues were all negative (*lower image*). All samples, including control RNA, were positive for β-actin (*upper image*).

The detection of HHV-6 in the eye might not be clinically relevant. HHV-6 can latently reside in cells of the lymphoid and myeloid lineage and it may have entered the inflamed eye via immune cells, similar to EBV and human immunodeficiency virus.[3,22,23] Thus, HHV-6 DNA has been detected in circulating T cells, monocytes, and leukocytes and may simply have been carried into the eyes in the inflammatory cells as a result of destruction of the blood-retina barrier. Our data indicate that most HHV-6 DNA in intraocular fluids of inflamed eyes might be a consequence of the release of HHV-6 DNA from resident ocular cells caused by intraocular inflammation. A high copy number of HHV-6 DNA was detected in patients with severe ocular inflammation, pan- or posterior uveitis, or endophthalmitis (Tables 1 and 2). This is supported by the findings of Arao et al.,[24] who showed that HHV-6 can infect human retinal pigment epithelial cells.

We detected HHV-6 in only one patient with an ocular surface inflammatory disorder. The patient was a young healthy donor suffering from atopic dermatitis. Okuno et al.[9] recently reported that 14 of 22 patients with corneal inflammation were positive for HHV-6, suggesting that the association of HHV-6 with disease was more frequent than with other herpes viruses, such as HSV-1. Thus, HHV-6 may be another sole causative agent of corneal inflammation.

HHV-6 reactivation frequently accompanies CMV reactivation,[25] and the presence of HHV-6A DNA in the eye may simply reflect the immunocompromised state of the patient. Case 6 in this study was a patient with CMV retinitis who was also found to be HHV-6A DNA-positive; however, with the exception of this patient, our HHV-6 PCR-positive patients were neither young nor immunosuppressed. We previously used multiplex PCR to search for HHV-6 in ocular fluids from 100 patients with uveitis and detected HHV-6A DNA in one patient with severe unilateral uveitis (case 1).[7] This patient's ocular fluid also contained antibodies to *Toxocara canis* larvae and we finally diagnosed ocular toxocariasis and HHV6-related pan-uveitis.[7] In this study, 7 patients were found to have other infectious agents, including bacteria, other herpes viruses (HSV-1), and parasites (*Toxocara*); however, it is unclear whether HHV-6 was the predominant pathogen. It is assumed that HHV-6 infections play a secondary role in the pathogenesis of ocular inflammation. Therefore, we tested intraocular samples for the presence of HHV-6 mRNA. Additional tests for HHV-6 RNA or protein in ocular tissues would have been more definitive and provided evidence of HHV-6 replication. We found HHV-6A mRNA and a high copy number of HHV-6 DNA in the same sample from a patient with ocular toxocariasis (case 1). As far as we know, this is the first report of detection of both HHV-6 DNA and mRNA in an ocular sample. The RT-PCR assay can reliably differentiate between latent and actively replicating HHV-6 and its use should allow an insight into the pathogenesis of this ubiquitous virus as previously reported.[21]

In conclusion, ocular samples collected from patients with infectious ocular disorders can contain a high copy number of HHV-6 DNA. The HHV-6–positive case was found to have HHV-6 DNA and mRNA in the inflamed eye. We are currently conducting experiments to determine whether HHV-6 type A and type B can infect ocular cells, such as retinal pigment epithelium, in vitro. Infected ocular cells can produce inflammatory cytokines and chemokines that differ from those in normal uninfected cells.

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Multicenter Study of Infliximab for Refractory Uveoretinitis in Behçet Disease

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Objective: To describe the effects of infliximab on refractory uveoretinitis in patients with Behçet disease during the first year of treatment.

Methods: Data were collected prospectively at 8 tertiary uveitis centers. Safety was analyzed in 63 patients. Efficacy was analyzed in 50 patients, after exclusion of those who had received infliximab for various reasons before the study.

Results: Eighty-nine percent (56 of 63) of the patients were male, with 70% (44 of 63) of the patients aged 25 to 44 years. The safety analysis demonstrated that 34 episodes of adverse effects occurred in 46% (29 of 63) of patients during 1 year, including 3 episodes of infusion reactions. No adverse effects were deemed serious. The efficacy analysis at 1 year showed that uveoretinitis had improved in 69% (33 of 48), had improved somewhat in 23% (11 of 48), was unchanged in 8% (4 of 48), and had worsened in no patients. The mean number of ocular attacks per 6-month period decreased from 2.66 at baseline to 0.44 during months 1 through 6 of infliximab therapy and to 0.79 during months 7 through 12. Forty-four percent (21 of 48) of patients had no ocular attacks during the 1-year period. Efficacy was best for patients with uveoretinitis duration of less than 5 years. The mean best-corrected visual acuity improved logarithm of the minimum angle of resolution from 0.736 at the first infliximab infusion to 0.616 at the end of 1 year ($P = .01$).

Conclusions: Infliximab treatment for Behçet disease uveoretinitis was well tolerated, with nonserious adverse effects occurring in about half of the patients. At the end of 1 year, uveoretinitis had improved or improved somewhat in 92% (44 of 48) of patients, accompanied by improvement in the mean visual acuity.

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Group Information: The Ocular Behçet's Disease Research Group of Japan members are listed at the end of this article.

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I N 2008, BEHÇET DISEASE AFfected more than 17 000 individuals in Japan[1] and in 2002 represented 6.2% of new uveitis referrals to specialty clinics in Japan.[2] The 4 major manifestations of Behçet syndrome are oral aphthous ulcers, genital ulcers, skin lesions, and recurrent bouts of anterior or posterior uveitis.[3] In many patients, uveitis can be reduced using standard immunosuppressive drugs.[4] However, recurrent bouts of uveitis, particularly if the macula or optic disc is involved, lead to a visual acuity of 0.1 or less within 10 years of ocular symptom onset in 38.7% of eyes, despite immunosuppressive therapy.[5] Therefore, recurrent uveoretinitis must be regarded as vision-threatening ocular inflammation and warrants an aggressive approach.

Biologic agents have increasingly been used in patients with refractory ocular inflammation due to various origins, including sarcoidosis, birdshot chorioretinopathy, and Behçet disease.[6-8] However, the application of these biologic agents has represented off-label use in most cases. Infliximab is a chimeric monoclonal antibody against tumor necrosis factor, a cytokine whose production from peripheral blood monocytes was increased in patients having Behçet disease with active

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uveitis vs those without active uveitis or control subjects.[9] An open-label trial of infliximab for refractory uveoretinitis in patients with Behçet disease showed a significant decrease in the mean number of ocular attacks compared with conventional therapy.[10] Based on this trial and an extension clinical trial, infliximab was approved by the Japanese Ministry of Health, Labour, and Welfare (MHLW) in January 2007 for the treatment of refractory

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uveoretinitis associated with Behçet disease.[11] As a condition of approval, the MHLW mandated the collection of data on all patients with Behçet disease who were prescribed infliximab. The present study pools these data for the first year of treatment from 8 tertiary centers in Japan that specialize in this disease. The study objective was to describe the effects of infliximab on refractory uveoretinitis in patients with Behçet disease during the first year of treatment.

METHODS

PARTICIPANTS

Data were collected prospectively using prepared data forms among patients receiving their first infliximab infusion between January 26, 2007, and August 23, 2008, for the indication of refractory uveoretinitis associated with Behçet disease. During this period, 97 patients with Behçet disease were consecutively registered as having started infliximab treatment at 8 participating centers. Data forms for the first year of treatment were returned on 63 patients. The diagnosis of Behçet disease was made based on established criteria by the Behçet's Disease Research Committee of Japan.[3] Participating centers and physicians involved are listed at the end of this article. Data on various aspects of some patients included in the present study have been reported previously by individual centers.[12-17] Standard informed consent was obtained from patients by the treating physicians.

No criteria were given by the MHLW at the time of infliximab approval and data collection to define refractoriness in patients. In general, the uveitis specialists involved in this study interpreted refractory uveoretinitis to imply that a patient continued to have ocular attacks, despite the use of systemic immunosuppression, or was intolerant of such treatment.

PRETREATMENT EVALUATION

Before initiating infliximab treatment, all patients underwent complete ophthalmological and internal medicine examinations, including tuberculin skin testing and a chest radiograph. Blood tests included baseline complete blood cell count, chemistries, liver function enzymes, and hepatitis B virus serologic testing. The administration of tuberculosis prophylaxis was left to the discretion of the treating physicians.

INFLIXIMAB DOSING AND INFUSION REACTIONS

As per the MHLW treatment protocol, patients received intravenous infusions of infliximab (5 mg/kg) at weeks 0, 2, and 6 and every 8 weeks thereafter. Infusion reactions were defined as any adverse reaction developing during the infliximab infusion or after completion of the infusion for up to 2 hours.

DATA COLLECTION AND STATISTICAL ANALYSIS

Forms were filled out by treating physicians at each participating center and were sent to a central data collection office for collation and analysis. As per the standardized MHLW criteria, adverse effects were severe if they resulted in death, were life threatening, required hospitalization or prolongation of hospitalization for treatment, or caused irreversible disability or dysfunction, congenital abnormalities or defects, or other severe medical conditions. Physicians were asked to report adverse effects in

Table 1. Baseline Characteristics and Pertinent Medical Histories Among 63 Patients

Characteristic	Value
Sex, No. (%)	
Male	56 (89)
Female	7 (11)
Age, y	
No. (%)	
<15	1 (2)
15 to <25	6 (10)
25 to <35	22 (35)
35 to <45	22 (35)
45 to <55	4 (6)
55 to <65	7 (11)
≥65	1 (2)
Mean (SD)	37.4 (11.6)
Medical history, No. (%)[a]	
Tuberculosis	6 (10)
Hepatitis B infection	1 (2)
Other disease[b]	38 (60)
Known drug allergies	9 (14)

[a]Some patients had more than 1 medical condition.
[b]Includes ocular and nonocular disease.

an open-ended question, as opposed to choosing from a list of potential adverse events known to be associated with infliximab.

Categorization of patients' uveoretinitis severity and treatment efficacy was left to the discretion of the treating physicians; no severity or efficacy terminology was specified. The consensus among the uveitis specialists participating in this study was that uveoretinitis was mild if it involved only the anterior segment without hypopyon, was moderate if it involved the anterior segment with hypopyon or involved the retina but not the macula or optic disc, and was severe if it involved the macula or optic disc. Similarly, the consensus on efficacy after initiation of infliximab therapy was that uveoretinitis had improved if no ocular attacks were observed, had improved somewhat if attacks were of decreased frequency and involved only the anterior segment, was unchanged if ocular attacks occurred at the same frequency or severity, and had worsened if ocular attacks occurred at a higher frequency or severity. Acute ocular attacks were managed at the discretion of the treating physicians, and this information was not collected.

Best-corrected visual acuity was assessed using Landolt ring charts. Decimal best-corrected visual acuity was converted to logarithm of the minimum angle of resolution before statistical analysis. Statistical comparisons were made using the Wilcoxon signed rank test.

RESULTS

BASELINE CHARACTERISTICS

As summarized in **Table 1**, 89% (56 of 63) of the patients were male, and 70% (44 of 63) of the patients were aged 25 to 44 years. Ten percent (6 of 63) of patients had a known history of tuberculosis, while 14% (9 of 63) of patients had known drug allergies. As summarized in **Table 2**, most patients had Behçet disease duration of less than 10 years, with a similar uveoretinitis duration. Uveoretinitis was severe in 83% (52 of 63) of patients. At least 3 ocular attacks in the 6-month period before starting infliximab had been documented in 33% (21 of 63)

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Table 2. Behçet Syndrome Characteristics Among 63 Patients

Characteristic	Value
Behçet syndrome duration, y	
No. (%)	
<5	23 (37)
5 to <10	23 (37)
10 to <15	10 (16)
≥15	4 (6)
Unknown	3 (5)
Mean (SD)	6.6 (4.6)
Uveoretinitis duration, y	
No. (%)	
<5	26 (41)
5 to <10	23 (37)
10 to <15	11 (18)
≥15	3 (5)
Mean (SD)	6.9 (4.7)
Severity of uveoretinitis, No. (%)	
Mild	0
Moderate	11 (18)
Severe	52 (83)
No. of ocular attacks in the 6-mo period before starting infliximab, No. (%)	
0	12 (19)
1	10 (16)
2	13 (21)
≥3	21 (33)
Unknown	7 (11)
Cyclosporine use before starting infliximab, No. (%)	
Yes	31 (49)
No	18 (29)
Unknown	14 (22)

Table 3. Adverse Effects of Infliximab Therapy Among 63 Patients

Adverse Effect	No. of Episodes	No. of Patients[a]
General systemic		
Headache	1	1
Fever	1	1
Sweats	1	1
Chills	1	1
Nausea	1	1
Blood pressure elevation	2	1
Bradycardia	1	1
Decreased white blood cell count	1	1
Respiratory		
Coughing	1	1
Difficulty breathing	1	1
Upper airway inflammation	3	3
Dermatological		
Flushing	2	2
Urticaria	6	3
Rash, unspecified	4	4
Purigo	1	1
Pruritus	1	1
Infectious		
Tonsillitis	1	1
Infectious colitis	1	1
Infusion related		
Paresthesia at infusion site	1	1
Infusion reaction	3	2
Total	**34**	**29**

[a] Some patients experienced more than 1 adverse effect or more than 1 episode of the same adverse effect.

of patients. Almost half of the patients were using cyclosporine. Of those, many immediately discontinued their cyclosporine at the initiation of infliximab therapy, while others tapered to a lower maintenance dose.

INFLIXIMAB INFUSION AND TUBERCULOSIS PROPHYLAXIS

Infliximab infusions were administered initially in the outpatient clinic in 35 of 63 patients (56%) and in an inpatient setting in 28 of 63 patients (44%). Of those who received infusions as inpatients, 24 patients were subsequently given their infusions in the outpatient clinic. Seventeen of 63 patients (27%) received pharmacological tuberculosis prophylaxis.

SAFETY

As summarized in **Table 3**, 34 episodes of adverse effects were documented, occurring in 29 of 63 patients (46%) during 1 year. The most common events were dermatological, such as urticaria and rash. Infusion reactions were reported in 2 of 63 patients (3%) and consisted of 1 episode of transient dyspnea in one patient and 2 episodes of decreased blood pressure in the other patient. No adverse effects were judged to be serious by the MHLW criteria.

During the 1-year study period, infliximab therapy was discontinued in 3 patients (the last infusions were ad-

ministered at 154, 170, and 358 days, respectively, after the initial infusions). The reasons given for stopping infliximab were adverse effects in 2 patients and adverse effects and inadequate efficacy in 1 patient.

OVERALL CLINICAL EFFICACY

To assess efficacy only in patients who were infliximab naive, the efficacy analysis was performed after the exclusion of 11 patients who had previously received infliximab as part of the phase 2 clinical trial for Behçet disease[10] or who had received infliximab for other clinical indications. An additional 2 patients at 6 months and 4 patients at 12 months were excluded because of incomplete data.

At 6 months of infliximab therapy (n=50), uveoretinitis had improved in 33 patients (66%), had improved somewhat in 10 patients (20%), was unchanged in 6 patients (12%), and had worsened in 1 patient (2%). For the most part, these percentages remained stable at the 12-month evaluation (n=48), when uveoretinitis had improved in 33 patients (69%), had improved somewhat in 11 patients (23%), was unchanged in 4 patients (8%), and had worsened in no patients.

FREQUENCY OF OCULAR ATTACKS

As summarized in **Table 4**, the mean number of ocular attacks per patient decreased significantly from 2.66 (median, 2; range, 0-10) during the 6 months before start-

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Table 4. Efficacy of Infliximab by Location and Severity of Ocular Inflammatory Attacks[a]

| | No. (%) of Attacks [Mean No. of Attacks per Patient] | | | | | |
| | 6-Month Period Before Starting Infliximab (n = 50) | Months 1-6 After Starting Infliximab (n = 50) | | Months 7-12 After Starting Infliximab (n = 48) | | |
Inflammatory Attacks		Value	P Value[b]	Value	P Value[b]	P Value[c]
Total	**133** [2.66]	**22** [0.44]	<.001	**38** [0.79]	<.001	.02
Location						
Anterior uveitis	16 (12) [0.32]	2 (9) [0.04]	.008	10 (26) [0.21]	.32	.07
Posterior uveitis	50 (38) [1.00]	7 (32) [0.14]	<.001	14 (37) [0.29]	<.001	.05
Panuveitis	65 (49) [1.30]	12 (55) [0.24]	<.001	14 (37) [0.29]	<.001	.39
Unknown	2 (2) [0.04]	1 (5) [0.02]	. . .	0 [0.00]	. . .	. . .
Severity						
Mild	35 (26) [0.70]	15 (68) [0.30]	.04	24 (63) [0.50]	.49	.18
Moderate	56 (42) [1.12]	3 (14) [0.06]	<.001	5 (13) [0.10]	<.001	.22
Severe	40 (30) [0.80]	4 (18) [0.08]	<.001	9 (24) [0.19]	<.001	.07
Unknown	2 (2) [0.04]	0 [0.00]	. . .	0 [0.00]	. . .	. . .

[a] The Wilcoxon signed rank test was used for statistical comparisons.
[b] Compared with the 6-month period before starting infliximab.
[c] Compared with months 1 through 6 after starting infliximab.

ing infliximab (baseline) to 0.44 (median, 0; range, 0-4) during months 1 through 6 of infliximab therapy and to 0.79 (median, 0; range, 0-5) during months 7 through 12 ($P < .001$ vs baseline for both). The difference in the mean number of ocular attacks per patient between months 1 through 6 vs months 7 through 12 was statistically significant ($P < .02$).

Thirty-eight of 50 patients (76%) had no ocular attacks during months 1 through 6, and 28 of 48 patients (58%) had no ocular attacks during months 7 through 12. Among 48 patients with complete efficacy data at 12 months, 21 (44%) had no ocular attacks, and 27 (56%) had at least 1 ocular attack during the 1-year period. Among 27 patients with at least 1 ocular attack, uveoretinitis had improved or improved somewhat in most of them according to the treating physicians; at 12 months, uveoretinitis had improved in 18 patients (67%), had improved somewhat in 7 patients (26%), was unchanged in 2 patients (7%), and had worsened in no patients.

EFFICACY BASED ON UVEORETINITIS DURATION

As shown in the **Figure**, the efficacy of infliximab during 1 year was best for patients with uveoretinitis duration of less than 5 years. However, infliximab therapy was judged to have improved uveoretinitis in more than half of the patients with a uveoretinitis duration of 5 years to less than 10 years or 10 years to less than 15 years.

EFFICACY BASED ON LOCATION AND SEVERITY OF UVEORETINITIS

Table 4 gives the number of ocular attacks at baseline and during months 1 through 6 and months 7 through 12 of infliximab therapy based on the location and severity of inflammatory attacks. During months 1 through 6, the mean number of ocular attacks per patient decreased markedly in all categories examined. However, during

months 7 through 12, a slight increase for all locations was observed compared with baseline, although these differences were not statistically significant. A small increase in the proportion of severe ocular attacks among all ocular attacks from months 1 through 6 (18% [4 of 22 attacks]) to months 7 through 12 (24% [9 of 38 attacks) was observed.

Examination of individual cases revealed that all 9 patients who had ocular attacks during months 1 through 6 had a reduction in severity relative to baseline. Of 19 patients who continued to have ocular attacks during months 7 through 12, a total of 14 had a reduction in severity relative to baseline, while 5 had no reduction (although 3 of these 5 patients had a decrease in the number of ocular attacks compared with baseline).

VISUAL ACUITY

The mean best-corrected visual acuity (logarithm of the minimum angle of resolution) for 50 patients (90 eyes) included in the efficacy analysis improved from 0.736 at the first infliximab infusion to 0.616 at the end of 1 year. This difference was significant ($P = .01$) using the last observation carried forward method.

COMMENT

Several case series have shown the efficacy of infliximab for treating uveitis associated with Behçet disease.[6,7,10,12-27] To our knowledge, the present study is the first to examine infliximab use for this disease in a prospective multicenter study among many patients.

As expected, most patients were male and were aged 25 to 44 years. What was somewhat surprising was that 10% (6 of 63) of the patients had a known history of tuberculosis; 27% (17 of 63) received pharmacological tuberculosis prophylaxis. In Japan, tuberculosis remains at moderately high incidence and prevalence levels[28]; there-
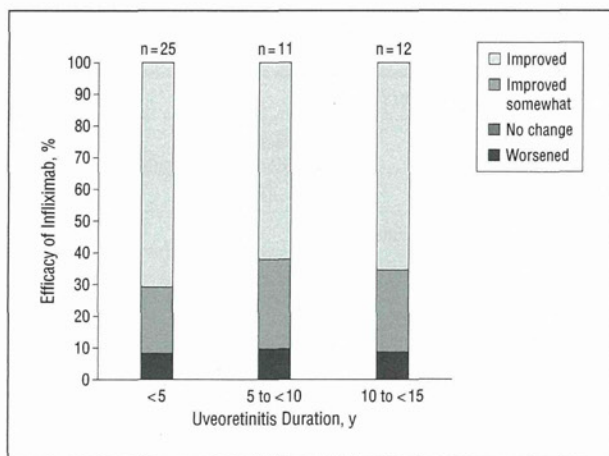
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Figure. Efficacy in 48 patients who completed 1 year of infliximab therapy by duration of uveoretinitis associated with Behçet syndrome. The number of patients who improved, improved somewhat, or did not change was 18, 5, and 2 for duration of less than 5 years; 7, 3, and 1 for a duration of 5 to less than 10 years; and 8, 3, and 1 for a duration of 10 to less than 15 years, respectively. No patients experienced worsening of their uveoretinitis.

fore, reactivation or new infection is of concern. Of note, in the phase 2 trial of infliximab for Behçet disease uveoretinitis in Japan, 1 of 13 patients was reported to have activation of latent tuberculosis.[10] The present analysis of 63 patients during 1 year revealed no cases of active tuberculosis or other severe infections. Severe pulmonary infections involving *Pneumocystis jiroveci* and *Histoplasma capsulatum* can occur during infliximab therapy, with numerous cases of the former reported in Japan.[29-31] However, these infections occurred in patients with diseases such as rheumatoid arthritis, most of whom were older; therefore, age-related comorbidities may have had a role. The mean age of our patients with Behçet disease was 37.4 years, with 81% (51 of 63) of patients younger than 45 years. Because Behçet disease uveoretinitis is a chronic disease, further observation of this cohort is needed to better evaluate the risk of opportunistic infection. Other possible severe adverse effects, such as thromboembolic events, have been reported in patients with uveitis[6] but were not observed in our study.

This study confirmed previous infliximab efficacy results among patients with Behçet disease uveoretinitis. The number of ocular inflammatory attacks was significantly reduced during months 1 through 6 and months 7 through 12 of infliximab treatment compared with baseline. Twenty-one of 48 patients (44%) had no ocular attacks during the entire 12-month period. Among patients in whom ocular attacks continued, uveoretinitis in most of them improved or improved somewhat. Furthermore, although efficacy was best for patients with uveoretinitis duration of less than 5 years, infliximab was effective in those who had had the disease for longer periods.

Although the overall frequency of ocular attacks declined compared with baseline, a statistically significant increase in the mean number of ocular attacks per patient was observed during months 7 through 12 (0.79 attacks) compared with months 1 through 6 (0.44 attacks). There was also a trend toward an increased proportion of severe attacks among all ocular attacks and a higher mean number of severe attacks per patient. Hu-

man antichimera antibodies (HACAs) have been implicated in the observed decline in therapeutic response to infliximab among patients with inflammatory bowel disease.[32,33] Although HACA titers were not measured in the present study, it is possible that the development of HACAs may have led to a gradual rise in frequency or severity of ocular attacks over time during the 1-year period. The development of HACAs may also be related to the occurrence of ocular attacks observed close to the next planned infliximab infusion in patients with Behçet disease,[12,15-17] a time when serum levels of infliximab are low.[16] Moreover, some patients in our study continued using cyclosporine, while others did not, and this may have influenced drug responses and the development of HACAs. Further analysis of our data relative to concomitant cyclosporine use is ongoing and will be the subject of a future publication.

It is difficult to compare our treatment outcomes with those of previous studies. However, a 1978 study[34] revealed that Japanese patients with Behçet disease had a mean of 1.61 ocular attacks per year when taking cyclophosphamide (50-100 mg/d) vs 3.94 ocular attacks per year when not receiving immunosuppression. A 1999 study[35] from Japan reported that patients with Behçet disease had a mean of 0.21 ocular attacks per 4-week period when using cyclosporine (5 mg/kg/d) compared with 0.60 ocular attacks per 4-week period when receiving no immunosuppression. The baseline patient characteristics and the reporting methods differed in these studies compared with our study; nonetheless, our infliximab results seem to compare favorably. It is known that the frequency of ocular attacks in Behçet disease decreases over time after onset of the disease. A Japanese study[5] examining this issue in patients receiving standard immunosuppression demonstrated that the mean frequency was 4.1 ocular attacks during the first year and 3.2 ocular attacks during the second year, with a subsequent decrease to 1.4 ocular attacks during the 10th year after onset of Behçet disease. Nevertheless, the decreased frequency of ocular attacks after 1 year of infliximab therapy in the present study greatly surpasses these reported rates of decline among patients receiving standard immunosuppression alone. Finally, perhaps the best support for the use of infliximab comes from the fact that the case series reporting efficacy involved patients having Behçet disease with refractory disease, who had recurrences despite standard immunosuppressive regimens.[6,7,10,18-27]

Strengths of our study include the collection of data among a large sample of patients in a prospective manner and the involvement of multiple centers, representing a wide spectrum of disease and treatment patterns. Furthermore, a single definition for diagnosing Behçet disease was used based on established criteria. Weaknesses of our study include the open-label design without a sham group for comparison, the absence of criteria for efficacy and severity ratings, and the short infliximab therapy follow-up period (1 year) given that this ocular disease usually continues with inflammatory recurrences for several years, if not decades. Furthermore, only acute inflammation that was clinically observable by the physicians at the time of clinic visits, whether scheduled or unscheduled, was considered an ocular attack. Therefore, the number or severity of ocular

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attacks may have been underestimated, although we assume that severe ocular attacks would have brought patients to the ophthalmologist for an unscheduled visit because of reduced vision. In addition, only 63 of 97 eligible patients from 8 centers were included in any analysis, and only 48 were included in the 1-year efficacy analysis. It is possible that patients not included in the present analysis had more adverse effects or worse efficacy. The influence of concomitant therapy also requires close examination, as already stated. Finally, the infliximab dose (5 mg/kg every 8 weeks during maintenance therapy) available for use in this study was limited by the MHLW guidelines. Patients with continued recurrence of ocular attacks, particularly those with severe attacks, may require more frequent or greater doses of infliximab, as has been reported for the treatment of various types of uveitis.[7,10,36]

New methods of assessing ocular inflammation would be useful to judge the severity of disease and the response to infliximab treatment. Fluorescein angiography has been used as a surrogate marker for the overall level of ocular inflammation by scoring retinal vascular and optic disc fluorescein leakage at times of clinical quiescence.[17] Other methods that may prove useful involve assessing the activation of various cytokines and other inflammation-related molecules by examining gene expression. Such analysis has been performed in autoimmune noninfectious uveitis,[37] in Behçet disease with uveitis,[38] and in rheumatoid arthritis and inflammatory bowel disease before and after the initiation of infliximab therapy.[39,40]

In summary, the present study among 63 patients having Behçet disease with refractory uveoretinitis demonstrated that infliximab was well tolerated, with nonserious adverse effects occurring in about half of the patients. At the end of 1 year, uveoretinitis had improved or improved somewhat in 92% (44 of 48) of patients, accompanied by improvement in the mean visual acuity. Forty-four percent (21 of 48) of patients had no ocular attacks during the 1-year period, while the remainder experienced a marked overall reduction in the frequency and severity of attacks compared with baseline.

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LETTER TO THE EDITOR

Enhanced depth imaging optical coherence tomography of the choroid in recurrent unilateral posterior scleritis

Wakako Taki · Hiroshi Keino · Takayo Watanabe · Annabelle A. Okada

Dear Editor,

Posterior scleritis is a painful and potentially destructive ocular inflammation caused by infectious agents or non-infectious immune reactions [1]. Recently, spectral-domain optical coherence tomography (OCT) using enhanced depth imaging (EDI) has been shown to reliably visualize the full thickness of choroid in normal and highly myopic eyes [2, 3]. In this report, we describe two patients with recurrent unilateral posterior scleritis in whom thinning of the choroid was observed by EDI–OCT.

Patient 1

A 33-year-old man was referred for scleritis refractory to systemic corticosteroids. On examination, visual acuities (VAs) were 1.2 OD without correction and 0.9 OS with correction of −0.50 diopter. The intraocular pressures (IOPs) were normal OU. The right eye was normal, but the left eye was mildly injected, with serous retinal detachment noted in the fundus (Fig. 1a). B-mode ultrasonography showed a thickened posterior eye wall (Fig. 1b). Syphilis serologies, antineutrophil cytoplasmic antibody, and angiotensin converting enzyme level were unrevealing. The tuberculin skin test (TST) was positive, but the QuantiFERON®-TB 2G (QFT; Cellestis, Carnegie, Australia) was negative. The

patient was diagnosed with posterior scleritis of unclear etiology, and started on 40 mg/day of prednisolone on a taper, and later on oral methotrexate (MTX) for steroid sparing. The patient was recurrence-free for over 2 years. However, 29 months after presentation while still on MTX 8 mg/week, ocular pain serous retinal detachment recurred OS (Fig. 1c). EDI–OCT was performed using the Heidelberg Spectralis instrument (Heidelberg Engineering, Heidelberg, Germany), with retinal scans performed along horizontal and vertical planes through the center of the fovea. Choroidal thickness was determined manually by measuring the
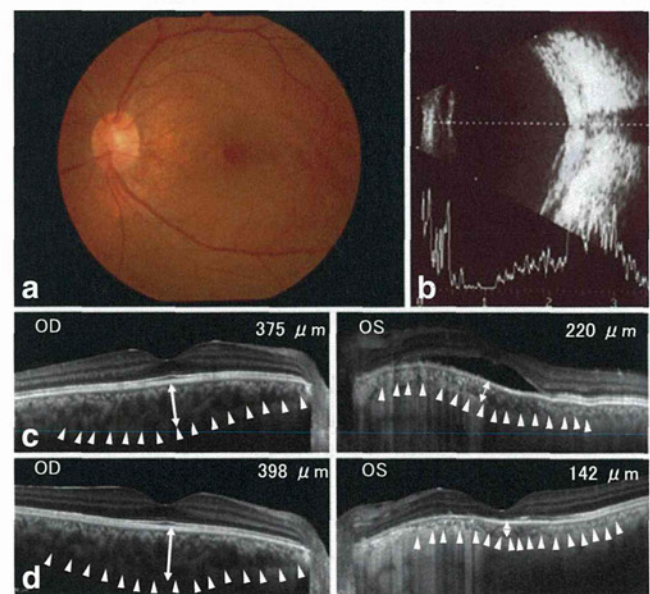

Fig. 1 **a** Fundus photograph of the left eye in patient 1, revealing serous retinal detachment and a hyperemic optic disc. **b** B-mode ultrasonography showed a thickened posterior eye wall. **c** EDI–OCT images at 29 months after presentation during a bout of active, recurrent inflammation. **d** EDI–OCT images at 35 months during a period of quiescence. *White arrowheads* delineate the external choroidal margins

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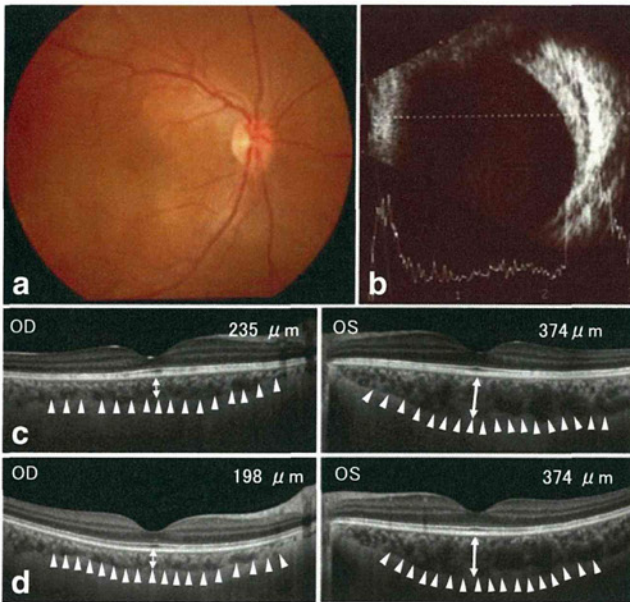e-mail: aokada@eye-center.org

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Fig. 2 **a** Fundus photograph of the right eye in patient 2, revealing serous retinal detachment and a hyperemic optic disc. **b** B-mode ultrasonography showed a thickened posterior eye wall. **c** EDI–OCT images at 53 months after presentation, at a time of resolution of recurrent posterior scleritis. **d** EDI–OCT images at 59 months after presentation during a period of quiescence. *White arrowheads* delineate the external choroidal margins

distance, under the center of the fovea, between the outer border of the hyper-reflective line corresponding to the retinal pigment epithelium and the outer border of the choroid. Subfoveal choroidal thicknesses were found to be 375 μm OD and 220 μm OS (Fig. 1c). The treatment regimen was increased to prednisolone 30 mg/day and MTX 10 mg/week, and the posterior scleritis again resolved. Follow-up EDI–OCT at 35 months revealed choroidal thicknesss of 390 μm OD and 143 μm OS (Fig. 1d). There was no difference in axial length between the two eyes (23.58 mm OD and 23.44 mm OS) as measured at 46 months after initial presentation.

Patient 2

A 21-year-old woman was referred for scleritis refractory to topical corticosteroids. On examination, the VAs were 0.06 OD with correction of +1.00 diopter and 0.8 OS with correction of −0.50 diopter. The IOPs were normal OU. The left eye was normal, but the right eye had a serous retinal detachment (Fig. 2a) and B-mode ultrasonography

showed a thickened posterior eye wall OD (Fig. 2b). Ancillary investigations were notable for presence of antinuclear antibodies and a positive TST, although the chest X-ray was normal; QFT testing was not available. The patient was diagnosed with posterior scleritis of unclear etiology, and treated with prednisolone 100 mg/day intravenously for 3 days, followed by an oral dose of 40 mg/day tapered to zero by 12 months. The posterior scleritis resolved within 1 month, with no recurrence for roughly 4 and a half years. However, at 53 months after presentation, the patient had recurrence of posterior scleritis OD, and was restarted on prednisolone 30 mg/day on a gradual taper. The posterior scleritis resolved by 1 month (Fig. 2c), and EDI–OCT performed at that time showed a subfoveal choroidal thickness of 235 μm OD and 374 μm OS. No recurrences were observed during the subsequent 6 months, and the choroidal thickness was noted to be 198 μm OD and 374 μm OS (Fig. 2d) at 59 months after presentation. Both eyes had the same axial length (22.5 mm OU) as measured 68 months after initial presentation.

In this report, EDI–OCT in two patients with recurrent unilateral posterior scleritis showed marked choroidal thinning in the eye with the scleritis compared to the non-involved eye. To our knowledge, this represents the first report to measure choroidal thickness in posterior scleritis. Scleral inflammation can readily spread to the choroid [4], and indocyanine green angiography has demonstrated diffuse zonal hyperfluorescence in the choroid with posterior scleritis [5]. We speculate that recurrent inflammation of the posterior sclera induces alterations to the adjacent choroid, gradually resulting in choroidal atrophy.

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