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CLINICAL INVESTIGATION

Acute retinal necrosis: factors associated with anatomic and visual outcomes

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Abstract
Purpose To examine the factors associated with anatomic and visual outcomes in Japanese patients with acute retinal necrosis (ARN).
Methods One hundred four patients with ARN who were followed for more than 1 year at nine referral centers were reviewed. Retinal involvement at initial presentation was classified into four groups: zone 1 (posterior pole, $n = 22$), zone 2 (midperiphery, $n = 54$), zone 3 (periphery, $n = 25$), and unknown ($n = 3$). Forty-eight eyes underwent prophylactic vitrectomy before development of retinal detachment (vitrectomy group); 56 eyes were treated conventionally without prophylactic vitrectomy (observation group).
Results The retina was attached in 28 of 48 eyes (58.3 %) in the vitrectomy group and 42 of 56 eyes (75.0 %) in the observation group at the final visit ($P = 0.071$). At 1 year, 56 eyes (53.8 %) had a best-corrected visual acuity (BCVA) of 20/200 or worse. Multivariate logistic regression analyses identified zone 1 disease (odds ratio = 4.983) and optic nerve involvement (odds ratio = 5.084) as significantly associated with BCVA of 20/200 or worse. Among the zone 3 eyes, significantly ($P = 0.012$) more eyes in the observation group than in the vitrectomy group had an attached retina.
Conclusions Prophylactic vitrectomy did not improve the final BCVA in any eyes. Zone 3 eyes had better outcomes without prophylactic vitrectomy.

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Keywords Acute retinal necrosis · Retinal detachment · Visual outcome · Prophylactic vitrectomy

Introduction

In 1971, Urayama et al. [1] first reported acute retinal necrosis (ARN), a devastating, potentially blinding, necrotizing retinitis. ARN is diagnosed on the basis of the clinical appearance and disease course as well as the standard diagnostic criteria proposed by the American Uveitis Society [2]. Because varicella zoster virus (VZV), herpes simplex virus (HSV), and Epstein-Barr virus are implicated in the pathogenesis of ARN [3–6], the standard treatment for ARN is intravenous acyclovir 10 mg/kg every 8 h or 1500 mg/m^2 daily for 5–10 days, followed by oral acyclovir 800 mg five times daily for 6 weeks [7]. Furthermore, systemic treatment with corticosteroids [8] and/or an antiplatelet agent [9] is often given empirically. Recently, prophylactic laser retinopexy [10] or prophylactic vitrectomy [11–13] before development of a retinal detachment has been reported to reduce the incidence of retinal detachment, which is predictive of the visual outcome in ARN [14, 15]. However, disappointing results have also been reported after these treatments [16, 17]. Conducting a study of treatments for ARN is difficult because the disease is rare. Indeed, ARN was diagnosed in only 53 of 3830 patients (1.4 %) with endogenous uveitis referred to university hospitals in Japan over a 1-year period [18].

In this study, we retrospectively investigated 104 patients diagnosed with ARN in Japan to determine the anatomic and visual outcomes as well as the indication for prophylactic vitrectomy.

Patients and methods

This study was a retrospective observational case series of 104 consecutive patients who were negative for the human immunodeficiency virus and who had been diagnosed as having ARN at nine referral centers in Japan (Hokkaido University, Kobe University, Kyorin University, Kyushu University, Osaka University, the University of Tokyo, Tokyo Medical University, Tokyo Medical and Dental University, and Yokohama City University) between 2002 and 2008 with a minimal follow-up time of 1 year. In cases of bilateral involvement, only the first involved eye was included. A clinical diagnosis of ARN was based on the standard diagnostic criteria proposed by the American Uveitis Society [2]. The institutional review board of each center approved the study protocol.

The patients' medical records were reviewed for age, sex, best-corrected visual acuity (BCVA) at the initial presentation, retinal necrotic lesions, optic nerve involvement (redness and/or edema), presence of prophylactic vitrectomy, BCVA at 1 year, retinal status at the final visit, and duration of follow-up. Eyes without silicone oil removal were considered to have a retinal detachment.

The sites of retinal necrosis were classified into three groups according to the classification of cytomegalovirus (CMV) retinopathy of Holland et al. [19]. Zone 1 was defined as the portion of the retina in which infection was immediately sight-threatening and corresponded to the area 3000 μm from the fovea or 1500 μm from the margins of the optic nerve head; zone 2 extended anteriorly from zone 1 to the clinical equator; zone 3 extended anteriorly from zone 2 to the ora serrata.

We compared the anatomic and functional outcomes of the two groups of patients. The vitrectomy group included patients who underwent prophylactic vitrectomy before the development of a retinal detachment. The observation group included patients who did not undergo prophylactic vitrectomy.

The VA was converted to logarithm of the minimum angle of resolution (logMAR) values for statistical analysis. On the basis of a previous report [20], the following logMAR values were assigned: counting fingers, 2.6 logMAR; hand motions, 2.9 logMAR; light perception, 3.1 log MAR; no light perception, 3.4 logMAR. According to the Standardization of Uveitis Nomenclature (SUN) criteria [21], patients were classified as having severe visual loss at a visual acuity of 20/200 or worse. Each variable with a significant association ($P < 0.05$) was introduced into a forward, stepwise, logistic regression model to identify the baseline factors that were independent predictors of ARN. The odds ratio (OR) and its 95 % confidence interval (CI) for each possible risk factor were also calculated. When appropriate, the Mann-Whitney and Fisher exact tests were used to compare the differences between the groups. Statistical analyses were performed using JMP version 8.0 for Windows (SAS Institute, Cary, NC, USA). Probability values less than 0.05 were considered significant.

Results

A total of 106 patients with a diagnosis of ARN were reviewed. Two patients did not meet the inclusion criteria because they were positive for the human immunodeficiency virus. One hundred four patients (61 men, 43 women) met the inclusion criteria. The median patient age at disease onset was 55 years (51.2 ± 15.5; range, 12–79 years). The causative virus was HSV in 18 eyes, VZV in 84 eyes, and unknown in 2 eyes. According to the

classification of CMV retinopathy of Holland et al. [19], the eyes were classified as having zone 1 disease (posterior) in 22 cases, zone 2 disease (midperiphery) in 54 cases, and zone 3 disease (periphery) in 25 cases; the zone was unknown in 3 cases because of vitreous opacity. Optic nerve involvement was detected in 79 eyes. The median follow-up time was 45 months (46.4 ± 23.1; range, 12–106 months).

Antiviral treatment (acyclovir or valacyclovir) was administered in all cases. A corticosteroid was prescribed for 95 eyes, and the initial doses of corticosteroid in prednisolone equivalents ranged from 7.5 to 1250 mg daily. Forty-eight eyes (46.2 %) underwent prophylactic vitrectomy before a retinal detachment developed on 0–186 days (median, 11 days) after the initial visit (vitrectomy group). Fifty-six eyes (53.8 %) did not undergo a prophylactic vitrectomy (observation group).

Development of retinal detachment in the observation group

In the observation group, 39 of 56 eyes (69.6 %) developed a retinal detachment during the follow-up period. The associations of six explanatory variables with development of RD (age, sex, causative virus, BCVA at presentation, site of retinal necrosis, and optic nerve involvement) were individually examined. Simple logistic regression analyses identified male sex, severe visual loss at the initial presentation, and optic nerve involvement as the factors associated with development of RD. In addition, stepwise multivariate logistic regression analyses identified optic nerve involvement as the sole predictor of development of RD ($P = 0.002$; OR, 9.481; CI 2.251–50.74). Of the 41 eyes with optic nerve inflammation at presentation, 32 (78.1 %) developed RD. In contrast, of the 11 eyes without optic nerve inflammation at presentation, 3 (27.3 %) developed RD.

Final retinal attachment

Retinal attachment was achieved in 70 eyes. In the vitrectomy group, 28 of 48 eyes (58.3 %) achieved retinal attachment. In the observation group, 42 of 56 eyes (75.0 %) achieved retinal attachment. In the 39 eyes that underwent reparative surgery in the observation group, 25 (64.1 %) achieved retinal attachment. Overall, 34 eyes did not achieve retinal attachment; they comprised 22 eyes that did not undergo silicone oil extraction and 12 eyes that developed a tractional retinal detachment after silicone oil removal. The associations of seven explanatory variables with final RD (age, sex, causative virus, BCVA at presentation, site of retinal necrosis, optic nerve involvement, and presence of prophylactic vitrectomy) were individually examined (Table 1). Simple logistic regression analyses identified older age, VZV infection, and severe visual loss at the initial presentation as the factors associated with final RD. In addition, stepwise multivariate logistic regression analyses identified severe visual loss (visual acuity of 20/200 or worse) at presentation as the sole predictor of final RD ($P = 0.004$; OR, 3.507; CI: 1.486–8.491).

Table 1 Univariate logistic regression analysis of potential predictors of final retinal detachment

	Odds ratio	95 % CI	*P* value
Age	1.042	1.012–1.076	0.006
Male	0.981	0.441–2.350	0.981
VZV infection	4.923	1.286–32.44	0.018
SVL at initial presentation	3.507	1.486–8.491	0.004
Zone 1 disease	2.302	0.857–6.144	0.097
Optic nerve involvement	2.207	0.730–8.247	0.168
Prophylactic vitrectomy	2.143	0.938–5.012	0.071

CI confidence interval, *SVL* severe visual loss, *VZV* varicella zoster virus

Visual acuity at 1 year

The mean (SD) BCVA at initial presentation was 0.76 ± 0.89 (range, −0.18 to 3.4) and at 1 year was 1.06 ± 1.01 (range, −0.18 to 3.4). The mean (SD) logMAR BCVAs at the initial presentation were 0.92 ± 0.99 in the vitrectomy group and 0.64 ± 0.78 in the observation group, which did not differ significantly ($P = 0.172$). The mean BCVAs at 1 year were 1.23 ± 1.09 in the vitrectomy group and 0.92 ± 0.93 in the observation group, which also did not differ significantly ($P = 0.129$). The BCVA in 23 patients gained more than three lines, remained unchanged in 34 patients, and lost more than three lines in 47 patients. Those who lost more than three lines of vision included 13 of the 22 patients with zone 1 disease (59.1 %), 26 of the 54 patients with zone 2 disease (48.1 %), 7 of the 25 patients with zone 3 disease (28.0 %), and 3 patients in which the zone was unknown. The associations of seven explanatory variables with severe visual loss at 1 year (age, sex, causative virus, BCVA at presentation, site of retinal necrosis, optic nerve involvement, and presence of prophylactic vitrectomy) were individually examined (Table 2). Simple multivariate logistic regression analyses identified VZV infection, severe visual loss at the initial presentation, zone 1 disease, and optic nerve involvement as related to severe visual loss at 1 year. In addition, stepwise multivariate logistic regression analyses identified two baseline factors: zone 1 disease ($P = 0.010$; OR, 4.983; CI 1.440–23.35) and optic nerve involvement ($P = 0.005$; OR, 5.084; CI 1.589–19.93; Table 3). The BCVAs of eyes without retinal attachment at the final visit are shown in Table 4.

Table 2 Univariate logistic regression analysis of potential predictors of severe visual loss at 1 year

	Odds ratio	95 % CI	*P* value
Age	1.012	0.987–1.038	0.354
Male	1.451	0.664–3.192	0.351
VZV infection	3.900	1.339–13.09	0.012
SVL at initial presentation	1.662	1.057–2.768	0.027
Zone 1 disease	7.741	2.400–34.82	0.0003
Optic nerve involvement	5.265	1.870–17.35	0.001
Prophylactic vitrectomy	1.400	0.645–3.068	0.395

CI confidence interval, *SVL* severe visual loss, *VZV* varicella zoster virus

Table 3 Multivariate logistic regression analysis of potential predictors of severe visual loss at 1 year

	Odds ratio	95 % CI	*P* value
Zone 1 disease	4.983	1.440–23.35	0.010
Optic nerve involvement	5.084	1.589–19.93	0.005

CI confidence interval

Table 4 Best-corrected visual acuity at 1 year in eyes that did not achieve retinal attachment

Eyes with tractional retinal detachment after silicone oil removal ($n = 12$)	
Better than 20/200	2
20/2000 to 20/200	8
Hand motions	1
Light perception	1
Eyes without silicone oil removal ($n = 22$)	
Better than 20/200	1
20/2000 to 20/200	9
Counting fingers	3
Hand motions	3
Light perception	3
No light perception	3

Prophylactic vitrectomy and extent of retinal necrosis

The efficacy of prophylactic vitrectomy was also evaluated on the basis of the extent of the retinal necrosis at the initial presentation. The anatomic and visual outcomes are shown in Table 5. Seven of 25 eyes with zone 3 ARN underwent prophylactic vitrectomy before a retinal detachment developed, and three of the seven eyes achieved retinal attachment. Nine of the 18 eyes in the observation group developed a retinal detachment, and 8 of the 9 eyes achieved retinal attachment following surgery. Overall, 3 of 7 eyes (42.9 %) in the vitrectomy group and 17 of 18 eyes (94.4 %) in the observation group achieved retinal attachment ($P = 0.012$). Zone 3 eyes had better anatomic outcomes without prophylactic vitrectomy. The anatomic and visual outcomes of zones 1 and 2 eyes did not differ significantly between the vitrectomy and observation groups.

Discussion

This is the first report on anatomic and visual outcomes of ARN in multiple uveitis centers in Japan. The visual prognosis of patients with ARN is generally poor and in Japanese patients is reported to be correlated with the presence of retinal detachment and VZV infection [14]. Simple logistic regression analyses showed that severe visual loss at 1 year in our patients was also correlated with VZV infection, severe visual loss at the initial presentation, zone 1 disease, and optic nerve involvement. Prophylactic vitrectomy was not correlated with the visual prognosis. These results suggest that irreversible damage to the retina and optic nerve before antiviral treatment strongly affects the visual prognosis.

In particular, the anatomic success rate of zone 3 eyes was better without prophylactic vitrectomy than with prophylactic vitrectomy. Three of 7 eyes (42.9 %) with zone 3 ARN in the vitrectomy group and 17 of 18 eyes (94.4 %) in the observation group achieved final retinal attachment ($P = 0.012$). Ishida et al. [22] also reported that all three eyes with zone 3 disease received only antiviral medical therapy and did not develop a rhegmatogenous retinal detachment. In eyes with zone 3 disease, the area of retinal necrosis was small, and intravenous antiviral treatment seemed sufficient to control the infection.

In the natural course of ARN, rhegmatogenous retinal detachment was observed in approximately 75 % of the untreated eyes [10]. RD also developed in about 70 % of the observation group eyes of our study. We found that eyes with optic nerve redness or edema had a risk of RD. In eyes with optic nerve redness or edema, the retinal necrosis extended posteriorly from the peripheral retina, suggesting longer duration of the necrosis after the onset of symptoms than in eyes without optic nerve redness and that this severe damage to the retina is related to the risk of RD development.

Previous studies have reported that prophylactic vitrectomy prevented retinal detachment [13, 22]; however, at the same time, prophylactic vitrectomy did not improve the mean final VA [13]. The eyes in these reports were treated from 1998 to 2006 [22] and from 1987 to 2008 [13]. Recent advances in retinal surgeries, i.e., vitrectomy using a high-speed vitreous cutter with intravitreal injection of triamcinolone acetonide to visualize the vitreous gel and locate vitreoretinal adhesions [23], are expected to reduce

Table 5 Anatomic status and logMAR BCVA at 1 year based on the extent of retinal necrosis at the initial presentation

Zone	RA	RD	Total
Vitrectomy group ($n = 48$)			
1	1.66 ± 1.02 ($n = 4$)	2.55 ± 0.83 ($n = 4$)	2.11 ± 0.98 ($n = 8$)
2	0.58 ± 0.56 ($n = 21$)	2.03 ± 0.96 ($n = 11$)	1.08 ± 0.72 ($n = 32$)
3	0.10 ± 0.09 ($n = 3$)	1.76 ± 1.31 ($n = 4$)	1.05 ± 1.28 ($n = 7$)

Zone	RA without RD development	RA after RD repair surgery	RD	Total
Observation group ($n = 56$)				
1	0.56 ± 0.51 ($n = 4$)	1.13 ± 0.26 ($n = 4$)	2.03 ± 1.05 ($n = 6$)	1.36 ± 0.96 ($n = 14$)
2	−0.01 ± 0.11 ($n = 4$)	1.10 ± 0.89 ($n = 13$)	1.52 ± 0.74 ($n = 5$)	1.00 ± 0.91 ($n = 22$)
3	0.11 ± 0.23 ($n = 9$)	0.54 ± 0.42 ($n = 8$)	1.00 ($n = 1$)	0.35 ± 0.46 ($n = 18$)

The zones of one eye in the vitrectomy group and of two eyes in the observation group were unknown because of vitreous opacity

BCVA best-corrected visual acuity, *RA* retinal attachment, *RD* retinal detachment

postoperative inflammation and achieve favorable anatomic success. Therefore, we limited our search to Japanese patients diagnosed as having ARN after 2002 and retrospectively evaluated their anatomic and visual outcomes. However, our results were similar to those of previous reports: prophylactic vitrectomy did not improve the visual prognosis in any eyes.

In the current study, we investigated the functional outcomes using the prediagnostic conditions and therapeutic approaches as variables. As a result, zone 1 disease and optic nerve involvement were associated with severe visual loss at 1 year. We predicted the visual prognosis of our patients from these factors but, unfortunately, could not improve the visual prognosis because we could not change those factors. The damage at the initial presentation might have mostly affected the outcome.

As with previous studies on ARN, the present study is limited by its relatively small sample size. The starting dose of steroid treatment, history of laser photocoagulation, timing of the prophylactic vitrectomy, and surgical procedures differed among the referral centers. The retrospective data collection may also have influenced the consistency of the available data. Nevertheless, we collected the clinical data of 114 cases from major referral centers in Japan, and our data described the characteristics of this rare disease in Japanese patients.

In conclusion, the anatomic and functional success in the treatment of ARN was determined primarily by the condition of the retina and optic nerve at the initial presentation. Regarding the indication for prophylactic vitrectomy, this therapy should not be administered for zone 3 ARN. Further studies with a larger number of patients with longer follow-up are needed to determine the treatment of ARN.

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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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Conflict of interest The authors have no proprietary interests or conflicts of interest in any aspect of this report.

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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

Human 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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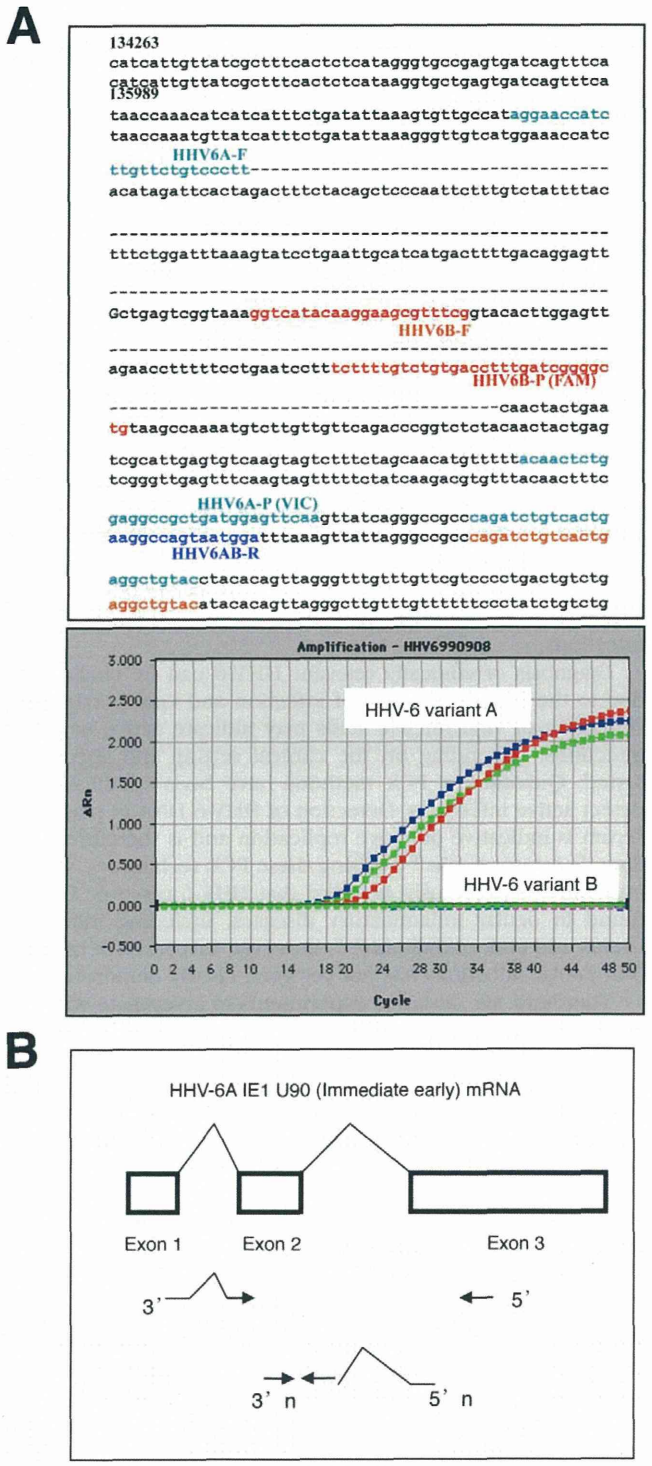

Figure 1. Amplification of HHV-6–specific DNA and mRNA. (**A**) TaqMan probes and primers used to amplify HHV-6 DNA (HHV-6A and HHV-6B). HHV-6 subtypes were identified using PCR with variant-specific primers and probes (*lower graph*). (**B**) Nested RT-PCR primers were designed to amplify HHV-6A mRNA.

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 endothelitis	0.5	33	Edema	Cell 2+	Mutton fat	None	None	PCR (-)	HSV-1 corneal endothelitis
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 syndrome	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: 2.0×10^4copies/mL, EBV: <50 copies/mL		
2	Aqh	HHV-6, HSV-1	HHV-6: 7.5×10^3copies/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.

ac.jp/ctr/index/htm with study number of R000002708. The study started in April 2006 and terminated in April 2010.

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

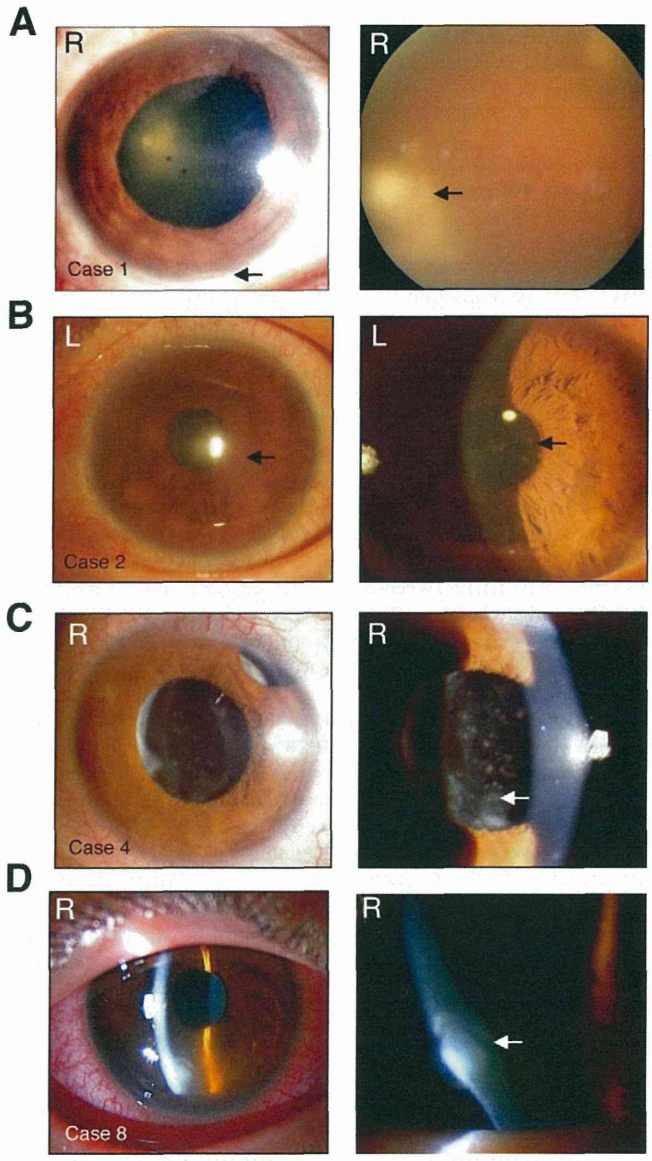

FIGURE 2. Slit-lamp and fundus photographs for HHV-6 infections. (**A**) Case 1: A case of ocular toxocariasis. Slit-lamp examination of right eye (RE) disclosed ciliary injection, moderate mutton-fat keratic precipitates (KPs), and severe anterior chamber cells with hypopyon (*arrow*). Funduscopic examination 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

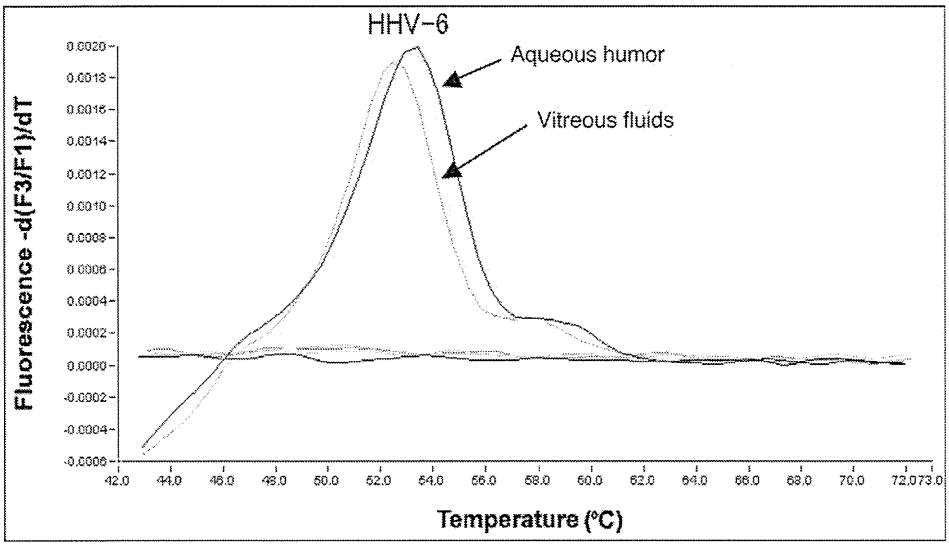

FIGURE 3. Results for multiplex PCR in a patient with HHV-6–positive uveitis. A significant positive curve was seen at 52°C, indicating detection of HHV-6 genomic DNA in the ocular fluids (case 1). DNA from other herpes viruses, such as HSV1, HSV2, VZV, EBV, CMV, HHV7, and HHV8, was not detected in this 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.

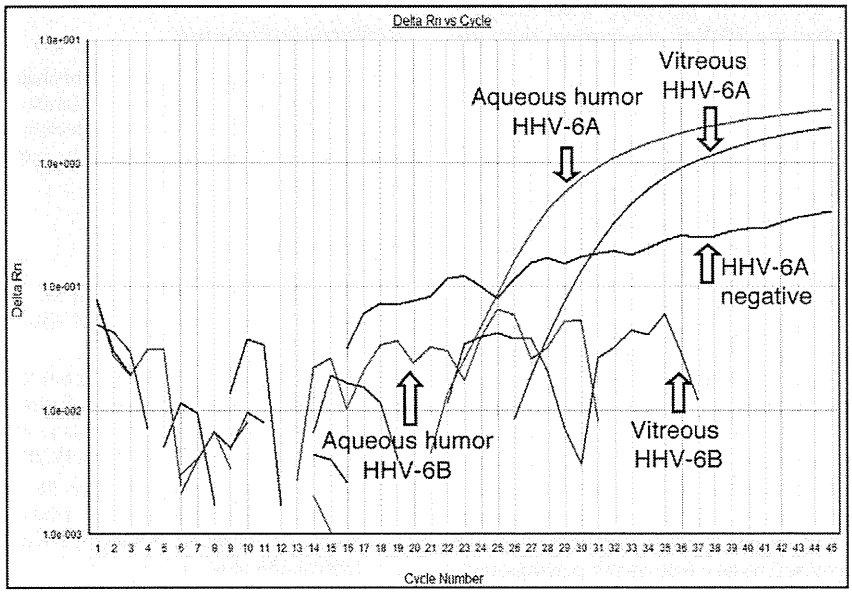

FIGURE 4. Detection of HHV-6 DNA by quantitative real-time PCR. The real-time PCR results for the samples from case 1 showed that intraocular samples, such as aqueous humor and vitreous fluids, contained a high copy number of HHV-6A DNA, but not HHV-6B DNA.

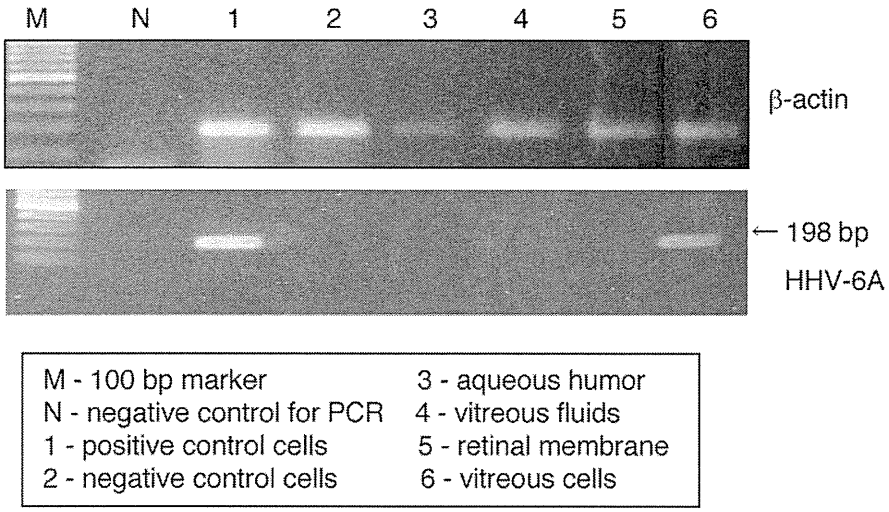

FIGURE 5. Detection of HHV-6 mRNA in intraocular samples. HHV-6A mRNA was detected in samples from vitreous cells, but other ocular samples, such as aqueous humor, vitreous fluids, and retinal membrane tissues were all negative (*lower image*). All samples, including control RNA, were positive for β-actin (*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 panuveitis.[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.

Acknowledgments

Kenji Nagata (Department of Ophthalmology, Kyoto Prefectural University of Medicine) and Yu Kaneko (Department of Ophthalmology, Yamagata University) collected and sent the samples used in this study. We are grateful for the expert technical assistance of Shizu Inoue.

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学会トピックス　第47回　日本眼炎症学会

会長：福島　敦樹（高知大学医学部眼科）

iPS細胞由来網膜色素上皮細胞の免疫抑制能解析

人工多能性幹細胞（induced pluripotent stem cells：iPS細胞）は，多種類の細胞・組織に分化することが可能な細胞として注目され，現在，ヒトiPS細胞由来の網膜色素上皮細胞（retinal pigment epithelial cells：RPE）の臨床試験が取り組まれ始めている．網膜疾患へのRPE移植時の問題の一つに拒絶反応があり，以前の報告ではT細胞免疫がその炎症の機序に深く関与していることが報告されている．今回，そのiPS細胞由来RPE（iPS-RPE）の免疫学的抑制能について検討した．

同意の得られた網膜疾患患者または健常人の皮膚組織から線維芽細胞を採取し，エピソーマルベクターを用いてプラスミド遺伝子導入（OCT3/4，SOX2，KLF4など6因子）してiPS細胞を樹立した．樹立したiPS細胞にRPE分化・誘導分子と特殊な培地を用いてRPEを樹立した．また標的細胞には健常人由来の活性化したT細胞を用いた．iPS-RPEのT細胞抑制は細胞増殖試験（BrdU，CFSE取り込み）およびサイトカイン産生能（ELISA，flow cytometry）で検討した．iPS-RPEのtransforming growth factor beta（TGFβ）発現は，flow cytometry，ELISA，免疫染色，定量reverse transcription-polymerase chain reaction（RT-PCR）で検討した．また，TGFβ発現抑制にsiRNA遺伝子導入したiPS-RPEを使用した．

その結果，樹立したヒトiPS-RPEは*in vitro*で活性化T細胞（ヘルパーCD4陽性T細胞，細胞傷害性CD8陽性T細胞）を有意に抑制した．iPS-RPEと共培養したCD4陽性T細胞は細胞増殖の低下や活性化マーカーの発現の低下がみられた．また，iPS-RPEと共培養したCD8陽性T細胞は細胞傷害物質グランザイムBの産生能が低下していた．iPS-RPEは構成的にTGFβ1，β2，β3を発現しており，iPS-RPEに曝露されたT細胞はTGFβ receptorやSmad2/3（TGFβ関連転写因子）を発現していた．si-RNA遺伝子導入によりTGFβ発現抑制したiPS-RPEはそのT細胞抑制能が明らかに減弱していた．加えて，リコンビナントTGFβ蛋白質は濃度依存的かつ有意に活性化T細胞を抑制した．

以上の結果から，iPS細胞由来RPEはTGFβを介してT細胞を抑制していることが判明した（図）．以前に我々は，正常RPE（primary cultured RPE）が同様にT細胞を抑制することを報告している[1]．近い将来ヒトに移植される予定のiPS細胞から分化・誘導したRPEは強い免疫学的抑制能力を有する細胞であることが示された．

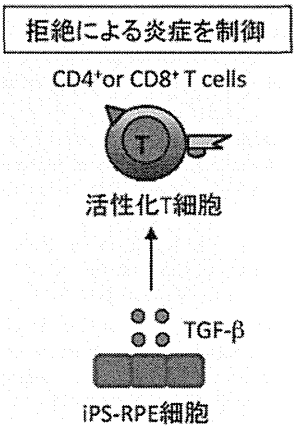

図　ヒトiPS由来の網膜色素上皮細胞（iPS-RPE）によるT細胞抑制．

文　献

1) **Sugita S, Streilein JW**：Iris pigment epithelium expressing CD86（B7-2）directly suppresses T cell activation *in vitro* via binding to cytotoxic T lymphocyte-associated antigen 4. J Exp Med 198：161-171, 2003.

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Vogt-小柳-原田病の慢性化に関与する因子の解析

Vogt-小柳-原田（VKH）病は適切に加療すればほとんどの症例で良好な予後が得られる疾患であるが，なかには通常どおりの治療を行っても再発する症例が一定数存在し，慢性化した症例はさまざまな合併症を伴い重度の視機能低下を来す．我々はVKH病の予後の向上を目指して，病態を理解するとともに，より良い投薬法を検討する目的で，VKH病の臨床データや末梢血リンパ球の解析を行ってきた．その一環として，2012年度の日本眼科学会総会および日本臨床眼科学会では，VKH病の年齢や病型（漿液性網膜剝離型と視神経乳頭炎型）と相関する因子について報告した．そのなかで，年齢，病型，および慢性化は互いに相関する因子であり，またその他にも発症から治療開始までの期間も経過と関連が強いことを報告した．

しかしながら，VKH病の慢性化の危険因子を検討す

るためには，年齢や病型など互いに相関している各因子の影響を考慮する必要がある．そこで今回我々は，2001年から2011年までに東京医科大学眼科で診断，加療し，半年間以上経過観察を行った連続104例について，ロジスティック多重回帰分析を行い，慢性化に関与する因子を解析した．年齢，性別，病型，発症から治療開始までの日数，髄液細胞数，加療前視力(logMAR)，臨床所見の重症度(前房炎症，漿液性網膜剝離，視神経乳頭炎，脈絡膜鄒襞)，加療開始後1か月以内の体重1kgあたりの副腎皮質ステロイド薬投与量について検討を行った．軽症例も含めて約35%の症例を慢性化と判断した．慢性化を目的変数とし，また年齢，性別，発症から治療開始までの期間および病型を説明変数としてロジスティック多重回帰分析を行ったところ，年齢のみが独立して慢性化と関連していた．

今回の解析結果を理解するうえでの注意点としては，軽度な前房炎症の再発も含めて慢性化と定義したため，大きな視機能障害がない症例も慢性化に含まれている．慢性化を真の治療抵抗性の症例に限定すれば，また違った結果となる可能性は否定できない．

VKH病において，年齢は慢性化の独立した危険因子であることが今回の解析で示された．VKH病患者の治療にあたっては，年齢が高いほど慢性化しやすいことに注意する必要がある．

奥貫　陽子[1)]，臼井　嘉彦[1)]，松本　知沙[2)]，
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急性網膜壊死のあたらしい診断基準の作成

急性網膜壊死(ARN)はヘルペスウイルスの眼内感染により生じるが，ウイルスの関与を考慮した診断基準はこれまでに作成されていない．我々は厚生労働科学研究費の助成を受けて本研究班を立ち上げ，診断基準(案)を作成した．次に，2009年1月から2011年12月の間に研究班員所属施設を受診したARN患者46名および対照患者(サイトメガロウイルス網膜炎，眼トキソプラズマ症，サルコイドーシス，Behçet病，結核，梅毒，眼内リンパ腫)409名について，臨床所見と経過，眼内液ウイルス検査結果について後方視的調査研究を行い，その調査結果を診断基準(案)に当てはめ算出した診断パラメータをもとに診断基準を改訂した．

その結果，初期眼所見項目(1a．前房細胞または豚脂様角膜後面沈着物，1b．周辺部網膜の網膜黄白色病変，1c．網膜動脈炎，1d．視神経乳頭発赤，1e．炎症による硝子体混濁，1f．眼圧上昇)，経過項目(2a．病巣は急速に円周方向に拡大，2b．網膜裂孔・網膜剝離，2c．網膜血管閉塞，2d．視神経萎縮，2e．抗ヘルペスウイルス薬に反応)ならびに眼内液検査〔単純ヘルペスウイルス(HSV)-1，HSV-2，帯状疱疹ウイルス(VZV)のいずれかが陽性〕を組み合わせ，確定診断群(1aと1b，経過項目の1項目，眼内液検査を満たす)，臨床診断群(眼内液検査は陰性または未施行だが，初期眼所見項目のうち1aと1bを含む4項目，経過項目の2項目を認め，他疾患を除外できるもの)からなる診断基準を作成した．

本診断基準の診断パラメータは，感度89%，特異度100%，陽性的中率100%，陰性的中率99%と，高いものとなった．本診断基準の妥当性をさらに多施設，多症例で行うために全国調査研究を実施中である．

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急性網膜壊死の診断基準に関する調査研究班
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新しいBehçet病ぶどう膜炎の活動性スコアによる評価の再現性の検討

これまでBehçet病ぶどう膜炎の活動性を評価する指標として，眼発作回数や医師の主観的評価，矯正視力などが用いられてきたが，いずれもぶどう膜炎の活動性を定量化する指標としては不十分な点がある．今回，新しいBehçet病ぶどう膜炎の活動性を定量化する指標としてBehçet's disease ocular attack score 24(BOS24)を作成し，その医師間での再現性を検討した．

BOS24は，眼発作が起きるたびに発作眼について，新たにみられた炎症所見を点数化するものである．片眼につき前房内炎症(最大4点)，硝子体混濁(最大4点)，網膜周辺部病変(最大8点)，後極部網膜病変(最大4点)，中心窩病変(最大2点)，視神経病変(最大2点)について炎症所見の程度を数値化し，最大24点で評価することとした．今回，50回分の眼発作の診療録について，ぶどう膜炎専門家5名がBOS24と主観的重症度(1〜10点)を用いて別々にスコアをつけ，それぞれの方法での医師間の評価の再現性を検討した．

5人の医師による主観的重症度の平均値とBOS24スコアの平均値の間には高い相関性がみられた($p<0.0001$, Spearman's rank-correlation coefficient test)．一方，主観的重症度およびBOS24による5名の評価の変動係数