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Ⅳ．研究成果の刊行物・別刷

Four cases of bilateral acute retinal necrosis with a long interval after the initial onset

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ABSTRACT

Aims To report the clinical features and causative virus of bilateral acute retinal necrosis (BARN) with a long interval after the initial onset.

Methods The causative virus and clinical features were retrospectively investigated in four patients with delayed-onset BARN with an interval of more than 3 years after the onset of the disease in the initially affected eye.

Results The intervals between the initially affected eye and the latter affected eye of the four cases were 8 years 7 months, 19 years 3 months, 9 years 7 months and 3 years 6 months. The fourth patient developed a second recurrence in the latter affected eye 17 years 6 months after the initial inflammation in the fellow eye. In all four cases, the same virus species, either varicella-zoster virus or herpes simplex virus, was detected in both eyes by PCR or antibody detection. In all cases, the final best-corrected visual acuity of the latter affected eye (20/20, 18/20, 20/20 and 12/20, respectively) was better than that of the initially affected eye (20 cm hand motion, light perception-negative, light perception-negative and light perception-positive, respectively).

Conclusion The present findings indicate that delayed-onset BARN in the fellow eye was caused by the same herpes virus species that induced the disease in the first affected eye.

Acute retinal necrosis (ARN) characterised by iritis, vitreitis and vaso-occlusive necrotising retinitis, develops in otherwise healthy patients of all ages and has a devastating visual outcome. The leading cause of ARN is the varicella-zoster virus (VZV) followed by the herpes simplex virus (HSV).[1 2] ARN was first reported in 1971 by Urayama.[3] The report described six cases of unilateral ARN. Bilateral cases were first reported by Willerson *et al* in 1977 as necrotising vaso-occlusive retinitis.[4] Young and Bird first used the term 'BARN' for bilateral cases of ARN in 1978.[5] In many cases of BARN, ocular inflammation in the fellow eye occurs several days or weeks after onset in the initially affected eye.[6–8] Although there are some reports of patients with BARN in which the disease developed in the fellow eye years after disease onset in the initially affected eye, these are single reports and, because delayed-onset BARN is quite rare, the characteristics of delayed-onset BARN have not been elucidated.[9–11]

In the present study, we report four cases of delayed-onset BARN. We investigated whether delayed-onset BARN in the fellow eye was caused by the same virus species as that in the initially affected eye. We also report clinical characteristics of these cases.

PATIENTS AND METHODS

Cases of BARN in which the disease developed in the fellow eye more than 3 years after the initial onset were chosen and retrospectively reviewed. All patients were diagnosed and treated at the Ophthalmology Clinic of Tokyo Medical University Hospital. Clinical features, causative virus and the interval between onset in the first eye and onset in the fellow eye were evaluated. All the patients were immunocompetent.

For detection of the causative virus, VZV, HSV and in some cases cytomegalovirus were examined. The causative virus was determined based on the results of immunoglobulin analysis with a Goldmann–Witmer coefficient of 6 or greater or positive PCR from the aqueous humour or vitreous fluid.[12] Quantitative PCR was used after 2006. All the examinations were performed by SRL (Tokyo, Japan).

RESULTS

All four patients who developed the disease in the fellow eye more than 3 years after onset in the first eye were male. Virus determination was performed in both the initially affected eye and the latter affected eye at the time of disease onset in each eye. In all four cases, the same virus species was detected in both eyes: VZV in two patients and HSV in two patients. In all cases, the final best-corrected visual acuity (BCVA) of the latter affected eye was better than that of the initially affected eye. The clinical course and patient characteristics are shown in the table 1.

Case 1

An 11-year-old boy first presented to an ophthalmologist on 19 December 1999, with complaints of redness in the right eye for 5 days. ARN was suspected by ophthalmologic examination. Treatment with intravenous acyclovir (1050 mg/day) and oral prednisolone (35 mg/day) was started. On 26 December, vitreous and cataract surgeries were performed due to the progression of retinal detachment. HSV-DNA was detected in the aqueous humour and vitreous fluid by PCR. Systemic administration of acyclovir and prednisolone was continued until 17 February. The final BCVA in the right eye was 20/200 at that time.

On 8 June 2008, he visited our department because of 5 days of blurred vision in the previously unaffected left eye. BCVA was 20-cm hand motion in the right eye and 20/20 in the left eye. Peripheral yellowish-white lesions in the temporal and inferior retina and mild retinal vasculitis in the peripheral fundus were observed (figure 1). ARN was suspected and treatment with intravenous

Table 1 Summary of four cases of delayed-onset bilateral acute retinal necrosis (BARN)

		First affected eye		Fellow eye			
Case	Sex	Age	Detected virus (method)	Age	Detected virus (method)	Interval	Final visual acuity: first affected eye/fellow eye
1	M	11	HSV (PCR)	19	HSV (qPCR)	8 years 7 months	HM: 20/20
2	M	47	VZV (GWC)	67	VZV (qPCR)	19 years 3 months	LP(−): 18/20
3	M	43	VZV (PCR)	52	VZV (PCR)	9 years 7 months	LP(−): 20:20
4	M	39	HSV (GWC)	43	HSV (PCR)	3 years 6 months	HM: 20/20
				60	HSV (qPCR)	17 years 6 months	LP(+): 12/20

GWC, Goldmann–Witmer coefficient; HM, hand motion; HSV, herpes simplex virus; LP, light perception; M, male; qPCR, quantitative PCR; VZV, varicella-zoster virus.

acyclovir (2250 mg/day) and betamethasone (6 mg/day) was immediately started. He was diagnosed with HSV-ARN because 1.5×10^4 copies/ml HSV-DNA was detected in the aqueous humour by quantitative PCR. Additional PCR experiments revealed that the HSV type was HSV-2. The necrotic lesions were diminished by systemic administration of acyclovir and corticosteroid, which was continued until 15 July and 23 July, respectively. BCVA in the left eye was maintained at 20/20.

Case 2

A 47-year-old man presented to the ophthalmologist on 17 February 1989, with blurred vision and redness in the right eye. Iridocyclitis and disc oedema were observed. BCVA in the right eye was 20/20 until 25 February, when he experienced a sudden loss of vision. When he was referred to our hospital on 2 March, his BCVA was light perception-negative in the right eye and 20/20 in the left eye. Funduscopic examination revealed diffuse oedema and pallor in the retina, narrowed retinal arteries and an oedematous optic disc with haemorrhage along the retinal vessels. Treatment with intravenous acyclovir (1500 mg/day), interferon-β (3 000 000 IU/day) and oral prednisolone (30 mg/day) was initiated. On 23 March, laser photocoagulation was performed on the localised white lesions that had appeared in the peripheral temporal retina of the left eye. He was diagnosed with VZV-ARN because the Goldmann–Witmer coefficient, calculated using the aqueous humour of the right eye, which was negative at the first visit, was 34.8. On April 4, vitreous surgery was performed on the right eye due to progressive retinal detachment, but the visual acuity of the right eye did not recover. Interferon-β, acyclovir and prednisolone were continued until 6 April, 14 April and 3 May, respectively. BCVA in the left eye was maintained at 20/20.

On 6 June 2008, he visited our department because of 7 days of blurred vision in the left eye. BCVA was light perception-negative in the right eye and 12/20 in the left eye. Anterior chamber cells, disc oedema, patchy granular lesions in the posterior retina and yellowish necrotic lesions between the previous photocoagulation scars were observed (figure 2). ARN was suspected and treatment with intravenous acyclovir (2250 mg/day) and betamethasone (6 mg/day) was immediately started. VZV-DNA was detected by quantitative PCR with 2.7×10^6 copies/ml in the aqueous humour, and he was diagnosed with VZV-ARN. Despite initial enlargement, the necrotic lesions diminished 2 weeks after hospitalised treatment. Systemic administration of acyclovir and corticosteroid was continued until 3 September. The BCVA of the left eye improved to 18/20.

Case 3

In 1995, a 43-year-old man had severe uveitis in the right eye and VZV-DNA was detected by PCR in the aqueous humour. He was diagnosed with VZV-ARN and treated with acyclovir. A cataract operation was performed in 1999, but his visual acuity decreased to hand motion in 2002 because of proliferative vitreoretinopathy. On 30 April 2005, he was referred to our department

Figure 1 Fundus photograph of patient 1. Peripheral white necrotic lesions and mild vasculitis were observed in the temporal and inferior retina.

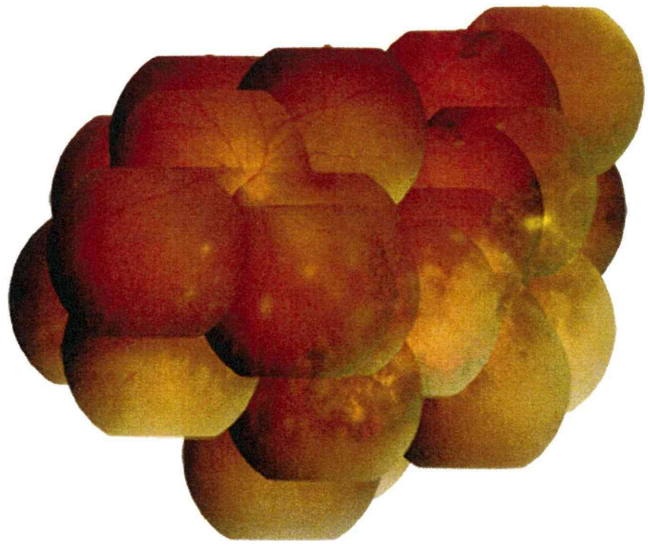

Figure 2 Fundus photograph of patient 2. Yellowish necrotic lesions between the previous photocoagulation scars were observed.

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because of a 2-day history of blurred vision in his previously unaffected left eye. His BCVA was light perception-negative in the right eye and 20/20 in the left eye. Mild inflammation in the anterior chamber and two areas of retinal necrosis were observed in the left eye by ophthalmoscopy. ARN was suspected and treatment with intravenous acyclovir (2250 mg/day) and betamethasone (4 mg/day) was started immediately. Laser photocoagulation was also performed to prevent retinal detachment (figure 3). VZV-DNA was detected in the aqueous humour and he was diagnosed with VZV-ARN. Betamethasone was administrated for 9 days and oral administration of acyclovir was continued until 1 June. The retinal lesions were diminished by the treatments and BCVA was maintained at 20/20.

Case 4

A 39-year-old man was referred to our department on 24 February 1988, due to ocular pain and blurred vision in the right eye starting 8 days earlier. BCVA was 20/50 in the right eye and 20/20 in the left eye. In the right eye, optic disc hyperaemia and oedema, and retinal haemorrhage along the whitish vessels and yellowish lesions were observed in the peripheral retina. He had a history of uveitis in the left eye 20 years prior, although the details were unknown. The left eye was free from active inflammation at this time, but posterior synechiae, cataract and peripheral retinal degeneration were observed. Treatment with intravenous acyclovir (1500 mg/day) and betamethasone (4 mg/day) was started. The Goldmann–Witmer coefficient of HSV examined using the aqueous humour was 18.9 and he was diagnosed with HSV-ARN in the right eye. Vitrectomy and scleral buckling were performed due to the development of retinal detachment. After cataract surgery performed the next year, BCVA was 8/200 in the right eye.

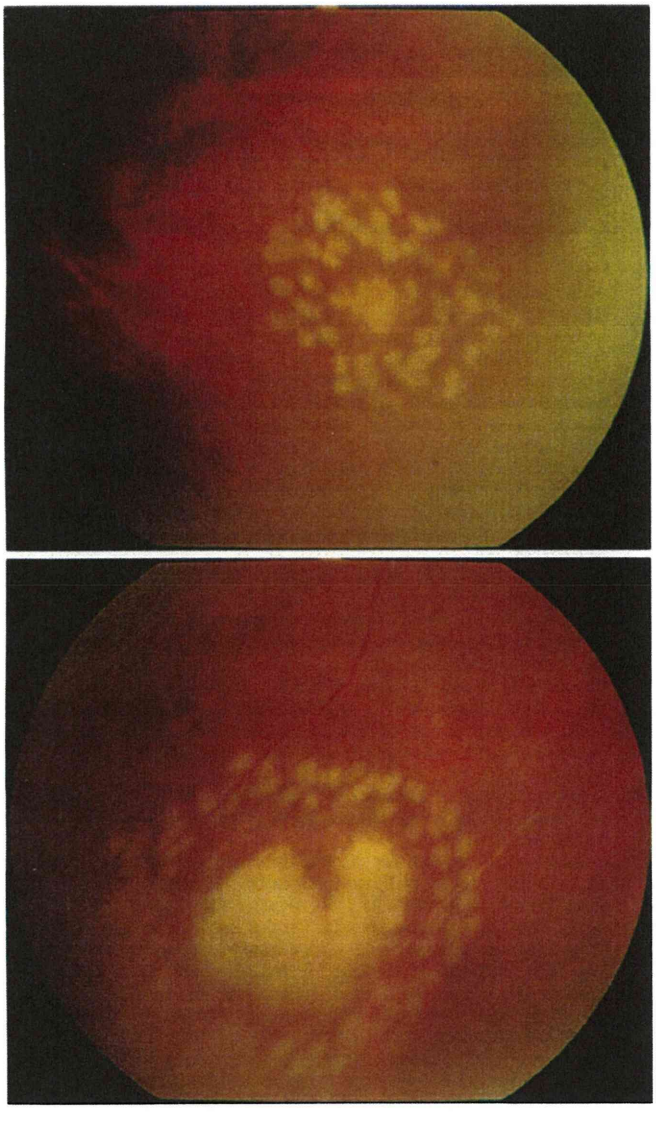

Figure 3 Fundus photograph of the fellow eye of patient 3 after photocoagulation. Two focal necrotising lesions were observed in the peripheral retina.

There was no remarkable change until August 1991. He visited our department on 16 August complaining of floaters and ocular pain for 5 days in the previously unaffected left eye. BCVA was 20/400 in the right eye and 20/20 in the left eye. A dense white retinal lesion was observed in the temporal peripheral retina. ARN was suspected and treatment with acyclovir (1200 mg/day) and interferon-β (3 000 000 IU/day) was started and continued until 11 September and 23 August, respectively. Laser photocoagulation around the retinal lesion was also performed. HSV-DNA was detected by PCR in the aqueous humour and he was diagnosed with HSV-ARN in the left eye. The retinal lesion diminished with the treatment and visual acuity was unchanged.

He was referred to our department again on 10 February 2009. His BCVA was light perception-positive in the right eye and 20/20 in the left eye. Mild anterior chamber cells, keratic precipitates and mild vitreous opacity were observed in the left eye. Because there was no retinal lesion, non-specific ocular inflammation was first suspected. By 3 March, however, small retinal lesions appeared and his BCVA decreased to 20/200 and HSV-DNA was detected by quantitative PCR in the aqueous humour at 4.7×10^2 copies/ml. Additional PCR experiments revealed that the HSV type was HSV-2. He was diagnosed again with HSV-ARN in the left eye. He was treated with intravenous acyclovir (2250 mg/day) and betamethasone (2 mg/day), which were administrated until 8 April and 11 March, respectively. Vitrectomy and scleral buckling were necessary due to the development of retinal detachment. On June 2009, his BCVA was 12/20 in the left eye.

DISCUSSION

In the present study, we report four cases of delayed-onset BARN in which the causative virus was diagnosed in both eyes. In our records of ARN between 1985 and 2009, nine patients developed BARN in addition to the four patients in the present report among 108 consecutive cases. All nine of these patients developed the disease in the fellow eye within 2 months after the initial onset. Consequently, we selected BARN patients who developed the disease in the fellow eye more than 3 years after the initial onset of delayed-onset BARN. In several previous reports of BARN,[1 8–11 13–17] the disease developed in the fellow eye either within a few months or more than 3 years after disease onset in the initially affected eye, and very few patients developed the disease in the fellow eye between a few months and 3 years. Consequently, it is reasonable that these four patients were considered to have delayed-onset BARN separately from so-called BARN in which the fellow eye disease develops within a few months.

Pathways of viral spread in BARN and the immune response in ARN are not fully understood. Based on studies of animal

models with ARN induced by HSV-1, several routes, such as the optic nerve and optic chiasm, parasympathetic pathways and the suprachiasmatic nucleus,[18–20] were suggested for viral spread to the contralateral eye. In experimental ARN, T cells, natural killer cells and neutrophils are thought to be important in the prevention of viral spread.[21–24] Although animal studies using HSV-1 do not fully apply to human ARN in which HSV-2 or VZV is also associated with the development, a similar mechanism of viral spread is suggested for human ARN.[25] After the initial active infection, the virus establishes latency in the sensory neurons. Although the part of the nervous system in which the virus causing delayed-onset BARN maintains latency is not clear, HSV-1 is reported to be latent not only in the trigeminal ganglion but also in dorsal root ganglia, sympathetic ganglia and brain.[26] Latency is thought to be maintained by a balance of the host immune responses mediated by CD8 T cells, immunologic status of the neuron and the virus condition.[27] Delayed-onset BARN might develop when the mechanism to maintain latency becomes imbalanced.

In the present report, the causative virus was detected in both the eye with the first onset and the eye with later onset, and the same virus species was detected in both eyes of each patient. Although a couple of previous reports of delayed-onset BARN describe the causative virus for the fellow eye, few reports describe the causative virus of the onset in the first affected eye.[10 11 15 16] Our findings that the same causative virus species was detected in both eyes in delayed-onset BARN suggest that the reactivated latent virus that affected the first eye is the causative virus of the disease in the fellow eye, rather than the fellow eye being affected by a different virus. Subtyping or genotyping of the virus, however, is needed to determine if the same virus induced the fellow eye disease, especially in cases 1 and 4, whose causative virus in the fellow eye was HSV-2, but the HSV subtype in the initially affected eye was unknown.

In general, inflammation and retinal necrosis in the fellow eye in BARN is less severe compared with that in the first affected eye. In the present report of delayed-onset BARN, the severity of the disease in the fellow eye was lower than that in the first affected eye in all four cases and none of the patients required surgical treatment for the fellow eye except case 4, who developed the disease twice in the fellow eye. In BARN, in which the fellow eye disease develops within a few months, one reason for the mildness of the fellow eye disease might be that antivirus treatment of the first affected eye effectively reduces the severity of the disease in the fellow eye. There might also be some mechanisms that reduce inflammation and retinal necrosis of the fellow eye in delayed-onset BARN, or earlier diagnosis and better treatment in the second affected eye may partly contribute to better prognosis. Although not investigated in retinal infection, vaccination is reportedly effective for reducing the severity of recurrent HSV-1 induced keratitis in mice and herpes zoster in humans.[28 29] In a murine model of HSV-1 reactivation in the trigeminal ganglion, the immune cells observed earliest in the trigeminal ganglion were T cells, which are suggested to be virus-specific memory cells,[30] and in vitro-activated virus-specific T lymphocytes have a protective effect on contralateral retinitis.[31] Consequently, in delayed-onset BARN, immune reactions occurring at the time of the initial inflammation might prime immune cells, working like a vaccination, which then functions to suppress the second infection.

In conclusion, the same virus species is suggested to be the causative virus of the disease in the fellow eye in delayed-onset BARN. Although delayed-onset BARN is very rare, physicians must consider the possibility of the development of ARN in the fellow eye, even many years after the initial onset.

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Ethics approval This study was conducted with the approval of the Ethics Committee in Tokyo Medical University Hospital.

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Four cases of bilateral acute retinal necrosis with a long interval after the initial onset

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Relation of Intraocular Concentrations of Inflammatory Factors and Improvement of Macular Edema After Vitrectomy in Branch Retinal Vein Occlusion

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• PURPOSE: To investigate the association of intraocular concentrations of inflammatory factors and improvement of macular edema after vitrectomy for patients with macular edema in branch retinal vein occlusion (BRVO).

• DESIGN: Retrospective case-control study.

• METHODS: Seventeen patients with BRVO who underwent vitreous surgery for macular edema and 15 control patients were enrolled from Hachioji Medical Center of Tokyo Medical University. The concentrations of eight inflammatory factors were measured in vitreous and aqueous fluids obtained at the time of vitrectomy using a flow cytometer. Macular thickness was measured by optical coherence tomography before and one, three, and six months after surgery. Correlations between the concentrations of inflammatory factors and macular thickness were statistically analyzed.

• RESULTS: Higher aqueous and vitreous concentrations of vascular endothelial growth factor (VEGF) and interleukin (IL)-8 were significantly correlated with a greater difference in macular thickness between before and six months after surgery (vitreous VEGF, $P = .047$; aqueous VEGF, $P = .032$; vitreous IL-8, $P = .016$; and aqueous IL-8, $P = .032$). Higher intraocular concentrations of monokine induced by interferon γ (Mig) were significantly correlated with a smaller degree of macular thickness six months after surgery (vitreous Mig, $P = .038$; aqueous Mig, $P = .009$).

• CONCLUSION: High preoperative VEGF, IL-8, and Mig concentrations were associated with improvement of macular edema six months after vitreous surgery in patients with macular edema attributable to BRVO. (Am J Ophthalmol 2011;151:610–616. © 2011 by Elsevier Inc. All rights reserved.)

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BRANCH RETINAL VEIN OCCLUSION (BRVO) IS A COMMON retinal vascular occlusive disease, characterized by vascular obstruction leading to intraretinal hemorrhage, exudation of fluid, and variable degrees of ischemia. Macular edema, the most frequent cause of visual impairment in BRVO, complicates approximately 60% of temporal branch retinal vein occlusions and becomes chronic in two-thirds of these cases.[1,2] Although a variety of treatments are applied for BRVO with macular edema, the effects of these treatments are not always satisfactory and are associated with some disadvantages.[3]

Intravitreous injection of triamcinolone acetonide (TA) provides short-term improvement of visual acuity (VA) and macular edema, but the effects do not persist for more than several months.[4] Increased intraocular pressure and posterior subcapsular cataracts are major side effects of TA therapy, whose frequencies are both approximately 40%.[4] Grid laser photocoagulation is best studied in the Branch Vein Occlusion Study. According to this study, the effect of grid laser photocoagulation is greater in patients with macular edema in BRVO than in patients with nontreated controls.[5] Reports by another group, however, indicate that the effect of grid laser photocoagulation in patients with BRVO differed little from that in control groups.[6,7] Intravitreal injection of anti–vascular endothelial growth factor (VEGF) antibody is currently used in the treatment for macular edema in BRVO. No ocular toxicity or adverse side effects have been observed,[8] and VEGF injection reduces macular thickness[9,10] and improves visual acuity to a greater degree than grid laser photocoagulation.[11] However, the high recurrence rate and short-term effectiveness are limitations of this treatment.[12,13]

Multiple chemokines, cytokines, and growth factors have been detected in the ocular fluids of patients with BRVO, such as VEGF, interleukin (IL)-1α, IL-6, IL-8, interferon-inducible protein-10 (IP-10), monocyte chemotactic protein (MCP)-1, erythropoietin, platelet-derived growth factor-AA, and pigment epithelium–derived factor.[14–17] In particular, aqueous humor and vitreous fluids obtained from patients with BRVO contain higher concentrations of IL-6, IL-8, MCP-1, and VEGF than those obtained from control patients.[16] In addition, Noma and associates reported that high vitreous and aqueous concentrations of VEGF and IL-6 are correlated with the

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TABLE 1. Intraocular Concentrations of Inflammatory Factors in Patients With BRVO and Control Patients

	Control (n = 15) Median (Range) (pg/mL)	BRVO (n = 17) Median (Range) (pg/mL)	*P* Value[a]
Vitreous VEGF	0 (0–70.7)	131.7 (0–741.5)	.001
Aqueous VEGF	80.46 (0–144.3)	108.7 (0–445.5)	.06
Vitreous IL-8	3.04 (1.4–7.9)	52.4 (1.4–140.2)	<.001
Aqueous IL-8	4.7 (1.9–7.4)	24.7 (2.6–96.6)	.001
Vitreous Mig	15.7 (1.3–74.3)	72.2 (13.3–243.2)	<.001
Aqueous Mig	16.6 (3.2–108.6)	55.1 (20.6–635.4)	<.001
Vitreous MCP-1	334.9 (67.3–966.2)	832.2 (24.7–1486.2)	.009
Aqueous MCP-1	389.2 (169.5–743.0)	562.5 (28.8–1812.3)	.04
Vitreous IP-10	27.8 (3.6–152.6)	143.6 (30.0–444.1)	<.001
Aqueous IP-10	28.1 (0–80.0)	76.9 (14.2–520.9)	.003

BRVO = branch retinal vein occlusion; IL = interleukin; IP = inducible protein; MCP = monocyte chemotactic protein; Mig = monokine induced by interferon γ; VEGF = vascular endothelial growth factor.

[a]Mann-Whitney *U* test.

preoperative severity of macular edema,[18,19] and they suggested that the two factors may be used as an index to evaluate the ischemic condition of macular edema in BRVO.[20] The levels of these factors alter the microcirculation, affecting macular edema and neovascularization in patients with BRVO. Vitrectomy is considered to be an effective treatment for macular edema in BRVO, and one of the mechanisms of the effects of vitrectomy for macular edema is considered to be the removal of a variety of chemical mediators contained in the vitreous gel.[21,22] In a previous report, central macular thickness decreased in 34.3% of patients with BRVO after vitrectomy, and best-corrected visual acuity (BCVA) improved in approximately 70% of the patients.[23–25] But vitrectomy is not always effective because BCVA fails to improve after vitrectomy in approximately 30% of patients with BRVO with macular edema.[23,24]

Although a single chemical factor is not sufficient to explain the pathogenesis of BRVO, the investigation of multiple inflammatory factors would be very helpful to understand the mechanism of improvement of macular edema after vitrectomy. In the present study, we evaluated multiple intraocular immune mediators in the vitreous gel and aqueous humor obtained at the time of vitreous surgery for macular edema in patients with BRVO using a cytometric bead array system,[26,27] and statistically compared the results with the degree of improvement in macular edema.

PATIENTS AND METHODS

• **PATIENTS:** Seventeen patients with BRVO (age 68.2 ± 7.8 years; 9 men, 8 women) and 15 control patients (age 65.3 ± 8.7 years; 7 men, 8 women) who underwent pars plana vitrectomy at Hachiouji Medical Center of Tokyo Medical University were enrolled in this study. Cataract surgery was also performed in all of the patients at the time of the vitrectomy. Inclusion criteria included BRVO patients with 1) clinically detectable macular edema persisting for more than three months, 2) BCVA worse than 0.7 on a decimal chart, and 3) prolonged macular edema even after photocoagulation. Control patients comprised 10 patients with a macular hole and five patients with an epiretinal membrane. None of the control patients had ocular inflammatory disease, macular edema, or vascular insufficiency with ischemia. Exclusion criteria included a history of vitreous hemorrhage or intraocular surgery. There were no significant differences in age or in the male-to-female ratio between BRVO and control patients. Mean interval between disease onset and vitrectomy was 8.3 ± 5.0 months for the patients with BRVO. Five patients with BRVO had undergone retinal photocoagulation before the surgery.

• **SURGICAL METHODS AND SAMPLE COLLECTION:** Vitreous fluid and aqueous humor samples were obtained at the time of surgery. Pars plana vitrectomy was performed using TA during surgery to visualize the vitreous. The inner limiting membrane was removed in all BRVO patients. The vitreous and aqueous samples were immediately frozen and stored at −80°C. The samples were assayed within six months of collection.

• **FLOW CYTOMETRIC ANALYSIS:** Vitreous and aqueous concentrations of human VEGF, IL-8, monokine induced by interferon γ (Mig), MCP-1, IP-10, basic fibroblast growth factor (basic-FGF), granulocyte-macrophage colony stimulating factor (GM-CSF), and regulated upon activation, normal T-cell expressed and secreted (RANTES)

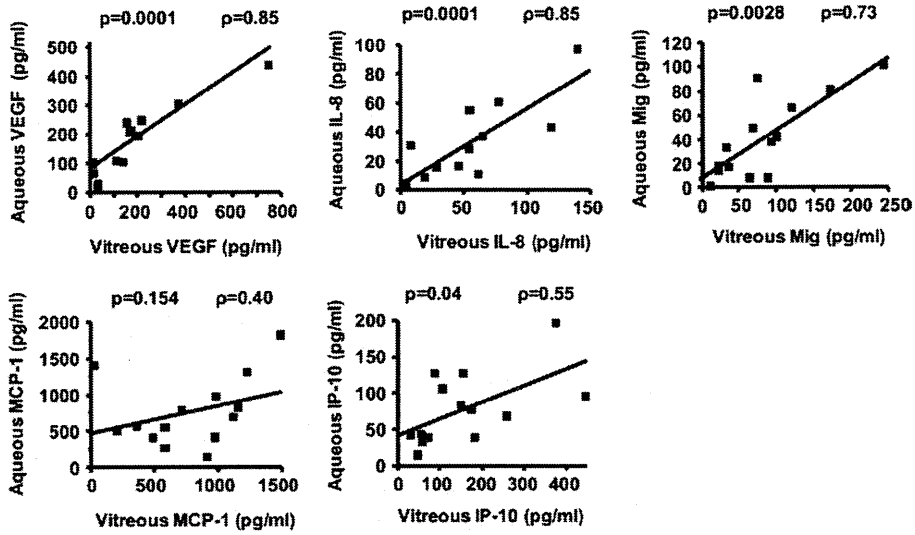

FIGURE. Correlations of vitreous and aqueous concentrations of inflammatory factors in branch retinal vein occlusion. Vitreous and aqueous concentrations of VEGF, IL-8, Mig, and IP-10 were statistically correlated. The data were analyzed by Spearman rank correlation test.

TABLE 2. Comparison of Macular Thickness and Best-Corrected Visual Acuity Before and After Vitreous Surgery

	Before Vitreous Surgery	After Vitreous Surgery			
		1 Month	3 Months	6 Months	1 Year
	(n = 17)	(n = 16)	(n = 15)	(n = 12)	(n = 14)
Macular thickness (μm)/median (range)	450 (238–867)	271 (131–715)	335 (164–650)	266.5 (144–581)	n.a.
P value[a]		.016	.012	.021	
	(n = 17)	(n = 17)	(n = 15)	(n = 15)	
logMAR VA/median (range)	0.52 (0.22–1.30)	0.52 (0.15–1.70)	0.40 (0.15–1.40)	0.40 (0.05–1.53)	0.46 (0.05–1.30)
P value[a]		.11	.006	.02	0.004

logMAR VA = logarithm of the minimal angle of resolution visual acuity; n.a. = not applicable.
[a]Wilcoxon signed rank test.

were quantified using the BD Cytometric Bead Array Flex Set System and BD Human Soluble Protein Master Buffer Kit (BD Bioscience-PharMingen, San Diego, California, USA) according to the methods recommended by the manufacturer. Briefly, samples were incubated with a mixture of capture beads specific to inflammatory factors and then with phycoerythrin-conjugated detection antibodies. Two-color flow cytometric analysis was performed using a FACSCalibur flow cytometer (BD Immunocytometry Systems, San Jose, California, USA).[26] Data were analyzed by FCAP Array (BD Immunocytometry Systems) analysis software.

• MEASUREMENT OF MACULAR THICKNESS: Macular thickness was measured by optical coherence tomography (OCT3000; Carl Zeiss, Dublin, California, USA) using the fast macular scan protocol. Measurement was performed within one week before surgery, and one, three, and six months after surgery.

• STATISTICAL ANALYSIS: The intraocular concentrations of inflammatory factors, macular thickness, and BCVA are expressed as median (range), and the data were analyzed using Mann-Whitney U test or Wilcoxon signed rank test. BCVA was measured using a decimal chart and was converted to the logarithm of the minimal angle of resolution. Correlations of inflammatory factors and macular thickness were analyzed by Spearman rank correlation test. A *P* value of less than .05 was considered significant. All analyses were performed using JMP statistical analysis software, version 7 (SAS Institute, Cary, North Carolina, USA).

RESULTS

• CONCENTRATION OF INFLAMMATORY FACTORS IN OCULAR FLUIDS FROM PATIENTS WITH BRANCH RETINAL VEIN OCCLUSION WITH MACULAR EDEMA: In patients with BRVO, vitreous and aqueous concentrations of

TABLE 3. Correlation Between Concentrations of Intraocular Inflammatory Factors and Macular Thickness

	Correlation With Macular Thickness Before Vitreous Surgery (n = 15)[a]	Correlation With Macular Thickness After Vitreous Surgery[a]		
		1 Month (n = 14)	3 Months (n = 13)	6 Months (n = 11)
	ρ/*P*	ρ/*P*	ρ/*P*	ρ/*P*
Vitreous VEGF	0.16/.58	−0.18/.54	−0.27/.37	−0.37/.29
Aqueous VEGF	0.08/.77	0.56/.037	−0.16/.86	−0.28/.40
Vitreous IL-8	0.33/.23	−0.16/.60	−0.16/.60	−0.31/.38
Aqueous IL-8	0.64/.010	0.26/.37	0.22/.44	0.009/.98
Vitreous Mig	0.30/.29	−0.17/.55	−0.40/.18	−0.66/.038[b]
Aqueous Mig	0.12/.67	0.40/.15	−0.60/.017[a]	−0.75/.009[b]
Vitreous MCP-1	0.20/.47	0.14/.62	0.071/.82	0.006/.99
Aqueous MCP-1	0.46/.081	−0.09/.76	0.46/.087	0.45/.16
Vitreous IP-10	0.10/.71	−0.09/.77	−0.006/.96	−0.18/.63
Aqueous IP-10	0.30/.29	0.07/.81	−0.040/.89	−0.12/.73

IL = interleukin; IP = inducible protein; MCP = monocyte chemotactic protein; Mig = monokine induced by interferon γ; VEGF = vascular endothelial growth factor.
[a]Data were analyzed by Spearman rank correlation test.
[b]$P < .05$.

VEGF (vitreous, 131.7 [0–741.5] pg/mL; aqueous, 108.7 [0–445.5] pg/mL), IL-8 (vitreous, 52.4 [1.4–140.2] pg/mL; aqueous, 24.7 [2.6–96.6] pg/mL), Mig (vitreous, 72.2 [13.3–243.2] pg/mL; aqueous, 55.1 [20.6–635.4] pg/mL), MCP-1(vitreous, 832.2 [24.7–1486.2] pg/mL; aqueous, 562.5 [28.8–1812.3] pg/mL), and IP-10 (vitreous, 143.6 [30.0–444.1] pg/mL; aqueous, 76.9 [14.2–520.9] pg/mL) were above the detection limit, and the concentrations of all of these factors were significantly higher in the aqueous and vitreous fluids of patients with BRVO than those of the control patients (Table 1). Concentrations of basic FGF, GM-CSF, and RANTES were below the detection limit in almost all vitreous and aqueous samples. The vitreous and aqueous concentrations of VEGF, IL-8, Mig, and IP-10 in patients with BRVO were statistically correlated (VEGF, $\rho = 0.85$, $P = .0001$; IL-8, $\rho = 0.85$, $P = .0001$; Mig, $\rho = 0.73$, $P = .0028$; IP-10, $\rho = 0.55$, $P = .04$) (Figure).

• CORRELATION OF MACULAR THICKNESS WITH INFLAMMATORY FACTORS: Macular thickness measured one, three, and six months after surgery (271 [131–715] μm, 335 [164–650] μm, and 266.5 [144–581] μm, respectively) was significantly decreased compared with macular thickness before surgery (450 [238–867] μm) in patients with BRVO ($P = .016$, $P = .012$, and $P = .021$, respectively). BCVA was not statistically improved at one month (0.52 [0.15–1.7]) after surgery; however, it was significantly improved at three months (0.40 [0.15–1.4]), six months (0.40 [0.05–1.53]), and one year (0.46 [0.05–1.3]) after surgery, compared with before surgery (0.52 [0.22–1.3]) ($P = .006$, $P = .02$, and $P = .004$, respectively) (Table 2). Macular thickness before surgery and one, three, and six months after surgery was statistically compared with vitreous and aqueous concentrations of VEGF, IL-8, Mig, MCP-1, and IP-10. High vitreous and aqueous Mig concentrations were significantly inversely correlated with macular thickness measured six months after surgery (vitreous Mig, $\rho = -0.66$, $P = .038$; aqueous Mig, $\rho = -0.75$, $P = .009$) (Table 3).

TABLE 4. Correlation of Concentrations of Intraocular Inflammatory Factors and Difference in Macular Thickness Before and After Vitreous Surgery

	Before/1 Month After (n = 14)[a]	Before/3 Months After (n = 13)[a]	Before/6 Months After (n = 11)[a]
	ρ/*P*	ρ/*P*	ρ/*P*
Vitreous VEGF	0.46/.10	0.45/.12	0.63/.047[b]
Aqueous VEGF	0.56/.04[b]	0.27/.4	0.65/.032[b]
Vitreous IL-8	0.48/.08	0.42/.15	0.73/.016[b]
Aqueous IL-8	0.26/.37	0.21/.47	0.65/.032[b]
Vitreous Mig	0.17/.56	0.45/.1	0.56/.09
Aqueous Mig	0.40/.15	0.37/.18	0.45/.16
Vitreous MCP-1	0.12/.70	0/>.999	0.39/.26
Aqueous MCP-1	−0.09/.76	−0.10/.73	0.19/.53
Vitreous IP-10	0.26/.37	0.12/.71	0.53/.12
Aqueous IP-10	0.07/.81	0.10/.73	0.36/.27

IL = interleukin; IP = inducible protein; MCP = monocyte chemotactic protein; Mig = monokine induced by interferon γ; VEGF = vascular endothelial growth factor.
[a]Difference in macular thickness between before and after surgery was analyzed by Spearman rank correlation test.
[b]$P < .05$.

Mean difference and range of macular thickness between before and after surgery are 133.8 (-321 to 468) μm at one month, 143.3 (-268 to 532) μm at three months, and 176.9 (-343 to 500) μm at six months after surgery. The relation between intraocular concentrations of VEGF, IL-8, Mig, MCP-1, and IP-10 and the change in macular thickness at one, three, or six months after surgery were evaluated (Table 4). At six months after surgery, the difference in macular thickness was significantly correlated with the vitreous and aqueous concentrations of VEGF (vitreous, $\rho = 0.63$, $P = .047$; aqueous, $\rho = 0.65$, $P = .032$), and IL-8 (vitreous, $\rho = 0.73$, $P = .016$; aqueous, $\rho = 0.65$, $P = .032$), although these correlations were not significant at one and three months after surgery, with the exception of aqueous VEGF at one month.

DISCUSSION

IN THE PRESENT STUDY, MACULAR THICKNESS OF THE patients with higher levels of intravitreous and aqueous VEGF and IL-8 was significantly reduced after vitreous surgery compared with macular thickness before surgery in patients with BRVO. Higher intraocular Mig concentrations were statistically associated with a smaller macular thickness measured six months after surgery. In the report by Yamasaki and associates, macular edema of patients with higher intravitreous VEGF concentrations improved more than that of patients with lower intravitreous VEGF in BRVO.[28] To our knowledge, there are no previous reports that present an association between intraocular IL-8 or Mig concentrations and postoperative macular thickness in patients with BRVO.

VEGF is an angiogenic protein and a potent vascular permeability factor that is associated with the formation of macular edema in ocular disease, particularly in diabetic retinopathy and BRVO.[29] Its expression increases in response to hypoxia in various intraocular tissues.[30] IL-8 and Mig are members of the CXC chemokine family, which includes IP-10. Among the CXC chemokine family members, IL-8 is an angiogenic chemokine with a proangiogenic glutamine-leucine-arginine (ELR) motif in the amino acid sequence, whereas Mig and IP-10 are angiostatic chemokines that lack the ELR motif.[31,32]

IL-8 was originally studied as a neutrophil attractant that mediates host immune response to injury and infection, but it also has a role as a mediator of vascular permeability and angiogenesis.[33,34] IL-8 is secreted by retinal pigment epithelial cells in response to proinflammatory factors, for example tumor necrosis factor-α, IL-1β, IL-7, and Toll-like receptors.[35–38] Its expression is also reported in retinal glial cells, retinal microvascular endothelial cells, macrophages, and endothelial cells.[39,40]

Mig is a critical component of many immune responses as a chemoattractant of activated natural killer and Th1 cells that express CXC chemokine receptor 3 (CXCR3),[41] which is a common receptor for Mig, IP-10, and inteferon-inducible T-cell α chemoattractant. Mig expression, which is activated by stimulation with IFN-γ,[42] is observed in a variety of tissues under inflammatory conditions including inflammatory and noninflammatory cells.[43,44] The angiostatic activity of Mig has been studied mainly in regard to antitumor therapies.[45,46] Although the intraocular expression of Mig and CXCR3 is unknown, CXCR3 and Mig, expressed in murine brain endothelial cells in the central nervous system, are suggested to inhibit inflammation-induced destruction of the blood-brain barrier.[47]

In the present report, high vitreous and aqueous VEGF and IL-8 concentrations were associated with decreased macular thickness measured six months after surgery. An association of higher intravitreous VEGF and greater improvement of macular edema after vitrectomy is consistent with the report by Yamasaki and associates.[28] In our study, IL-8 also correlated with the improvement of macular edema. This finding is consistent with the notion that one of the mechanisms by which vitrectomy reduces macular edema is the removal of vitreous gel containing chemical mediators, because the decrease in intraocular VEGF and IL-8 through removal of the vitreous improved the macular edema.

Unlike VEGF and IL-8, the intraocular concentration of Mig, an angiostatic factor, was not significantly correlated with the change in macular thickness after surgery. In contrast, a high presurgical intraocular Mig concentration was associated with a smaller macula thickness six months after surgery. Our group previously reported an increased level of intravitreous Mig in patients with diabetic macular edema.[48] Because Mig levels are high in patients with BRVO and diabetic retinopathy, it may have some function in intraocular ischemic diseases. Although the level of intraocular CXCR3 expression is not known, Mig is suggested to reduce macular edema by protecting the blood-retinal barrier, which is impaired in patients with BRVO, as Mig protects the blood-brain barrier.[47]

The present study revealed that intraocular concentrations of VEGF, IL-8, and Mig correlated with the improvement of macular edema in patients with BRVO after vitrectomy. Mig, however, inversely correlated with macular thickness. This might be attributable to the fact that VEGF and IL-8 are angiogenic factors and stimulators of vascular permeability that are supposed to stimulate each other.[49,50] On the other hand, the function of Mig is opposite to that of VEGF and IL-8 with respect to angiogenesis and vascular permeability. Consequently, inflammatory factors with different and opposite functions might be involved in the formation and improvement of macular edema in BRVO. Further studies are required to determine whether evaluation of inflammatory factors would be useful for preoperative assessment of the application of vitrectomy for macular edema in BRVO. In conclusion, the findings of the present study suggest that intraocular VEGF, IL-8, and Mig are associated with the improvement in macular edema after vitrectomy in patients with BRVO.

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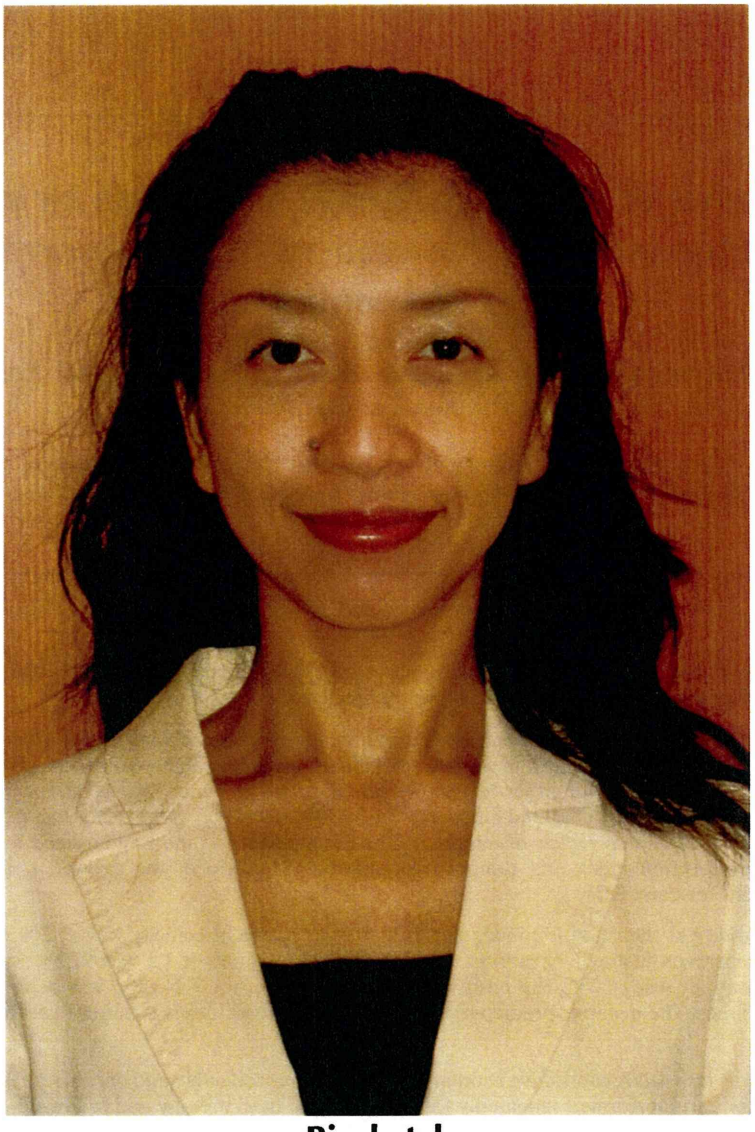

Biosketch

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CLINICAL INVESTIGATION

Diagnosis of Uveal Malignant Melanoma by a New Semiquantitative Assessment of *N*-isopropyl-*p*-[^{123}I]-Iodoamphetamine

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Abstract

Purpose: To semiquantify the uptake of *N*-isopropyl-*p*-[^{123}I]-iodoamphetamine (I-123 IMP) in patients with uveal malignant melanoma reproducibly and objectively.

Methods: Fifty-two patients were examined. Twenty-nine patients had malignant melanoma (group A), three were clinically diagnosed with metastatic choroidal tumor, and 20 patients were given either histological or clinical diagnoses of either benign pigmented lesion or tumor (group B). Early and delayed I-123 IMP images were obtained and standardized by Neurostat software. Using fusion software, we applied a three-dimensional region of interest (3D-ROI) template to the standardized I-123 IMP, and calculated the retention index and tumor-to-nontumor (T/N) ratio of the delayed phase using the maximum count for each ROI.

Results: Sensitivity at the retention index cutoff of 30 was 82.4%, specificity was 85.2%, and accuracy was 83.6%. Sensitivity at the T/N ratio of the delayed phase cutoff of 1.3 was 91.2%, specificity was 77.8%, and accuracy was 85.2%. The positive predictive value of the T/N ratio was better than that of the retention index. The negative predictive value of the retention index was better than that of the T/N ratio.

Conclusion: This new semiquantitative estimation method is reproducible and objective, especially when the examinations are performed repeatedly for evaluation of both therapy and follow-up. **Jpn J Ophthalmol** 2011;55:148–154 © Japanese Ophthalmological Society 2011

Keywords: I-123 IMP, malignant melanoma, semiquantification, uveal tumor

Introduction

The usefulness of *N*-isopropyl-*p*-[^{123}I]-iodoamphetamine (I-123 IMP) for the diagnosis of uveal malignant melanoma is well recognized.[1–4] However, the accurate estimation of its uptake is sometimes difficult when the images acquired 24 h after injection become so faint that the region of interest (ROI) established for semiquantification becomes arbitrary. Using Neurostat software (Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA),[5,6] we attempted to create a new globular template adjusted to bilateral eyeballs. This software package is widely used for statistical analysis of brain images and retrospectively semiquantifies the uptake of I-123 IMP in patients with uveal malignant melanoma.

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Materials and Methods

Materials

We evaluated 52 patients (27 women, 25 men) in a total of 61 examinations (Table 1). Histologically proven uveal malignant melanoma was found in 29 patients receiving 34 examinations (group A); three patients were clinically

diagnosed with metastatic choroidal tumor from the lungs and breast; and 21 patients (24 examinations) received a histological or clinical diagnosis of either a benign pigmented lesion or a tumor (group B). Benign lesions included hemangioma, adenoma (excised), melanocytoma, nevocellular nevus (excised), macular degeneration, and either postplaque radiotherapy or enucleated melanoma without recurrent lesion. In nontreated cases, the ocular manifestations had been stable for more than 1 year.

Table 1. Patient and examination numbers by tumor type

Tumor type	Number of patients (examinations)
Group A	
Malignant melanoma	29 (34)
Group B	
Metastasis from breast cancer	1
Metastasis from lung cancer	2
Melanocytoma	10 (11)
Nevocellular nevus	2
Macular degeneration	2
Medulloepithelioma	1
Mesectodermal leiomyoma of ciliary body	1
Ciliary epithelial adenoma	1
Hemangioma	2
Hematoma	1
Post-therapeutic malignant melanoma without recurrence	1 (3)

I-123 IMP Single Photon Emission Computed Tomography Studies

Early images were obtained 20 min after an intravenous injection of 111 MBq I-123 IMP, supplied by Nihon Medi-Physics (Nishinomiya, Japan). Delayed images were obtained after 24 h. In each phase, whole-body and single photon emission computed tomography (SPECT) images were obtained during a period of 30 min using an IRIX triple head gamma-camera (Picker International, Cleveland, OH, USA) equipped with a high-resolution parallel collimator. The acquisition parameters included a 20% energy window centered on 159 KeV, 120 projection angles over 360°, and a 128 × 128 matrix with a pixel width of 2.3 mm in the projection domain. The projection images were reconstructed by filtered backprojection and filtered by a low-pass filter. For uniform attenuation correction, Chang's first-order method was used. The projection data were profiltered through a Butterworth filter. No attenuation correction was performed.

Data Analysis

We first made a three-dimensional (3D) ROI template using the anatomically standardized magnetic resonance imaging (MRI) results of eight patients without any abnormal find-

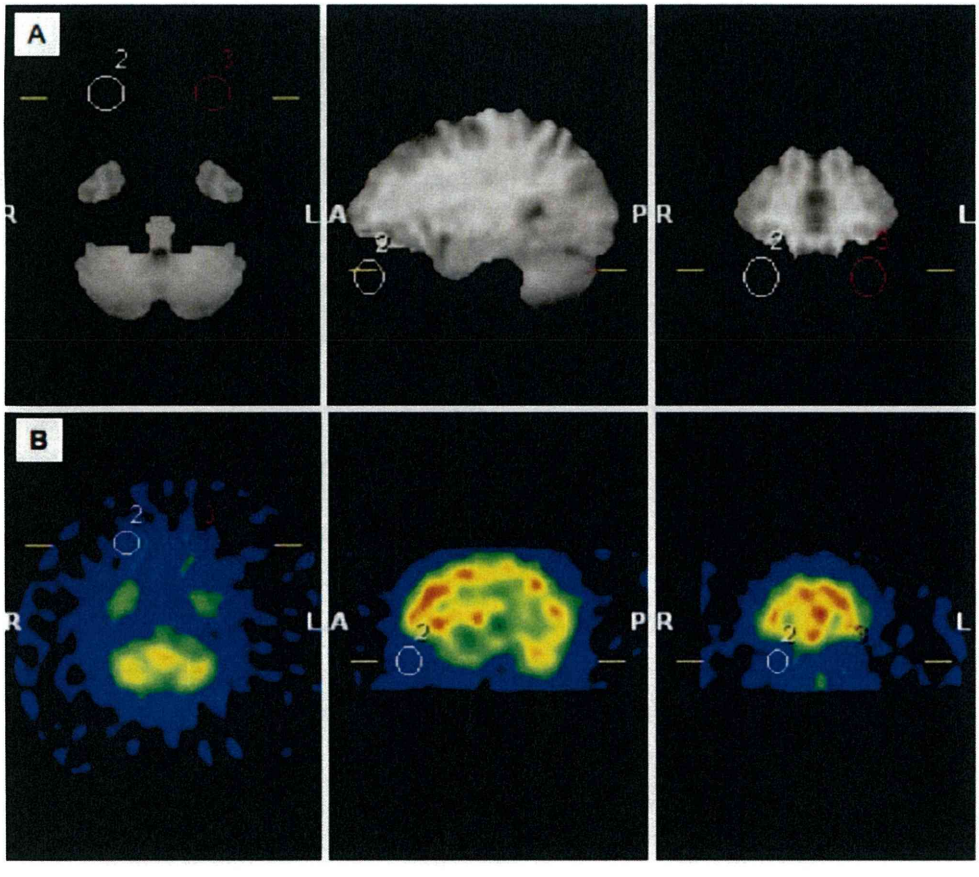

Figure 1. A Three-dimensional region of interest (3D-ROI) template drawn on a normalized brain magnetic resonance imaging scan. **B** The same template adjusted by Fusion Viewer to a 3D-volume *N*-isopropyl-*p*-[^{123}I]-iodoamphetamine (I-123 IMP), which was also standardized by Neurostat software.

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ings. MRI scanning was performed with a Signa, 1.5-T scanner (GE Yokogawa Medical Systems, Tokyo, Japan). A regular head coil and conventional T2-weighted image in 3D acquisition mode were used. Individual MRI scans were anatomically normalized with Neurostat software. This software uses linear scaling to correct each individual brain size and nonlinear warping to minimize regional anatomic variations of the subject. In the linear-scaling step, the anteroposterior length and width of the brain were measured on the MRI images, and the brain height was estimated by a contour-matching procedure using the midsagittal plane. In the nonlinear warping step, individual locations were matched with those of a standardized brain.[5,6] When the brain image was standardized in three dimensions, the eyeball image was standardized in three dimensions, too. We applied globular ROIs with a volume of 8.38 cm^3 each to all the standardized eyeballs of the eight cases. The 3D-ROI template determined from this standardized MRI was adjusted by fusion software, Fusion Viewer (Nihon Medi-Physics, Nishinomiya, Japan) to the 3D volume image of the I-123 IMP, which was also standardized with Neurostat software (Fig. 1).

The retention index [= (delayed ratio – early ratio)/early ratio × 100 (%)] and the tumor-to-nontumor ratio (T/N ratio) of each delayed phase were calculated using the maximum count of each ROI, that is, on the affected side of the eyeball as the tumoral lesion, and on the contralateral side as the nontumoral lesion. To determine the optimal cutoff point, a tentative cutoff point was established, ranging from 28 to 34 for the retention index, and from 1.2 to 1.5 for the T/N ratio of the delayed phase. A value equal to or greater than the cutoff point was defined as positive, and a value less than the cutoff point was defined as negative. Based on these values, the diagnostic accuracy was evaluated by means of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Differences in continuous variables between malignant melanoma (group A) and other lesions (group B) were assessed by unpaired Student's *t* test. Significance was set at the $P < 0.01$ level.

The experimental design of the study was approved by the local institutional ethics committee.

Results

Retention Index

The mean retention index of group A was 136.9 ± 162.5 (minimum value, –21.5; maximum value, 783.3), and that of group B was 8.0 ± 22.3 (minimum, –35.1; maximum, 58.7) (Fig. 2). It was significantly higher in group A than in group B ($P < 0.01$, *t* test). Sensitivity when the retention index cutoff was 30 was 82.4%, specificity was 85.2%, PPV was 87.5%, NPV was 79.3%, and accuracy was 83.6% (Table 2).

T/N Ratio of the Delayed Phase

The mean T/N ratio of the delayed phase in group A was 2.4 ± 1.3, with a range from 1.2 to 6.3. On the other hand, group B had a ratio of 1.1 ± 0.2 (minimum, 0.75; maximum, 1.7) (Fig. 3). The ratio was significantly higher in group A than in group B ($P < 0.01$, *t* test). Sensitivity when the T/N ratio of the delayed phase cutoff was 1.3 was 91.2%, specificity was 77.8%, PPV was 83.8%, NPV was 87.5%, and accuracy was 85.2% (Table 3).

Figure 4 shows a typical case of uveal malignant melanoma. The retention index was 176.7, and the T/N ratio of

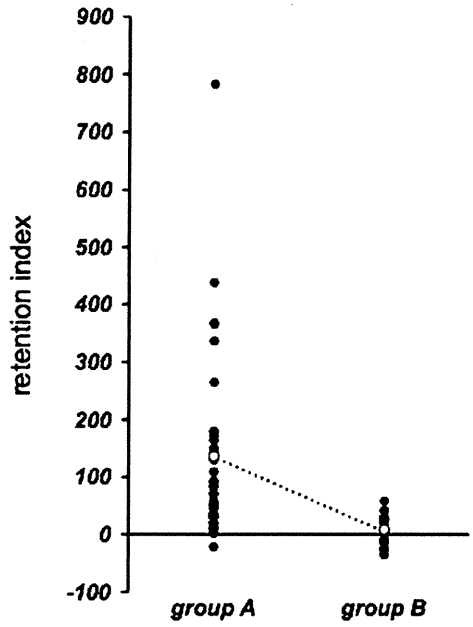

Figure 2. Retention index [= (delayed ratio – early ratio)/early ratio × 100 (%)]. The retention index was calculated from the early and delayed ratio of the affected eyeball. It was significantly higher in *group A* (patients with malignant melanoma) than in *group B* (patients with other lesions) ($P < 0.01$, *t* test).

Table 2. Diagnostic performance of the retention index

Retention index cutoff point	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
28	28	22	5	6	82.4	81.5	84.8	78.6	82.0
30	28	23	4	6	82.4	85.2	87.5	79.3	83.6
32	26	25	2	8	76.5	92.6	92.9	75.8	83.6
34	25	25	2	9	73.5	92.6	92.6	73.5	82.0

TP, true positive; TN, true negative; FP, false positive; FN, false negative; PPV, positive predictive value; NPV, negative predictive value.

the delayed phase was 2.5. A left uveal malignant melanoma 12 mm × 12 mm × 6 mm was excised. When the accumulation is obvious, setting the ROI is simple and semiquantification can be performed even without a 3D-ROI template. Figure 5 shows a choroidal pigmented tumor that had been followed as either a nevocellular nevus or another benign lesion for 8 years. It protruded above the choroid with a height of 5 mm, and its shape was atypical for a nevus. I-123 IMP was performed to exclude the possibility of a malignant melanoma. Both early and delayed images showed low attenuation, so it was very difficult to establish the ROI without a template. The retention index was 0.97, and the T/N ratio of the delayed phase was 1.03. This result reflects the natural course during 8 years of stability. Figure 6 shows the case of a 61-year-old woman with suspected right choroidal nevus with scleral and iris melanosis from the ocular manifestation, although high attenuation of the delayed image of the I-123 IMP examination was evident. After a month, the ocular manifestations progressed rapidly, suggesting malignancy, so transpupillary thermotherapy (TTT) with an infrared diode laser was performed. After the therapy, improvement of the ocular manifestation and a decrease of the attenuation of the I-123 IMP were confirmed, although the degree of the decrease was insufficient in both the retention index and the T/N ratio. Two years after the TTT, a relapse of the tumor was suspected from a follow-up I-123 IMP examination, and the excised tumor was confirmed to be a malignant melanoma. Applying the ROI template makes the semiquantification more reliable, especially when the same lesion is examined repeatedly.

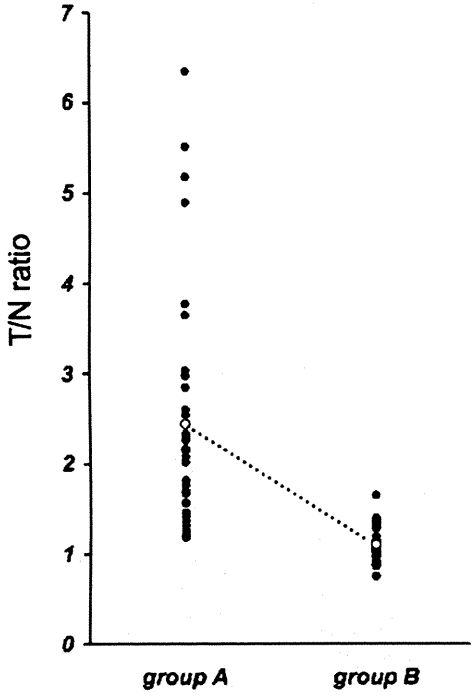

Figure 3. *T/N ratio* [= tumor count/nontumor count] of the delayed phase. The T/N ratio was calculated from both the affected and nonaffected uptake of the same phase image. It was significantly higher in *group A* than in *group B* ($P < 0.01$, t test).

Discussion

Several methods of estimating uveal malignant melanoma are now being attempted. F-18 FDG positron emission tomography does not seem to be sensitive enough for the diagnosis of uveal malignant melanoma, even though it is a sensitive and accurate technique for detecting primary and metastatic lesions of cutaneous malignant melanoma.[7,8] On the other hand, I-123 IMP is at times either the only or the best tool to estimate a malignant melanoma of the orbit,[1,9-11] especially when the ocular manifestations obtained by slit-lamp biomicroscopy, binocular fundus examination, ultrasonography, and MRI are not typical because of complications such as cataract, retinal detachment, or vitreous hemorrhage.[2,3] Moreover, after treatment such as brachytherapy (episcleral plaque radiotherapy) and TTT, the tumor size and contour seldom change; therefore, I-123 IMP is the most reliable follow-up instrument. We previously reported the usefulness of semiquantification with I-123 IMP by establishing the ROI manually. The accuracy with the retention index cutoff set at 30 was 80.0%, and with the T/N ratio cutoff set at 1.5 was 88.6%.[2] However, sometimes setting up the ROI is arbitrary, especially when the regional accumulation is faint. We therefore attempted an easier and more objective set-up of the ROI by creating a 3D-ROI template of the eyeballs from 3D eyeballs constructed from standardized MRI images. By applying this template to an I-123 IMP image standardized by the same method, we could semiquantify the I-123 IMP uptake of the eyeballs reproducibly

Table 3. Diagnostic performance of the T/N ratio of the delayed phase

T/N ratio cutoff point	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
1.2	34	19	8	0	100.0	70.4	81.0	100.0	86.9
1.3	31	21	6	3	91.2	77.8	83.8	87.5	85.2
1.4	29	24	3	5	85.3	88.9	90.6	82.8	86.9
1.5	27	26	1	7	79.4	96.3	96.4	78.8	86.9

T/N ratio, tumor count/nontumor count.

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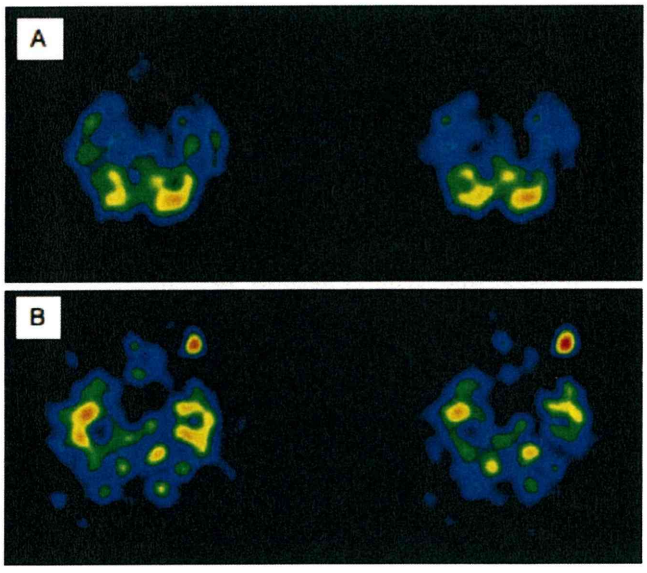

Figure 4. A, B I-123 IMP single photon emission computed tomography (SPECT) early and delayed images of a 69-year-old woman with left uveal malignant melanoma. As the accumulation is obvious, semiquantification can be performed even without a 3D-ROI template.

and objectively. Semiquantification with a 3D-ROI template is advantageous in three ways. First, other modalities such as computed tomography and MRI are unnecessary. Second, we can estimate the accumulation by using 3D volume data. Third, reproducing the results is easy. Fusion with other modalities is a common estimation method, but sometimes there is a mismatch of these modalities in spite of the individual data involved, mainly caused by the eyeballs trembling during the examination. Especially when the eyeballs move along the cephalocaudal axis, inaccurate estimation by counting loss becomes notable in axial planar fusion images. On the other hand, semiquantification by 3D-ROI can pick up all the accumulation by covering the whole eyeball. Moreover, even when the accumulation becomes blurred because of previous therapy or the eyeballs trembling, the 3D-ROI template makes it possible to semiquantify the same lesion reproducibly and objectively.

This time we employed two parameters, the retention index and the T/N ratio. Each has its advantages, and the two complement each other. The uptake of I-123 IMP is sometimes affected by the localization and the shape of the tumor. The frontal half of the lesion tends to be overestimated because the gantry for measurement is nearer. Flat

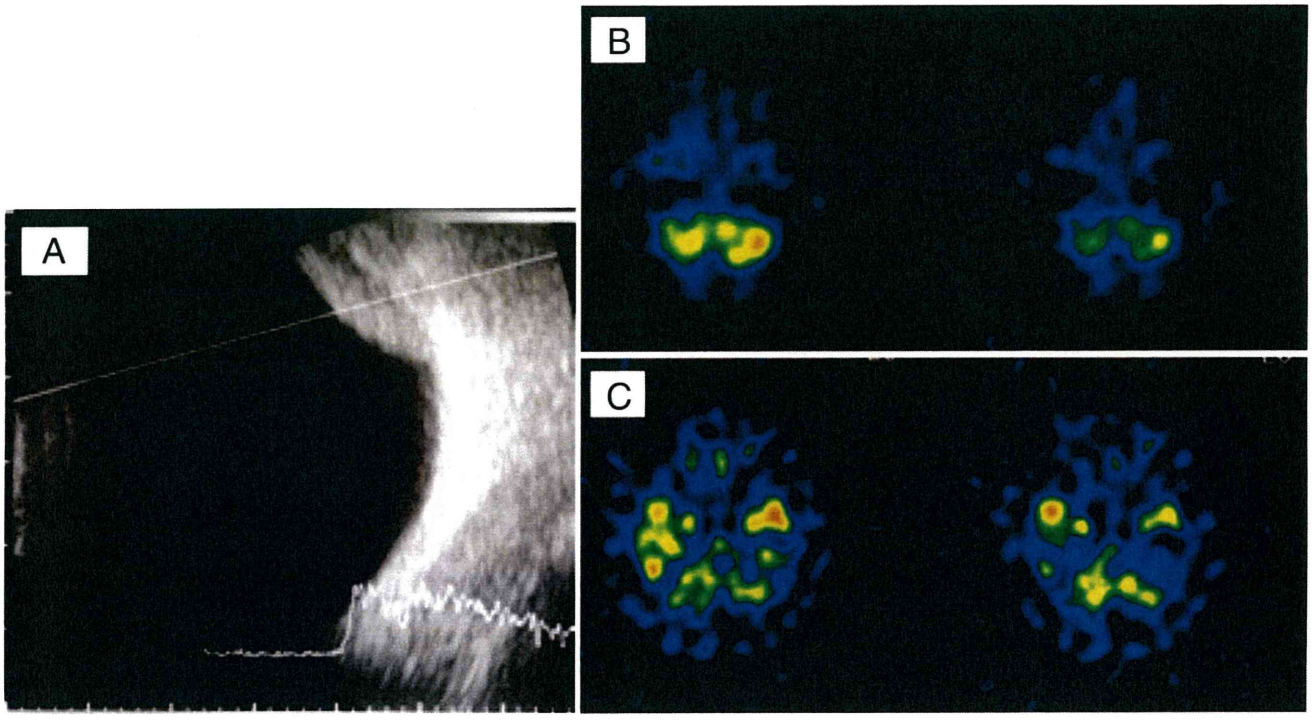

Figure 5. A Ultrasonography of a 65-year-old woman with left nevocellular nevus. It protruded to a height of 5 mm above the choroid. **B, C** I-123 IMP SPECT early and delayed images. Both images show low attenuation, so it is very difficult to establish the ROI without a 3D-ROI template. The retention index was 0.97, and the T/N ratio of the delayed phase was 1.03. This result reflects the natural course of 8 years of stability.