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*Original Article*

# Positron Emission Tomography/Computed Tomography after Immunocytochemical and Clonal Diagnosis of Intraocular Lymphoma with Vitrectomy Cell Blocks

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The purpose of this study is to report the role of whole-body 2-[$^{18}$F] fluoro-2-deoxy-D-glucose (FDG) positron emission tomography fused with computed tomography (PET/CT) after immunocytochemical and clonal diagnosis of intraocular lymphoma with vitrectomy cell blocks. Eleven patients with intraocular lymphoma were involved in this study : 6 patients presented with vitreous opacity in both eyes and 5 patients presented with unilateral involvement. The concurrent retinal lesions were present in 6 eyes of 5 patients. Brain lymphoma was diagnosed in 7 of the 11 patients : simultaneous with eye lesions in one patient, following the eye lesions in 3, and prior to the eye lesions in 3. Vitrectomy was done in 17 eyes of the 11 patients, and vitrectomy cell blocks were processed for immunocytochemical staining and clonality analysis by polymerase chain reaction amplification of the immunoglobulin heavy chain gene. The 7 most recent patients were evaluated with fluorodeoxyglucose whole-body PET/CT. Immunocytochemical staining of vitrectomy cell blocks in all patients showed large cells which were positive for CD20 and Ki-67 but negative for CD3, consistent with diffuse large B-cell lymphoma. The size and sequence of amplified fragments of the immunoglobulin heavy chain gene were different between the lesions of both eyes in one patient while they were the same in another patient. PET/CT after the diagnosis by vitrectomy revealed abnormal uptake in the cerebellum of two patients, in the eye as a recurrent lesion of one patient, and in both eyes as residual retinal lesions of one patient. In conclusion, PET/CT could be considered as a method to confirm brain lymphoma or as a reference for initiating additional therapy in the case of eye recurrence or residual lesions after vitrectomy. The clonality of lymphoma cells was variable between the lesions in both eyes. 〔*J Clin Exp Hematopathol 49(2) : 77-87, 2009*〕

**Keywords:** intraocular lymphoma, central nervous system lymphoma (cerebral lymphoma), clonality (clonal), vitrectomy cell block, fluorodeoxyglucose positron emission tomography (FDG-PET, PET/CT)

## INTRODUCTION

Primary intraocular malignant lymphoma is an established clinical entity but there is difficulty in making a prompt and correct diagnosis. It manifests as vitreous opacity or retinal or subretinal yellow-white lesions or their combinations and might be misdiagnosed as uveitis (intraocular inflammation) usually in the early phase and even in the late advanced phase.[1,2] Pathologically, primary intraocular lymphoma is diffuse large B-cell lymphoma. Primary central nervous system (cerebral) lymphoma is also an established clinical entity which presents as diffuse large B-cell lymphoma in pathological and immunohistochemical diagnosis. Primary intraocular lymphoma and primary central nervous system lymphoma occur concurrently or are followed or preceded by each other in the time course. The diagnostic term, primary intraocular-central nervous system lymphoma or oculocerebral lymphoma is used in such circumstances.

Recent advances in diagnosis and treatment of malignant lymphoma has revealed the same clonality or different clonalities between the primary lesion and the recurrent lesion as well as between the simultaneously developed different lesions.[3,4] Primary intraocular lymphoma frequently involves both eyes. Taken together, the fact that intraocular lymphoma is also associated with central nervous system lymphoma,

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multiple sites of the involvement raises the question of whether clonality of lymphoma cells are the same or different among the lesions.[5-8]

In this study, we made immunocytochemical diagnosis using vitrectomy cell blocks and also analyzed the clonality of lymphoma cells. After the establishment of the diagnosis, 2-[$^{18}$F] fluoro-2-deoxy-D-gluocose (FDG) positron emission tomography/computed tomography (PET/CT) was undertaken to test its usefulness in screening brain lesions and the residual eye lesions.

## METHODS

### *Patients*

This retrospective study was in accordance with the Declaration of Helsinki and the Ethical Guidelines for Clinical Study published by the Ministry of Health, Labor and Welfare of the Japan Government. Informed consent for the procedure was obtained from each patient in written form. Eleven consecutive patients with primary intraocular malignant lymphoma seen from January 2005 to May 2008 at the Ophthalmology Department of Okayama University Hospital were involved in this study (Table 1). The patients were 4 men and 7 women, with the age at presentation ranging from 68 to 82 (mean, 73) years. Six patients showed vitreous opacity in both eyes (Fig. 1) while 5 patients showed unilateral involvement. The concurrent retinal lesions (Fig. 2 & 3) were present in 6 eyes of 5 patients. Vitrectomy was done in 17 eyes of the 11 patients.

Brain lymphoma was diagnosed in 7 of the 11 patients. The brain lesion developed simultaneously with eye lesions in one patient, occurred one month after the onset of eye lesions in 2 patients or 11 months after the onset of eye lesions in one

**Table 1.** Clinical features of 11 consecutive patients with intraocular malignant lymphoma

| Case/Age*/ Sex | First visit | Laterality | Retinal lesion | Vitreous opacity | Vitrectomy | Brain lesion | Onset of brain lesion# | Treatment | Other features |
|---|---|---|---|---|---|---|---|---|---|
| 1/68 /Male | January 2005 | Right eye<br>Left eye | No<br>No | Yes<br>Yes | Yes<br>Yes | Yes | simultaneous | Chemotherapy | Alive in October 2008 |
| 2/72 /Male | September 2005 | Right eye<br>Left eye | No<br>Yes | Yes<br>Yes | Yes<br>Yes | Yes | 1 mon after | Chemotherapy | Died of brain lymphoma in January 2008 |
| 3/68 /Male | October 2005 | Right eye<br>Left eye | No<br>No | Yes<br>Yes | Yes<br>Yes | No | | None | On hemodialysis after nephritis from 2001<br>Died of renal failure in May 2006 |
| 4/74 /Female | December 2005 | Right eye<br>Left eye | Yes<br>No | Yes<br>No | Yes<br>No | Yes | 3 mon previously | Chemotherapy<br>Brain radiation | Alive in October 2008 |
| 5/72 /Female | April 2007 | Right eye<br>Left eye | No<br>Unknown | Yes | Yes | No | | None | Left sphenoid ridge to orbital meningioma 26 years previously<br>Alive in October 2008 |
| 6/69 /Female | May 2007 | Right eye<br>Left eye | No<br>No | Yes<br>Yes | Yes<br>Yes | Yes | 1 year 10 mon previously | Chemotherapy | Alive in October 2008 |
| 7/76 /Male | June 2007 | Right eye<br>Left eye | Yes<br>Yes | Yes<br>Yes | Yes<br>Yes | Yes | 10 mon previously | Chemotherapy<br>Brain radiation (40 Gy) | Alive in October 2008 |
| 8/70 /Female | August 2007 | Right eye<br>Left eye | No<br>No | Yes<br>No | Yes<br>No | Yes | 1 mon after | Chemotherapy | Alive in October 2008 |
| 9/74 /Female | October 2007 | Right eye<br>Left eye | No<br>Yes | Yes<br>Yes | Yes<br>Yes | Yes | 11 mon after | Chemotherapy | Alive in October 2008 |
| 10/79 /Female | April 2008 | Right eye<br>Left eye | Yes<br>No | Yes<br>No | Yes<br>No | No | | Right eye radiation (40 Gy) | Vitrectomy for rhegmatogenous retinal detachment in left eye in March 2007<br>Alive in October 2008 |
| 11/82 /Female | May 2008 | Right eye<br>Left eye | No<br>No | No<br>Yes | No<br>Yes | No | | None | Alive in October 2008 |

* : Age (years) at initial visit for eye examination

\# : Onset of brain lesion relative to onset of eye manifestation

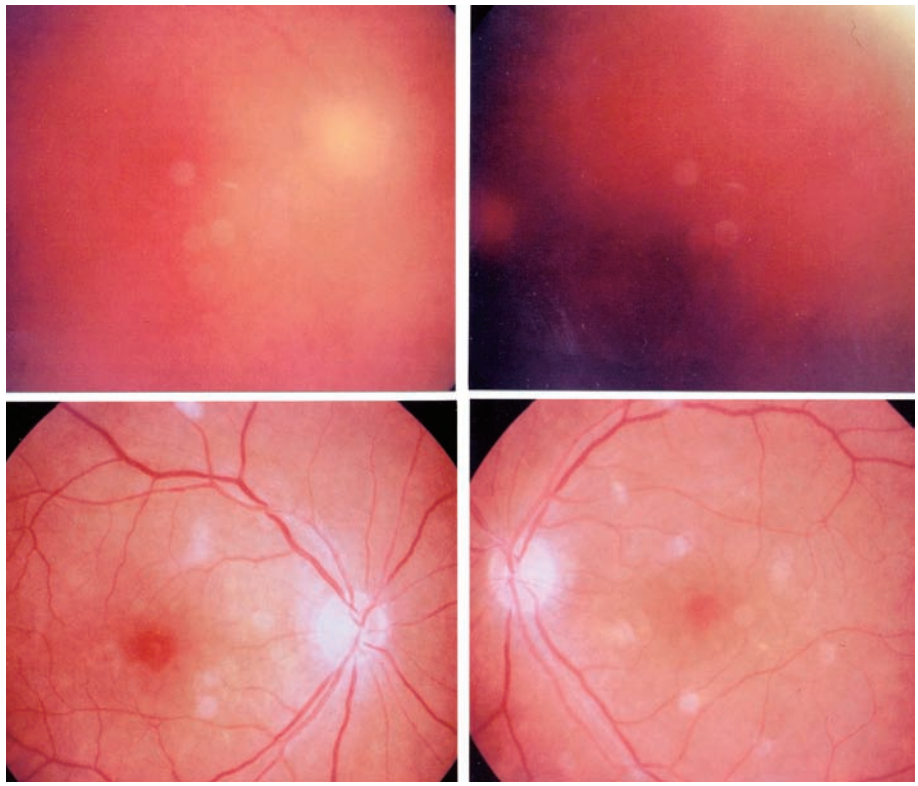

**Fig. 1.** Case 3. A 68-year-old man, showing vitreous opacity in both eyes (*top row* and *bottom row*, before and after vitrectomy ; *left column* and *right column*, right eye and left eye). White spots in bottom row are artifacts of fundus camera.

patient, and had preceded the onset of eye lesions by 3, 10 or 22 months in 3 patients. The 7 patients with brain lymphoma underwent chemotherapy, combined with brain radiation in 2 patients.

### *Histopathology and immunohistochemistry*

Vitrectomy fluid was transferred to one or two 50-mL tube(s) with the addition of 4% paraformaldehyde solution in an adequate volume, and centrifuged at 3,500 rpm for 20 min. The pellets were suspended in a small volume of 4% paraformaldehyde solution and transferred to a 15-mL tube to pellet by centrifugation at 3,000 rpm for 5 min.

The final pellet was embedded in paraffin, paraffin sections were cut and deparaffinized with xylene and graded ethanol series. The sections were stained with hematoxylin-eosin and also by immunochemistry. The brain biopsy specimens were processed as the same. In brief, the sections were incubated with 3% hydrogen peroxide for 5 min to inactivate endogenous peroxidase, and blocked with 10% normal goat serum for 10 min. The sections were then incubated with primary antibodies overnight at 4℃, washed with 0.05% Tween 20-containing phosphate buffered saline three times, incubated with the secondary antibody at room temperature for 30-60 min, and washed. The color was developed with diaminobenzidine, and the nuclei were counterstained with hematoxylin.

### *Immunoglobulin heavy chain gene rearrangement*

Immunoglobulin heavy chain gene rearrangement was detected by polymerase chain reaction (PCR).[9,10] Briefly, unstained, paraformaldehyde-or formaldehyde-fixed, paraffin sections placed on slide glasses were deparaffinized with xylene and graded ethanol series. Samples for DNA isolation were cut out from at least two different areas of the deparaffinized section of the brain tissue while DNA was isolated from the combined 4 sections of a vitrectomy cell block. The amplification of immunoglobulin heavy chain genes was performed by semi-nested PCR, using primers directed to the framework 2 region (FR2A : 5'-TGGRTCCGMCAGSCYYCNGG-3' for both the first and the second PCR) and to the joining region (LJH : 5'-TGAGGAGACGGTGACC-3' for the first PCR, and VLJH : GTGACCAGGGTNCCTTGGCCCCAG-3' for the second PCR). At least two DNA samples from different paraffin sections were separately subjected to PCR with TAKARA Ex Taq (Takara Bio Inc., Otsu, Japan). The amplified products from each patient were electrophoresed in

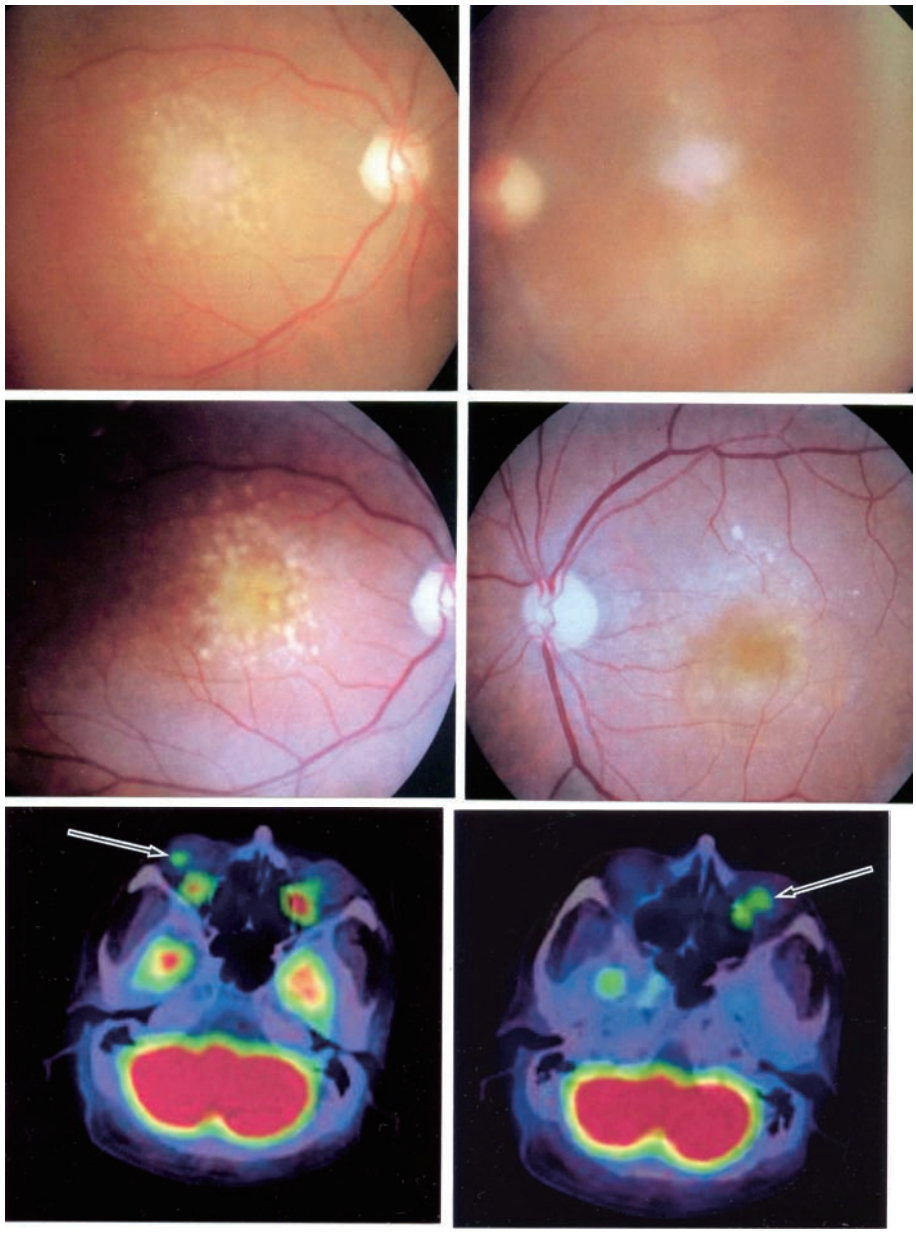

**Fig. 2.** Case 7. A 76-year-old man, showing vitreous opacity and macular lesions in both eyes (*top row* and *middle row*, before and after vitrectomy ; *left column* and *right column*, right eye and left eye). PET/CT (*bottom row*) more than two months after vitrectomy shows abnormal uptake in both eyes (*arrows*, SUV max = 5.0 in the right eye and SUV max = 5.6 in the left eye).

parallel in a 3% agarose gel. The determination of 'clonal' was made only when a single or dominant discrete band was consistently reproduced from different specimens.[10] The process was repeated twice to check reproducibility of the results.

For the sequencing of clonal bands, the PCR products were purified with ExoSAP-IT (USB, Cleveland, OH, USA) and used as a template for direct sequencing with the ABI 310 Genetic Analyzer (Perkin-Elmer, Foster, CA, USA) using the BigDye Terminator Cycle Sequencing Kit (Perkin-Elmer) and either of the two primers (FR2A and VLJH) to sequence in both directions. At least two PCR products from different samples derived from the same tissue were sequenced in both directions. Nucleotide changes were defined as those which repeatedly occurred between the two different tissues.

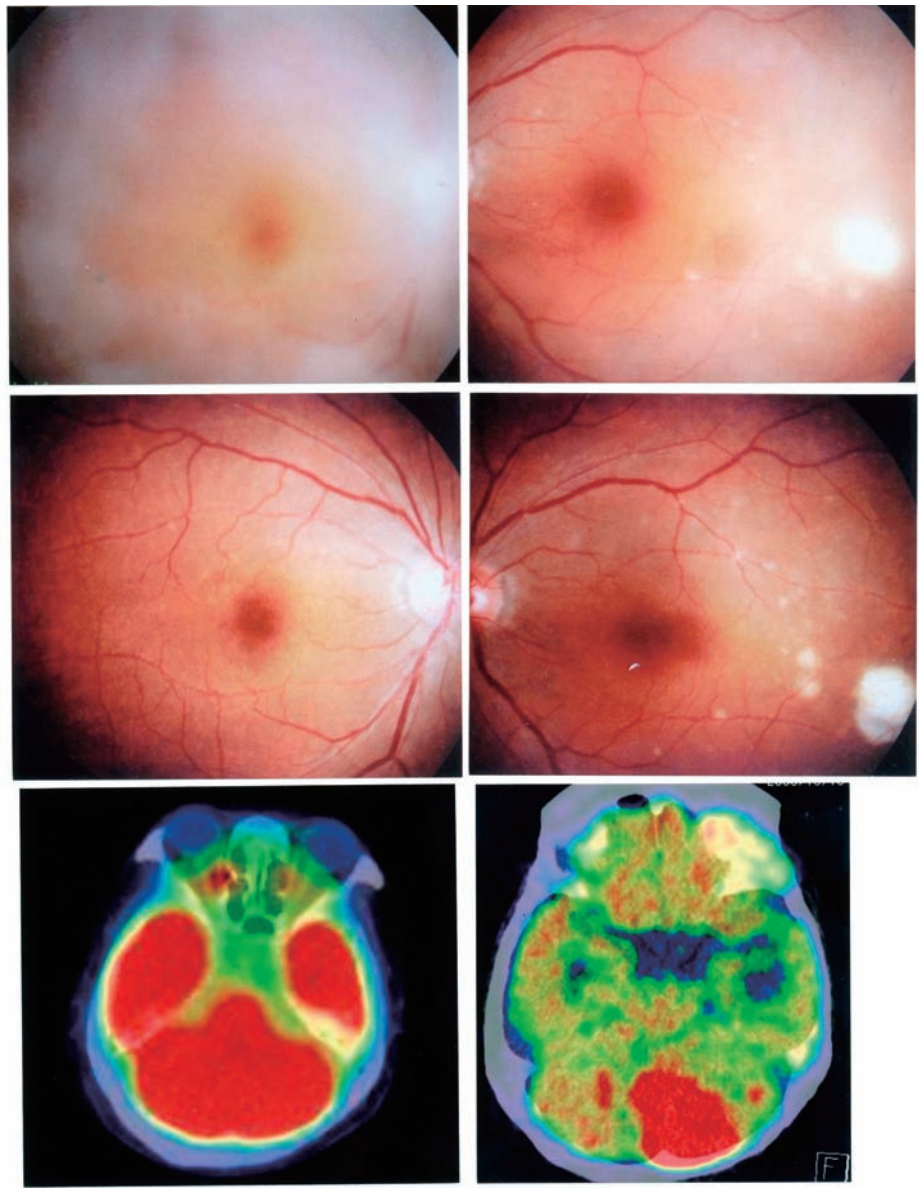

**Fig. 3.** Case 9. A 74-year-old woman, showing vitreous opacity in both eyes and retinal lesions in the left eye (*top row* and *middle row*, before and after vitrectomy ; *left column* and *right column*, right eye and left eye). PET/CT one month after vitrectomy shows no abnormal uptake (*bottom left*), and 11 months later, she developed a left cerebellar lesion with high uptake (SUV max = 18.2, *bottom right*) .

***Positron emission tomography/computed tomography (PET/CT)***

PET was taken in the recommended standard condition with PET/CT Scanner Biograph LSO/Sensation 16 (Siemens, Munchen, Germany) at Okayama Diagnostic Imaging Center. In brief, after at least 5-hour fasting, the patients received intravenous injection of FDG at 0.1 mCi (37 MBq)/kg of body weight. The FDG-PET images were obtained 90 minutes after the FDG injection, and reconstructed with an ordered subset expectation maximization (OSEM) iterative reconstruction algorithm. The technical parameters for a 16-detector row helical CT included a section thickness of 3 mm. Areas of focally increased glucose uptake were evaluated by standardized uptake values (SUVs). The maximum SUV (SUV max) for each site was calculated by the conventional formula normalized for body weight. The 7 most recent patients underwent PET/CT.

## RESULTS

### *Immunochemical diagnosis*

Paraffin sections of vitrectomy cell blocks in 17 eyes of 11 patients showed large cells in hematoxylin-eosin stain and immunocytochemical staining revealed the cells positive for CD20 and Ki-67 but negative for CD3, CD5, or CD10, compatible with the diagnosis of diffuse large B-cell malignant lymphoma (Fig. 1 & 4, Table 2). Brain biopsy was done in 4 of 7 patients with brain lesions : 3 patients with brain lesions (Cases 2, 8 and 9) did not undergo brain biopsy since preceding vitrectomy cell blocks established the diagnosis of malignant lymphoma. Immunohistochemical staining of brain biopsy specimens showed that large cells infiltrating around the vessels and brain parenchyma were positive for CD20 and Ki-67, but negative for CD3, CD5, or CD10, supporting the diagnosis of diffuse large B-cell lymphoma.

### *Clonality analysis*

Genomic DNA was extracted from 17 samples derived from 17 vitrectomy cell blocks and 3 samples from 3 brain biopsy specimens. A single discrete band was generated by polymerase chain reaction amplification of the immunoglobulin heavy chain gene from 5 samples only (Fig. 5, Table 2). Single discrete bands in different sizes were generated separately from vitrectomy cell blocks of both eyes in one patient (Case 3, Fig. 1) while single discrete bands in the same size were generated from vitrectomy cell blocks of both eyes in another patient (Case 9, Fig. 3). The brain biopsy specimen from only one patient (Case 7, Fig. 2) gave rise to a single discrete band by polymerase chain reaction amplification of the immunoglobulin heavy chain gene. By sequencing, the two bands in the different sizes derived from both eyes in Case 3 had different DNA sequences while the two bands in the same size derived from both eyes in Case 9 had the same sequences.

### *Positron emission tomography/computed tomography (PET/CT)*

PET/CT imaging was done in the 7 most recent patients at least one month after the diagnosis of lymphoma by vitrectomy (Table 2). One patient (Case 8, Fig. 6) showed a large high-uptake lesion (SUV max = 19.7) in the cerebellum which was evident on magnetic resonance imaging one month after vitrectomy (Fig. 6). Another patient (Case 9, Fig. 3) initially showed no abnormalities on PET/CT imaging one month after the diagnosis of intraocular lymphoma, and 11 months later, developed a left cerebellar lesion with high uptake (SUV max = 18.2). These two patients underwent chemotherapy for

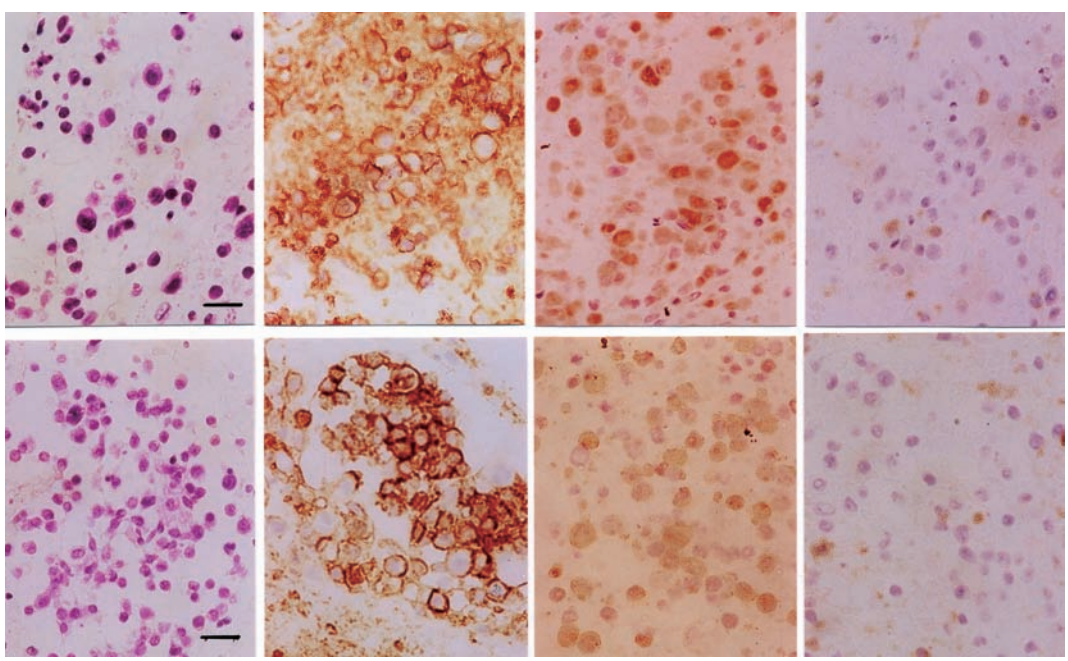

**Fig. 4.** Case 3. Cytopathological and immunocytochemical staining of vitrectomy cell blocks (*top row* and *bottom row*, right eye and left eye). Hematoxylin-eosin stain shows large cells (*leftmost column*) which are positive for CD20 (*middle left column*) and Ki-67 (*middle right column*), but negative for CD3 (*rightmost column*). These results are consistent with the diagnosis of diffuse large B-cell lymphoma. Counterstaining of nuclei by hematoxylin in immunocytochemical plates. *Bar* = 20 $\mu$m.

**Table 2.** Immunocytochemical or immunohistochemical results, polymerase chain reaction and sequencing of immunoglobulin heavy chain gene, and positron emission tomography with computed tomographic scan (PET/CT) of 11 patients with intraocular malignant lymphoma

| Case No. | Location | Eye lesion | Brain lesion | Brain biopsy | Pathology or Cytology | Immunocyto (histo)-Chemistry | | | Immunoglobulin heavy chain gene PCR | | PET/CT* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | CD20 | CD3 | Ki-67 | Single band | Sequence | |
| 1 | Right eye | Yes | | | Large cells | Yes | No | Yes | No | | |
| | Left eye | Yes | | | Large cells | Yes | No | Yes | No | | |
| | Brain | | Yes | Available | Diffuse large cells | Yes | No | Yes | No | | Not done |
| 2 | Right eye | Yes | | | Large cells | Yes | No | Yes | No | | |
| | Left eye | Yes | | | Large cells | Yes | No | Yes | No | | |
| | Brain | | Yes | Not done | | | | | | | Not done |
| 3 | Right eye | Yes | | | Large cells | Yes | No | Yes | Yes | Yes | |
| | Left eye | Yes | | | Large cells | Yes | No | Yes | Yes | Yes | |
| | Brain | | No | | | | | | | | Not done |
| 4 | Right eye | Yes | | | Large cells | Yes | No | Yes | No | | |
| | Left eye | No | | | | | | | | | |
| | Brain | | Yes | Done, Not available | Diffuse large cells | | | | | | Not done |
| 5 | Right eye | Yes | | | Large cells | Yes | No | Yes | No | | No uptake |
| | Left eye | Unknown | | | | | | | | | No uptake |
| | Brain | | No | | | | | | | | No uptake |
| 6 | Right eye | Yes | | | Large cells | Yes | No | Yes | No | | No uptake |
| | Left eye | Yes | | | Large cells | Yes | No | Yes | No | | No uptake |
| | Brain | | Yes | Available | Diffuse large cells | Yes | No | Yes | No | | No uptake |
| 7 | Right eye | Yes | | | Large cells | Yes | No | Yes | No | | Uptake SUVmax = 5.0 |
| | Left eye | Yes | | | Large cells | Yes | No | Yes | No | | Uptake SUVmax = 5.6 |
| | Brain | | Yes | Available | Diffuse large | Yes | No | Yes | Yes | | No uptake |
| 8 | Right eye | Yes | | | Large cells | Yes | No | Yes | No | | No uptake |
| | Left eye | No | | | | | | | | | No uptake |
| | Brain | | Yes | Not done | | | | | | | Uptake SUVmax = 19.7 |
| 9 | Right eye | Yes | | | Large cells | Yes | No | Yes | Yes | Yes | No uptake |
| | Left eye | Yes | | | Large cells | Yes | No | Yes | Yes (weak) | Yes | No uptake |
| | Brain | | Yes | Not done | | | | | | | Uptake SUVmax = 18.2 |
| 10 | Right eye | Yes | | | Large cells | Yes | No | Yes | No | | Uptake SUVmax = 5.8 |
| | Left eye | No | | | | | | | | | No uptake |
| | Brain | | No | | | | | | | | No uptake |
| 11 | Right eye | No | | | | | | | | | No uptake |
| | Left eye | Yes | | | Large cells | Yes | No | Yes | No | | No uptake |
| | Brain | | No | | | | | | | | No uptake |

* ; PET/CT was done at least one month after the diagnosis of intraocular lymphoma was established by vitrectomy.
PCR ; polymerase chain reaction, SUVmax ; the maximum of standardized uptake value

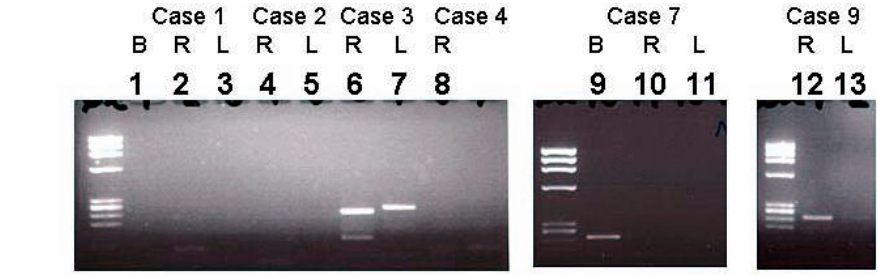


**Fig. 5.** Polymerase chain reaction of the immunoglobulin heavy chain gene. *B*, brain ; *R*, right eye ; *L*, left eye. No band is amplified in Case 1 and Case 2. Bands in different sizes were generated in Case 3 between the right eye and left eye while bands in the same size were generated in Case 9. The band from the left eye of Case 9 is faint but discrete and single. In case 7, a band only from the brain, but not from either eye, was generated. Size markers in leftmost columns : phage × 174 DNA Hae III digest (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 base pairs).

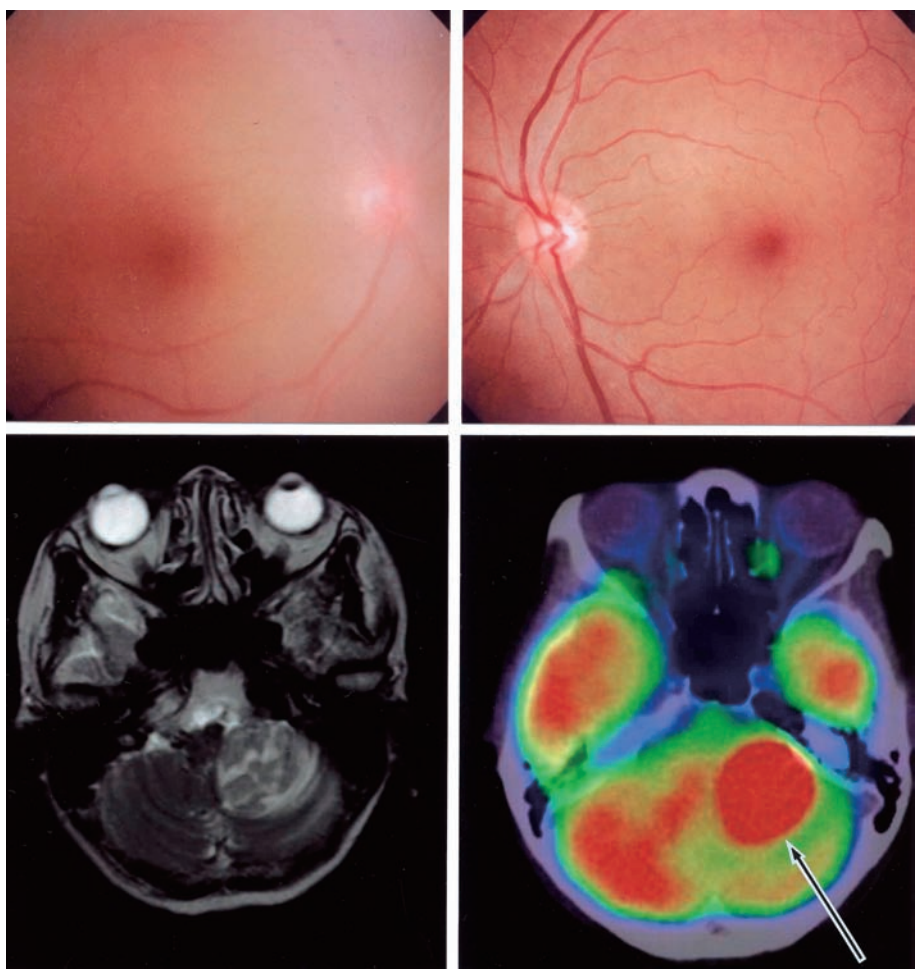

**Fig. 6.** Case 8. A 70-year-old woman, showing vitreous opacity only in the right eye (*top left*). *Top right* shows the normal fundus in the left eye. One month later, magnetic resonance imaging (*bottom left*) discloses a large left cerebellar lesion and PET/CT (*bottom right*) shows high uptake in the cerebellum (*arrow*, SUV max= 19.7). No other abnormal uptake is found in PET/CT.

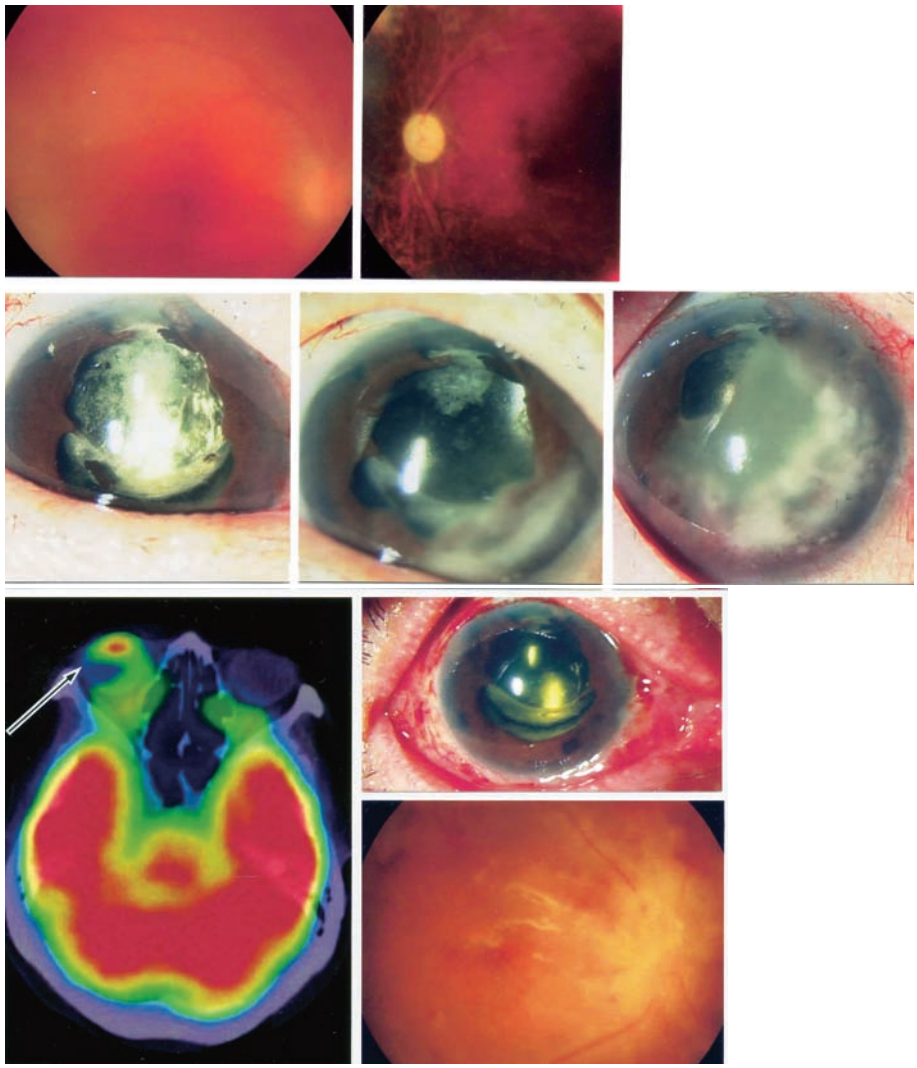

**Fig. 7.** Case 10. A 79-year-old woman, showing vitreous opacity and retinal lesions in the right eye (*top left*). The left eye has retinal degeneration and optic disc atrophy after vitrectomy for rhegmatogenous retinal detachment (*top right*). After vitrectomy and gas tamponade for vitreous opacity and rhegmatogenous retinal detachment in the right eye, she shows the recurrence in the iris, anterior chamber, and vitreous (*middle row* ; *left*, immediately after vitrectomy ; *middle*, 3 weeks after ; *right*, 6 weeks after). PET/CT reveals abnormal uptake (*arrow*, SUV max = 5.8) in the right eye one month after vitrectomy (*bottom left*). No abnormal uptake is found in the brain. She undergoes 40 Gy radiation to the right eye, leading to the remission (*bottom right*, *upper panel*). The patient develops retinal detachment again, and undergoes vitrectomy and silicone oil tamponade (*bottom right, lower panel*).

brain lymphoma without brain biopsy, leading to remission. One patient (Case 10, Fig. 7) who showed recurrence in the iris and the anterior chamber after vitrectomy showed abnormal uptake (SUV max = 5.8) in the anterior part of the right eye globe. This patient underwent radiation at 40 Gy to the right eye globe, leading to the remission.

One patient (Case 7, Fig. 2) with macular retinal lesions showed abnormal uptake in both eye globes (SUV max = 5.0 in the right eye and SUV max = 5.6 in the left eye) on PET/CT scan more than two months after vitrectomy for vitreous opacity in both eyes. The macular lesions became degenerated in a few months without radiation. Another patient (Case 9, Fig. 3) also had retinal lesions only in the left eye but PET/CT did not show any uptake in the left eye globe one

month after vitrectomy for vitreous opacity in both eyes. The retinal lesions in this patient also became degenerated spontaneously.

## DISCUSSION

The goals of this study were two fold : 1) to evaluate the effectiveness of immunocytochemical diagnosis using vitrectomy cell blocks in patients with intraocular lymphoma, and 2) to establish the role of PET/CT imaging after the establishment of diagnosis as lymphoma. In addition to the pathological diagnosis, we also analyzed the clonality of lymphoma cells arising in different locations such as both eyes and the brain.

Cytological diagnosis with cell blocks is superior to cell smear cytology since cells are accumulated by centrifugation and stored as paraffin blocks.[11,12] A larger number of cells in a compacted area of one section on a slide glass, compared with sparse cells on the smear, leads to a more accurate diagnosis. Furthermore, paraffin sections can be used later for immunocytochemical diagnosis and clonal analysis. The key for preparing vitrectomy cell blocks was that pellets from several tubes for a large volume of vitrectomy fluid were combined to make a single large pellet in an appropriate configuration for paraffin embedding. In addition, the vitrectomy fluid was an intraocular irrigating solution (BSS PLUS) used for cataract surgery and vitrectomy, and contained salts and glucose as well as phosphate and bicarbonate for buffering action. Cells can be kept in better condition in vitrectomy fluid and the addition of paraformaldehyde solution does not cause an adverse effect on cells.

In the present series of patients, we could make a diagnosis of large B-cell lymphoma with the aid of immunocytochemical characteristics of cells. The hallmark was that cells were positive for CD20, B-cell marker, and also positive for Ki-67, marker for cell division. Therefore, vitrectomy cell blocks were proven useful and effective in making a diagnosis of intraocular malignant lymphoma.

In clonality studies using vitrectomy cell blocks, two patients with bilateral eye involvement gave conflicting results : one patient (Case 3) showed different clonality of lymphoma cells between the lesions of the right eye and the left eye while another patient (Case 9) showed the same clonality of the lesions in both eyes. The different clonality of lymphoma cells indicates that lymphoma cells arising in one eye are independent of lymphoma cells arising in the other eye. These lesions in both eyes might develop simultaneously in an independent fashion or either lesion might develop later. This study is the first to show the evidence of different clonality between both eyes in oculocerebral lymphoma.[5-8] The same clonality of lymphoma cells indicates that lymphoma cells in both eyes derived from the same clone. In other words, lymphoma cells in one eye might travel to the other eye.

In the present series of patients, DNA fragments were generated from the immunoglobulin heavy chain gene by polymerase chain reaction only in a limited number of patients. Therefore, this method is not universal to search for clonality in diffuse large B-cell lymphoma. Too small an amount of vitrectomy specimens might be the main reason for this failure. We chose the framework 2 region as a location of mixed primers for amplification by polymerase chain reaction and did not use primers located in the framework 1 or the framework 3 of the immunoglobulin heavy chain gene. The reasons for choosing only the framework 2 region are that primers in this region give rise to a single discrete band at a higher rate than primers located in the other framework regions,[13,14] and that the vitrectomy samples are too limited to conduct polymerase chain reaction for all framework regions. The combination of polymerase chain reaction with primers located in three framework regions is known to increase the rate of getting a single discrete band with any of the primers.[13,14]

The role of PET/CT imaging has been reported in ocular adnexal malignant lymphoma and is recommended for staging of lymphoma.[15,16] In addition, a recent study has shown PET/CT as a useful method for staging primary central nervous system lymphoma.[17] In this study, for the first time, the role of PET/CT in intraocular lymphoma was evaluated and it was confirmed by PET/CT that no other lesions were present systemically except for those located in the brain or in the eyes. Thus, the tendency of lymphoma in the patients to involve the central nervous system and the eyes only is consistent with the term “oculocerebral lymphoma”.

The PET/CT findings among the present 7 patients with intraocular lymphoma were used to show brain lymphoma and also to confirm the recurrence of ocular lymphoma. Of course, the brain lesions detected by PET/CT were detected by magnetic resonance imaging and the eye lesions were observed by standard ophthalmic examinations. It should be noted that one patient with retinal lesions in both eyes (Case 7) showed unusual uptake of fluorodeoxyglucose in both eyes after vitrectomy for removing vitreous lesions. The remaining retinal lesions in the macular area would be responsible for this uptake. Radiation was not scheduled for this patient because degeneration would be anticipated to follow after radiation, leading to worse visual acuity in both eyes. Indeed, the patient maintained good visual acuity after spontaneous resolution of the macular lesions in a few months.

In conclusion, vitrectomy cell blocks would be useful and thus recommended for the diagnosis of intraocular lymphoma. Immunocytochemical staining of sections from paraffin-embedded cell blocks could establish the diagnosis of large B-cell lymphoma. Paraffin sections could be also used for clonality analysis of lymphoma cells derived from different lesions such as both eyes. The clonality was different be-

tween both eyes in one case while it was the same in both eyes of another case. Whole-body PET/CT is a new tool to confirm brain lesions of lymphoma and residual or recurrent lesions in the eyes after the lymphoma can be diagnosed with vitrectomy cell blocks.

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## REFERENCES

1 Matsuo T, Yamaoka A, Shiraga F, Matsuo N : Two types of initial ocular manifestations in intraocular-central nervous system lymphoma. Retina 18 : 301-307, 1998
2 Coupland SE, Hummel M, Müller HH, Stein H : Molecular analysis of immunoglobulin genes in primary intraocular lymphoma. Invest Ophthalmol Vis Sci 46 : 3507-3514, 2005
3 Nishiuchi R, Yoshino T, Teramoto N, Sakuma I, Hayashi K, *et al*.: Clonal analysis by polymerase chain reaction of B-cell lymphoma with late relapse : a report of five cases. Cancer 77 : 757-762, 1996
4 Matsuo T, Ichimura K, Shinagawa K, Yoshino T : Different histopathological types of orbital lymphoma 16 years after systemic follicular lymphoma : immunohistochemical and immunogenetic analyses of two cases. J Clin Exp Hematop 48 : 17-24, 2008
5 Pilozzi E, Talerico C, Uccini S, Addesso M, Rossi R, *et al*.: B cell clonality in multiple localizations of primary central nervous system lymphomas in AIDS patients. Leuk Lymphoma 44 : 963-966, 2003
6 Coupland SE, Hummel M, Stein H, Willerding G, Jahnke K, *et al*.: Demonstration of identical clonal derivation in a case of "oculocerebral" lymphoma. Br J Ophthalmol 89 : 238-239, 2005
7 Higashide T, Takahira M, Okumura H, Torisaki M, Sakurai M, *et al*.: Bilaterally identical monoclonality in a case of primary intraocular lymphoma. Am J Ophthalmol 138 : 306-308, 2004
8 Ohta K, Sano K, Suzuki T, Hidaka E, Yoshida A, *et al*.: B cell clonality of primary central nervous system and primary intraocular lymphomas. Jpn J Ophthalmol 51 : 147-149, 2007
9 Matsuo T, Yoshino T : Long-term follow-up results of observation or radiation for conjunctival malignant lymphoma. Ophthalmology 111 : 1233-1237, 2004
10 Mannami T, Yoshino T, Oshima K, Takase S, Kondo E, *et al*.: Clinical, histopathological, and immunogenetic analysis of ocular adnexal lymphoproliferative disorders : characterization of MALT lymphoma and reactive lymphoid hyperplasia. Mod Pathol 14 : 641-649, 2001
11 Mayall F, Chang B, Darlington A : A review of 50 consecutive cytology cell block preparations in a large general hospital. J Clin Pathol 50 : 985-990, 1997
12 Karma A, von Willebrand EO, Tommila PV, Paetau AE, Oskala PS, *et al*.: Primary intraocular lymphoma : improving the diagnostic procedure. Ophthalmology 114 : 1372-1377, 2007
13 van Dongen JJ, Langerak AW, Brüggemann M, Evans PA, Hummel M, *et al*.: Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations : report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 17 : 2257-2317, 2003
14 Evans PA, Pott Ch, Groenen PJ, Salles G, Davi F, *et al*.: Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia 21 : 207-214, 2007
15 Roe RH, Finger PT, Kurli M, Tena LB, Iacob CE : Whole-body positron emission tomography/computed tomography imaging and staging of orbital lymphoma. Ophthalmology 113 : 1854-1858, 2006
16 Valenzuela AA, Allen C, Grimes D, Wong D, Sullivan TJ : Positron emission tomography in the detection and staging of ocular adnexal lymphoproliferative disease. Ophthalmology 113 : 2331-2337, 2006
17 Mohile NA, Deangelis LM, Abrey LE : The utility of body FDG PET in staging primary central nervous system lymphoma. Neuro Oncol 10 : 223-228, 2008