• Clinical Research •

B-scan ultrasound and cytology of the vitreous in primary central nervous system lymphoma with vitreoretinal involvement

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Abstract

• AIM: To evaluate the diagnostic value of B-scan ultrasound and explore the cytological characteristics of patients with vitreoretinal lymphoma (VRL) and primary central nervous system lymphoma (PCNSL).

• METHODS: The clinical data and pathologic specimens from patients with VRL diagnosed at the North Huashan Hospital from 2016 to 2017 were retrospectively reviewed. The patients were diagnosed by slit lamp ophthalmoscopy, B-scan ultrasound, cytology of the vitreous, which was obtained by vitrectomy, and cytokine measurements of interleukin (IL)-10 and IL-6.

• RESULTS: Twenty-six eyes (19.4%) out of 134 eyes of 67 patients (47 men and 20 women) with PCNSL were diagnosed with VRL by B-scan ultrasound, and 14 eyes (10.4%) were diagnosed by slit lamp ophthalmoscopy. Twenty-four eyes (17.9%) of 17 patients were confirmed as having VRL with cytology. No difference in the association between intracranial lesion location and ocular involvement was found. VRL patients had higher levels of vitreous IL-10 and IL-10/IL-6 when compared with macular hole cases, but the difference was not statistically significant.

• CONCLUSION: A total of 25.4% of the PCNSL patients had VRL, B-scan ultrasound examination had characteristic features and is recommended over slit lamp ophthalmoscopy for the screening diagnosis of PCNSL with intraocular involvement. Moreover, the cytological and immunohistochemical analyses performed after 25-gauge diagnostic vitrectomy were accurate diagnostic techniques.

• KEYWORDS: primary central nervous lymphoma; intraocular lymphoma; B-scan ultrasound; vitrectomy; interleukin-10
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INTRODUCTION

Primary central nervous system lymphoma (PCNSL) is a rare non-Hodgkin lymphoma that occurs in the brain, pia mater (dura mater), spinal cord and eye and accounts for 2%-6% of the incidence of intracranial tumors[1-2]. Because of the aggressiveness and high pathological and morphological heterogeneity of this disease, PCNSL exhibits a poor prognosis and a strong possibility of recrudescence[3]. Although PCNSL is categorized as a rare disease, there has been a significant increase in the incidence of PCNSL in the past two decades, and together with glioma, they have become the two most common primary brain tumors[4].

Intraocular involvement occurs in approximately 15%-25% of PCNSL cases[5]. The most common phenotype of intraocular involvement is vitreoretinal lymphoma (VRL). In the United States, approximately 380 new VRL cases are reported each year[6]. VRL patients usually have blurred vision that affects their quality of life. In the PCNSL Guidelines for Baseline Evaluation for Clinical Trials published by the International PCNSL Collaborative Group (IPCG), slit lamp examination and indirect ophthalmoscopy are suggested for evaluation in clinical practice, whereas invasive examinations, including

vitreous biopsy, subretinal fine-needle aspiration biopsy (FNAB) and/or central serous biopsy, are suggested as diagnostic techniques[7-8]. MYD88 gene analysis is a helpful ancillary tool for diagnosing VRL as well[9]. If involvement of the optic nerve is suspected, then optic nerve biopsy should be conducted according to the patient's clinical condition[10].

Although the slit lamp exam is still advised for diagnostic examination by the IPCG Guidelines, the experience and technical skill of ophthalmologists differ; thus, the results lack objectivity and reproducibility. Moreover, the slit lamp examination is not a good choice for follow-up because it is not quantitative. B-scan ultrasound is a useful adjunctive diagnostic technique for the detection and differential diagnosis of degeneration in the vitreous[11]. B-scan ultrasound can reveal retinal detachment and additional mass lesions. Recently, B-scan ultrasound has become a routine examination for PCNSL cases to diagnose intraocular involvement and rule out other conditions, such as uveal melanoma, metastatic carcinoma and choroidal hemangioma[12]. Biopsy remains one of the most important diagnostic methods. Specimens are obtained by fine needle vitreous aspiration or pars plana vitrectomy (PPV), and 25-gauge PPV is the primary choice. However, the role of B-scan ultrasound in the diagnosis of PCNSL has yet to be undetermined.

The aim of our study was to determine the value of B-scan ultrasound compared with slit lamp ophthalmoscopy for the screening diagnosis of VRL and to describe the biological characteristics in PCNSL with vitreoretinal involvement.

SUBJECTS AND METHODS

Ethical Approval This study received approval from the Ethics Committee of the Institutional Review Board of Huashan Hospital, Fudan University and was performed in compliance with the tenets of the Declaration of Helsinki. All the patients voluntarily participated in this study and provided informed consent.

Subjects In this study, 67 patients who pathologically confirmed PCNSL at the Huashan Hospital from April 2016 to January 2017 were recruited. The patients' demographic and clinical data were collected, including age, gender, date of cerebral diagnosis and ophthalmic diagnosis, eye (left/right), visual acuity, intraocular pressure, clinical symptoms and duration of ocular symptoms.

B-scan Ultrasound Examination All patients received eye examinations, including slit lamp (MediWorks, S350), indirect ophthalmoscopy examinations, B-scan ultrasound examinations (SOUER, SW-2100, B-10MHz), visual field tests, optical coherence tomography (OCT) and funduscopy. All the examinations were performed by doctors who were blinded to the patients' diagnoses. When yellow deposits in the retina were observed by slit lamp, clusters of moderately condensed punctate echoes or eccentric masses were observed on ultrasound, it indicated suspected PCNSL with intraocular involvement.

Diagnostic Vitrectomy and Pathology A 25-gauge diagnostic vitrectomy was performed in patients with suspected intraocular involvement. Within one hour after the vitrectomy, the vitreous cells were sent to the cytology laboratory for further examination.

Wright's staining and immunohistochemical staining were performed on 1 mL of undiluted vitreous humor or 5 mL of diluted sediment of vitreous humor (when 1 mL of vitreous humor could not be pipetted). Primary antibodies, including those for CD3, CD20, PAX-5, BCL-2 and BCL-6 (Shanghai Sangon Biotech, Shanghai, China), secondary antibodies and chromogen (DAKO, Denmark) were obtained. The EnVision two-step method and Diaminobenzidine (DAB) color development were adopted. The appearance of brown particles in the cell membrane or cytoblast was considered to indicate a positive result. Due to the limited cell count in the vitreous humor, a semi-quantitative method was applied to analyze the results. If the number of tumor cells was greater than 10% of the total cell count, the PCNSL patient was considered to have intraocular involvement.

The concentrations of IL-10 and IL-6 in the supernatant fluid of the vitreous humor of 13 eyes that were confirmed to have vitreoretinal involvement were measured. The enzyme-linked immunosorbent assay (ELISA) kits (Guangzhou LDEBIO Co., Guangzhou, China) were used according to the manufacturer's instructions. Vitreous from whom had macular hole collected as negative controls.

Statistical Analysis We conducted a descriptive statistical analysis to determine the demographic, tumor, and treatment characteristics, including a 2-tailed *t*-test, analysis of variance, a Chi-squared test and Fisher's exact test. The data were analyzed using SPSS Statistics version 23.0. The null hypothesis was rejected if the *P*-value was less than 0.05. Continuous data are presented as the median (range) or number (%) as applicable.

RESULTS

Clinical Features Among the 67 PCNSL patients, 47 were male patients, and 20 were female patients. The median age was 55y (range 20-76y). Only a few PCNSL patients complained of specific ocular symptoms, and their courses of disease were varied. The most common ocular symptom was blurred vision, with 14 of the 67 patients (20.90% of the total number of patients) reporting this symptom. The median duration of the disease was 11mo. As for other ocular symptoms, 5 patients (7.47%) reported fundic hemorrhage or exudate, 4 (5.97%) had conjunctival congestion, and 2 (2.99%)

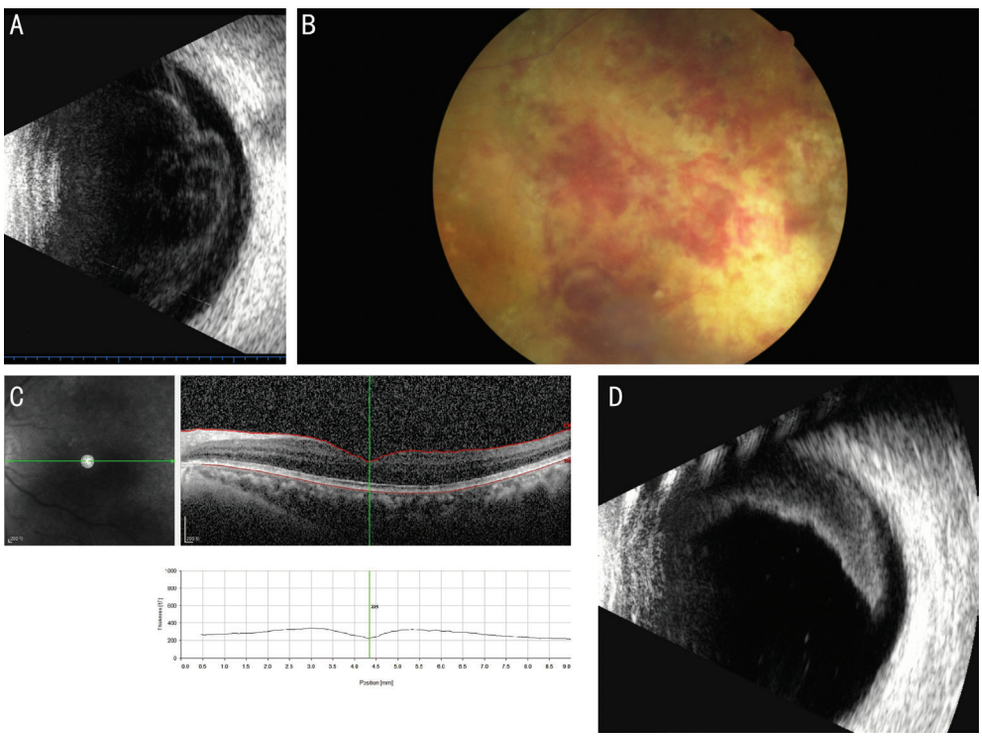

Figure 1 Photos of PCNSL with intraocular involvement A: Clusters of moderately or highly condensed punctate echoes were observed in the B-scan ultrasound examination of the eyes; B: Yellow deposits in the retina were observed; C: Normal central foveal thickness was detected through OCT scanning; D: Suspected eccentric masses were observed on ultrasound but not on slit lamp examination.

Table 1 Clinical characteristics of PCNSL patients

Parameters	Total (*n*=67)	Vitreoretinal involved (*n*=17)	Non-ocular involved (*n*=50)
Gender (M/F), *n*	47/20	9/8	38/12
Age (y), median (range)	55 (20-76)	58 (45-71)	55 (20-76)
Ocular symptoms, *n* (%)			
Blurred vision	14 (20.9)	10 (58.8)	4 (8.0)
Conjunctival congestion	4 (5.97)	4 (23.5)	0
Fundic hemorrhage or exudate	5 (7.46)	5 (29.4)	0
Limitations of eye movement	2 (2.99)	2 (11.8)	0

had limitations in eye movement. In most cases, the vitreous body and retina were involved in the PCNSL. Only nebulous and flaky turbidity of the vitreous body was observed, whereas no abnormalities except for yellow subretinal lesions were detected on OCT scanning (Figure 1).

Two patients had anterior segment involvement in the PCNSL and actively sought medical advice at the Ophthalmology Department. Examinations indicated that the intraocular pressure was > 21 mm Hg. Additionally, a deposit was detected in the posterior cornea, and wavy creases of the iris occupying the ciliary body were also observed (Table 1).

Even the PCNSL patients who received systemic chemotherapy plus monocular chemotherapy were likely to exhibit invasion of the other eye during the course of treatment. One patient was diagnosed with VRL during received systemic chemotherapy. Two patients were diagnosed with VRL during follow-up period when successfully relieved the clinical intracranial symptoms. One patient presented with involvement of the left eye during the first visit to the Ophthalmology Department. However, after four months of local chemotherapy of the left eye, the right eye was also found to be involved.

Diagnosis Rate of B-scan Ultrasound Compared with Slit Lamp Ophthalmoscopy Vitreous flocculence and nebulous turbidity were observed in 12 cases (15 eyes) with slit lamp indirect ophthalmoscopy in patients with dilated pupils. Yellow fundic lesions were also detected, indicating suspected intraocular infiltration of PCNSL, and 14 eyes were diagnosed with intraocular infiltration. Among these 14 eyes, 13 were diagnosed with VRL by cytology. The diagnosis rate of slit lamp ophthalmoscopy was 10.4%, which was lower than the cytological diagnosis rate (17.9%). However, 26 eyes were observed to have clusters of condensed punctate echoes (vitreous hemorrhage was not observed) on B-scan ultrasound, indicating suspicion for vitreoretinal infiltration of PCNSL. The diagnosis rate of B-scan ultrasound was 19.4% and was similar to the cytological diagnosis rate.

Table 2 Characteristics of PCNSL patients and intracranial lesion locations *n* (%)

Location	Total (*n*=67)	Vitreoretinal involvement (*n*=17)	No ocular involvement (*n*=50)	*P*
Single lesion	34 (50.7)	10 (58.8)	24 (48.0)	
Multiple lesions	33 (49.3)	7 (41.2)	26 (52.0)	0.58
Intracranial distribution				0.65
Hemisphere	48 (71.6)	13 (76.5)	35 (70.0)	
Ventricle	14 (20.9)	2 (11.8)	12 (24.0)	
Basal ganglia	12 (17.9)	3 (17.6)	9 (18.0)	
Corpus callosum	12 (17.9)	2 (11.8)	10 (20.0)	
Brainstem	12 (17.9)	5 (29.4)	7 (14.0)	
Thalamus	10 (14.9)	2 (11.8)	8 (16.0)	
Right or left hemisphere distribution				0.41
Right hemisphere	16 (23.9)	3 (17.6)	13 (26.0)	
Left hemisphere	22 (32.8)	8 (47.1)	14 (28.0)	
Both hemispheres	10 (14.9)	2 (11.8)	8 (16.0)	
Hemisphere distribution				0.99
Frontal lobe	31 (46.3)	8 (47.1)	23 (46.0)	
Temporal lobe	16 (23.9)	4 (23.5)	12 (24.0)	
Parietal lobe	18 (26.9)	4 (23.5)	14 (28.0)	
Occipital lobe	8 (11.9)	2 (11.8)	6 (12.0)	
Insular lobe	1 (1.5)	0	1 (2.0)	

Relevance of PCNSL Ocular Involvement with Location of Intracranial Lesion When comparing VRL with cases without ocular involvement, we found no difference in the location of the intracranial lesions (Table 2). Regardless of whether or not there was ocular involvement, almost one-half of the patients presented with a single intracranial lesion, and the other half presented with multiple lesions. The highest proportion of patients presented with hemispheric lesions when we analyzed the different intracranial distributions. However, we found no difference between the two groups ($P>0.5$). Similarly, the different locations of the hemispheric lesions were not different between the two groups.

Cytology A 25-gauge diagnostic vitrectomy was performed on 17 PCNSL patients (26 eyes) based on the diagnosis made with B-scan ultrasound, including 3 patients who reported postoperative complications during the early stages of sutureless vitrectomy. After the operation, the intraocular pressure was tested. Patients with lower intraocular pressure were required to have sclerotic sutures placed, and no postoperative complications were reported.

Among the 17 patients with vitreoretinal involvement, 24 eyes contained atypical lymphomas in the vitreous humor based on Wright's staining. Additionally, a significant multiplication of mononuclear macrophages, primitive lymph cells or abnormally shaped lymphoblasts were observed, and the proportions of these cells were 51%, 30% and 19%, respectively. A typical lymphoid cell had large, irregular, nuclear, loosely arranged chromatin, prominent nucleoli and scant basophilic cytoplasm (Figure 2). In terms of immunohistochemical staining, the results for the specific lymph cells were as follows: CD20 was positive, PAX-5 was positive, CD3 was negative, and Bcl-2 was partially positive. All 67 cases were diffuse large B cell-lymphomas, and this finding was consistent with the pathological results of the intracranial lesions.

IL-10 and IL-10/IL-6 Levels in the Vitreous The level of IL-10 in the vitreous humor of the VRL patients was significantly higher than that of PCNSL patients without ocular involvement (252.40 pg/mL and 29.81 pg/mL, respectively). However, there was no significant difference between the two groups. In the VRL patients, the ratio of IL-10/IL-6 was 48.85, which was more than double the value of the PCNSL patients without ocular involvement; however, the values were not significantly different (Table 3, Figure 3). However, when compared with non-PCNSL cases, the level of IL-10 and the ratio of IL-10/ IL-6 were statistically higher.

DISCUSSION

Our study indicated that PCNSL with intraocular involvement had a slow onset and resembled non-infectious or infectious uveitis, white dot syndrome or other metastatic carcinomas, and these patients experienced progressively blurred vision. However, the visual acuity of these patients generally exceeded expectations. The typical symptoms, including vitreous opacity and yellow subretinal lesions, could have led to retinal detachment and affected the visual perception of these patients. For PCNSL patients, if only the posterior segment of the

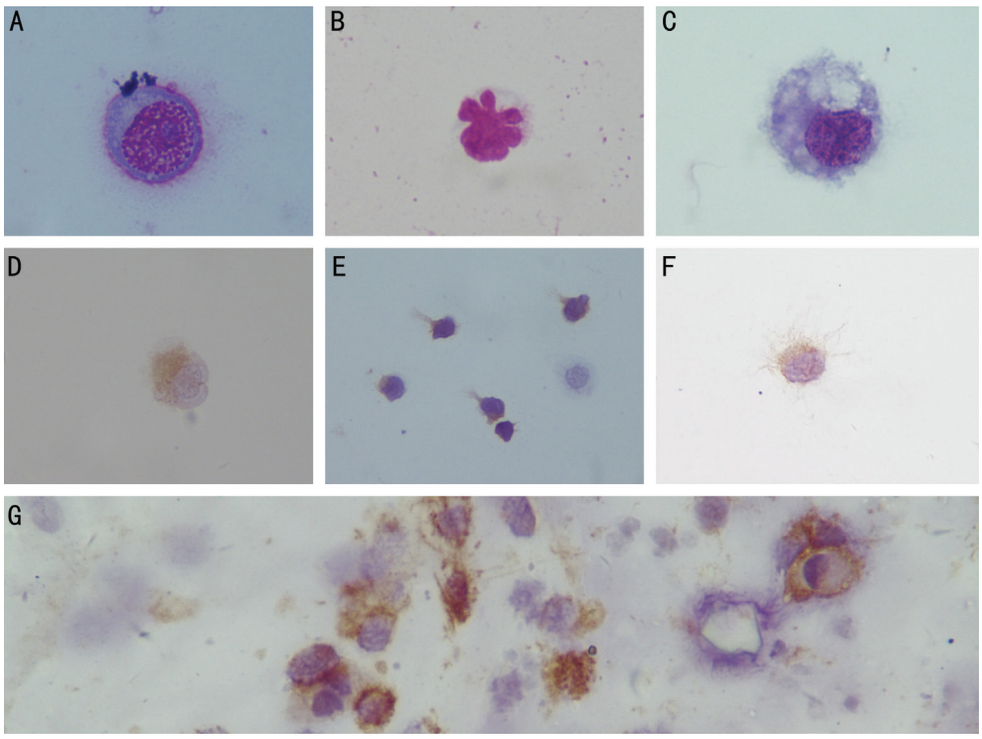

Figure 2 Wright's staining (100×) and immunohistochemical staining (40×) of vitreous cells A: Abnormally large B-cells with clear nucleoli and loosely arranged chromatin; B: T-cell; C: Macrophage; D-G: Immunohistochemical staining showing PAX-5+, CD3+, Bcl-2+ and CD20+ expression.

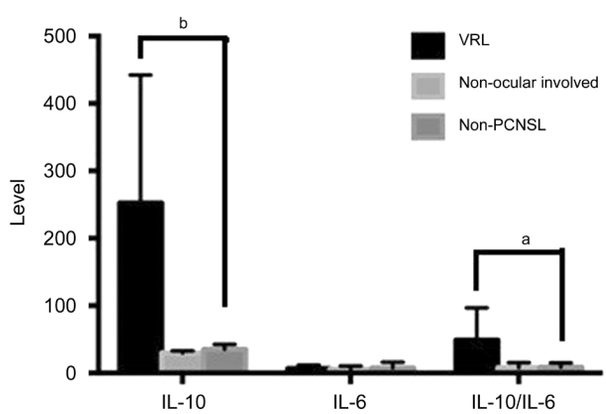

Figure 3 Levels of IL-10 and IL-10/IL-6 in the vitreous The levels of IL-10 and IL-10/IL-6 were statistically higher in the VRL patients than those in the non-PCNSL cases. When compared with the PCNSL patients without ocular involvement, the levels increased in the VRL patients; however, they were not significantly different. [a]P<0.01, [b]P<0.001.

Table 3 IL-10 and IL-10/IL-6 levels

Cytokine	PCNSL patients		Non-PCNSL patients (n=14)
	VRL (n=13)	Non ocular involvement (n=2)	
IL-10 (pg/mL)	252.40	29.81	35.29
IL-6 (pg/mL)	6.81	5.81	7.96
IL-10/IL-6	48.85	8.16	8.85

eye is involved, then they are unlikely to complain of ocular discomfort. Similarly, this study also found that intraocular involvement in PCNSL patients could occur at any time during the development of the disease, including during treatment with systemic chemotherapy, during the follow-up process after clinical relief of central nervous system signs or even during systemic chemotherapy plus monocular chemotherapy. At present, the pathogenesis of PCNSL remains unclear. The eyes are likely to become sites for the "storage" of lymphomas, as observed in human immunodeficiency virus (HIV)[13]. In general, due to the development of intraocular disease, both patients and clinicians should attach greater importance to follow-up examinations of the eyes[13-14].

Presently, according to the guidelines introduced in the United States, a slit lamp examination and indirect ophthalmoscopy are recommended methods for screening for PCNSL with intraocular involvement. However, in practice, many other routine ocular examinations can facilitate the diagnosis. Because slit lamp and indirect ophthalmoscope examinations largely depend on the experience and technical skill of the ophthalmologist, and the results cannot be recorded for filing, these techniques fail to support the follow-up clinical diagnosis of intraocular involvement in PCNSL patients, which adversely affects the diagnosis and treatment of these patients. Therefore, a larger study must be conducted to determine a screening method with an expanded range of application for PCNSL with intraocular involvement. In this study, B-scan ultrasound examination demonstrated a higher diagnosis rate than slit lamp ophthalmoscopy, but the diagnosis rate was not much different than that achieved by cytology. In addition,

B-scan ultrasound allows objective measurement with good repeatability, descriptiveness and ease of follow-up. Therefore, the B-scan ultrasound examination of the eyes should provide a basis for the clinical diagnosis and follow-up of PCNSL with intraocular involvement as a cost-effective method with high accuracy and efficiency for diseases that have a high level of malignancy and low incidence.

Various diagnostic methods are available for PCNSL with intraocular involvement, including cytological examination of the vitreous humor, immunohistochemical detection, FNAB, flow cytometry analysis, PCR-based monitoring of immunoglobulin gene rearrangement and cytokine detection. However, MRI or CT scans were less useful for the diagnosis of ocular lymphoma. Cytological examination is considered the gold standard, and the other methods are considered auxiliary methods to improve the diagnosis rate[12]. Sufficient specimens for the cytological diagnosis of VRL can obtained through vitrectomy, and 25-gauge diagnostic vitrectomy tends to outperform 20-gauge diagnostic vitrectomy in terms of diagnosis rate; furthermore, genetic mutations can be assessed in vitrectomy samples as a valuable tool to improve the diagnostic yield of vitreous aspirates[14-15]. Diffuse B-cell lymphomas are the main pathological type; however, the limited cell counts in the vitreous humor and the effects of chemotherapy and vitrectomy on the tumor cells have become major obstacles to diagnosis. Jiang *et al*'s[16] study showed that corticosteroids could decrease the viability of lymphomas and destroy the cellular structure, and the speed of vitrectomy could also affect the viability of the cells. Specifically, the viability began to fall as the speed reached 600 cpm and remained at its lowest level at 2500 cpm. Thus, during diagnostic vitrectomy, the speed and pressure should remain low, and the specimens should be collected quickly and immediately sent for preparation for smears and staining[12,17].

Malikova *et al*[1] studied in the characteristics of cranial MRI in 54 PCNSL patients. They showed that PCNSL presented either as multiple lesions that enhanced homogenously or as diffuse infiltrative brain involvement, often with involvement of the basal ganglia and optic pathways. However, there are no reports about whether the location of the intracranial lesions is a risk factor for intraocular involvement. Our studies suggested no correlation between the distribution of intracranial lesions and ocular involvement.

At present, vitreous cytokine examinations are known to effectively facilitate the diagnosis of primary intraocular lymphoma (PIOL). The high expression of IL-10 in PIOL serves as a growth and conversion factor for malignant B-cell lymphomas, and the expression of IL-10 in PIOL is significantly higher than in cases of uveitis or T-cell lymphomas[18-19]. However, IL-6 tends to indicate inflammation. During the development of follicular lymphomas, accessory T-cells tend to facilitate the generation of chemotactic factors through the secretion of cytokines, which play a key role in tumor immune escape and growth[20-21]. IL-10 may have similar effects on the incidence of PCNSL[1,16,18-19]. It was reported that IL-10 concentrations above 150 pg/mL in undiluted vitreous and above 50 pg/mL in diluted vitreous were diagnostic for PIOL[18]. Increased IL-10 levels in the vitreous had a sensitivity of 89% and a specificity of 93% for the diagnosis of PIOL; therefore, measurement of IL-10 levels in the vitreous was suggested as a screening test[19]. In addition, PIOL was reported to exhibit a higher ratio of IL-10/IL-6 (>1.0). The higher IL-10/IL-6 ratio can directly support the diagnosis of PIOL with a diagnostic susceptibility of 74.0%-88% and a specificity of 75.0%-85%[22-23]. In our studies, the IL-10 levels and IL-10/ IL-6 ratio of the PCNSL patients with ocular involvement were 252.40 pg/mL and 48.85, respectively, and were higher than those of patients without ocular involvement. In addition, the levels were statistically higher than those of the non-PCNSL patients. There were limitations in our studies. First, with regard to ethics, patients who did not present with vitreous opacities on slit lamp ophthalmoscopy or B-scan ultrasound did not undergo surgery. We may have failed to confirm the diagnosis by cytology. However, we excluded patients with ocular involvement during follow-up. Even if slit lamp and indirect ophthalmoscopy are advised by the IPCG Guidelines, some patients may still not be diagnosed with PCNSL with ocular involvement. Second, to verify the increased values of IL-10 and IL-10/ IL-6, a larger sample size is needed.

In conclusion, the analysis of the clinical data, screening and pathological features of PCNSL with intraocular involvement showed that PCNSL with intraocular involvement accounted for 25.37% of the PCNSL cases, with VRL being the most common cause of PCNSL with intraocular involvement. Additionally, this study showed that B-scan ultrasound examination increased the potential for diagnosis of intraocular lymphoma in PCNSL cases. The use of 25-gauge diagnostic vitrectomy combined with a cytological examination and immunohistochemical staining represents a safe and effective method for the diagnosis of intraocular involvement in PCNSL patients.

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