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Multicentre clinical study of the herpes simplex virus immunochromatographic assay kit for the diagnosis of herpetic epithelial keratitis

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ABSTRACT

Background/aims The novel immunochromatographic assay (ICGA) kit was recently developed to diagnose herpes simplex virus (HSV) infection. This multicentre study aimed to evaluate the value of the ICGA kit for the diagnosis of herpetic epithelial keratitis by comparing it with immunofluorescence assay (IFA) and real-time PCR.

Methods Corneal scrapings were collected from 117 patients, including 77 with herpetic keratitis as their final clinical diagnosis as well as 40 others at 21 facilities. These samples were tested by the ICGA kit, IFA and real-time PCR.

Results The positive concordance between final clinical diagnosis and ICGA was 46.7% (35/75 cases) and the negative concordance was 100% (39/39). The positive and negative concordance between real-time PCR and ICGA were 57.4% (35/61 cases) and 100% (53/53), respectively. The positive and negative concordance between IFA and ICGA were 61.1% (22/36 cases) and 83.3% (55/66), respectively. In 92 cases where anti-HSV drugs were not prescribed prior to corneal scraping, the positive and negative concordance between final clinical diagnosis and ICGA were 55.0% (33/60 cases) and 100% (32/32), respectively.

Conclusions The ICGA kit has moderate sensitivity and high specificity, indicating clinical utility in the diagnosis of herpetic epithelial keratitis.

INTRODUCTION

Herpetic epithelial keratitis has been diagnosed mostly based on clinical manifestation as it is easily recognised as dendritic keratitis with terminal bulb in typical textbook cases. However, in actual clinical settings, there are plenty of atypical cases in which diagnosis is difficult by clinical manifestation alone, sometimes leading to misdiagnosis.

For accurate diagnosis, the detection of herpes simplex virus (HSV) is essential. The gold standard of HSV detection is culture of live HSV; however it is very low sensitivity¹⁻⁴ and time-consuming. It requires constant preparation of indicator cells and special equipment and expertise, and is therefore not suitable for clinical examination. Immunofluorescence assay (IFA)^{4,5} and PCR⁵⁻⁸ have been available as practical means of HSV detection in clinical settings; however both necessitate expensive equipment and special expertise and are difficult

procedures for general ophthalmologists to perform. In addition, PCR is too sensitive to detect only pathological-level HSV. HSV is constantly shedding in the ocular surface, and can be detected in normal individuals by highly sensitive PCR.⁹⁻¹¹

Recently the novel immunochromatographic assay (ICGA) kit (Checkmate Herpes Eye, Wakamoto Pharmaceutical Co., Tokyo, Japan) has been developed to diagnose HSV keratitis.

This ICGA kit utilises the monoclonal antibody against HSV glycoprotein D, which is expressed in the virions, infected cells in all HSV strains; it is essential for infectivity,¹² and highly conserved between HSV-1 and HSV-2 as a type-common antigen.^{13,14} This kit can be performed as an 'in-the-office' diagnostic test within 15 min, and does not require specialised equipment and expertise.

The aim of this study was to evaluate the utility of the newly developed ICGA for the diagnosis of herpetic epithelial keratitis by comparing it with IFA and real-time PCR in a multicentre study.

MATERIALS AND METHODS

Subjects

A total of 117 patients with corneal epithelial lesions necessitating differential diagnosis of herpetic keratitis at 21 ophthalmological facilities belonging to the HSV ICGA Kit Study Group participated in this study from June 2008 to August 2009. There were 66 men and 51 women, with a mean age of 60.3±18.2 years. Corneal scrapings from 48 right eyes and 69 left eyes were collected by cotton swab after topical anaesthesia was applied; these scrapings were used in ICGA (Checkmate Herpes Eye), real-time PCR and IFA.

The criteria for the clinical diagnosis of herpetic epithelial keratitis were defined as follows: typical dendritic keratitis was defined as a dendritic form epithelial defect with terminal bulb, intraepithelial infiltration on the border and normal appearance of the epithelium surrounding the area of the lesion, and diagnosed as herpetic epithelial keratitis in cases where there was a response to anti-HSV treatment. In addition, atypical dendritic form, stellate form, and geographic form epithelial lesions were diagnosed as herpetic epithelial

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keratitis in cases where there was a response to anti-HSV treatment and there were no other causes of the keratitis.

This study was approved by Institutional Review Boards of Tottori University Faculty of Medicine, Kinki University Faculty of Medicine, Ehime University Graduate School of Medicine, Kanazawa Medical University, Hokkaido University Graduate School of Medicine, the University of Tokushima Graduate School, Nihon University School of Medicine in Tokyo, Okamoto Eye Clinic in Ehime, Ideta Eye Hospital in Kumamoto, and Tokushima Eye Clinic in Tokyo. All the subjects provided informed consent before the examination. Final clinical diagnosis was determined by clinical manifestation, ruling out of other causes of disease and the effects of anti-HSV drugs independent to the results of the tests.

Tests for HSV detection

The different parts of the affected area of the cornea were scraped with two swabs. One swab was examined by ICGA and the other examined by IFA. DNA was extracted from the remains of the ICGA and IFA samples and real-time PCR was carried out.

For ICGA, the swab was inserted in 500 µl of diluent buffer of ICGA, antigens were extracted to this diluent buffer and 150 µl of the extracted solution was dropped on the well, that is, the specimen area of the test strips, and incubated for 15 min at room temperature. The extracted solution developed on the anti-HSV antibody-fixed area. The results were considered positive in cases where both control and sample lines appeared in the test window, and negative in cases where only the control line appeared in the test window in accordance with the principles of ICGA measurements (figure 1).

IFA testing was carried out with the commercial kit, Herpes (1, 2) FA Reagent 'SEIKEN' (Denka Seiken Co., Tokyo, Japan),

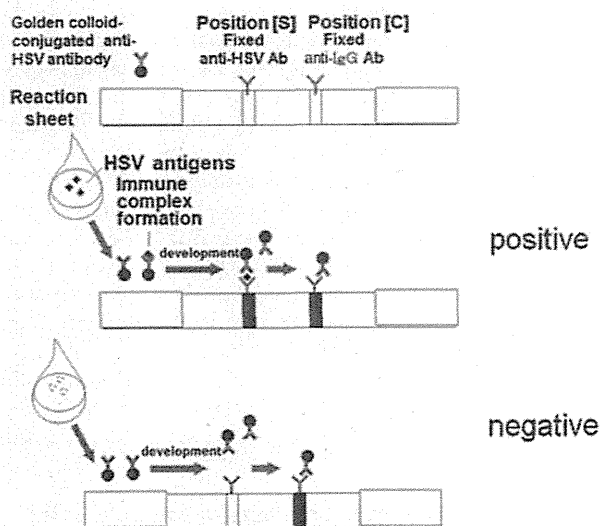


Figure 1 Principles of measurements of immunochromatographic assay kit. The anti-herpes simplex (HSV) monoclonal antibody recognises glycoprotein D expressed on the HSV envelope and HSV-infected cell surface. If there are HSV antigens in the samples, these antigens are reacted with golden colloid-conjugated anti-HSV antibody; the antigen-antibody complexes are trapped at the first position of fixed anti-HSV antibody, and the remaining free golden colloid-conjugated antibodies are trapped in the second position of fixed anti-IgG antibody. Two bands are observed in such cases. If there are no HSV antigens, all free golden colloid-conjugated antibodies are trapped in the second position, and only one band is observed.

using corneal scrapings in accordance with the manufacturer's instructions. In brief, corneal scrapings were smeared on to a glass slide, air-dried, and fixed with acetone. After incubation with FITC-conjugated anti-HSV-1 or anti-HSV-2 monoclonal antibody at 37°C for 15 min, this glass slide was observed under a fluorescence microscope.

For real-time PCR, total DNA was isolated from the remains of both the ICGA and IFA samples. (SmiTest EX-R&D, MBL, Nagoya, Japan). The DNA was amplified and quantified using a thermocycler (Real-time PCR 7300, Life Technologies, Carlsbad, California, USA, with STBR green PCR master mix, Life Technologies). The sequences of the real-time PCR primer pairs were: forward 5'-CAT CAC CGA CCC GGA GAG GGA C-3', reverse 5'-GGG CCA GGC GCT TGT TGG TGT A-3'. The cut-off value was set as 1500 copies according to the reproducibility and reliability of our real-time PCR system.

Statistical analysis

The κ statistical method was used with regard to the real-time PCR agreement between residual samples from ICGA and IFA. A κ value of more than 0.75 was considered to indicate excellent reproducibility.

To assess factors associated with ICGA results, one multivariate logistic regression model including four changes as covariates—clinical manifestation (typical or atypical), anti-HSV drug prescription prior to corneal scraping, days after onset, and viral copy numbers—was generated and used to provide an estimated OR. To assess factors associated with false negative results of ICGA, another multivariate logistic regression model including three changes as covariates—anti-HSV drug prescription before corneal scrapings, days after onset, and viral copy numbers—was generated and used to provide estimated ORs.

The results from multivariate logistic regression analyses were presented with 95% CI for the ORs. SPSS V.15.0 for Windows was used for the data analysis.

RESULTS

Final clinical diagnoses

There were 77 final clinical diagnoses of herpetic keratitis and 40 other clinical diagnoses, including eight of simple corneal erosion, four of acanthamoeba keratitis, three of varicella zoster virus keratitis, three of persistent epithelial defect, two of dry eye, two of drug-induced keratopathy, one of staphylococcal allergy, one of cicatricial keratoconjunctival disorder, one of Thygeson's superficial punctate keratitis, one of bacterial keratitis and 14 of corneal epithelial problems of indeterminate causes.

Comparison of final clinical diagnosis, real-time PCR, IFA and ICGA

Real-time PCR was carried out using remains from both ICGA and IFA samples. The maximum viral copy number of ICGA samples was 1.7×10^8 /specimen, and the minimum viral copy number was 1.7×10^4 . The maximum viral copy number of IFA samples was 1.9×10^9 /specimen, and minimum viral copy number was 2.3×10^4 . Both results of real-time PCR were significantly correlated with excellent agreement ($\kappa=0.853$), indicating ICGA and IFA had an equal chance of detecting HSV. After this confirmation, as the results of real-time PCR, we used those from the remains from ICGA in the comparative analysis of three methods.

The results of final clinical diagnosis and three methods (ICGA, IFA and real-time PCR) were compared in all 117 cases (table 1). The positive concordance with final clinical diagnosis

Table 1 Comparison of three methods and final clinical diagnosis

	Herpetic keratitis (n=77)	Others (n=40)
ICGA		
Positive	35	0
Negative	42	39
Failure	0	1
IFA		
Positive	36	0
Negative	32	37
Failure	9	3
Real-time PCR		
Positive	64	1
Negative	11	39
Failure	2	0

ICGA, immunochromatographic assay; IFA, immunofluorescence assay.

in both ICGA and IFA were almost equal (35/77 cases, 36/77 cases) while the positive concordance in real-time PCR was higher (64/77). There were 12 cases of test failure in IFA.

Statistical analysis comparing three methods

A total of 114 cases were statistically analysed. Three of the 117 cases were excluded from statistical analysis due to ICGA failure in one case and real-time PCR failure in two cases with insufficient sample amounts.

In 114 cases, the positive concordance between final clinical diagnosis and ICGA was 46.7% (35/75 cases), the negative concordance was 100% (39/39), and the total concordance was 64.9% (74/114). The positive, negative and total concordance between real-time PCR and ICGA were 57.4% (35/61 cases), 100% (53/53) and 77.2% (88/114), respectively. Twelve cases were excluded in the comparison between IFA and ICGA because of IFA failure. In 102 cases, the positive, negative and total concordance between IFA and ICGA were 61.1% (22/36 cases), 83.3% (55/66) and 75.5% (77/102), respectively.

Multivariate analyses were carried out to further analyse the factors associated with the results of ICGA. In multivariate analysis, the virus copy number had significantly high OR (3.696) as a factor associated with the results of ICGA (table 2). Furthermore, anti-HSV drug prescription prior to corneal scraping had significantly high OR (4.079) as a factor associated with false negative results of ICGA (table 3).

In consideration of the association of anti-HSV drug prescription prior to corneal scraping with false negative results, statistical analyses were carried out in the 92 cases where anti-HSV drugs had not been prescribed prior to corneal scraping.

In these 92 cases, the positive concordance between final clinical diagnosis and ICGA was 55.0% (33/60 cases), the

Table 2 Associated factors with results of immunochromatographic assay

	OR	95% CI	p Value
Clinical manifestation (typical or atypical)	2.087	0.512 to 8.508	0.305
Anti-HSV drug prescription before corneal scraping	0.873	0.106 to 7.192	0.899
Days after onset	1.008	0.945 to 1.074	0.816
Viral copy number*	3.696	1.913 to 7.141	<0.001

*Log viral copy number/specimen.
HSV, herpes simplex virus.

Table 3 Associated factors with false negative results of immunochromatographic assay

	OR	95% CI	p Value
Anti-HSV drug prescription before corneal scraping	4.079	1.464 to 11.364	0.007
Days after onset	0.989	0.943 to 1.037	0.635
Viral copy number*	1.082	0.903 to 1.296	0.394

*Log viral copy number/specimen.
HSV, herpes simplex virus.

negative concordance was 100% (32/32) and total concordance was 70.7% (65/92). The positive, negative and total concordance between real-time PCR and ICGA were 62.3% (33/53 cases), 100% (39/39) and 78.3% (72/92), respectively. Five cases were excluded in the comparison between IFA and ICGA because of IFA failure. In 87 cases, the positive, negative and total concordance between IFA and ICGA were 63.6% (21/33 cases), 81.5% (44/54) and 74.7% (65/87), respectively (table 4).

Furthermore, 32 cases of known other causes of keratitis were excluded from these 92 cases, and the remaining 60 cases were divided into typical cases and atypical cases according to diagnosis criteria. In 47 cases of typical epithelial keratitis, ICGA was positive in 28 cases (59.6%), and in 13 cases of atypical epithelial keratitis, ICGA was positive in five cases (38.5%).

DISCUSSION

The words 'sensitivity' and 'specificity' are not suitable in cases of herpetic keratitis because viral culture, although being the gold standard of herpetic keratitis diagnosis, is very low sensitivity itself, and PCR is too sensitive and has the possibility of detecting shedding virus, which is not a causative pathogen.¹¹ Therefore, the words 'positive concordance' and 'negative concordance' are used in this study. Roughly speaking, positive concordance is similar to sensitivity and negative concordance is similar to specificity. For instance, negative concordance between the ICGA kit and final clinical diagnosis or real-time PCR is 100%, indicating high specificity, while their positive concordance is 55.0% and 62.3%, respectively, indicating moderate sensitivity. With gross simplification, ICGA is a diagnostic method of herpetic keratitis with moderate sensitivity and high specificity similar to adenovirus ICGA in which sensitivity has been reported as 54.7% and specificity as 97.1% in the earlier study.¹⁵ There are some limitations of sensitivity in cases of antigen detection as compared with DNA detection, and this holds true in this ICGA kit.

The setting of sensitivity is difficult to begin with in cases of herpetic keratitis because of viral shedding in normal individuals.¹¹ This is the reason PCR is not the standard laboratory

Table 4 Comparison of immunochromatographic assay with clinical diagnosis, real-time PCR and immunofluorescence assay (IFA) in cases without anti-HSV drug prior treatment

	Positive concordance (%)	Negative concordance (%)	Total concordance (%)
Versus clinical diagnosis	55.0	100	70.7
Versus real-time PCR	62.3	100	78.3
Versus IFA	63.6	81.5	74.7

HSV, herpes simplex virus.

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test in the diagnosis of herpetic keratitis. However real-time PCR is more reliable than usual PCR because information regarding the DNA amount is available. In our report, the detection of more than 10^4 copies of HSV by real-time PCR is likely to be pathogenic.⁹ In this ICGA kit study, the lowest copy number that can be detected by the kit is 2.6×10^4 copies (data not shown)—a similar level to viral DNA judged as clinically reliable by real-time PCR. These results indicate the ICGA kit is appropriate for clinical use.

The diagnostic power of slit lamp examination is strong in cases of typical dendritic keratitis in contrast to cases of adenoviral keratoconjunctivitis. In such cases, laboratory tests including ICGA as well as IFA and PCR are not valuable. In atypical cases, however, laboratory tests are very useful in making a diagnosis. This ICGA kit returned 38.5% positive results even in atypical herpetic keratitis cases, indicating its clinical utility.

The ICGA kit and IFA, both of which recognise HSV-specific antigens, attained a similar positive rate, however there was some inconsistency between the ICGA kit and IFA. This discrepancy is probably due to the differences in recognising antigens. HSV kit recognises glycoprotein D while IFA recognises other HSV-1 specific antigens. The antigen recognised by IFA is not disclosed by the manufacturer, however it is presumed to be glycoprotein C or G considering IFA's ability to differentiate between HSV-1 and HSV-2.¹⁶ Differentiation of types of HSV is not an issue in cases of herpetic keratitis as it is caused almost exclusively by HSV-1.¹⁷

In this study, two samples were taken separately from small lesions by not-so-fine cotton swabs. Therefore, there is the possibility that sampling was not done properly in some cases. This is probably the main reason for the negative laboratory results in 11 cases of clinically diagnosed herpetic epithelial keratitis. As a consequence of this defect in the study, we compared ICGA with not only IFA and PCR but also clinical diagnosis. In real clinical settings, all the lesions can be provided to the ICGA kit, and this defect can be avoided.

Other types of keratitis such as stromal keratitis and endotheliitis are also important in herpetic keratitis. PCR can detect viral DNA from aqueous humours and tear films in such cases,⁶⁻⁸ however it is presumed to be difficult to detect viral antigens from aqueous humours and tear films by the ICGA kit because it requires a sufficient amount of infected cells and virions. On the other hand, the ICGA kit is able to detect HSV antigens in herpetic conjunctivitis, which is very difficult to differentiate from adenoviral conjunctivitis clinically and is a causative pathogen in 1.4–7% of epidemic keratoconjunctivitis cases.¹⁸ This necessitates a future study.

In conclusion, the ICGA kit has a high level of negative concordance and moderate level of positive concordance with other methods, which means positive results from the ICGA kit can be interpreted as a definite diagnosis of HSV keratitis.

Although the improvement of sensitivity is desirable for this kit to be of more value in clinical use, this ICGA kit is useful for the diagnosis of herpetic epithelial keratitis as it is faster and easier to use than IFA or real-time PCR.

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Contributors YI: contributed to acquisition of patient data, data analysis and manuscript preparation. YS: contributed to acquisition of patient data, data analysis, interpretation and manuscript revision. MF: contributed to acquisition of patient data, data collection and interpretation. Other members of HSV Immunochromatographic Assay Kit Study Group contributed to acquisition of patient data. All authors approved the final version to be published.

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

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Abstract

Purpose To investigate etiologic data on intraocular inflammation in Japan collected in the 2009 epidemiologic survey of uveitis in Japan and assess the current state of etiology compared with that reported in a previous survey. **Methods** Thirty-six university hospitals participated in this prospective etiologic study. Patients who visited the outpatient uveitis clinic of each hospital for the first time between 1 June 2009 and 31 May 2010 were enrolled in the study. Uveitic diseases were diagnosed according to the guidelines when available or following commonly accepted diagnostic criteria.

Results A total of 3,830 patients were enrolled in the survey and 2,556 cases of uveitis were identified, of which 1,274

cases were described as unclassified intraocular inflammation. In the identified cases, the most frequent intraocular inflammatory disease was sarcoidosis (10.6 %), followed by Vogt–Koyanagi–Harada disease (7.0 %), acute anterior uveitis (6.5 %), scleritis (6.1 %), herpetic iridocyclitis (4.2 %), Behçet’s disease (3.9 %), bacterial endophthalmitis (2.5 %), masquerade syndrome (2.5 %), Posner–Schlossman syndrome (1.8 %), and retinal vasculitis (1.6 %).

Conclusions The current etiology of uveitis in Japan was elucidated by means of a multi-center prospective survey. Conducting such surveys on a periodic basis may help clinicians in their management of uveitis.

Keywords Epidemiology · Survey · Intraocular inflammation · Uveitis

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Introduction

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

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

Materials and methods

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

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

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

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

Results

A total of 3,830 patients were enrolled in the study, among whom 2,556 cases of uveitis were identified with a specific etiology and 1,274 cases were recorded with unclassified

intraocular inflammation. Among the identified cases, 75.4 % were non-infectious diseases and 24.6 % were infectious diseases.

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

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

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

Table 2 Cause of scleritis

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

Table 3 Methods for diagnosing herpetic iritis

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

Table 4 Causes of viral retinitis

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

Table 5 Background of masquerade syndrome

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

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

Table 2 summarizes the causes of scleritis, the fourth most frequent disease in the 2009 survey. Many cases were associated with rheumatoid arthritis; however, some cases were identified as infectious. In herpetic iridocyclitis, the PCR assays detected similar numbers of cases of herpes simplex virus, varicella-zoster virus (VZV), and

cytomegalovirus (Table 3). VZV was the most frequent disease entity detected by PCR assays in acute retinal necrosis (Table 4), while about 50 % of the disease entity in masquerade syndrome was primary intraocular lymphoma (Table 5).

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

Discussion

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

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

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

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

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

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

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

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微量検体の網羅的解析による眼炎症性疾患の診断

高瀬 博

〔要 約〕

感染性ぶどう膜炎や眼内リンパ腫の診断を行う際に必要な検査項目には、ぶどう膜炎等の眼感染症の病因となり得る各種病原微生物や、リンパ腫細胞に特異的な遺伝子配列などが含まれる。我々は、眼局所から得られた微量検体に対する、多項目の PCR 同時検査を可能とする網羅的解析シス

テムを開発、運用している。これを用いた多施設共同前向き研究では、約 9 割の症例で確定診断および除外診断に本システムが有用であった。今後、本システムが、より広く簡便に行われる様になる事が期待される。

はじめに

ぶどう膜炎の原因は多岐にわたり、その病因により治療法は大きく異なる。特に感染性ぶどう膜炎と眼内リンパ腫に対しては疾患特異的な治療が適切に行われる必要があるが、その診断には眼局所から得られる検体の解析が最も直接的かつ重要な情報を提供する。しかし眼から得られる検体は微量であり、その一方で候補となりえる病因は多岐にわたるため、従来の解析法では診断が困難な事が多い。一部の感染性ぶどう膜炎は急速に不可逆的な視機能障害を生じ、また眼内リンパ腫は生命予後に直結する疾患であるため、これらが疑われるものには迅速な診断が要求される。

近年、ポリメラーゼ鎖反応 (polymerase chain reaction, PCR)⁽¹⁾ (図 1) の臨床使用が広く普及しており、病原微生物やリンパ腫細胞に特異的な遺伝子配列を増幅、検出する事で診断に役立てられている。我々は、ヒトヘルペスウイルス (HHV) をはじめとして、ぶどう膜炎の病因として想定される各種病原微生物やリンパ腫細胞に特異的な遺伝子

配列を、微量な眼局所検体から同時に検出する網羅的解析システムを確立し、これまでに多くの眼内検体を解析した。本稿では、当科の微量眼局所検体の網羅的解析システムを紹介し、これを用いた多施設共同研究の結果について概説する。

I. 眼局所検体の網羅的解析システムの概要

1. 感染症に対する網羅的解析

解析対象となりえる眼組織検体には、涙液、結膜、角膜擦過物および搔爬組織、前房水、虹彩、線維柱帯、硝子体液などがある。このうち、液性検体は約 100 μ l の検体量での解析が可能である。これらの検体から抽出した DNA に対して、4 種類の感染症検査セットを用いて同時にスクリーニング検査を行う (図 2)。

「ウイルスセット」として HHV 1 (単純ヘルペスウイルス (HSV) 1 型)、HHV 2 (HSV 2 型)、HHV 3 (水痘・帯状疱疹ウイルス (VZV))、HHV 4 (Epstein-Barr (EB) ウイルス)、HHV 5 (サイトメガロウイルス (CMV))、HHV 6~8 とヒト T リンパ球向性ウイルス (HTLV)-1 のプロウイルス

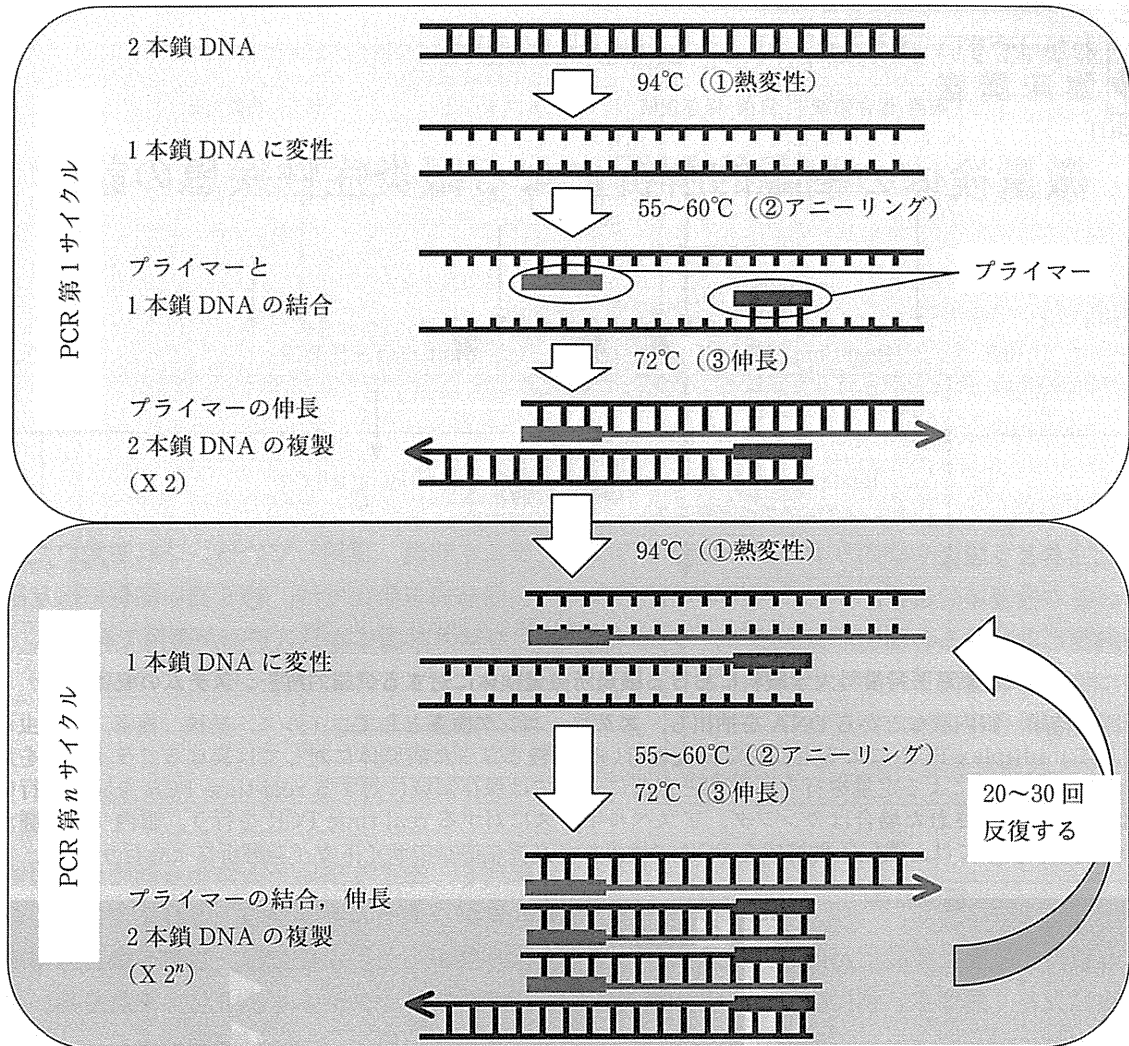


図1 PCRの原理

反応チューブ内に、増幅対象とするDNA、プライマー（増幅対象となる遺伝子領域に特異的に結合する一対の短い核酸断片）、DNA合成酵素、DNA合成のための基質などを混合し、PCR装置で以下の3つのステップで温度を変化させる。①熱変性：94℃に加熱すると、2本鎖構造であるDNAが変性して1本鎖DNAとなる。②アニーリング：60℃に急速冷却すると1本鎖DNAとプライマーが結合する。③伸長：72℃まで加熱するとDNA合成酵素が活性化し、それによりプライマーがDNA合成を開始し、2本鎖DNAが複製される。この①～③を一つのサイクルとして、20～30サイクル程度反応を繰り返す事で、DNAを大量に複製する事ができる。

DNAに対して、また「ぶどう膜炎、角膜炎セット」として結核、梅毒、バルトネラ、トキソカラ、トキソプラズマ、アカントアメーバに対して Multiplex PCR^(用語解説2)による定性的PCRを同時に施行する¹⁻³⁾。これらのスクリーニング検査で陽性に検出されたものには、real-time PCR^(用語解説3)による定量的PCRを行い、その遺伝子コピー数を測定する¹⁻³⁾。

細菌と真菌に対しては、それぞれの種のリボゾームRNA (rRNA) に保存されている種特異的な遺伝子領域を標的にしたブロードレンジ PCR^(用語解説4)を行い、細菌もしくは真菌全般の存在を検出する事

が可能である^{4),5)}。真菌陽性例に対しては、眼科領域でしばしば原因菌として検出されるカンジダまたはアスペルギルスの18S rRNAを標的としたreal-time PCRを施行し、さらなる原因菌の特定を行う⁶⁾。またPCRで増幅したDNAの配列を解析し、それを既知のDNA情報のデータベースと照合すれば菌種を同定する事が可能であり⁴⁾、今後のさらなる臨床応用が期待される。

2. 眼内リンパ腫に対するPCRおよびサイトカイン解析

眼内リンパ腫が疑われる患者の眼内液検体に対し

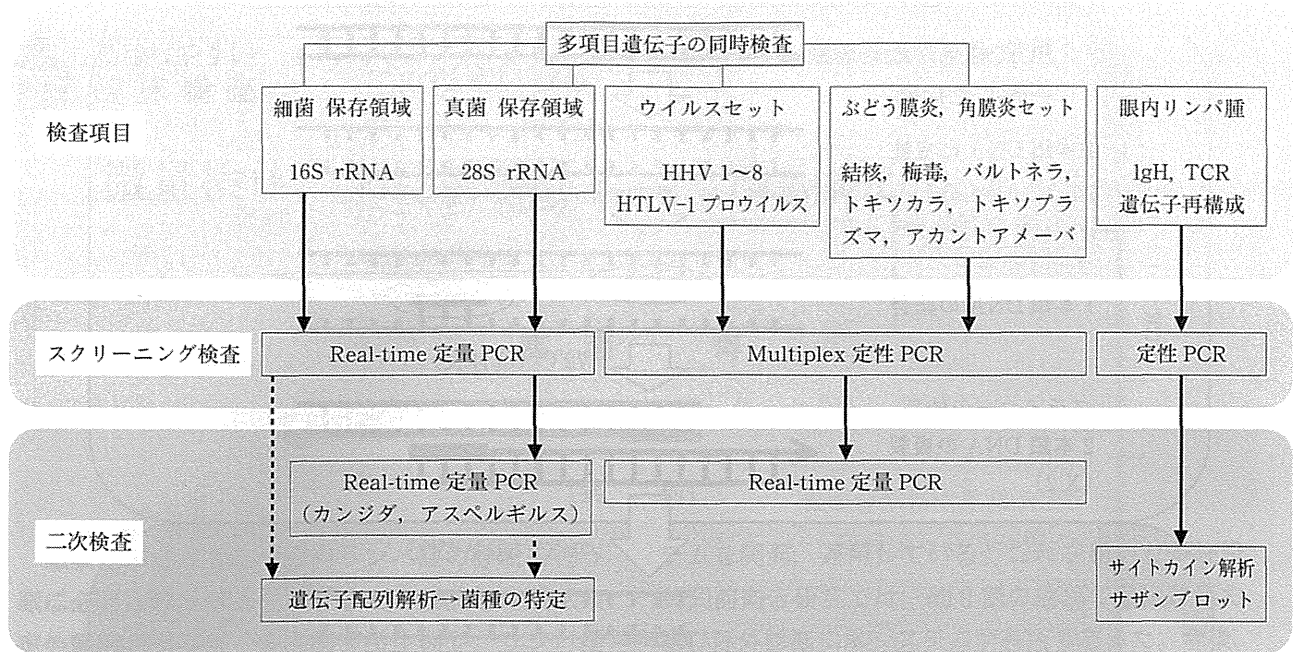


図2 東京医科歯科大学眼科における眼局所微量検体に対する網羅的解析システムの概要

涙液、角結膜、眼内液などからDNAを抽出し、スクリーニング検査としてウイルス、結核、梅毒、寄生虫などに対してmultiplex PCRによる定性的な検査を行い、陽性となった病原体に対しては異なるプライマーを用いてreal-time PCRによる定量を行う。細菌と真菌に対しては保存領域に対するreal-time PCRを同時に行い、真菌が陽性に検出された場合はカンジダ、アスペルギルスに対するreal-time PCRを行う。眼内リンパ腫が疑われる症例については、遺伝子再構成をPCRで検出し、必要に応じてサイトカイン解析などを行う。

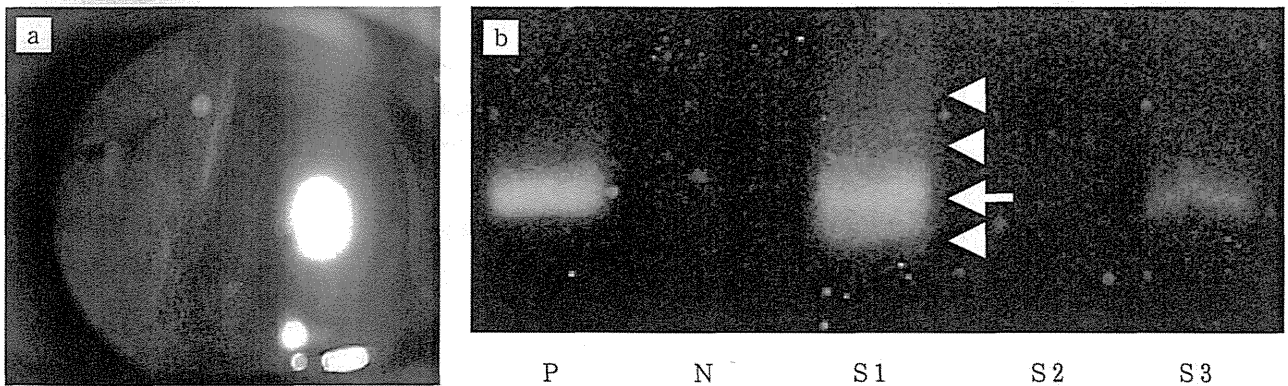


図3 80歳女性、右眼の眼内リンパ腫

a 前部硝子体の混濁

近医で右眼白内障手術後に硝子体混濁を発症し、精査目的で紹介された。大型の細胞浸潤を伴う硝子体混濁がみられた。

b 硝子体液のPCR写真

診断的治療目的で、右眼の硝子体手術を施行した。手術時に得られた硝子体液の病理細胞診は悪性と判定、サイトカイン測定ではIL-10/IL-6比が上昇しており、またPCRで免疫グロブリン重鎖遺伝子の単クローン性増幅(S3)を検出し、眼内リンパ腫と診断した。P:陽性コントロール N:陰性コントロール S1:陰性と判定された症例。バンド(矢印)の周囲にスメア(矢頭)が形成されており、非腫瘍性の様々な免疫グロブリンが硝子体中に存在する事を示す。ウイルス感染性疾患などで見られる事が多い。S2:陰性症例。硝子体液から免疫グロブリン遺伝子は検出されなかった。S3:本症例。単一バンドの存在は単クローン性に増殖した免疫グロブリンの存在を示し、それを産生するB細胞が腫瘍性増殖を生じている事を示唆する。

では、B細胞の免疫グロブリン重鎖(IgH)遺伝子や、T細胞受容体(TCR)遺伝子を標的にしたプライマーを用いてPCRを施行する(図1, 図3)⁷⁻⁹⁾。一

方、B細胞性の眼内リンパ腫ではサイトカイン^(用語解説5)に特徴的なパターン、すなわちインターロイキン(IL)-10濃度の上昇と、IL-10/IL-6比の1以上の

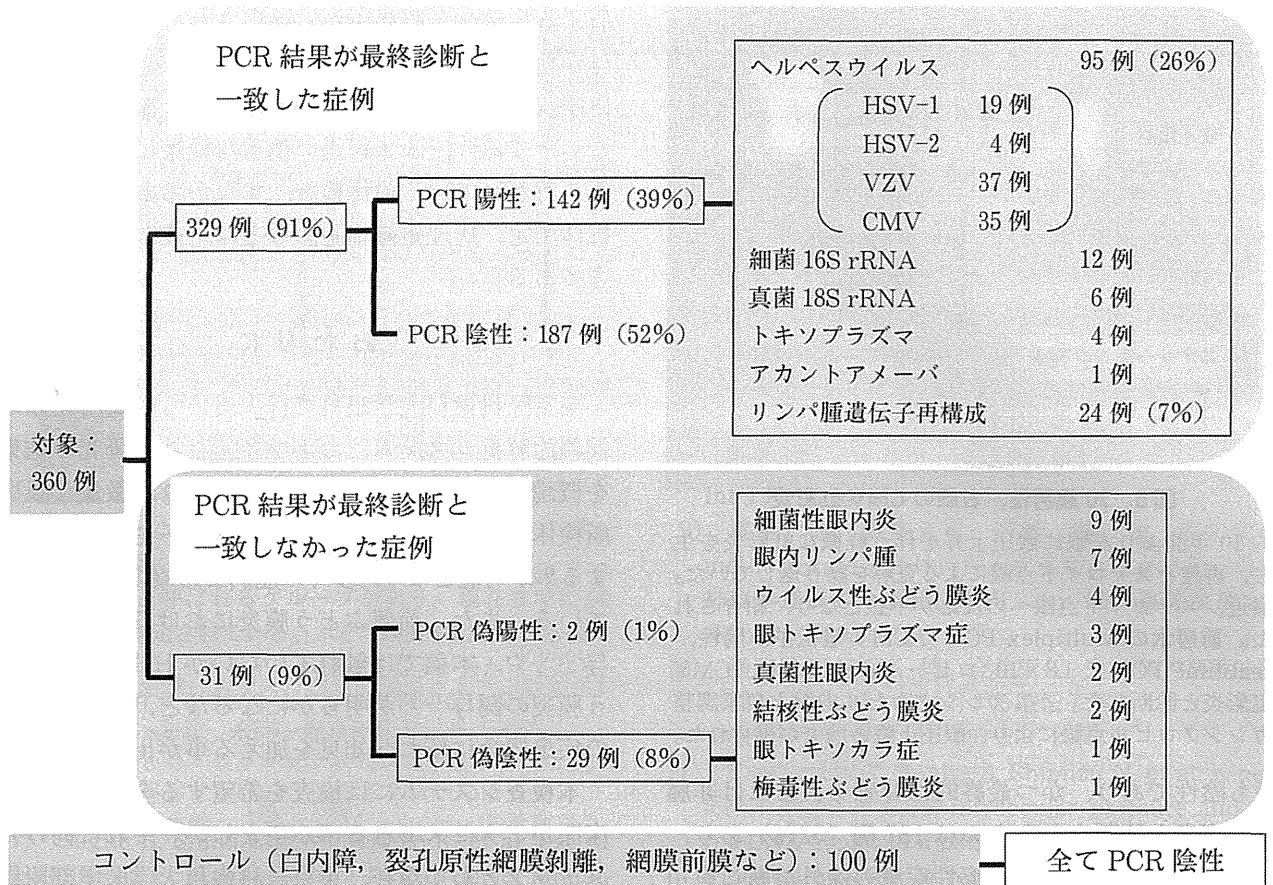


図4 網羅的解析システムの多施設共同研究結果

上昇がみられる⁷⁻¹⁰⁾。これはB細胞性眼内リンパ腫に対して、高い感度と特異度を持つため⁷⁾、本疾患が疑われる症例に対しては適宜行っている。

II. ぶどう膜炎患者に対する 網羅的解析システムの多施設共同研究

2009年4月から2011年10月にかけて、東京医科歯科大学眼科を中心に、東京医科大学眼科、京都府立医科大学眼科、大阪大学眼科、九州大学眼科と共同で、網羅的迅速診断システムの有用性を検討する事を目的として、各施設の倫理審査委員会承認のもとに多施設前向きの共同研究を行った。

1. 対象と方法

対象となった患者は、眼感染症が疑われる患者が292例、眼内リンパ腫が疑われる患者が68例の合計360例で、それに加えて眼内炎症を伴わない白内障、裂孔原性網膜剝離、増殖糖尿病網膜症、網膜静脈閉塞症などに対して原疾患治療を目的に手術を施行した100例を対照とした(図4)。これらの患者から検査目的に採取、または手術時に回収された前

房水、硝子体液、角膜擦過物、涙液などの眼局所検体を用いて、図2に示す各種検査を網羅的に施行した。

2. 網羅的解析システムの有効性の検討(図4)

まず、本検査の対照疾患として、眼内炎症を伴わない白内障、裂孔原性網膜剝離などの手術時に採取した眼内液100検体に対して網羅的PCRを施行したところ、いずれの病原微生物遺伝子も非炎症性疾患の眼内液では陰性であった。

つぎに、眼感染症または眼内リンパ腫が疑われた患者眼局所検体360例を解析した。このうち、臨床経過、治療への反応、他の眼局所および全身検査結果などから総合的に判定し、PCR陽性の結果が最終診断と一致したと考えられたものは142例(39%)あった。この中で最も多くを占めたのはHSV、VZV、CMV(図5)などのヘルペスウイルス属の95例(26%)だった。また、IgH遺伝子およびTCR遺伝子再構成の単クローン性の検出により眼内リンパ腫と診断された症例は合計24例(7%)あり、高い割合を示した。また本検査ではいずれのPCR結



図5 70歳男性、右眼のCMV虹彩炎

約10年前より左眼に眼圧上昇を伴う軽微な虹彩炎を生じ、再発とステロイド点眼による寛解を繰り返していた。最近、ステロイド点眼への反応が不良となり、紹介された。前房水のMultiplex PCRでCMV遺伝子が陽性、real-time PCRで 1.8×10^5 コピー/ml検出され、CMV虹彩炎と診断した。バルガンシクロビル内服と自家調整ガンシクロビル点眼により、眼圧下降と消炎が得られた。

果も陰性であり、かつ最終的に非感染性または非腫瘍性疾患と診断されたものは187例(52%)あり、本検査が感染性疾患や腫瘍性疾患の除外診断に有用である可能性が示された。これら、PCR陽性例の診断一致率とPCR陰性例の除外診断率を合わせると、329例(91%)で本検査が最終診断と矛盾しないものとなった。

3. 網羅的解析システムの問題点

一方、今回の眼検体検査ではPCR陰性だったものの、最終的には感染性眼疾患もしくは眼内リンパ腫と診断されたものが29例、また逆にPCRが偽陽性と判断されたものが2例あり、計31例(9%)でPCR結果と最終診断に明らかな解離がみられ、本システムが無効であったと判断された(図4)。また、今回の検討で同一検体から複数の病原微生物遺伝子が検出される例が散見された。急性網膜壊死の一例では、VZV遺伝子に加えてCMV遺伝子が陽性と検出された。しかし、その典型的な臨床経過からはCMVの病態への関与は小さいものと考えられた。またEBウイルスが8検体から、HHV-7が2検体から検出されたが、これらはEBウイルス陽性の1検体を除いて、全て他の病原微生物と同時に検出され、これらのウイルスの眼内炎症への関与は小さいものと判断された。

このように、PCRの結果が実際の臨床経過に矛

盾する症例や、眼局所から検出されても病態への関与が明らかに出来ないものも複数ある。従って、PCRの結果を直ちに診断の根拠とする事は厳に慎むべきであり、臨床経過の慎重な観察に加え、従来から行われている検体検査、すなわち塗抹、培養、抗体測定、病理組織細胞診などは可能な限り行うべきである。

おわりに

東京医科歯科大学眼科で行っている眼局所検体の網羅的解析システム、および多施設共同研究の結果を概説した。本システムの使用により、微量な眼局所検体から複数の病原微生物の遺伝子を同時に検索する事が可能となり、多くの症例で診療の一助となっている。また、前部ぶどう膜炎におけるCMVの関与^{11),12)}や、本稿では触れていないがHHV-6のぶどう膜炎の関与^{13),14)}が明らかになるなど、ぶどう膜炎の疾患概念に新たな知見を加える事が出来た。

本検査システムには検査を希望する全ての眼科臨床医がアクセスできる事が望ましい。それに向けたステップとして現在、東京医科歯科大学医学部附属病院において、先進医療として本システムを使用できる様に厚生労働省に申請中である。将来、同様の手続きを経て、国内の複数の拠点施設で本システムが運用されるようになる事が望ましいと考えられる。また理想的には、本検査をDNAチップの形で施行するなど、より簡便かつ迅速に行える検査システムの確立が望まれるが、その為には未だ多くの課題が残っている。

【用語解説】

- 1 PCR: 生物の遺伝情報を担うDNAの、ある特定の領域を増幅する手法(図1参照)。PCRで増幅されるDNAの領域を決定するのはプライマーと呼ばれる一対の短い核酸断片であり、その塩基配列は増幅したいDNAの塩基配列情報を基にコンピュータで設計される。ヒトの組織検体に外来微生物、例えばウイルスの感染が無いかを調べるには、そのウイルスだけに結合するプライマーを用いてPCRを行う。感染が生じていないヒト検体にウイルスに対するプライマーを用いてPCRをかけてもDNAは増幅されないが、ヒト検体にウイルスが感染していれば、そのDNAが増幅、検出される。
- 2 Multiplex PCR: 複数の異なるプライマーを一本の反応チューブ内で同時に反応させ、定性PCRを行う方法。微量なヒト検体から、複数の病原微生物のDNA

の有無を一度に調べる事ができる。

- 3) Real-time PCR : 蛍光発色するプライマーを用いて PCR を行い, DNA の増幅に伴う蛍光量の増加を経時的 (リアルタイム) に測定し, 増幅対象となる DNA の量を算出する方法。例えば, ヒトの組織にウイルスが感染している事を検出するだけでなく, そのウイルス量を調べる事ができる。
- 4) ブロードレンジ PCR : 細菌または真菌それぞれに共通に存在する DNA 保存領域に結合するプライマーを用いて PCR を行う方法。細菌は数千種, 真菌は数万種の多様な菌種の存在が知られている。これら細菌や真菌の DNA は, 全ての菌種に共通して存在する保存領域と, 菌種によって異なる可変領域で構成されている。この保存領域に対して PCR を行う事で, 既知の菌種の 60~80% でその存在を検出する事ができる。
- 5) サイトカイン : 細胞間の情報伝達に働くタンパク質。代表的なものに, 白血球から主に分泌されるインターロイキンと呼ばれるグループがあり, 現在までに 30 種以上が同定されている。ぶどう膜炎を始めとする様々な眼疾患にサイトカインが関与する事が分かっており, 眼内に存在するサイトカインの種類や量は疾患によって様々なパターンを示す事が報告されている¹⁰⁾。

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特集◇眼科疾患の病態と診断・治療 (II)

ぶどう膜炎

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Key words : ぶどう膜炎, サルコイドーシス, ヘルペスウイルス

はじめに

ぶどう膜炎は眼内炎症性疾患の総称であり、虹彩、毛様体、脈絡膜および網膜に生じるさまざまな疾患群により構成されている。その原因は多彩であり、それ故に病態と治療法も多岐に渡る。本稿では、ぶどう膜炎の分類と、それを構成する代表的な疾患の診断と治療について概説する。

I. ぶどう膜炎の構成疾患

ぶどう膜炎の構成疾患は、居住地域、民族、年齢、性別などで異なることが知られている。わが国では2005年に日本眼炎症学会がぶどう膜炎の原因疾患についての後ろ向き全国調査を行った。それによれば、ぶどう膜炎の原因疾患として従来よく知られているサルコイドーシス、Vogt-小柳-原田病、ベーチェット病の三大ぶどう膜炎疾患に加えて、細菌性眼内炎、ヘルペス性の虹彩炎や網膜炎などが挙げられる(表1)¹⁾。これを約20年前の報告²⁾と比較すると、当時第一位だったベーチェット病の患者の減少、寄生虫感染症である眼トキソカラ症の登場などの変化があり、ぶどう膜炎の構成疾患が調査時期によっても変化していることがわかる。これは、生活習慣の変化や公衆衛生の改善、分

表1 ぶどう膜炎全国統計 (2005年)¹⁾

疾患名	頻度 (%)
サルコイドーシス	13.3
Vogt-小柳-原田病	6.7
ベーチェット病	6.2
細菌性眼内炎	3.8
ヘルペス性虹彩炎	3.6
ポスターシュロスマン症候群	1.9
糖尿病性虹彩炎	1.6
HLA-B27 関連ぶどう膜炎	1.5
急性網膜壊死	1.3
眼トキソプラズマ症	1.1
眼トキソカラ症	1.1
HTLV-I 関連ぶどう膜炎	1.1
真菌性眼内炎	1.0
眼内悪性リンパ腫	1.0
膠原病関連ぶどう膜炎	1.0
CMV 網膜炎	0.8
眼結核	0.7
IBD 関連ぶどう膜炎	0.6
若年性ぶどう膜炎 (JRA を除く)	0.5
フックス虹彩異色性虹彩毛様体炎	0.5
JRA 関連ぶどう膜炎	0.5
その他	11.1
分類不能	38.9
計	100

HLA: ヒ白血球抗原, HTLV: ヒト T リンパ球向性ウイルス, CMV: サイトメガロウイルス, IBD: 炎症性腸疾患, JRA: 若年性関節リウマチ (文献¹⁾より許可を得て引用, 改変)

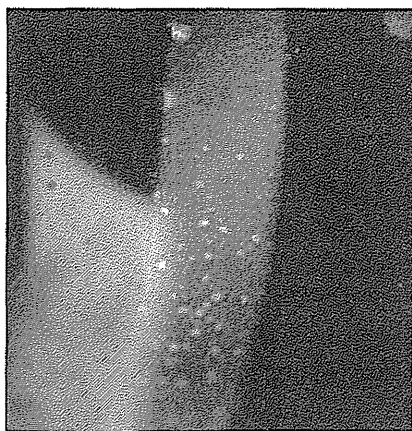


図1 サルコイドーシスの細隙顕微鏡写真（多数の豚脂様角膜後面沈着物が観察される。）

子生物学的診断法の進歩などが関係するものと考えられる。

II. ぶどう膜炎の分類

様々な疾患で構成されているぶどう膜炎を分類する際には、その病因による分類、炎症の解剖学的主座による分類、そして炎症の性状による分類を用いる。

病因による分類では、ぶどう膜炎は非感染性ぶどう膜炎と感染性ぶどう膜炎に大別される。炎症の主座による分類では、①虹彩やその周囲組織に炎症の主座があるものを前部ぶどう膜炎、②毛様体と硝子体に炎症の主座があるものを中間部ぶどう膜炎、③網膜や脈絡膜に炎症の主座があるものを後部ぶどう膜炎と呼び、④前眼部から後眼部にかけて眼内全体に炎症が波及

しているものを汎ぶどう膜炎と呼ぶ。炎症の性状による分類では、肉芽腫性ぶどう膜炎と非肉芽腫性ぶどう膜炎に分類される。肉芽腫性ぶどう膜炎は眼内のさまざまな組織における結節形成を特徴とし、細隙顕微鏡検査や隅角検査、倒像鏡による眼底検査などで直接観察できる。代表的なものにサルコイドーシス、ヘルペス感染によるぶどう膜炎などが挙げられる。非肉芽腫性ぶどう膜炎にはベーチェット病などが挙げられる。

これらの疾患分類を組み合わせ、眼所見をもとに「肉芽腫性汎ぶどう膜炎」などと記述することで、ぶどう膜炎の原因疾患の類推、病態の理解に役立てることができる。

III. 非感染性ぶどう膜炎

非感染性ぶどう膜炎は、原因が明らかなぶどう膜炎の大半を占める疾患分類である。その診断は眼所見や全身検査所見の特徴からなされ、そのためには詳細な眼科的精査はもちろんのこと、患者の生活歴、既往歴、前駆症状などの問診、類推される鑑別診断のための必要十分な全身検査の施行とその解釈が重要となる。代表的な原因疾患にはサルコイドーシス、Vogt-小柳-原田病、ベーチェット病などがあり、これらは順位の変動はあるものの常にわが国のぶどう膜炎原因疾患の上位3位を占める。

1. サルコイドーシス

原因不明の非乾酪壊死性類上皮肉芽腫が全身多臓器に生じ、眼では主に肉芽腫性ぶどう膜炎（図1）を生じる。わが国で最も頻度の高いぶどう膜炎の原因疾患である。前部ぶどう膜炎のみ

表2 サルコイドーシスの診断基準と診断の手引き-2006より、眼病変を強く示唆する臨床所見

- | |
|------------------------------------|
| 1) 肉芽腫性前部ぶどう膜炎（豚脂様角膜後面沈着物、虹彩結節） |
| 2) 隅角結節またはテント状周辺虹彩前癒着 |
| 3) 塊状硝子体混濁（雪玉状、数珠状） |
| 4) 網膜血管周囲炎（主に静脈）および血管周囲結節 |
| 5) 多発するろう様網脈絡膜滲出斑または光凝固斑様の網脈絡膜萎縮病巣 |
| 6) 視神経乳頭肉芽腫または脈絡膜肉芽腫 |

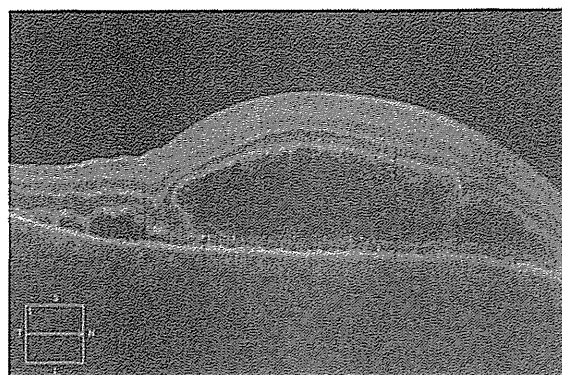


図2 Vogt-小柳-原田病発症初期の網膜光干渉断層像(漿液性網膜剝離を生じている。)

のものから汎ぶどう膜炎までその病態はさまざまであるが、サルコイドーシスによるぶどう膜炎と診断するためには「サルコイドーシスの診断基準と診断の手引き」⁹⁾に示される「眼病変を強く示唆する臨床所見」(表2)の6項目中2項目以上を満たす必要がある。治療にはベタメタゾン0.1%の点眼やトリアムシノロンアセトニドの眼周囲注射、重篤なものにはステロイド薬の内服をプレドニゾン換算で1 mg/kg から開始する。一部の症例は早期に寛解するが、しばしば炎症は遷延し、時に重篤化する。

2. Vogt-小柳-原田病

全身のメラニン産生細胞を標的とした自己免疫性疾患であり⁴⁾⁵⁾、漿液性網膜剝離(図2)を伴う肉芽腫性汎ぶどう膜炎を呈する。色素に富んだ網脈絡膜組織の他に内耳や髄膜にも炎症を生じるため、難聴や耳鳴り、無菌性髄膜炎などの全身症状を呈し、これらは原田病の診断のために重要な所見となる。そのため本症が疑われるぶどう膜炎症例には、早期の聴力検査や髄液検査を行うことが診断に必須である⁶⁾。晩期には白髪や皮膚の白斑、眼底には夕焼け状眼底(図3)と呼ばれる網脈絡膜の脱色素を生じる。本症に罹患する患者素因として、HLA-DR4, DR53, DQ4などの関与が報告されている⁷⁾。治療は原則的にステロイドパルス療法を行い、後療法にはプレドニゾン0.5~1 mg/kg から長期間を

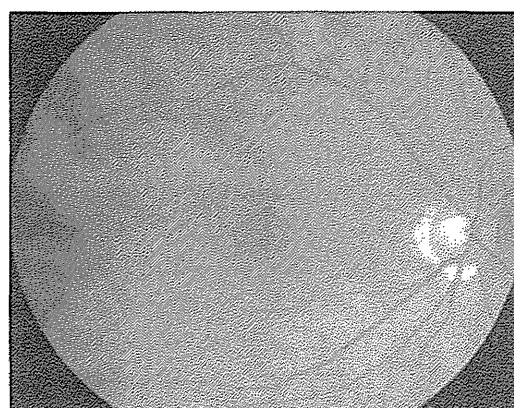


図3 Vogt-小柳-原田病回復期の眼底写真(網脈絡膜の脱色素による夕焼け状眼底を生じている。)

かけた注意深い漸減が再発防止のために重要である⁸⁾。ステロイドに対する反応不良な遷延例にはシクロスポリン⁹⁾¹⁰⁾、メトトレキサート¹¹⁾などの免疫抑制剤を用いる。

3. ベーチェット病

反復する前房蓄膿性虹彩毛様体炎(図4)と網脈絡膜炎を特徴とする、非肉芽腫性汎ぶどう膜炎である。再発性口腔内アフタ性潰瘍、外陰部潰瘍、結節性紅斑様皮疹や皮下血栓性静脈炎などの皮膚症状、それにぶどう膜炎を加えた4つの主症状と、関節炎、副睾丸炎、回盲部潰瘍、血管病変、中枢病変等の副症状から成る全身性の炎症性疾患である。本症はHLA-B51との相



図4 ベーチェット病眼発作時の細隙鏡顕微鏡写真（前房蓄膿が二ポーを形成している。）

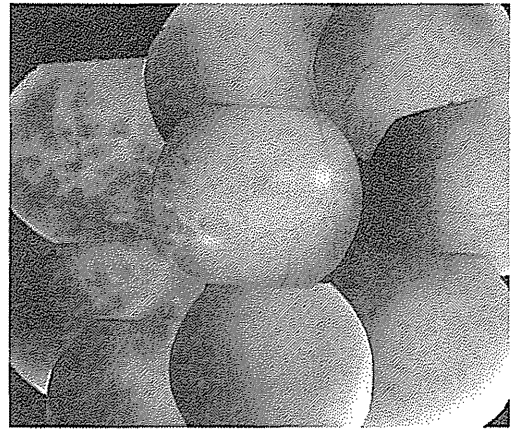


図5 ベーチェット病眼発作時の眼底写真（閉塞性網膜血管炎に網膜出血を伴っている。）

関¹²⁾が以前より知られており、本症患者の約60%が陽性とされる。本症に生じる網脈絡膜炎は網膜動静脈血管の閉塞性病変（図5）が主体であり、それを急性発作性に反復することで黄斑変性、増殖性眼底変化、視神経萎縮などの重篤な網膜視神経障害を生じ、失明に至る症例も多い。そのため治療は炎症発作の抑制が主眼となる。炎症発作時にはトリアムシロンアセトニドやデキサメサゾンなどのステロイド薬の眼球周囲注射による速やかな消炎を行い、視神経や黄斑部網膜に重篤な炎症発作を生じた場合には、水溶性プレドニゾロンの大量点滴静注やステロイドパルス療法を行うこともある。炎症発作の抑制にはコルヒチン1mg/日の内服、それでも炎症発作を反復する例ではシクロスポリンをトラフ値で100 ng/mL前後に調整して内服を行うが、これらの治療の無効例に対しては抗TNF- α 抗体であるインフリキシマブ（レミケード[®]）がベーチェット病による網膜ぶどう膜炎に対して2010年に追加承認され、高い効果をあげている¹³⁾。

IV. 感染性ぶどう膜炎

病原微生物の眼内感染と、それに対する免疫反応により生じる病態であり、細菌、真菌、ウイルス、寄生虫と、さまざまなものが原因となる。感染様式には他臓器からの伝播による内因性感染と、内眼手術や穿孔性眼外傷により生じ

る外因性感染がある。内因性感染によるぶどう膜炎はあらゆる病原微生物で生じ得る。一方、外因性感染によるぶどう膜炎は、主に細菌や真菌で生じる。診断には、それぞれの感染症に特徴的な眼所見、全身検査所見、全身既往歴、生活歴に加えて、全身的な感染症検索、何より眼内局所からの病原微生物の分離、PCR法による遺伝子検索や、病原微生物に対する特異的抗体の眼内局所における産生を証明することが診断の決め手となる。

1. 細菌、真菌によるぶどう膜炎

細菌感染によるぶどう膜炎は、その多くで急性進行性の重篤な眼内炎症を生じ、時に数日の経過で失明に至ることもある。フィブリン析出を伴う激しい汎ぶどう膜炎（図6）を生じ、網膜に感染が成立すれば短期間に網膜壊死と網膜剝離を生じる。内因性細菌性眼内炎は肝膿瘍や腎盂腎炎などから血行性に眼内に伝播し、突然の充血、眼痛、視力低下で発症する。眼内炎発症の時点ではその原因となる他臓器の感染巣が明らかでない場合も多く、原発巣の検索、原因菌や抗生剤感受性の同定などに時間を費やしている間に不可逆的な網膜障害をきたすため、本症が強く疑われた際には速やかに眼内液採取、血液培養提出を行い、それらの結果を待たずに硝子体内へのバンコマイシンとセフトジジムの混合