Research article

The impact of aqueous humor polymerase chain reaction and serological test results for establishing infectious uveitis diagnosis: An Indonesian experience

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ABSTRACT

Objective: To assess the clinical value of aqueous humor real-time polymerase chain reaction (RT-PCR) and serological antibody tests among uveitis patients in Indonesian cohort.
Methods: In this prospective cohort study, single-plex RT-PCR analysis of aqueous samples from 86 new uveitis patients was performed to detect *Mycobacterium tuberculosis*, *Toxoplasmosis gondii*, cytomegalovirus, herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, and rubella virus. Specific serological antibodies for suspected pathogens were also obtained. Comparison of PCR and serological antibodies with the initial and final diagnosis were presented.
Results: The diagnostic positivity of aqueous RT-PCR in our cohort was 20% (17/86). The rate of infection as final etiological classification was higher after RT-PCR was performed (45 patients, 52%) compared to initial diagnosis based on clinical presentation alone (38 patients, 44%). In particular, the RT-PCR positivity among patients with infection as the final etiological classification was 33.33% (15/45). A significant difference in the IgG but not IgM toxoplasma value among those with ocular toxoplasmosis as the final diagnosis compared to the other etiologies were observed (3953 (IQR 2707–19562) IU/mL vs 428 (IQR 82–1807) IU/mL; p < 0.0001).
Conclusion: RT-PCR analysis of aqueous fluid from uveitis patients helped confirm a third of infectious uveitis cases in Indonesia. In ocular toxoplasmosis, high IgG but not IgM antibody value might help differentiate those with other etiology.

1. Introduction

Uveitis is a spectrum of multiple ocular inflammation entities contributing up to 20–25% of blindness [1]. It is estimated that the prevalence of uveitis is around 75 to 714 cases per 100,000 in the population [1, 2, 3]. Unlike non-infectious uveitis, which is more common in the developed world, infection accounted for a higher proportion as the cause of uveitis in the developing countries [1, 4], including Indonesia. Our previous study revealed that approximately one-third of new uveitis cases were due to infection [5]. *Mycobacterium tuberculosis* (Mtb) and

Toxoplasma gondii (*T. gondii*) were the two commonest causes of infectious etiology in our population [5].

The diagnosis of uveitis, particularly infectious uveitis, is challenging with its variation of phenotypes. Suspicion of infection in the first place needs a high index of clinical suspicion based on the clinical presentation [6, 7, 8]. The ancillary investigations are not mandatory but are often considered beneficial to guide the diagnosis even in the era of advanced diagnostic procedures. These include multimodal imaging, serological tests for a specific immunoglobulin, and even intraocular fluid polymerase chain reaction (PCR) addressing a specific pathogen based on

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clinicians' preferences [7, 9]. However, firm diagnostic criteria are lacking for most infectious uveitis entities; thus, it leads experts to make a consensus for giving a specific treatment instead, as in ocular tuberculosis [10]. In practice, clinicians mostly rely on demographic data and morphological lesions to diagnose infectious uveitis. Interestingly, inter-observer agreement among experts in establishing uveitis diagnosis is not high (Kappa 0.39) [11]. Thus, the SUN (Standardization of Uveitis Nomenclature) Working Group tried to establish classification criteria in which intraocular fluid and serological positivity could help but not necessarily be positive to classify a specific infectious uveitis entity. However, they warned that the classification criteria are merely not the same as diagnostic criteria [12].

Recent advances in molecular detection lead to the increased utility of intraocular fluid PCR using only a small amount of samples [13, 14]. It was reported that the positive result of intraocular fluid PCR could change initial diagnosis and therapy in more than one-third of patients with suspicion of infectious uveitis [13]. Even though it is considered a rapid, sensitive, and specific method, a combination with Goldmann-Witmer Coefficient (GWC) analysis is sometimes employed to increase its utility further [15]. On the other hand, the performance of the immunological tests for detecting indirect evidence of infections, i.e., IgG and IgM, has gained less attention even though they may still be employed in clinical settings. Seropositivity across populations could be varied, and the interpretation of such positive results could be misleading, especially in only positive IgG [16].

Hereby, we presented our experience dealing with intraocular fluid RT-PCR and serological tests in confirming the diagnosis of infectious uveitis in our population. The selection of pathogens tested for RT-PCR was based on our epidemiological data that Mtb and *T. gondii* were the two most frequent causes of infectious uveitis [5]. Besides, additional five common causes of viral uveitis based on the existing literature: cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and Rubella virus, were also evaluated [17]. To our knowledge, this is the first study reporting on the results of standardized RT-PCR in uveitis cases in Indonesia. This result would offer diagnostic value of the aqueous humor examination, confirming the diagnosis in suspected infection and excluding the possibility of infection in suspected non-infectious cases.

2. Materials and methods

This was a prospective study of RT-PCR analysis recruiting suspected infectious uveitis cases between October–December 2016 with a median follow-up duration of 5.10 (IQR 0.93–15.07) months at Clinical Microbiology Laboratory, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital. Informed consent was obtained from all recruited patients. Aqueous samples were taken in the outpatient clinic of the Department of Ophthalmology, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital. A comprehensive ocular and systemic review were performed on all patients. The tailored laboratory investigations were performed as needed to support the diagnosis based on the attending uveitis specialist's preferences. Demographic profiles and presenting visual acuity based on the Snellen visual acuity test were noted. Patients with a shallow anterior chamber (von Herrick grade <2) or already on an antimicrobial drug for uveitis were excluded. Initial clinical diagnosis was recorded based on the clinical judgment of the uveitis consultant in charge at the initial visit based on systematic history taking and clinical appearance from the slit-lamp examination. HIV positivity was recorded based on a qualitative antibody screening test result. Viral load data for patients in this study were not recorded.

Serological toxoplasma, CMV, rubella, HSV-1, and HSV-2 IgM and IgG tests were performed on all patients. Following their first visit to our center, patients were subjected to a 3-ml venous blood sample collection using BD vacutainer serum collection tubes (Becton Dickinson, CA, USA) and then centrifuged (3000 rpm) for 10 min. Sera were subsequently processed to both qualitative and quantitative assays against pathogen-

specific IgM and IgG antibodies. Sera were examined using Roche Elecsys Toxo, CMV, and Rubella (IgM and IgG) assays (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations by an automated electrochemiluminescent immunoassay (ECLIA) system on Roche Elecsys and Roche (Cobas e411) immunoassay analyzers. For HSV-1 and HSV-2, the enzyme-linked immunosorbent assays (ELISA) method was used using Herpelisa 1 and Herpelisa 2 (IgM and IgG; Indec Diagnostics, Jakarta, Indonesia). All interpretation results were based on the manufacturer's criteria. Serological assays were all performed under clinical pathologist supervision in the general laboratory of Cipto Mangunkusumo General Hospital, Jakarta, Indonesia (ISO certification number: 15189:2012).

Anterior chamber paracentesis was performed in a procedural room. About 100–200 μl of aqueous samples were obtained using a 30-gauge needle on a 1 mL syringe under topical anesthesia and topical 5% povidone-iodine solution. Samples were then stored in a 1.5 ml microtube and transported immediately to the diagnostic laboratory (Department of Microbiology, Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo General Hospital) within 2 h under 4 °C storage. Samples were then centrifuged (12,000 rpm) for 5 min. Pellet was separated for Mtb and *T. gondii* detection while the supernatant was stored under −80 °C before the DNA extraction.

We performed the extraction using QIAamp Viral RNA Kit (Qiagen, Cat. No: 52904) or QIAamp DNA Mini Kit (Qiagen, Cat. No: 51304) according to the manufacturer's protocol with final elution of 40 μl (DNA) and 60 μl (RNA) and stored at −80 °C for below 48 h. The elution was used as a template for single-plex RT-PCR targeting *T. gondii*, Mtb, HSV, VZV, CMV, EBV, dan Rubella virus (primers used for PCR are listed in Table 1). A cycle threshold <40 was considered a positive PCR result (Supplementary file 1). The final diagnosis was established after analyzing clinical presentation, laboratory examination, systemic evaluation, RT-PCR results, and a positive response toward the particular treatment prescribed by the attending uveitis specialist. In general, toxoplasmosis, TB, CMV, HSV, VZV, and syphilis were treated with appropriate anti-infective drugs (Supplementary file 2). Rubella and EBV uveitis required no specific treatment. Non-infectious uveitis was treated with systemic corticosteroid with additional immunosuppressive agents under the supervision of a clinical immunologist or rheumatologist if necessary. Masquerades syndromes were treated according to the specific cause (i.e., retinal surgery for retinal detachment and lymphoma protocol for intraocular lymphoma with ocular oncologist referral). Topical corticosteroid (prednisolone acetate 1%) was prescribed for anterior chamber inflammation control and tapered according to the clinical response.

This study was approved by the Ethical Committee of the Faculty of Medicine Universitas Indonesia (No. 737/UN2.F1/ETIK/2016) and adhered to the tenets of the Declaration of Helsinki. Descriptive statistics were computed using statistical software (Statistical Package for Social Science version 25 for windows). A comparison of two independent continuous variables with non-parametric distribution was performed using the Mann-Whitney test. *P*-value < 0.05 was considered to be significant.

3. Results

3.1. Patients' characteristics and anatomical inflammation site

A total of 86 patients along with their aqueous samples were collected during the study period. The median age was 36 (28–49) years, and more female patients were analyzed (48 patients, 56%). The median time from onset of symptoms to the first visit to our center was 65.5 days (interquartile range [IQR] 30–211.5 days). We found that twelve patients were HIV positive. Positive TST results occurred in 83% of patients with HIV, which is significantly higher compared to the HIV-negative group (39%). More than half (55%) of patients presented unilateral ocular involvement with panuveitis as the most common inflammation location, both in HIV-

Table 1. Primers used in the analysis of polymerase chain reactions.

Mycobacterium tuberculosis [44, 45]	Forward	5′-CCT GCG AGC GTA GGC GTC GG-3′
	Reverse	5′-CTC GTC CAG CGC CGC TTC GG-3′
Toxoplasma gondii [46]	Forward	5′-CTA GTA TCG TGC CGC AAT GTG-3′
	Reverse	5′-GGC AGC GTC TCT TCC TCT TTT-3′
	Probe	5′-FAM-CCA-CCT-CGC-CTC-TTG-G-3′
CMV [47]	Forward	5′-CAT GAA GGT CTT TGC CCA GTA C-3′
	Reverse	5′-GGC CAA AGT GTA GGC TAC AAT AG-3′
	Probe	5′-TGG CCC GTA GGT CAT CCA CAC TAG G-3′
HSV [48]	Forward	5′-CCG TCA GCA CCT TCA TCG A-3′
	Reverse	5′-CGC TGG ACC TCC GTG TAG TC-3′
	Probe	5′-CCA CGA GAT CAA GGA CAG CGG CC-3′
VZV [48]	Forward	5′-TCT TTC ACG GAG GCA AAC AC-3′
	Reverse	5′-TCC AAG GCG GGT GCA TAT CT- 3′
	Probe	5′-FAM-TAA CGT GGC TCG AGA ACG GTT TGG GTT T-3′
EBV [47]	Forward	5′-CGG AAG CCC TCT GGA CTT C-3′
	Reverse	5′-CCC TGT TTA TCC GAT GGA ATG-3′
	Probe	5′-TGT ACA CGC ACG AGA AAT GCG CC-3′
Rubella	Forward	5′-CCT AHY CCC ATG GAG AAA CTC CT- 3′
	Reverse	5′-AAC ATC GCG CAC TTC CCA- 3′
	Probe	5′-CCG TCG GCA GTT GG -3′

positive and negative patients. Posterior and panuveitis accounted for more than four-fifth of uveitis cases (Table 2).

3.2. Aqueous humor PCR and serological examination results

Overall, the positivity of RT-PCR from aqueous samples in our setting was 20% (17/86). The details of PCR positivity based on the anatomical involvement of inflammation and initial suspicion of the causative pathogen were presented in Table 3 and Figure 1, respectively. Based on anatomical involvement, the highest positivity of aqueous RT-PCR was observed in posterior uveitis (30%), followed by anterior uveitis (18%). The percentage of positive PCR results was considerably higher among HIV-positive patients: 60% in posterior uveitis and 40% in panuveitis cases with infectious uveitis found to be the predominant type in this particular group (10/12; 83.3%), and half of them (5/10; 50%) showed positive PCR result. Interestingly, only one-third of patients having infectious uveitis as the final diagnosis had PCR positive for one of the pathogens. The proportion of positive PCR results for a specific pathogen that matches with each final infectious etiological diagnosis is further displayed in Figure 2. All patients with VZV (2 patients), EBV (2 patients), and rubella (2 patients) as the final etiological cause of uveitis were confirmed by PCR results.

Table 4 elaborates on the final clinical diagnosis of infectious uveitis and their association with serology results. There was no difference in the proportion of serological positivity with the final infectious etiological diagnosis. However, there was a significant difference in the IgG toxoplasmosis values among those with toxoplasmosis as the final diagnosis compared to the other etiologies (3953 (IQR 2707–19,562) IU/mL vs 428 (IQR 82–1807) IU/mL; p < 0.0001).

3.3. Changes in diagnosis before and after work-up

Table 5 shows the crossmatch of initial and final uveitis diagnosis among recruited cases. Initially, 38/86 (44%) cases were classified as infectious uveitis. After work-up, the number increased to 45/86 (52%). Undetermined etiology contributed to most of that addition (5/7: 4 TB and 1 HSV, Table 5). At first, uveitis due to toxoplasmosis was thought to be occurred in nearly half (44.7%) of infectious uveitis cases, followed by TB (31.5%). After work-up, including PCR and serological test, TB uveitis

was the primary infectious uveitis in our center (18/45, 40%), followed by toxoplasmosis (12/45, 26.7%). Even after the PCR analysis, uveitis of unknown/undetermined etiology remained as the second most prevalent type of uveitis in our center (19% of all cases).

Because of the rapid results obtained by PCR and serological assays, switching treatments occurred immediately. Of note, one patient with an initial clinical diagnosis of toxoplasmosis switched to valganciclovir due to positive PCR for CMV, which then well responded to the anti-CMV treatment. Three patients switched to anti-toxoplasma treatment based on high IgG results (1831 IU/ml, 963 IU/ml, and 995 IU/ml).

The sensitivity and specificity for single-plex RT-PCR of each pathogen are shown in Table 6. Discordance results between aqueous humor PCR positivity with the final infectious uveitis diagnosis were observed in three patients. A case initially considered as an undetermined cause of uveitis had a positive CMV result which was finally diagnosed as an unknown cause of uveitis. The patient had a clinical picture not compatible with classic CMV retinitis, visual acuity of no light perception, tested negative for HIV screening, and no other immunocompromised issue. With those findings, this case was very unlikely to be a CMV uveitis case. The other observed discordance was a female patient with posterior uveitis having positive *T. gondii* PCR. This patient had recurrent inflammation with anti-toxoplasma treatment and then switched to antitubercular treatment, which then the inflammation was well-controlled. Among non-infectious uveitis entities in the final diagnosis, only one patient diagnosed with Behcet's disease (non-infectious systemic disease) had a positive PCR result (positive for Rubella), which the result was then put aside for further disease management.

4. Discussion

One of the most important factors to consider when treating uveitis is determining whether the underlying cause is infectious or non-infectious [18]. In developing countries, including Indonesia, where ancillary testings are not easily accessible in all centers, implementing a diagnostic approach based on clinical pattern recognition is essential in managing uveitis cases [6, 7, 8]. Determining a specific pathogen that causes infectious uveitis is paramount to guide an appropriate antimicrobial treatment administration [19]. We found that eighty percent (36 out of

Table 2. Demographic characteristics of patients.

Characteristic	Total	HIV Positive	HIV Negative
	N = 86	N = 12	N = 74
Age (median, IQR), years	36 (28–49)	34 (29–36)	41 (25–49)
Gender			
Male	38 (44%)	10 (83%)	28 (38%)
Female	48 (56%)	2 (17%)	46 (62%)
TST positive*	25/57 (44%)	5/6 (83%)	20/51 (39%)
Follow up duration (median, IQR), days	153 (28–470)	153 (30–538)	114 (21–445)
HIV positive	12 (14%)		
Laterality			
Unilateral	47 (55%)	5 (42%)	42 (57%)
Bilateral	39 (45%)	7 (58%)	32 (43%)
Location of inflammation			
Anterior uveitis	11 (13%)	1 (8%)	10 (14%)
Intermediate uveitis	2 (2%)	0 (0%)	2 (3%)
Posterior uveitis	30 (35%)	5 (42%)	25 (33%)
Panuveitis	43 (50%)	6 (50%)	37 (50%)

IQR: interquartile range, TST: Tuberculin skin test, IGRA: Interferon Gamma Release Assay: tuberculosis, VKH: Vogt Koyanagi Harada, FUS: Fuchs uveitis syndrome. *TST positive cut-off ≥ 10 mm and ≥ 5 mm in HIV-negative and HIV-positive, respectively; 29 patients were not tested for TST: 6 HIV positive and 23 HIV negative.

Table 3. PCR positivity in each pathogen based on the anatomical location of uveitis.

| Anatomical location of uveitis	N (patients)	Breakdown of PCR with positive results							Total PCR positivity (N = 86)	Total PCR positivity in HIV positive (N = 12)
		Toxo-plasma	Tuber-culosis	CMV	HSV	VZV	Rubella	EBV		
Anterior uveitis	11/86 (13%)	0/11 (0%)	1/11 (9%)	0/11 (0%)	0/11 (0%)	0/11 (0%)	1/11 (9%)	0/11 (0%)	2/11 (18%)	0/1 (0%)
Intermediate uveitis	2/86 (2%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/0 (0%)
Posterior uveitis	30/86 (35%)	2/30 (7%)	0/30 (0%)	3/30 (10%)	0/30 (0%)	2/30 (7%)	1/30 (3%)	1/30 (3%)	9/30 (30%)	3/5 (60%)
Panuveitis	43/86 (50%)	1/43 (2.5%)	2/43 (5%)	1/43 (2.5%)	0/43 (0%)	0/43 (0%)	1/43 (2.5%)	1/43 (2.5%)	6/43 (14%)	2/6 (40%)
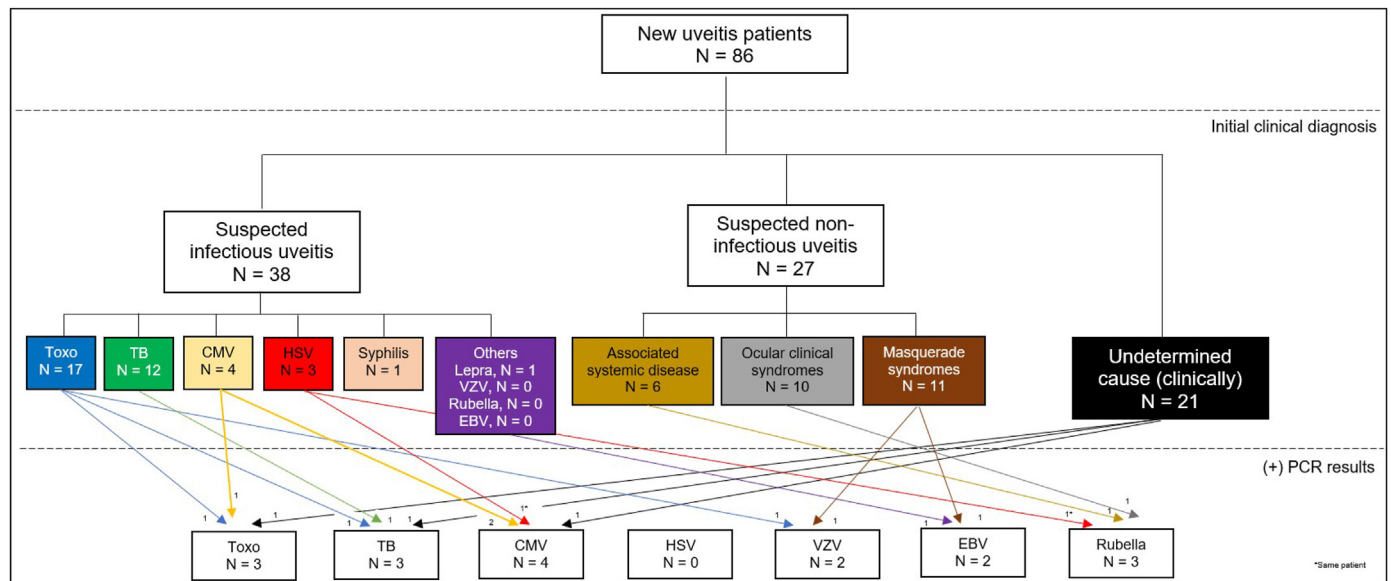
Figure 1. Diagram flow of PCR positivity results based on initial uveitis clinical diagnosis. Notes: Colored arrows describe the shifting flow from initial clinical diagnosis to the final infectious ethiological diagnosis made based on aqueous PCR results. Numbers next to each arrow indicate the number of patients whose diagnosis shifted according to the direction of the arrow. *One patient initially suspected of HSV showed positive PCR results for both CMV and Rubella.

45) cases in this study have been correctly diagnosed as infectious uveitis before work-up. Besides, the most common type of uveitis encountered in our center is still infectious uveitis, consistent with our previous study conducted in 2014–2015 [20]. In this study, we also found that a comparison of initial and final diagnosis after aqueous humor PCR in cases with suspicion of non-infectious systemic disease (100%), ocular clinical syndromes (82%), and masquerade syndromes (90%) showed consistency, thus confirming the specificity of aqueous humor PCR in such situations as this is a benchmark study for intraocular PCR in uveitis cases in Indonesia. As for the serological assay, IgM antibodies appear at the end of the first week of infection, begin to fall in 4–8 weeks, and may persist in low levels up to a year, while IgG antibodies appear after 2 weeks, peak by 3 months of infection and may persist throughout life [21]. Despite the low sensitivity, the specificity of the IgM test is claimed to be relatively high [22]. Combination of several work-up methods is stated to increase the diagnostic yield, especially in atypical lesions [22]. The current study found that TB was the leading cause of infectious uveitis, followed by toxoplasmosis. Interestingly, compared to initial clinical diagnosis, the increasing number of cases between pre-and post-work-up in this study only occurred in TB. This implies that ocular TB is difficult to recognize only from the clinical presentation and often resembles other etiologies [23].

In the context of ocular TB, PCR from intraocular fluids was often negative [23], and in most cases, the diagnosis of ocular TB was only presumptive [24]. In our experience, we only found three cases with positive PCR for Mtb (PCR Mtb sensitivity 16.7%, specificity 100%), out of 18 cases established as ocular TB after considering the results of other investigations and observing the clinical course response to treatment.

The distribution of Mtb in the retinal pigment epithelium (RPE) [25] could contribute to this result. The sensitivity of PCR was reported to be lower when the sample was obtained not in the main localization site of inflammation [26]. In our study, the ocular fluid samples were limited to aqueous fluid, thus potentially contributing to the low sensitivity of PCR for Mtb. Besides, the inflammation in ocular TB could also be associated with latent tuberculous infection (LTBI) instead of the direct invasion from a viable organism in ocular tissue [27]. This mechanism will theoretically limit the utility of ocular fluid PCR in ocular TB cases [28]. Two tests are currently available for the diagnosis of LTBI: tuberculin skin test (TST) and interferon (IFN)-gamma release assay (IGRA) [29]. In this study, 57 patients underwent TST, and 25 (44%) had positive results. In particular, the percentage of positive TST results was much higher in the HIV-positive group (83%) than in the HIV-negative group (39%). However, among the HIV-infected group in this study, none of them had ocular TB as their final diagnosis despite the positive TST result, suggesting its low utility and a need for corroborative pieces of evidence to diagnose ocular TB [28].

Our study also found low positivity for *T. gondii* PCR (overall: 3/86, 3.5% and specific at final diagnosis: 2/12, 17.0%) with a sensitivity of 16.7% and specificity of 98.6%. Previous reports showed that a routine PCR for toxoplasmosis yielded 7–8% positivity rate among uveitis patients [30, 31]. It has been suggested that the positivity rate may be increased if the nested PCR method was performed [32, 33]. Moreover, the vitreous may be more suitable for PCR analysis in toxoplasmosis as the posterior part of the eye is thought to be primarily involved in the inflammation process [34, 35]. In addition, the necrotizing lesions in toxoplasmosis cases could also be caused by immunological mechanisms
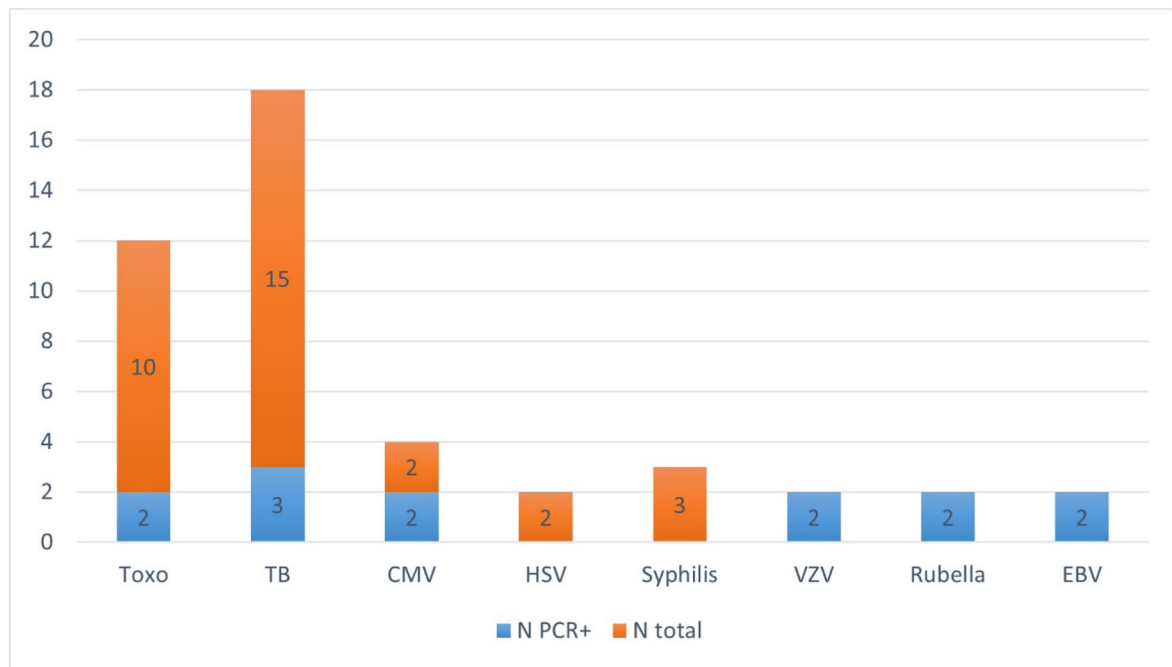
Figure 2. Proportion of positive PCR results that match with final infectious uveitis diagnosis. *One of the patients was diagnosed as syphilis and CMV.

instead of the presence of the parasite itself [36]. Interestingly, we found that serological analysis plays a vital role in establishing the diagnosis of ocular toxoplasmosis. A negative result for toxoplasmosis antibodies is generally believed to be more informative than a positive result [37, 38]. However, more than a merely positive or negative result of the test, a high IgG serum antibody titer (3–4 times higher than normal level or increased titer in three weeks intervals) was reported to indicate an active infection [16, 39]. In our population, the IgG value in patients with ocular toxoplasmosis was about nine-fold higher than in patients not diagnosed with ocular toxoplasmosis (3953 IU/ml vs 428 IU/ml, respectively). This result is in accordance with previous studies in South Korea (Park et al) [39] and Switzerland (Papadia et al) [40] populations; nonetheless, the IgG value in our study is found to be much higher than

those two previous reports. In Park et al study, they found that the average toxoplasma IgM and IgG titers were 2.29 IU/mL and 223.4 IU/mL, respectively [39]. We presumed that different populations in different geographic areas might be attributed to this phenomenon. However, there is still no clear explanation for such a high increase in IgG titer was found in cases of ocular toxoplasmosis in our population. Furthermore, we found that the high value of IgG seems more critical than the IgM positivity in the context of establishing the diagnosis of ocular toxoplasmosis, potentially due to more cases related to reactivation in nature [35].

With regard to viral etiologies, previous reports showed that viral pathogens had been regarded as the common cause of anterior uveitis [41, 42]. In our cohort, one case (1/11, 9.1%) had a final diagnosis of

Table 4. Peripheral blood serological test role in the final diagnosis.

Final diagnosis	Total N = 86	Specific IgG value IU/ml (median; IQR)	Specific IgM value (median; IQR)	Specific IgG positivity (N, %)		Specific IgM positivity (N, %)		Specific PCR positivity (N, %)	
				Positive	Negative	Positive	Negative	Positive	Negative
Toxoplasmosis[a]									
Yes	12 (14%)	3953; 2707-19,562*	0.2; 0-2-0.4	11/75 (15%)	0/75 (0%)	1/70 (1.5%)	9/70 (13%)	2/86 (2.5%)	10/86 (11.5%)
No	74 (86%)	428; 82-1807	0.3; 0.2-0.4	51/75 (68%)	13/75 (17%)	3/70 (4%)	57/70 (81.5%)	1/86 (1%)	73/86 (85%)
Rubella[a]									
Yes	2 (2.5%)	43; 27-60	NA	2/71 (3%)	0/71 (0%)	0/65 (0%)	1/65 (1.5%)	2/86 (2.5%)	0/86 (0%)
No	84 (97.5%)	70; 20-216	0.3; 0.2-0.3	57/71 (80%)	12/71 (17%)	4/65 (6%)	60/65 (92.5%)	1/86 (1%)	83/86 (96.5%)
CMV[a]									
Yes	4 (5%)	1407; 356-2457	0.85; 0–1.7	2/74 (3%)	0/74 (0%)	1/68 (1.5%)	1/68 (1.5%)	3/86 (3.5%)	1/86 (1%)
No	82 (95%)	210; 102-408	0.3; 0.2-0.4	68/74 (92%)	4/74 (5%)	5/68 (7.5%)	61/68 (90%)	1/86 (1%)	81/86 (94.5%)
HSV (for HSV1 serology)[a]									
Yes	2 (2.5%)	4.2; (3.7–4.7)	0.3; (0.3–0.4)	2/73 (3%)	0/73 (0%)	0/72 (0%)	2/72 (2.5%)	0/86 (0%)	2/86 (2.5%)
No	84 (97.5%)	2.5; (0.4–4)	0.4 (0.3–0.6)	49/73 (67%)	22/73 (30%)	4/72 (5.5%)	66/72 (92%)	0/86 (0%)	84/86 (97.5%)
HSV (for HSV2 serology)[a]									
Yes	2 (2.5%)	0.2; (0.2–0.3)	0.1; 0.1-0.1	0/71 (0%)	2/71 (3%)	0/70 (0%)	2/70 (3%)	0/86 (0%)	2/86 (2.5%)
No	84 (97.5%)	0.2; (0.2–0.3)	0.3; 0.2-0.5	7/71 (10%)	62/71 (87%)	4/70 (6%)	64/70 (91%)	0/86 (0%)	84/86 (97.5%)

* p value < 0.0001; NA data is not available.
[a] We exclude unavailable data and indeterminate results. Toxoplasma IgG N = 75, toxoplasma IgM N = 70, rubella IgG N = 71, rubella IgM N = 65, CMV IgG N = 74, CMV IgM N = 68, HSV1 IgG N = 73, HSV1 IgM N = 72, HSV2 IgG N = 71, HSV2 IgM N = 70.

Table 5. Cross tabulation showing changes in uveitis diagnosis before and after work-up.

Initial clinical uveitis diagnosis	Final infectious (N = 45), %									Final non-infectious systemic diseases (N = 4), %	Final ocular clinical syndromes (N = 11), %	Final masquerade syndromes (N = 10), %	Final undetermined (N = 16), %
	Toxo (N = 12)	TB (N = 18)	CMV (N = 4)	HSV (N = 2)	Syphilis (N = 3)	Lepra (N = 0)	VZV (N = 2)	Rubella (N = 2)	EBV (N = 2)				
Infectious (N = 38)	**12/12 (100%)**	**14/18 (78%)**	**4/4 (100%)**	**1/2 (50%)**	**2/3 (67%)**	**0/0 (0%)**	**1/2 (50%)**	**1/2 (50%)**	**1/2 (50%)**	**0/4 (0%)**	**2/11 (18%)**	**0/10 (0%)**	**0/16 (0%)**
Toxo (N = 17)	11/12 (91.7%)	3/18 (16.7%)	0/4 (0%)	0/2 (0%)	1/3 (33.3%)	0/0 (0%)	1/2 (50.0%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	1/2 (50%)	0/0 (0%)	0/0 (0%)
TB (N = 12)	0/12 (0%)	11/18 (16.1%)	0/4 (0%)	0/2 (0%)	0/3 (0%)	0/0 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	1/2 (50%)	0/0 (0%)	0/0 (0%)
CMV (N = 4)	1/12 (8.3%)	0/18 (0%)	3/4 (75%)*	0/2 (0%)	0/3 (0%)	0/0 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)
HSV (N = 3)	0/12 (0%)	0/18 (0%)	1/4 (25%)	1/2 (50%)	0/3 (0%)	0/0 (0%)	0/2 (0%)	1 (50.0%)	0/2 (0%)	0/0 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)
Syphilis (N = 1)	0/12 (0%)	0/18 (0%)	0/4 (0%)	0/2 (0%)	1/3 (33.3%)	0/0 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)
Lepra (N = 1)	0/12 (0%)	0/18 (0%)	0/4 (0%)	0/2 (0%)	0/3 (0%)	0/0 (0%)	0/2 (0%)	0/2 (0%)	1/2 (50.0%)	0/0 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)
VZV (N = 0)	0/12 (0%)	0/18 (0%)	0/4 (0%)	0/2 (0%)	0/3 (0%)	0/0 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)
Rubella (N = 0)	0/12 (0%)	0/18 (0%)	0/4 (0%)	0/2 (0%)	0/3 (0%)	0/0 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)
EBV (N = 0)	0/12 (0%)	0/18 (0%)	0/4 (0%)	0/2 (0%)	0/3 (0%)	0/0 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)
Associated with non-infectious systemic diseases (N = 6)	**0/12 (0%)**	**0/18 (0%)**	**0/4 (0%)**	**0/2 (0%)**	**1/3 (33%)**	**0/0 (0%)**	**0/2 (0%)**	**0/2 (0%)**	**0/2 (0%)**	**4/4 (100%)**	**0/11 (0%)**	**1/10 (10%)**	**0/16 (0%)**
Ocular clinical syndromes (N = 10)	**0/12 (0%)**	**0/18 (0%)**	**0/4 (0%)**	**0/2 (0%)**	**0/3 (0%)**	**0/0 (0%)**	**0/2 (0%)**	**1/2 (50%)**	**0/2 (0%)**	**0/4 (0%)**	**9/11 (82%)**	**0/10 (0%)**	**0/16 (0%)**
Masquerade syndromes (N = 11)	**0/12 (0%)**	**0/18 (0%)**	**0/4 (0%)**	**0/2 (0%)**	**0/3 (0%)**	**0/0 (0%)**	**1/2 (50%)**	**0/2 (0%)**	**1/2 (50%)**	**0/4 (0%)**	**0/11 (0%)**	**9/10 (90%)**	**0/16 (0%)**
Undetermined (N = 21)	0/12 (0%)	4/18 (22%)	0/4 (0%)	1/2 (50%)	0/3 (0%)	0/0 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/4 (0%)	0/11 (0%)	0/10 (0%)	16/16 (100%)

* One of the patients was diagnosed as syphilis and CMV.

Table 6. Sensitivity and specificity of aqueous PCR for in infectious uveitis.

	Toxo	TB	CMV	HSV	VZV	Rubella	EBV
Sensitivity	2/12 (16.7%)	3/18 (16.7%)	3/4 (75.0%)	0/2 (0%)	2/2 (100%)	2/2 (100%)	2/2 (100%)
Specificity	73/74 (98.6%)	68/68 (100%)	81/82 (98.8%)	84/84 (100%)	84/84 (100%)	83/84 (98.8%)	84/84 (100%)

Rubella anterior uveitis. Meanwhile, a larger proportion ended up with idiopathic cause/unknown (5/11, 45.4%). Furthermore, we found that posterior uveitis yielded a higher PCR positivity compared to anterior uveitis (9/30, 30% vs 2/11, 18%), with both CMV and VZV as predominant findings in posterior uveitis (5/9 cases). Based on our results, the PCR analysis generally showed high sensitivity and specificity for Rubella, EBV, CMV, and VZV. Thus, PCR might be beneficial in suspected posterior viral uveitis. On the other hand, our result also supports that the serological test is insignificant in diagnosing viral uveitis. As most parts of the world have high seroprevalences for most causes of viral uveitis, routine viral antibodies have no added value [41].

Our study has several limitations. The current study recruited patients from a single national referral eye hospital in Indonesia. Multisite or multi-center studies from across regions of Indonesia could broadly represent the Indonesian population even though it is difficult to be conducted due to limited facilities in many settings outside Jakarta, the capital city of Indonesia. Also, paired aqueous and vitreous to compare the diagnostic value of each sample was not applicable in our study. This may be beneficial in patients presenting with posterior or panuveitis. The aqueous but not vitreous value as the intraocular fluid selected for PCR in posterior uveitis had been reported elsewhere and yielded useful utility [43]. Moreover, we acknowledge that our study could not combine PCR with GWC. Previously, GWC might be more useful, especially in posterior uveitis with focal necrotizing lesions. Overall, it was estimated that PCR testing alone could potentially miss 66% of diagnosis in posterior uveitis, particularly associated with ocular toxoplasmosis [43]. However, at the time of this study, GWC was not available yet. Interestingly, we found the utility of quantitative serum IgG value to aid the diagnosis of ocular toxoplasmosis, thus helping to overcome the shortcomings of PCR and unavailable GWC in our clinical situation.

In Indonesia, infection is the leading cause of uveitis. RT-PCR analysis of aqueous fluid yielded low positivity, including both TB and toxoplasmosis. However, its sensitivity and specificity were high for viral pathogens. In the case of suspected ocular toxoplasmosis, serum IgG but not IgM antibody has a significant role in indicating active inflammation. Corroborative evidence other than aqueous PCR is necessary to establish the diagnosis of ocular TB. The implementation of GWC can be considered in further study along with its cost-benefit analysis.

Declarations

Author contribution statement

Ikhwanuliman Putera; Priscilla Jessica: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Rina La Distia Nora: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nunik Utami; Andi Yasmon: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Anis Karuniawati: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Dewi Wulandari; Mei Riasanti: Conceived and designed the experiments; Analyzed and interpreted the data.

Lukman Edwar; Ratna Sitompul: Conceived and designed the experiments; Performed the experiments.

Made Susiyanti; Yulia Aziza: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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References

[1] E. Miserocchi, G. Fogliato, G. Modorati, F. Bandello, Review on the worldwide epidemiology of uveitis, Eur. J. Ophthalmol. 23 (5) (2013) 705–717.

[2] L. Dandona, R. Dandona, R.K. John, C.A. McCarty, G.N. Rao, Population based assessment of uveitis in an urban population in southern India, Br. J. Ophthalmol. 84 (7) (2000) 706–709.

[3] K.M. Saari, T. Päivönsalo-Hietanen, H. Vaahtoranta-Lehtonen, J. Tuominen, M. Sillanpää, Epidemiology of endogenous uveitis in south-western Finland, Acta Ophthalmol. Scand. 73 (4) (1995) 345–349.

[4] T. Tsirouki, A. Dastiridou, C. Symeonidis, O. Tounakaki, I. Brazitikou, C. Kalogeropoulos, et al., A focus on the epidemiology of uveitis, Ocul. Immunol. Inflamm. 26 (1) (2018) 2–16.

[5] R. La Distia Nora, R. Sitompul, M. Bakker, M. Susiyanti, L. Edwar, S. Sjamsoe, et al., Tuberculosis and other causes of uveitis in Indonesia, Eye (Lond). 32 (3) (2018) 546–554.

[6] K.F. Tabbara, Infectious uveitis: a review, Arch. Soc. Esp. Oftalmol. 75 (4) (2000) 215–259.

[7] A. Patel, A. Kelgaonkar, H. Kaza, M. Tyagi, S. Murthy, A. Pathengay, et al., Recent advances in diagnosis and treatment of infectious uveitis prevalent in Asia-Pacific region, Asia-Pac. J. Ophthalmol. (Philadelphia, Pa) 10 (1) (2021) 99–108.

[8] A. Agarwal, K. Aggarwal, V. Gupta, Infectious uveitis: an Asian perspective, Eye (Lond.). 33 (1) (2019) 50–65.

[9] S.R. Rathinam, M. Babu, Algorithmic approach in the diagnosis of uveitis, Indian J. Ophthalmol. 61 (6) (2013) 255–262.

[10] R. Agrawal, I. Testi, S. Mahajan, Yuen Y. Sen, A. Agarwal, O.M. Kon, et al., Collaborative ocular tuberculosis study consensus guidelines on the management of tubercular uveitis-report 1: guidelines for initiating antitubercular therapy in tubercular choroiditis, Ophthalmology 128 (2) (2021) 266–276.

[11] D.A. Jabs, A. Dick, J.T. Doucette, A. Gupta, S. Lightman, P. McCluskey, et al., Interobserver agreement among uveitis experts on uveitic diagnoses: the standardization of uveitis nomenclature experience, Am. J. Ophthalmol. 186 (2018) 19–24.

[12] Development of classification criteria for the uveitides, Am. J. Ophthalmol. 228 (2021) 96–105.

[13] A. Chronopoulos, D. Roquelaure, G. Souteyrand, J.D. Seebach, J.S. Schutz, G. Thumann, Aqueous humor polymerase chain reaction in uveitis - utility and safety, BMC Ophthalmol. 16 (1) (2016) 1–7.

[14] M. Mochizuki, S. Sugita, K. Kamoi, H. Takase, A new era of uveitis: impact of polymerase chain reaction in intraocular inflammatory diseases, Jpn. J. Ophthalmol. 61 (1) (2017) 1–20.

[15] H.N.V. Dos Santos, E. Ferracioli-Oda, T.S. Barbosa, C.S.V. Otani, T. Tanaka, L. de CS. da Silva, et al., Usefulness of aqueous and vitreous humor analysis in infectious uveitis, Clinics 75 (2020) 1–6.

[16] S.R. Rathinam, I. Tugal-Tutkun, M. Agarwal, V. Rajesh, M. Egriparmak, G. Patnaik, Immunological tests and their interpretation in uveitis, Indian J. Ophthalmol. 68 (9) (2020) 1737–1748.

[17] P. Lin, Infectious Uveitis, . Curr Ophthalmol Rep. 3 (3) (2015) 170–183.

[18] K. Maruyama, Current standardized therapeutic approach for uveitis in Japan, Immunol. Med. 42 (3) (2019) 124–134.

[19] A.B. Sallam, K.A. Kirkland, R. Barry, M.K. Soliman, T.K. Ali, S. Lightman, A review of antimicrobial therapy for infectious uveitis of the posterior segment, Med. Hypothesis, Discov. Innovation Ophthalmol. J. 7 (4) (2018) 140–155.

[20] R. La Distia Nora, R. Sitompul, M. Bakker, M. Susiyanti, L. Edwar, S. Sjamsoe, et al., Tuberculosis and other causes of uveitis in Indonesia, Eye 32 (3) (2018) 546–554.

[21] S.R. Rathinam, I. Tugal-Tutkun, M. Agarwal, V. Rajesh, M. Egriparmak, G. Patnaik, Immunological tests and their interpretation in uveitis, Indian J. Ophthalmol. Ophthalmol. 68 (9) (2020) 1737–1748.

[22] W. Choi, H.G. Kang, E.Y. Choi, S.S. Kim, C.Y. Kim, H.J. Koh, et al., Clinical utility of aqueous humor polymerase chain reaction and serologic testing for suspected infectious uveitis: a single-center retrospective study in South Korea, BMC Ophthalmol. 20 (1) (2020) 1–7.

[23] G.G. Alvarez, V.R. Roth, W. Hodge, Ocular tuberculosis: diagnostic and treatment challenges, Int. J. Infect. Dis. 13 (4) (2009) 432–435.

[24] F.I. Shakarchi, Ocular tuberculosis: current perspectives, Clin. Ophthalmol. 9 (2015) 2223–2227.

[25] K.V. Chalam, S.K. Gupta, S. Vinjamaram, V a Shah, Tuberculous uveitis: distribution of Mycobacterium tuberculosis in the retinal pigment epithelium, Arch. Ophthalmol. 119 (3) (2006) 409–410.

[26] H.N. Madhavan, K.L. Therese, P. Gunisha, U. Jayanthi, J. Biswas, Polymerase chain reaction for detection of Mycobacterium tuberculosis in epiretinal membrane in Eales' disease, Investig. Ophthalmol. Vis. Sci. 41 (3) (2000) 822–825.

[27] M. Ang, A. Hedayatfar, R. Zhang, S.P. Chee, Clinical signs of uveitis associated with latent tuberculosis, Clin. Exp. Ophthalmol. 40 (7) (2012) 689–696.

[28] R. Agrawal, I. Testi, B. Bodaghi, T. Barisani-Asenbauer, P. McCluskey, A. Agarwal, et al., Collaborative ocular tuberculosis study consensus guidelines on the management of tubercular uveitis—report 2: guidelines for initiating antitubercular therapy in anterior uveitis, intermediate uveitis, panuveitis, and retinal vasculitis, Ophthalmology 128 (2) (2021) 277–287.

[29] D. Hinthorn, M.S. Bader, Tuberculin skin testing: indication, interpretation, and management, Adv. Stud. Med. 4 (10) (2004) 534–542.

[30] K. Pathanapitoon, N. Kongyai, W. Sirirungsi, J.D.F. de Groot-Mijnes, P. Leechanachai, J. Choovuthayakorn, et al., The diagnostic value of intraocular fluid analysis by polymerase chain reaction in Thai patients with uveitis, Trans. R. Soc. Trop. Med. Hyg. 105 (11) (2011) 650–654.

[31] M.A. Scheepers, K.A. Lecuona, G. Rogers, C. Bunce, C. Corcoran, M. Michaelides, The value of routine polymerase chain reaction analysis of intraocular fluid

[32] Q. Liu, Z.D. Wang, S.Y. Huang, X.Q. Zhu, Diagnosis of toxoplasmosis and typing of Toxoplasma gondii, Parasites Vectors 8 (1) (2015) 1–14.

[33] A. Farhadi, A. Haniloo, A. Fazaeli, S. Moradian, M. Farhadi, PCR-based diagnosis of Toxoplasma parasite in ocular infections having clinical indications of toxoplasmosis, Iran. J. Parasitol. 12 (1) (2017) 56–62.

[34] V. Greigert, F. Bittich-Fahmi, A.W. Pfaff, Pathophysiology of ocular toxoplasmosis: facts and open questions, PLoS Neglected Trop. Dis. 14 (12) (2020) 1–18.

[35] G. Bou, M.S. Figueroa, P. Martí-Belda, E. Navas, A. Guerrero, Value of PCR for detection of Toxoplasma gondii in aqueous humor and blood samples from immunocompetent patients with ocular toxoplasmosis, J. Clin. Microbiol. 37 (11) (1999) 3465–3468.

[36] R.B. Nussenblatt, K.K. Mittal, S. Fuhrman, S.D. Sharma, A.G. Palestine, Lymphocyte Proliferative responses of patients with ocular toxoplasmosis to parasite and retinal Antigens, Am. J. Ophthalmol. 107 (6) (1989) 632–641.

[37] M.D. Becker, J.T. Rosenbaum, Essential laboratory tests in uveitis, Dev. Ophthalmol. 31 (February) (1999) 92–108.

[38] P.D. Majumder, S. Sudharshan, J. Biswas, Laboratory support in the diagnosis of uveitis, Indian J. Ophthalmol. 61 (6) (2013) 269–276.

[39] S.W. Park, S.H. Kim, H.J. Kwon, S.M. Lee, I.S. Byon, J.E. Lee, Diagnostic value of positive findings of toxoplasma gondii-specific immunoglobulin M serum antibody in uveitis patients to confirm ocular toxoplasmosis, Ocul. Immunol. Inflamm. 27 (4) (2019) 583–590.

[40] M. Papadia, R. Aldigeri, C.P. Herbort, The role of serology in active ocular toxoplasmosis, Int. Ophthalmol. 31 (6) (2011) 461–465.

[41] F. Groen-Hakan, K. Babu, I. Tugal-Tutkun, K. Pathanapithoon, J.H. de Boer, J.R. Smith, et al., Challenges of diagnosing viral anterior uveitis, Ocul. Immunol. Inflamm. 25 (5) (2017) 710–720.

[42] Y.T. Hsiao, M.T. Kuo, W.Y. Chiang, T.L. Chao, H.K. Kuo, Epidemiology and clinical features of viral anterior uveitis in southern Taiwan - diagnosis with polymerase chain reaction, BMC Ophthalmol. 19 (1) (2019) 4–9.

[43] A. Rothova, J.H. de Boer, N.H. Ten Dam-van Loon, G. Postma, L. de Visser, S.J. Zuurveen, et al., Usefulness of aqueous humor analysis for the diagnosis of posterior uveitis, Ophthalmology 115 (2) (2008) 306–311.

[44] K. Sharma, A. Sharma, S.K. Sharma, R.K. Sen, M.S. Dhillon, M. Sharma, Does multiplex polymerase chain reaction increase the diagnostic percentage in osteoarticular tuberculosis? A prospective evaluation of 80 cases, Int. Orthop. 36 (2) (2012) 255–259.

[45] A.K. Asthana, M. Madan, Study of target gene IS6110 and MPB 64 in diagnosis of pulmonary tuberculosis, Int. J. Curr. Microbiol. App. Sci. 4 (8) (2015) 856–863.

[46] R. Belfort, J. Isenberg, B.F. Fernandes, S. DiCesare, R. Belfort Jr., M.N. Burnier Jr., Evaluating different methods of Toxoplasma gondii detection in peripheral blood, Cureus (January 2013) (2013).

[47] S. Sugita, N. Shimizu, K. Watanabe, M. Mizukami, T. Morio, Y. Sugamoto, et al., Use of multiplex PCR and real-time PCR to detect human herpes virus genome in ocular fluids of patients with uveitis, Br. J. Ophthalmol. 92 (7) (2008) 928–932.

[48] K.R. Jerome, M. Huang, A. Wald, S. Selke, L. Corey, Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR, J. Clin. Microbiol. 40 (7) (2002) 2609–2611.

specimens in the diagnosis of infectious posterior uveitis, Sci. World J. 2013 (2013), 545149.