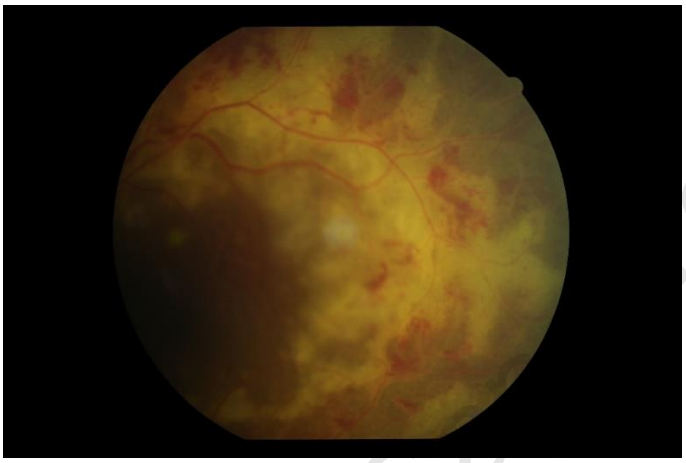
Co-infection in HIV positive patients with retinitis: A case series of dual positive intraocular fluid polymerase chain reaction



by

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Student number: HYSMOR001

Submitted to the University of Cape Town

In fulfilment of the requirements for the degree

MMed (Ophthalmology)

Faculty of Health Sciences

University of Cape Town

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1. Introduction

Infectious retinitis is a potentially devastating cause of permanent visual loss in both immunocompetent and immunocompromised patients (1). Infectious retinitis typically presents as a posterior uveitis characterized by confluent areas of white retinal necrosis with associated retinal haemorrhages. There are multiple etiologies, including, but not limited to: herpes simplex virus (HSV); cytomegalovirus (CMV); varicella zoster virus (VZV); and Toxoplasma gondii (1). However, cases of dual pathology are seldom reported. Urgent diagnosis and institution of proper therapy is needed to improve visual outcomes in all cases of infectious retinitis (1). Blindness and visual impairment caused by infectious uveitis can be prevented by early identification of the responsible pathogen and prompt treatment accordingly (2).

In this literature review, we will discuss the reported challenges associated with identifying the etiology of infectious retinitis using clinical examination alone. We will also discuss making a diagnosis by identifying the causative pathogen, with special consideration given to the immunocompromised patient. This of particular relevance to the South African context, due to the high prevalence of patients with Human Immunodeficiency Virus (HIV).

2. Limitations of the use of clinical examination alone to identify the cause of infectious retinitis

Several studies have shown that there are disadvantages in using clinical examination alone to identify the cause of infectious retinitis. For example, in their 2019 study, Knox et al. found that different pathogens may present with a very similar clinical picture (3). This is especially true for patients with HIV. In these cases, the retinitis may be atypical compared to the usual presentation in their immunocompetent counterparts (3).

Another 2019 study by del-a-Torre et al. concluded that uveitis secondary to a non-infectious cause may also present with similar clinical characteristics and non-specific symptoms (4). It was emphasized that multiple differential diagnoses need to be considered when dealing with patients with suspected uveitis. This is of particular relevance as immunosuppressive medications which are frequently used to treat noninfectious causes of uveitis may not only prolong but also worsen an infectious process (5). This leads to the clinician having diagnostic doubts (4). It is also important to be aware that different geographical strains of pathogens may manifest with varied ocular presentations, depending on the intraocular immune response elicited (4). Furthermore, the characteristics of the retinal appearance are often difficult to assess due to associated vitreous inflammation, which obscures the view (4). Scheepers et al. further discuss the fact that media opacity or poor pupil dilation which frequently occur in uveitis secondary to posterior synechiae may mask the clinical features (6). This is another disadvantage to using clinical examination alone to identify the cause of the infection.

3. Making a diagnosis

In their 2013 article, Majumder et al. emphasised that an adapted approach to laboratory diagnosis of uveitic cases should be based not only on clinical examination, but should also be focused on the patient's history and specific symptoms as well (7). Only after direct evidence of the pathogen in the aqueous or vitreous is identified, can a definitive diagnosis be made. This was emphasized by de Groot-Mijnes et al. who suggested that due to similar clinical signs and symptoms of patients with uveitis of infectious and non-infectious causes, a distinction between these aetiologies based on ocular findings alone is not feasible (5). Matos et al. also concluded from their 2007 study that Polymerase Chain Reaction (PCR) is a supplementary diagnostic procedure that should be evaluated together with ophthalmological aspects of the patient (8). Bodaghi and LeHoang concluded from their 2002 study that diagnostic yield is significantly increased when PCR and local antibody production are associated, especially in viral infections (9).

Polymerase chain reaction of intraocular fluids (both aqueous and vitreous) has become standard of care to identify the pathogen in infectious retinitis and infective posterior uveitis (3), (8). PCR is a reliable investigation that can identify most of the common causes of infection (8). This then allows for the clinician to tailor treatment accordingly (3).

In their 2011 study, Santos et al. analyzed the sensitivity and specificity of real-time PCR to detect the etiological agent from blood, plasma, vitreous and aqueous humor and compared this with the diagnostic hypothesis (10). They concluded that Real-time PCR was able to detect and to confirm diagnostic hypothesis in uveitis. Vitreous humor was found to be the best source for molecular diagnosis of infectious uveitis (10). However, aqueous humor samples were also found to be appropriate when tested by Real-time PCR (10). This was also illustrated by De Groot-Mijnes et al who found that despite the posterior location of infectious retinitis, PCR testing of a small volume of aqueous humor revealed a high frequency of positive results with no complications (5).

In their 2013 study, Scheepers et al. reported that routine PCR analysis on intraocular fluid from patients presenting with a first episode of suspected active infectious posterior uveitis in a population with a high prevalence of human immunodeficiency virus infection were found to be valuable (6). They found that PCR testing changed the diagnosis in a quarter of cases (6). Based on their findings, it was recommended that PCR analysis of ocular fluids be done early in the disease, even in under-resourced settings. When not treated correctly, infections such as these can lead to long term complications such as irreversible blindness (6).

Aqueous and vitreous PCR have been shown to have a high positive (98,7%) and negative (67,9%) predictive value in the diagnosis of infectious retinitis (1). These methods are also helpful in that they also rule out other similar infectious diseases (11).

4. Pathogen identification

Mandelcorn reported in his 2013 article that the more common infectious causes of posterior uveitis include syphilis, toxoplasmosis, tuberculosis, endogenous endophthalmitis, and viral causes (including herpes simplex virus, herpes zoster virus, and cytomegalovirus) (12). Harper et al. reported that patients with vascular or optic nerve inflammation, extensive retinitis, or immunocompromise are more likely to have positive PCR results (1).

It is rare to have more than pathogen identified on a sample. In a large retrospective case-series of 433 PCRs on 133 patients, Harper et al. similarly reported only 5 cases of dual-positive PCR. Of these dual-positive cases, only 4 (0.92%) were considered to be a true co-infection (1). To the best of our knowledge there is currently no published case series on the phenomenon of true co-infection in patients with dual-positive PCR.

When more than one pathogen is isolated it may be misleading. Many clinicians assume that the result may be a false positive due to the high sensitivity of the PCR technique. False-positives can result from sample-to-sample contamination, however a more serious source of false-positives is the carryover of DNA from a previous amplification of the same target (13). It has also been postulated that certain viruses in dormant states can be liberated in an inflamed eye leading to dual-infection (6). Scheepers et al. believed that the false positive results found in their study may have been due to previous resolved infection with a small number of "old" viruses still being present in the eye. They also suggested that it may have been due to virus in the systemic circulation leaking into the eye across a compromised blood ocular barrier, but not causing active infection in the eye (6).

5. HIV positive patients

Patients with HIV are known to present with more than one infectious etiology due to their poor immune response. HIV positive patients with low CD4 counts commonly present with multiple concurrent infections. Vago et al. evaluated the frequency and histopathological features of concomitant infections of the central nervous system (CNS) with CMV and herpes simplex viruses type 1 or 2 (HSV1/2) in a large series of patients who had died from AIDS (14). They documented concomitant CMV/HSV central nervous system infections in 16% of patients who had died from AIDS (14). Given that the retina is known as an extension of the central nervous system both anatomically and developmentally (15), it is plausible that HIV positive patients may also have more than 1 concomitant intraocular infection. Similarly, Senya et al. demonstrated that 50% of HIV patients hospitalized with an infectious diagnosis had an additional concurrent infectious etiology on further investigation (16). It can then be deduced that patients with HIV who present with retinitis may present atypically and require a careful differential diagnosis. As highlighted in de-la-Torre et al.'s study, making a definite diagnosis may be challenging in these cases, as causes may share similar and overlapping clinical characteristics (4).

6. Other considerations

The presence of viral DNA does not necessarily indicate that an organism is causing pathology. Epstein-Barr virus (EBV) virus, for example, may remain dormant in both B and T lymphocytes and has be shown to be ubiquitous in its distribution in cadaveric tissues (17) as well as in aqueous humor of immunocompetent donors (18).

In their 2015 study, Laaks et al. analysed aqueous PCR results in patients diagnosed with undifferentiated uveitis. They documented an increased presence of EBV DNA in ocular fluid samples from immunocompromised patients where another infectious agent had been identified as the cause of the uveitis (19). They also recommended PCR testing for Herpesviridae as a valuable test for patients with undifferentiated uveitis (19). However, Ongkosuwito et al.'s 1998 study concluded that testing for EBV does not have to be included in the routine management of patients with uveitis, since their study showed no significant indications for this virus in the pathogenesis of intraocular inflammation (18).

7. Conclusion

In reviewing the literature on this topic, it has been found that there are limited studies documenting co-infection of patients with retinitis. Although many forms of posterior uveitis share similar clinical characteristics, it is of paramount importance to distinguish infectious from non-infectious uveitis. More specifically, it is necessary to determine the underlying etiology of infectious retinitis as treatment differs accordingly. As highlighted by Scheepers et al. in their 2013 study, the outcome of incorrectly treated infective uveitis can result in irreversible blindness (6).

Dual positive intraocular fluid polymerase chain reaction is a rare phenomenon which is often overlooked. Clinicians need to be aware that, in rare cases, true dual pathology may occur. This is of particular importance within the South African context, where a significant portion of the population is immunocompromised.

8. References

1. Harper TW, Miller D, Schiffman JC, Davis JL,. Polymerase Chain Reaction Analysis of Aqueous and Vitreous Specimens in the Diagnosis of Posterior Segment Infectious Uveitis. American journal of ophthalmology. 2009;147(1):140-7.
2. London NJS, Rathinam SR, Cunningham ET. The epidemiology of uveitis in developing countries. International Ophthalmology Clinics. 2010;50(2): 1-17
3. Knox CM, Chandler D, Short GA, Margolis TP. Polymerase chain reaction—based assays of vitreous samples for the diagnosis of viral retinitis. Ophthalmology. 2019;105(1):37-45.

4. de-la-Torre A, Valdés-Camacho J, Mesa CLd, Uauy-Nazal A, Zuluaga JD, Ramírez-Páez LM, et al. Coinfections and differential diagnosis in immunocompetent patients with uveitis of infectious origin. BMC Infectious Diseases. 2019;19(91):1-10.
5. Rothova A, de Boer JH, ten Dam-van Loon NH, Postma G, de Visser L, Zuurveen SJ, Schuller M, Weersink AJL, van Loon A, de Groot-Mijnes J. Usefulness of Aqueous Humor Analysis for the Diagnosis of Posterior Uveitis. Ophthalmology. 2008;115 (2):306-311.
6. Scheepers MA, Lecuona KA, Rogers G, Bunce C, Corcoran C, Michaelides M. The value of routine polymerase chain reaction analysis of intraocular fluid specimens in the diagnosis of infectious posterior uveitis. The scientific world journal. 2013.
7. Majumder PD, Sudharshan S, Biswas J. Laboratory support in the diagnosis of uveitis. Indian Journal of Ophthalmology. 2013;61(6):269-276.
8. Matos K, Muccioli C, Belfort Junior R, Rizzo LV. Correlation between clinical diagnosis and PCR analysis of serum, aqueous, and vitreous samples in patients with inflammatory eye disease. *Arq Bras Oftalmol*. 2007;70(1):109-114
9. Bodaghi B, LeHoang P. Testing ocular fluids in uveitis. *Ophthalmol Clin North Am*. 2002;15(3):271-279.
10. Santos FF, Commodaro AG, Souza AV, et al. Real-time PCR in infectious uveitis as an alternative diagnosis. *Arq Bras Oftalmol*. 2011;74(4):258-261
11. Fardeau C, Romand S, Rao NA, Cassoux N, Bettembourg O, Thulliez P , et al. Diagnosis of toxoplasmic retinochoroiditis with atypical features. American journal of Ophthalmology. 2002;134:196-203.
12. Mandelcorn ED. Infectious causes of posterior uveitis. Canadian Journal of Ophthalmology. 2013;48(1):31-39.
13. Kwok S. Procedures to minimize PCR-product carry-over. PCR Protocols: A guide to methods and applications. 1990. Harcourt Brace
14. Vago L, Nebuloni M, Sala E, Cinque P, Bonetto S, Isella A, et al. Coinfection of the central nervous system by cytomegalovirus and herpes simplex virus type 1 or 2 in AIDS patients: autopsy study on 82 cases by immunohistochemistry and polymerase chain reaction. Acta neuropathologica. 1996;92(4):404-
15. London, A., Benhar, I. & Schwartz, M. The retina as a window to the brain—from eye research to CNS disorders. Nat Rev Neurol. 2013; 9: 44–53.
16. Senya C, Mehta A, Harwell JI, Pugatch D, Flanigan T, Mayer KH. Spectrum of opportunistic infections in hospitalized HIV-infected patients in Phnom Penh, Cambodia. International journal of STD & AIDS. 2003;14(6):411-6.
17. Chodosh J, Gan Y-J, Sixbey JW. Detection of Epstein—Barn Virus Genome in Ocular Tissues. Ophthalmology. 1996;103(4):687-90.
18. Ongkosuwito JV, Van der Lelij A, Bruinenberg M, Wienesen-van Doorn M, Feron EJ, Hoyng CB, et al. Increased presence of Epstein–Barr virus DNA in ocular fluid samples from HIV negative immunocompromised patients with uveitis. Brit J Ophthalmol. 1998;82(3):245-51.
19. Laaks D, Smit DP, Harvey J. Polymerase chain reaction to search for Herpes viruses in uveitic and healthy eyes: a South African perspective. African health sciences. 2015;15(3):748-54.

CHAPTER 2: MANUSCRIPT

Co-infection in HIV positive patients with retinitis: A case series of dual positive intraocular fluid polymerase chain reaction

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Key words
Co-infection, Retinitis, HIV, Intraocular fluid, PCR, Dual positive

Abstract

Objective
To report 10 cases of dual-positive intraocular fluid PCR results in infectious retinitis where both pathogens may be clinically relevant.

Methods
A retrospective observational case series including 10 patients with infectious retinitis who demonstrated more than 1 positive result on PCR testing over a 10-year period at a single referral centre.

Results
Of 619 patients who underwent intraocular fluid PCR testing for infectious retinitis, we identified 10 patients (1.62%) where 2 organisms were isolated. All 10 patients were HIV positive with profound immunosuppression (mean CD4 count 67cells/mm^3) and extensive retinitis. CMV was identified in all 10 cases whilst the additional pathogen was VZV in 6 cases, *Toxoplasma gondii* in 3 cases and HSV in 1 case.

Conclusions
PCR analysis of ocular fluids is important in this clinical scenario since more than one pathogen may be present and clinically relevant. Clinicians should be aware of this rare phenomenon to ensure that, when it does occur, consideration be given to adjusting treatment to cover both organisms.

Introduction

Infectious retinitis is a potentially devastating cause of permanent visual loss in both immunocompetent and immunocompromised patients. Causes include herpes simplex virus (HSV), cytomegalovirus (CMV), varicella zoster virus (VZV), or *Toxoplasma gondii*.(1) Urgent institution of effective therapy is needed to ensure optimal visual outcomes in these cases.(1) However, identification of the causative organism based on clinical examination alone may be challenging. The different pathogens may be indistinguishable, particularly in HIV patients where the retinitis may be atypical compared to the usual presentation in their immunocompetent counterparts.(2) Furthermore, vitritis may obscure the characteristics of the retinitis. Hence it has become standard of care in many institutions to investigate infectious retinitis with aqueous or vitreous polymerase chain reaction (PCR) to aid in the diagnosis of the pathogen and guide treatment selection.(3)

Aqueous and vitreous PCR has a high positive predictive value (98,7%) and negative predictive value (67,9%) in the diagnosis of infectious retinitis.(1) It is rare to identify more than one pathogen from a single a sample.(1) In such cases it is often assumed that the result is a false positive related to the high sensitivity of the PCR technique.(1) We report 10 cases of dual-positive intraocular fluid PCR results in infectious retinitis where both pathogens may be clinically relevant.. To the best of our knowledge, this is the largest case series in the English literature on this phenomenon.

Methods

Patients were identified retrospectively from all intraocular fluid PCR specimens performed for infectious retinitis at Groote Schuur Hospital, a university-based referral hospital in Cape Town, South Africa. The database of the National Health Laboratory Service (NHLS) at the hospital was used to identify cases between January 2009 and August 2019. All patients with more than one pathogen identified on an intraocular fluid PCR sample were included in the study.

Patients were examined by ophthalmology consultants and registrars at Groote Schuur hospital and the initial pre-PCR clinical diagnoses were recorded . Investigations included HIV serology, CD4 count, syphilis serology and chest X-ray. Informed consent was taken and vitreous samples were obtained by ophthalmology registrars in the outpatient department. Patients received topical anaesthesia, followed by cleaning with povidone-iodine 5% solution instilled for more than 3 minutes prior to the procedure. Adhering to strict aseptic techniques, a sterile lid speculum was used and the vitreous sample as well as intravitreal ganciclovir injection was given using a 28-gauge 12.7mm needle. All patients were phakic, thus the needle insertion site was 4mm from the limbus. Povidone-iodine 5% was instilled again following the procedure.

Laboratory detection of HSV, VZV, CMV and *Toxoplasma gondii* was performed in an ISO 15189 accredited diagnostic laboratory (Groote Schuur National Health Laboratory Service, Cape Town, South Africa). At various time points during the period described, different nucleic acid isolation and PCR methods were used.

Total nucleic acid isolation from vitreous fluid prior to 1 November 2015 was performed using the NucliSens EasyMAG platform (bioMerieux, Boxtel, The Netherlands) according to the manufacturer's protocol, and nucleic acid was eluted in 50 µL elution buffer. From 1 November 2015 onwards, the Abbott m2000sp (Abbott Laboratories, Abbott Park, Illinois, USA) was used according to the manufacturer's protocol, and nucleic acid was eluted in 120 µL elution buffer. Nucleic acid was stored at 4 °C until testing, when 10 µl input volume was used for all in-house and commercial kit-based PCRs. When in-house end-point nested PCR was used for diagnostic testing for each organism, the protocols for each organism were as previously described.(4)

For herpes simplex virus detection, the in-house PCR was used until 1 May 2016; from 1 May 2016 onwards the RealStar HSV PCR Kit 1.0 (Altona Diagnostics GmbH, Hamburg, Germany) was used according to the manufacturer's protocol. For CMV detection, the in-house PCR was used until 1 November 2015; from 1 November 2015 onwards the Abbott Realtime CMV kit (Abbott Laboratories, Abbott Park, Illinois, USA) was used according to the manufacturer's protocol. For varicella zoster virus and *Toxoplasma gondii* detection, the in-house PCR was used throughout the entire time period.(4) Patients with equivocal PCR results (a value of <62 international units/mL) were excluded from the study to limit the possibility of false positives due to contamination or replication of dormant organisms.

Treatment of the infectious retinitis was initiated according to the initial clinical diagnosis and then tailored according to the results of the PCR. Due to a lack of access to valganciclovir, CMV was treated with weekly intravitreal ganciclovir injections. VZV and HSV were treated with intravenous acyclovir, and Toxoplasma *gondii* was treated with oral co-trimoxazole. Additional oral steroids were given at the discretion of the treating ophthalmologist. HIV positive patients who were not on antiretrovirals were referred to an HIV physician for initiation of treatment.

The following parameters were recorded: age, sex, HIV status, CD4 count, initial clinical diagnosis, time until presentation, visual acuity at presentation, PCR results, complications related to vitreous sampling, initial treatment based on clinical diagnosis and alterations in treatment according to PCR results.

Ethics approval was obtained from the Human Research Ethics Committee at the University of Cape Town (HREC reference number 592/2019). Data analysis was performed using Stata version 12 statistical software. Parametric data was presented by means and confidence intervals. Non-parametric data was presented by medians and ranges.

Results

A total of 619 patients underwent intraocular fluid PCR testing for infectious retinitis during this time period of which 234 patients (37.8%) had a positive result. Ten patients had more than one organism identified on PCR testing and were included in the study. Thus, these dual-positive cases occurred in 1.62% of all cases where PCR was performed and 4.27% of cases where there was a positive result on PCR.

Table 1 describes the clinical characteristics and PCR results of the 10 cases. All the patients with dual-positive PCR were HIV positive and most were profoundly immunocompromised. The median CD4 count was 21cells/mm^3 (range 1-300cells/mm^3) and 7 patients (70%) had a CD4 count of less than 100cells/mm^3. Three patients (30%) were on antiretroviral treatment at the time of presentation.

The mean age was 32,9 years (95% CI [26.96, 38.83]). Nine out of 10 cases (90%) were female. The average time between onset of symptoms and presentation was 4.1 weeks (2 days – 8 weeks). Five cases demonstrated bilateral involvement (50%). One patient (case 9) had no visual acuities documented at presentation. The presenting best corrected Snellen visual acuity of the remaining 13 involved eye/-s ranged from 6/9 to perception of light, with 7 eyes (53.8%) presenting with a visual acuity of counting fingers or worse. All except one of the patients had an initial clinical diagnosis of CMV retinitis. The other patient was diagnosed as posterior uveitis of uncertain aetiology and although CMV retinitis was considered in the differential diagnosis it was thought to be less likely as this patient had a CD4 count of 300cells/mm^3.

PCR revealed CMV as one of the pathogens in all 10 cases. The additional pathogen was VZV in 6 cases (60%), *Toxoplasma gondii* in 3 cases (30%) and HSV in 1 case (10%). One patient (case number 8) had dual-positive PCR for CMV and VZV demonstrated on 2 separate specimens at the same presentation. Similarly, one patient (case number 3) had CMV and *Toxoplasma gondii* confirmed on PCR at original presentation, which was treated with intravitreal ganciclovir injections and oral co-trimoxazole. Ten months later the patient represented with a posterior uveitis and again was found to have CMV and *Toxoplasma gondii* on vitreous PCR.

Treatment was adjusted to include the additional organism identified on PCR and all eyes responded to treatment. The duration of follow up ranged from 2 weeks to 26 months (mean 8.5 months). No complications related to vitreous fluid aspiration procedures were identified and no rhegmatogenous retinal detachments were documented.

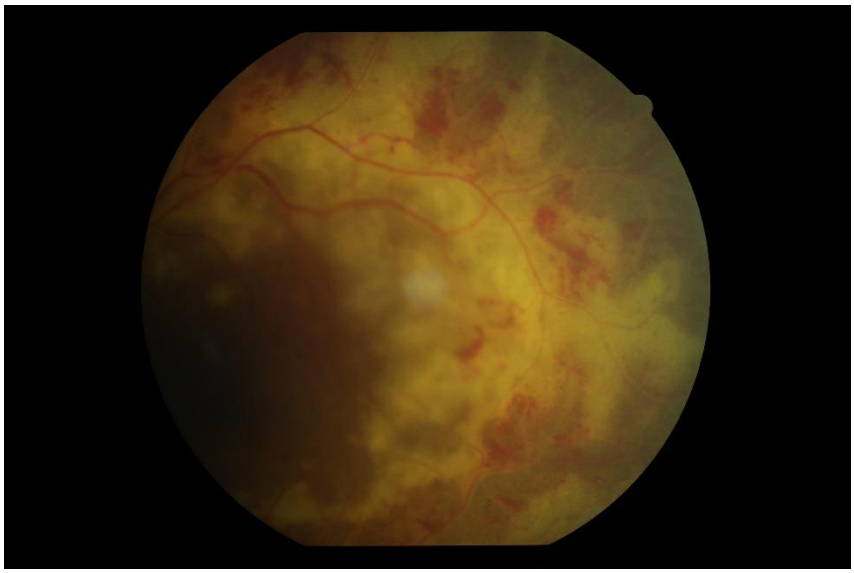

Figure 1: Fundus photograph of the left eye of case nr 6. A 42-year-old female. HIV positive with CD4 count of 1 cell/mm^3. Diagnosed as CMV retinitis on presentation. PCR showed CMV+ and VZV+.

Table 1: Patient characteristics and PCR results

Patient	**Age**	**Sex**	**HIV Status**	**CD4 Count (mm^3)**	**Initial Clinical Diagnosis**	**Laterality**	**Presenting Snellen Visual Acuity in Affected Eye/-s**	**PCR Positive Results**
1	39	Female	Positive	53	CMV Retinitis	Right	HM	CMV + Toxo
2	30	Female	Positive	9	CMV Retinitis	Left	6/18	CMV + VZV
3	50	Male	Positive	130	CMV Retinitis	Bilateral	CF both eyes	CMV + Toxo
4	28	Female	Positive	79	CMV Retinitis	Bilateral	Right 6/18 Left NPL	CMV + Toxo
5	31	Female	Positive	21	CMV Retinitis	Right	PL	CMV + HSV
6	42	Female	Positive	1	CMV Retinitis	Left	PL	CMV + VZV
7	28	Female	Positive	300	Posterior uveitis ?cause	Right	6/24	CMV + VZV
8	31	Female	Positive	2	CMV retinitis	Bilateral	Right 6/9 Left 6/18	CMV + VZV
9	28	Female	Positive	Not done	CMV retinitis	Bilateral	Unknown	CMV + VZV
10	22	Female	Positive	12	CMV retinitis	Bilateral	Right NPL Left 6/60	CMV + VZV

NPL: Nil perception of light, PL: Perception of light, HM: Hand movements, CF: Counting fingers
CMV: Cytomegalovirus, Toxo: Toxoplasma gondii, VZV: Varicella zoster virus, HSV: Herpes simplex virus

Discussion

We report 10 cases of infectious retinitis with more than one organism identified on vitreous PCR. This is a rare occurrence (1.42% of all PCRs performed for infectious retinitis at our institution). In a large retrospective case-series of 433 PCRs on 133 patients, Harper *et al.* similarly reported only 5 cases of dual-positive PCR of which 4 (0.92%) were considered true co-infection.(1) To the best of our knowledge in the English literature to date, our case series is the largest identifying potential co-infection on ocular fluid PCR.

Previously it has been assumed that the second organism identified on PCR is a false positive result related to the high sensitivity of the PCR technique.(1) It is notable that all the patients in our case series were HIV positive with profound immunosuppression and presented with extensive retinitis. We propose that, in this specific clinical scenario, dual-positive PCRs should be considered as true co-infection.

False positive results would be expected also to occur in HIV negative patients and HIV positive patients with higher CD4 counts. HIV positive patients with low CD4 counts commonly present with multiple concurrent infections. Vago *et al.* documented concomitant CMV/HSV central nervous system infections in 16% of patients who had died from AIDS.(5) Similarly Senya *et al.* demonstrated that 50% of HIV patients hospitalized with an infectious diagnosis had an additional concurrent infectious aetiology on further investigation.(6) Therefore it is not unexpected that the dual-positive patients in this case series were all HIV positive with low CD4 counts.

Additionally, two cases had dual positive PCR's confirmed on more than one occasion which reduces the possibility of the results being false positives due to contamination. Case number 8 was clinically diagnosed as bilateral CMV retinitis. Vitreous tap PCR was positive for CMV and VZV. Due to the dual positive result the vitreous tap was repeated 4 days later and the PCR was again positive for both. Similarly, case number 3 was clinically diagnosed as bilateral CMV retinitis. The PCR was positive on the right eye for *Toxoplasma gondii*, and positive on the left eye for CMV and *Toxoplasma gondii*. Ten months after successful treatment for both organisms, it was noted that the retinitis reactivated in the right eye. Vitreous tap of the right eye was now positive for CMV as well as *Toxoplasma gondii*.

The presence of viral DNA does not necessarily indicate that an organism is causing pathology. Epstein-Barr virus (EBV) virus, for example, may remain dormant in both B and T lymphocytes and has be shown to be ubiquitous in its distribution in cadaveric tissues(7) as well as in aqueous humor of immunocompetent donors.(8) Laaks *et al.* documented an increased presence of Epstein–Barr virus DNA in ocular fluid samples from immunocompromised patients where another infectious agent had been identified as the cause of the uveitis.(9) Therefore, EBV PCR testing was not performed in our patients.

In immunocompromised patients CMV, HSV, VZV and *Toxoplasma gondii* may all present with clinically indistinguishable atypical retinitis. Ocular fluid PCR may provide specific aetiological orientation and guide treatment.(1, 10) In the presence of dual-positive PCRs where both organisms could be causative and require different treatment strategies, management becomes more difficult. Although repeating the sample would be judicious, delay in instituting early

treatment may result in permanent visual loss. Therefore, it may be prudent to initiate treatment to cover both organisms from the outset.

Limitations include the small size and retrospective nature of our case series. Furthermore, a percentage of the PCR results could still be false positives. Larger, multicentric studies are needed to better evaluate this rare phenomenon and establish its true prevalence.

In conclusion, this case series has shown that more than one pathogen may be present and clinically relevant in significantly immunocompromised HIV positive patients with infectious retinitis. It is important for clinicians to be aware of this rare phenomenon to ensure that, when it does occur, consideration be given to adjusting treatment to cover both pathogens.

Acknowledgements. We thank Stephen Korsman for assistance with data collection.
Author contributions. MPH: literature review, data collection, manuscript preparation; JS: study design, data collection supervision, manuscript preparation.
Funding. None.
Conflicts of interest. None.

REFERENCES

1. Harper TW, Miller D, Schiffman JC, Davis JL, . Polymerase Chain Reaction Analysis of Aqueous and Vitreous Specimens in the Diagnosis of Posterior Segment Infectious Uveitis. American journal of ophthalmology. 2009;147(1):140-7.e2.
2. Knox CM, Chandler D, Short GA, Margolis TP. Polymerase chain reaction—based assays of vitreous samples for the diagnosis of viral retinitis. Ophthalmology. 2019;105(1):37-45.
3. de-la-Torre A, Valdés-Camacho J, Mesa CLd, Uauy-Nazal A, Zuluaga JD, Ramírez-Páez LM, et al. Coinfections and differential diagnosis in immunocompetent patients with uveitis of infectious origin. BMC Infectious Diseases. 2019;19(1):1-10.
4. Scheepers MA, Lecuona KA, Rogers G, Bunce C, Corcoran C, Michaelides M. The value of routine polymerase chain reaction analysis of intraocular fluid specimens in the diagnosis of infectious posterior uveitis. The scientific world journal. 2013.
5. Vago L, Nebuloni M, Sala E, Cinque P, Bonetto S, Isella A, et al. Coinfection of the central nervous system by cytomegalovirus and herpes simplex virus type 1 or 2 in AIDS patients: autopsy study on 82 cases by immunohistochemistry and polymerase chain reaction. Acta neuropathologica. 1996;92(4):404-8.
6. Senya C, Mehta A, Harwell JI, Pugatch D, Flanigan T, Mayer KH. Spectrum of opportunistic infections in hospitalized HIV-infected patients in Phnom Penh, Cambodia. International journal of STD & AIDS. 2003;14(6):411-6.
7. Chodosh J, Gan Y-J, Sixbey JW. Detection of Epstein—Barn Virus Genome in Ocular Tissues. Ophthalmology. 1996;103(4):687-90.
8. Ongkosuwito JV, Van der Lelij A, Bruinenberg M, Wienesen-van Doorn M, Feron EJ, Hoyng CB, et al. Increased presence of Epstein–Barr virus DNA in ocular fluid samples from HIV negative immunocompromised patients with uveitis. Brit J Ophthalmol. 1998;82(3):245-51.
9. Laaks D, Smit DP, Harvey J. Polymerase chain reaction to search for Herpes viruses in uveitic and healthy eyes: a South African perspective. African health sciences. 2015;15(3):748-54.

10. Tran T, Rozenberg F, Cassoux N, Rao N, LeHoang P, Bodaghi B. Polymerase chain reaction analysis of aqueous humour samples in necrotising retinitis. Brit J Ophthalmol. 2003;87(1):79-83.

APPENDICES

1. Tables

Patient characteristics and PCR results

Patient	Age	Sex	HIV Status	CD4 Count (mm^3)	Initial Clinical Diagnosis	Laterality	Presenting Snellen Visual Acuity in Affected Eye/-s	PCR Positive Results
1	39	Female	Positive	53	CMV Retinitis	Right	HM	CMV + Toxo
2	30	Female	Positive	9	CMV Retinitis	Left	6/18	CMV + VZV
3	50	Male	Positive	130	CMV Retinitis	Bilateral	CF both eyes	CMV + Toxo
4	28	Female	Positive	79	CMV Retinitis	Bilateral	Right 6/18 Left NPL	CMV + Toxo
5	31	Female	Positive	21	CMV Retinitis	Right	PL	CMV + HSV
6	42	Female	Positive	1	CMV Retinitis	Left	PL	CMV + VZV
7	28	Female	Positive	300	Posterior uveitis ?cause	Right	6/24	CMV + VZV
8	31	Female	Positive	2	CMV retinitis	Bilateral	Right 6/9 Left 6/18	CMV + VZV
9	28	Female	Positive	Not done	CMV retinitis	Bilateral	Unknown	CMV + VZV
10	22	Female	Positive	12	CMV retinitis	Bilateral	Right NPL Left 6/60	CMV + VZV

NPL: Nil perception of light, PL: Perception of light, HM: Hand movements, CF: Counting fingers
CMV: Cytomegalovirus, Toxo: Toxoplasma gondii, VZV: Varicella zoster virus, HSV: Herpes simplex virus

2. Ethics approval letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: olivia.langenhoven@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

06 September 2019

HREC REF: 592/2019

Dr J. Steffen
Ward D4
Department of Ophthalmology
Main Building
New Groote Schuur Hospital

Dear Dr Steffen

PROJECT TITLE: ***CO-INFECTION IN HIV POSITIVE PATIENTS WITH RETINITIS: A CASE SERIES OF DUAL POSITIVE INTRAOCULAR FLUID POLYMERASE CHAIN REACTION. (MMED DEGREE - DR MORGAN HAYES)***

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 September 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Dr Morgan Hayes will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

3. Instructions to authors of the journal “Ocular Immunology and Inflammation”.

Instructions for authors

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