Title page

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University of Cape Town, South Africa

Degree: Master of Medicine (MMed) in Ophthalmology

This research is based on independent work performed by Marius Anton Scheepers. This research has not been previously published. There is no part of this research, which is being, or is to be submitted for another degree to any other university.

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ABSTRACT

Diagnostic and clinical value of routine polymerase chain reaction analysis of intraocular fluid specimens in the diagnosis of suspected infectious posterior uveitis

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Groote Schuur Hospital Ophthalmology, Cape Town, South Africa

Objective: To assess the diagnostic and clinical value of routinely performing polymerase chain reaction (PCR) analysis on intraocular fluid samples in patients with suspected infectious posterior uveitis in a population with a high prevalence of human immunodeficiency virus infection.

Design: Retrospective, interventional consecutive case series of 159 patients presenting with suspected active infectious posterior uveitis.

Methods: Patients presenting with a first episode of suspected infectious posterior uveitis underwent PCR testing of ocular fluid samples in a tertiary care hospital over a five year period. PCR analysis was performed for cytomegalovirus (CMV), varicella zoster virus (VZV), herpes simplex virus type 1 and 2 (HSV), toxoplasma gondii (TG) and mycobacterium tuberculosis (MTB).

Results: The prevalence of the commonest causes of infectious posterior uveitis based on PCR studies was CMV in 47% of patients, VZV in 11% and TG in 10%. HSV was not identified. PCR analysis confirmed the initial clinical diagnosis in 55 patients (35%) and altered the initial clinical diagnosis in 36 patients (23%). The clinical diagnosis prior to

PCR testing was non specific (uncertain) in 51 patients (32%), with PCR providing a definitive final diagnosis in 20 of these patients (39%); necrotizing herpetic retinopathy and ocular toxoplasmosis were particularly difficult to diagnose correctly without the use of PCR analysis. The overall PCR sensitivity was 84%, specificity was 99%, positive predictive value was 97% and negative predictive value was 95%.

Conclusion: The clinical phenotype alone was unreliable in diagnosing the underlying infectious cause in a quarter of patients in this study. Since the outcome of incorrectly treated infective uveitis can be blinding, PCR analysis of ocular fluids is recommended early in the disease even in resource poor settings.

Financial disclosure(s): The authors have no proprietary or commercial interest in any materials discussed in this article.

Polymerase Chain Reaction analysis of ocular fluid in the diagnosis of suspected infectious posterior uveitis.

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1.0 Introduction

This study aims to assess the usefulness of performing a vitreous biopsy or anterior chamber tap of ocular fluids for polymerase chain reaction (PCR) analysis in patients presenting clinically with suspected infectious posterior uveitis at Groote Schuur Hospital in Cape Town, South Africa.

Infectious uveitis occurs commonly in South Africa, especially in those patients who are immune compromised. It is a potentially devastating disease which often results in loss of vision, and treatment can help prevent further destruction of eye tissues. Knowing the causative organism is important because it allows the institution of early appropriate treatment to prevent complications and spread of infection.

The identification of the aetiological pathogens responsible for infectious posterior uveitis has historically been based on clinical presentation. Since the advent of PCR tests for common infectious agents, PCR analysis has become a useful adjunct in determining the organism responsible.
In posterior uveitis caused by Cytomegalovirus, Herpes Simplex virus and Varicella Zoster virus, PCR analysis has reported sensitivities greater than 90% and specificities greater than 95%[1]. Toxoplasma gondii posterior uveitis PCR analysis has reported sensitivities of 40 – 60%.[2]
The sensitivity of PCR analysis in the diagnosis of Mycobacterium Tuberculosis posterior uveitis is undetermined at present.

The usefulness of PCR in clinical practice has not previously been investigated.

2.0 Study objective

The primary objective is to determine how useful PCR is in establishing an aetiological diagnosis in patients with suspected infectious posterior uveitis, seen at the uveitis clinic at Groote Schuur Hospital.

3.0 Study design

Retrospective case review.

4.0 Study population

All patients suffering from a first episode of suspected infectious posterior uveitis not previously investigated, presenting between May 2004 and June 2009 at the Groote Schuur Hospital Eye clinic in Cape Town.

4.1 Inclusion criteria

Patients presenting with active, or inactive("old") chorioretinitis or a choroidal granuloma over a five year period from May 2004 to June 2009 who had PCR analysis of either vitreous or anterior chamber ocular fluid. PCR analysis was performed for common causative organisms namely Cytomegalovirus (CMV), Herpes simplex virus (HSV), Varicella Zoster Virus (VZV), Toxoplasma Gondii, and

Mycobacterium Tuberculosis (TB). Tests for CMV, HSV, VZV, and Toxoplasma Gondii were performed commonly whereas TB tests were performed less commonly. Patients who had a poor retinal view from posterior synaechiae or media opacity, and who were suspected to have infectious posterior uveitis will be included in the study.

4.2 Exclusion criteria

1. Previous episodes of chorioretinitis.
2. Absence of chorioretinitis on fundoscopy if the retina was visible.

5.0 Material and methods

5.1 Patient recruitment

A list of all patients who had ocular fluid PCR analysis over a five year period from May 2004 to June 2009 was obtained from the virology laboratory at Groote Schuur Hospital.
Patient folders will be requested from medical records and the Ophthalmology case notes will be reviewed by 2 Ophthalmologists in order to complete data sheets with the required patient information (Please see Appendix 1).

5.2 Vitreous biopsy procedure according to standard department protocol

Vitreous biopsies are performed under local anaesthesia using a 23 gauge needle under sterile conditions in a minor operation theatre in the out-patient department or in main operation theatre. Anterior chamber samples are obtained under sterile conditions using either a 28 gauge or 30 gauge half inch needles depending on availability.

5.3 Study aims

5.3.1 The primary aim of the study is to determine the proportion of cases in which PCR confirmed, altered or did not contribute to making a final aetiological diagnosis.

5.3.2 To determine whether there is a statistically significant difference in the PCR positive rate between vitreous and anterior chamber aspirates.

5.3.3 To look for features associated with a positive PCR result, including anterior chamber inflammation, vitritis, relative afferent pupil defect, and time from onset of disease to PCR testing.

6.0 Statistical analysis

6.1 Statistical analysis will be performed by Catey Bunce from the Moorfields Eye Hospital Statistics and Research department.

7.0 Ethical and regulatory considerations

Ethical approval to be obtained by the Groote Schuur Hospital research committee.

7.0 References

1. Van Gelder RN. CME review: Polymerase chain reaction diagnostics for posterior segment disease. Retina 2003;23:445–452.

2. Fardeau C, Romand S, Rao NA, et al. Diagnosis of toxoplasmic retinochoroiditis with atypical clinical features. Am J Ophthalmol 2002; 134: 196–203.

Appendix to protocol - Data capture spreadsheet

Study number	Patient name	Patien folder number	Date of birth	Age at present-ation	Date of death	Gender	Race B=Black W=White M=Mixed I=Indian	HIV status	MTB STATUS T=CURRENT T.B ON Rx	OTHER Current active systemic disease S=SYPHILIS C=CRYPTOCOCCAL MENINGITIS D=DIABETES MELLITIS	CD4 COUNT 0-2000 U=Unknown	Date of ONSET of SYMPTOMS of Disease	PRESENTATION AND PCR SAMPLING DATES
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RAPD grade (0-4) N=NOT DONE S=SLUGGISH BOTH EYES OR SLUGGISH ONLY EYE	OD VA @ PRESENTATION 1= 6/6-6/18 2= 6/24-4/60 3= 3/60-NPL	OS VA @ PRESENTATION 1= 6/6-6/18 2= 6/24-4/60 3= 3/60-NPL	EYE infected OD/OS/OU	PCR - EYE SAMPLED (OD/OS/OU)	PCR site A=AC V=VIT AV=BOTH N=NOT SPECIFIED	AC cells 0-4 N=NOT DONE	AC flare Y=YES	POST SYNECHIAE Y=YES	Hypopion Y=YES N=NO ND=NOT DOCUMENTED	Vitritis Y=YES H=HAZY VIEW N=NOT DOCUMENTED	Retino- choroiditis A=ACTIVE I=INACTIVE G=GRANULOMA H=HAZY VIIEW

POST TREATMENT VA OD 1= 6/6-6/18 2= 6/24-4/60 3= 3/60-NPL	POST TREATMENT VA OS 1= 6/6-6/18 2= 6/24-4/60 3= 3/60-NPL	DIAGNOSIS PRE PCR	DIAGNOSIS POST PCR	TREATMENT PRIOR TO PCR TESTING	TREATMENT POST PCR TESTING	FOLLOW UP PERIOD	TOXO PCR RESULT	CMV PCR RESULT	HSV PCR RESULT	VZV PCR RESULT	MTB PCR RESULT	SYPHILIS SEROLOGY

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06 July 2010

HREC REF: 309/2010

Dr M Scheepers
c/o Dr K Lecuona
Ophthalmology

Dear Dr Scheepers

PROJECT TITLE: POLYMERASE CHAIN REACTION ANALYSIS OF OCULAR FLUID IN THE DIAGNOSIS OF SUSPECTED INFECTIOUS POSTERIOR UVEITIS.

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

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

Approval is granted for one year till the 15th July 2011.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

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

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
pp **CHAIRPERSON, HSF HUMAN ETHICS**
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

S Thomas

This serves to confirm that the University of Cape Town 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) and Declaration of Helsinki guidelines.

The 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.

S Thomas

Literature review

Objectives of literature review:

1. To explore the current knowledge of the causes of infectious posterior uveitis in a population with a high prevalence of human immunodeficiency virus
2. To document current diagnostic methods used in establishing an aetiological diagnosis in infectious posterior uveitis
3. To compare the value of different diagnostic techniques currently used to determine the aetiology of infectious posterior uveitis, in particular the value of polymerase chain reaction (PCR) analysis of intraocular fluid.
4. To explore the effect of different intraocular fluid sampling sites on PCR test accuracy

Literature search strategy:

a. An online pubmed search was performed using the following search words.

   1. Diagnosis AND posterior uveitis OR chorioretinitis OR uveitis OR cytomegalovirus OR herpes simplex virus OR varicella zoster virus OR toxoplasma gondii OR toxoplasmosis OR mycobacterium tuberculosis OR syphilis.
   2. Polymerase chain reaction analysis AND posterior uveitis OR chorioretinitis OR uveitis OR cytomegalovirus OR herpes simplex virus OR varicella zoster virus OR toxoplasma gondii OR toxoplasmosis OR mycobacterium tuberculosis OR syphilis.

b. Reference lists from pubmed cited articles were cross referenced

c. The South African Department of Health online database was searched for human immunodeficiency virus prevalence statistics, which are based on antenatal clinic data.

All pubmed cited articles relevant to infectious posterior uveitis and its diagnosis were included in the literature search.

The search was limited to papers published in English

Quality criteria:

More weight was given to larger studies and less weight to case reports.

Introduction

In high income countries, uveitis affects approximately 200 per 100,000 in the population, and uveitis and its complications accounts for up to 35% of severe visual impairment.[1] In low income countries, uveitis and it's complications are thought to be even more common, affecting an estimated 714 per 100,000 and contributing to 25% of blindness.[1] Posterior uveitis is thought to comprise approximately 5% of all uveitis entities.[2] The commonest pathogens responsible for infectious posterior uveitis and panuveitis are Herpes Simplex Virus (HSV) type 1 and 2, Varicella-Zoster Virus (VZV), Cytomegalovirus (CMV), Toxoplasma Gondii (TG), Treponema Pallidum and Mycobacterium Tuberculosis (MTB).[2] In high income countries, the most common infectious aetiologies are TG, HSV, and VZV, whereas CMV is a common pathogen in countries with a high prevalence of human immunodeficiency virus (HIV) / acquired immune deficiency syndrome (AIDS).[3-5] Blindness and visual impairment caused by infectious uveitis can be prevented by early identification of the correct aetiological pathogen responsible for infectious uveitis, and the prompt administration of specific antimicrobial therapy.[6] This is particularly so in immunocompromised patients.[3,6]

The etiological diagnosis of infectious uveitis is initially made on the clinical features of the phenotypic expression of the disease, but there is often significant overlap between the phenotypic expressions of these different pathogens.[7] Simultaneous infection of the retina with multiple agents in patients with AIDS has been reported, making it almost impossible to make a correct clinical diagnosis.[8] Establishing a diagnosis based on clinical findings alone is also difficult in cases where media opacity or poor pupil dilation may mask clinical features. Under these circumstances an incorrect diagnostic decision not only causes a delay of appropriate treatment and prevention of loss of vision, but also exposes patients to side effects of an unnecessary medication.[9]

DIAGNOSTIC METHODS USED IN ESTABLISHING AN ETIOLOGICAL DIAGNOSIS IN INFECTIOUS POSTERIOR UVEITIS

a. *Peripheral blood analysis*

Analysis of peripheral blood samples to detect antigens and antibodies is of limited value in most cases. This is because peripheral blood analysis does not necessarily reflect disease activity in the eye.

Positive serological results may be incidental, especially if the prevalence of a particular infection is high in a given population.[3,6] Negative serological results make the chances of an infection less likely, but cannot rule out the possibility of that infection.[10]

Peripheral blood analysis to determine the aetiology of infectious uveitis is thus not very useful in most cases.

b. *Intraocular fluid analysis*

1. Culture of intraocular fluids

Culturing pathogens which commonly cause uveitis is a difficult task in many cases.[11-14] Viruses are obligatory intracellular pathogens, and they therefore require susceptible host cells in order to culture. Many susceptible specific viral strain host cells are not available for the purpose of culturing and as a result there are many viruses we are unable to culture. Fungi and bacteria are generally easier to culture than viruses, although some bacteria and fungi have specific nutritional requirements making them more challenging to culture (for example mycobacterium tuberculosis & treponema pallidum.)[15]

Pathogenic load in sampled ocular fluid is important, as low pathogenic load results in a lower sensitivity. Pathogens that are liberated into the ocular humours as part of the disease process are usually easier to culture.

Another consideration is the stability of the pathogen in transit from clinic to the laboratory. Specific transport medium requirements and the time interval from sampling

to testing can affect culture sensitivity. Specialised diagnostic laboratories are not available at all health care facilities, and thus transporting samples to laboratories can result in considerable time delay before culturing is initiated. This is important for viral culture, as some viruses are unstable in cell free environments, and the infectious viral load may drop considerably from sampling time to culture time.

Results from culturing take longer than PCR or antigen / antibody analysis, and this is unfavourable, as rapid diagnoses are required.

2. Intraocular antibody analysis

The detection of intraocular antibodies to a particular pathogen may indicate possible intraocular infection, but as the blood ocular barrier is often broken down in uveitis, immunoglobulins may cross from the peripheral blood to the intraocular fluid. Infection elsewhere in the body causing antibody production may thus lead to a false positive result.

3. Goldman Witmer Coefficient

Testing for antibodies is more useful if the levels of intraocular antibody are compared to that of peripheral blood serum antibody production.

The Goldman Witmer coefficient (GWC) corrects for the leakage of immunoglobulins from the peripheral blood into intraocular fluid. The GWC compares the ratio of specific antibody in the eye and peripheral blood, to the ratio of total IgG in the eye and peripheral blood. The formula is: (Specific IgG in aqueous / specific IgG in serum) / (Total IgG in aqueous / Total IgG in serum). When leakage occurs, division of the ratios approximate one.[6,16-17] A Goldman Witmer coefficient of greater than 3 is generally considered positive, and therefore indicative of probable intraocular infection.[10,16-18]

False positives may however result from polyclonal B-Cell activation by certain pathogens that are able to produce ‘super antigens’. When testing for only one pathogen,

polyclonal B Cell activation may be missed. Testing for antibodies against two pathogens and calculating the C' coefficient comparing specific aqueous / serum ratios can help one identify polyclonal B cell activation, but one has to consider the rare possibility of multiple infections.[10,19]

False negatives may occur when high serum antibodies combined with extensive blood aqueous barrier breakdown may mask a positive coefficient. [10,18]

GWC has been described for the most common causes of posterior uveitis including Toxoplasma gondii, CMV, HSV, VZV, Rubella Virus and Toxocara canis.[3,6,20-21]

4. Polymerase chain reaction (PCR) analysis

PCR is a technique whereby a single or a few copies of a piece of DNA are amplified across several orders of magnitude, generating millions of copies of a particular nucleic acid sequence. Several different PCR techniques are available, all of which provide different advantageous additional information.

Quantitative real time polymerase chain reaction is used to amplify and simultaneously quantify a targeted nucleic acid sequence. For one or more specific sequences in a DNA sample, real time PCR enables both detection and quantification. The quantity can be either an absolute number of copies or a relative amount when normalized to DNA input or additional normalizing genes. Real time PCR is thus able to provide information regarding viral loads.[22] Real time PCR has been used to measure viral loads and disease activity in patients with herpetic uveitis as well as CMV retinitis.[23-25]

Multiplex PCR uses multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences, i.e. different transgenes. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform.[26]

Nested polymerase chain reaction is a modification of the polymerase chain reaction intended to reduce the contamination in products due to the amplification of unexpected primer binding sites. Nested polymerase chain reaction involves two sets of primers, used in two successive runs of polymerase chain reaction, the second set intended to amplify a secondary target within the first run product. Nested PCR has been shown to be useful in the diagnosis of ocular Mycobacterium Tuberculosis.[27]

False positives PCR results are however possible if contamination occurs, or if pathogens enter the eye from the peripheral blood, but are not causing any active infection in the eye.[28-29]

False negative results may occur in cases of low specimen antigenic load or polymorphism (genetic variability or mutation).[29]

PCR analysis of intraocular fluid to detect viral infection in infectious posterior uveitis has been shown to be a sensitive and highly specific test. From previous studies, PCR CMV retinitis sensitivities range from 91% to 95% and for VZV or HSV causing necrotizing herpetic retinopathy sensitivities range from 79% to 100%. [6,30-34]

PCR analysis in patients with ocular toxoplasmosis is generally less sensitive than viral retinitis. Studies have shown variable sensitivity ranging from 27% to 85%.[6,20,35-39]

PCR procedures are generally more sensitive than cultures and allow more rapid detection.

Although the identification of the pathogens responsible for infectious posterior uveitis has historically been based on clinical presentation, the advent of PCR tests for common infectious agents has become a useful adjunct in determining the organism responsible. De Boer and colleagues showed that in patients with AIDS & CMV retinitis, polymerase chain reaction analysis is preferable above local antibody production in detecting the inciting agent of retinitis. In cases of Toxoplasma chorioretinitis, the combination of both techniques can make a valuable contribution to the diagnosis. De Boer's study also

showed that in Acute Retinal Necrosis the PCR sensitivity was higher at 2 weeks after the onset of disease compared to an acute sample. (81% versus 100%; n = 16)[6]

PCR is very useful in cases of media opacity where the retina isn't visible.

COMPARISON OF DIFFERENT DIAGNOSTIC TECHNIQUES CURRENTLY USED TO DETERMINE THE AETIOLOGY OF COMMON INFECTIOUS POSTERIOR UVEITIS SYNDROMES

As mentioned before, some of the most common pathogens responsible for infectious posterior uveitis and panuveitis include Cytomegalovirus (CMV), Varicella-Zoster Virus (VZV), Herpes Simplex Virus (HSV) type 1 and 2, Toxoplasma Gondii, Treponema Pallidum and Mycobacterium Tuberculosis.[2]

CMV retinitis (CMVR)

CMV retinitis usually affects patients who are immune compromised. CMV retinitis is the most common cause of acquired viral retinitis, primarily because of the acquired immunodeficiency syndrome (AIDS).[40]

Ocular CMV most commonly presents as a viral necrotizing retinitis, which typically starts in the mid-periphery and can progress in a "brush fire" pattern.[41] CMV retinitis may also present as an indolent type CMV retinitis that presents with atrophic central lesions with granular whitish active borders.[42]

Before the advent of PCR, the diagnosis of CMV retinitis relied predominantly on fundal appearance. In the early stages of disease and in patients with atypical features, it can be difficult to differentiate between retinitis caused by CMV, and retinitis associated with the other herpes viruses. Discrimination between viral and non-viral pathogens such as Toxoplasma gondii can be particularly difficult by clinical examination alone as their clinical presentation can be similar. Rapid, accurate diagnosis of ocular CMV infection

and the prompt initiation of appropriate therapy is essential both for the preservation of sight and the improved survival of the patient. Furthermore, the personal cost to the patient and the waste of resources associated with the use of multiple antibiotic and antiviral therapies prompts the need for rapid, sensitive, and specific diagnostic tests for ocular pathogens such as CMV.[43]

In AIDS patients, the clinical diagnosis of CMV retinitis can be even more difficult if multiple agents are co-infecting the retina, which underlines the importance of intraocular fluid analysis.[44]

Many studies have showed both PCR and GWC to be useful in confirming the diagnosis of CMV retinitis. There are however no large-scale concurrent studies on PCR and GWC. PCR for CMV retinitis has been shown to be up to 95 % sensitive in detecting untreated CMV retinitis and 48% sensitive in detecting CMV retinitis that had been treated with systemic gancyclovir or foscarnet, or both.[30] PCR for CMV retinitis is highly sensitive for untreated CMV retinitis, and also highly specific.

Necrotising herpetic retinitis

The necrotizing herpetic retinopathies, induced by viruses of the herpes family (HSV, VZV and CMV), includes acute retinal necrosis (ARN) and progressive outer retinal necrosis (PORN).[45]

Necrotizing herpetic retinitis is a continuous spectrum of diseases induced by herpes viruses, whose clinical expression depends on the immune state of the host, presenting as classical ARN at one end in patients with non-detectable or slight immune dysfunction, to PORN in severely immunosuppressed patients at the other end, and with intermediary forms between these extremes.[45]

ARN was first described by Urayama in 1971 and was termed Kirisawa uveitis.[46] ARN is clinically described as the classical triad of (1) an arteritis and phlebitis of the retinal and

choroidal vasculature (2) a confluent, necrotizing retinitis that preferentially affects the peripheral retina, and (3) a moderate to severe vitritis.[47]

PORN was first described by Forster and colleagues in 1990. They described two patients with unilateral fulminant retinal necrosis involving the outer retinal layers with sparing of the inner retina and retinal vasculature.[48] In 1994 Engstrom and colleagues reported on thirty-eight patients (65 eyes) with PORN.[49] They noted that all their patients suffered from the acquired immune deficiency syndrome and that the median CD4 lymphocyte count was 21/mm^3. A history of cutaneous zoster infection was documented in 67% of patients, and anterior chamber reaction and vitreous inflammatory reactions were absent or minimal in all patients. They also noted typical retinal lesions to be multifocal, deep opacities scattered in the retinal periphery, and 32% of eyes had macular involvement at presentation.

VZV is the most frequent cause of ARN. HSV is the second most common cause of ARN. HSV associated ARN occurs more commonly with meningo-encephalitis than VZV associated ARN.[50,51]

However, the typical phenotype of necrotizing herpetic retinitis may also be caused by treponema pallidum (syphilis), toxoplasma gondii and CMV.[9,31,52-53] It is therefore important to establish a laboratory based identification of the pathogen.

Polymerase chain reaction based assays of ocular fluid samples are often used for the diagnosis of acute retinal necrosis syndrome, and to determine the specific pathogen causing the syndrome.[6,31,54-56] The sensitivity of PCR for HSV and VZV is in excess of 90% and the specificity exceeds 95%.[31,33,57]

The results for PCR analysis is highly sensitive and specific, as demonstrated by Pendergast, who found zero false positives for Herpes DNA in 75 intraocular specimens (35 aqueous and 40 vitreous samples) from 75 patients undergoing scleral buckling or vitrectomy.[58] Each specimen was tested using a PCR-based assay for CMV, HSV 1 & 2,

VZV and EBV. Of the 75 samples tested, none were found to be PCR positive. This was despite the percentage of patients with positive herpes virus serology ranging from 86% to 100%.[58]

The false-negative rate is however difficult to determine because it is usually compared with cultures, and viral cultures are among the least efficient diagnostic tests for intraocular fluid. In the case of infectious uveitis, the final clinical diagnosis is therefore used as gold standard.[59] For this reason, validation in consecutive patients typical of those who usually undergo the testing is needed to assess the usefulness of the method for routine diagnostic use.

In cases where acute retinal necrosis is due to Toxoplasma Gondii, GWC determination in addition to PCR is particularly useful in establishing a diagnosing.

Toxoplasma Gondii related posterior uveitis

Ocular toxoplasmosis (OT) caused by the parasite toxoplasma gondii is a common cause of posterior uveitis that can be contracted congenitally or through postnatal infection.[60] OT may arise from a primary infection or reactivation.

OT most frequently presents as a unilateral focal retinochoroidal lesion.[61-62] OT may however have varied atypical phenotypic expressions, particularly in immunocompromised patients, where OT lesions may be multifocal, or where OT may present as diffuse areas of retinal necrosis mimicking ARN.[53,62-63] It may thus be very difficult to determine the most likely etiological pathogen based on clinical appearance alone, and diagnostic testing is most valuable in these situations.

There is a high prevalence of TG antibody seropositivity in the general population in Southern Africa, as many adults have had infection with T. Gondii.[64] Peripheral blood analyses for antibody production is therefore not very useful in proving active ocular infection.

GWC analysis for TG has been shown to be one of the most sensitive tests for T Gondii. (Up to 93% positive results) PCR analysis of intraocular fluid is another useful test to detect T. Gondii. In primary OT PCR & GWC analysis appear to contribute equally to the diagnosis.[3,20-21,62]

PCR appears to be particularly valuable in cases of atypical OT in immunocompromised patients.[6,62] Improved PCR techniques such as qualitative multiplex PCR used by Sugita and colleagues have also improved sensitivities.[39]

Van Gelder noted that vitreous PCR might be more sensitive than aqueous PCR due to the size of the TG organism and the paucity of the organism.[29] Fekkar noted the combination of GWC & PCR to increase diagnostic sensitivity to 93% in a study of 34 patients with OT.[35]

Early in the disease process viral nucleic acids are easier to determine, whereas late in the disease process PCR positive rates are lower and GWC positive rates are higher as antibodies are being produced.[6]

Mycobacterium tuberculosis induced posterior uveitis

The reported incidence of ocular tuberculosis among patients with systemic tuberculosis varies from 1.46% to 18%, and is increasing in areas with a high HIV / AIDS prevalence.[65-70]

Posterior segment involvement in immunocompetent individuals may manifest a spectrum of clinical manifestations, from mild nummular focal and multifocal choroiditis to severe endogenous endophthalmitis. The overlying vitreous may have little or no vitreous cells. Multifocal choroidal tubercles, which are foci of granuloma in the choroid, frequently occur with involvement of the retina. These tuberculomas appear to reach the choroid via a haematogenous spread. The choroidal tubercles appear to be bilateral or unilateral and measure between 0.2 and 3 mm. Although they appear commonly in the posterior pole they can be anywhere in the posterior segment of the eye. The retina is

frequently involved and sometimes serous detachment may occur. Retinal vasculitis may occur in the absence of choroiditis or retinitis. This form of predominant phlebitis occurring in patients with healed tuberculosis may represent an immune-mediated reaction to tuberculoprotein. Rapid progression of the disorder and even more varied presentations may occur in immunocompromised individuals.[71]

Serpiginous choroiditis like chorioretinal lesions may also be observed. Tuberculous serpiginous-like choroiditis often presents with significant vitritis, and lesions are usually multifocal in the posterior pole and periphery, whereas true serpiginous choroiditis most often reveals minimal vitritis and frequently shows bilateral involvement with larger solitary lesions extending primarily from the juxtapapillary area and sparing the periphery.[72] Optic nerve papillitis and juxtapapillary chorioretinitis may occur as well.[73] Ocular TB is a great mimicker, and hence may give rise to varied presentations.

Initial diagnosis may involve performing a mantoux tuberculin skin test, a chest x ray and sputum analysis for acid fast bacilli / culture. As most patients in South Africa are immunized with the Bacillus Calmette-Guerin strain vaccine, false positive results are common although the false positive rates due to BCG vaccination are unknown. The interferon-gamma release assays (IGRAs) such as the commercially available T SPOT-TB and the QuantiFERON-TB GOLD tests have been shown to be more specific and sensitive in populations with a low incidence of tuberculosis, but in a country like South Africa with a higher TB burden the IGRAs have not been shown to be superior to the tuberculin skin test.[74-76] It is also important to note that negative IGRA's do not exclude ocular TB as they may be negative in patients with very low CD4 counts.[77]

Although IGRA's can distinguish exposure to M. tuberculosis from the Bacillus Calmette-Guérin vaccine strain, they currently lack the specificity to distinguish between latent tuberculosis infection and active tuberculosis.[78] Similarly, testing for serum antibodies to TB does not prove active TB.

A very low sensitivity of acid-fast bacilli smear and culture in ocular fluid specimens, and a long time period (6–8 weeks) for this bacteria to grow in culture are major limitations in ocular fluid analysis in cases of suspected ocular TB.[79]

PCR diagnosis of ocular TB has emerged as a potential powerful tool for rapid detection. It has been shown to be highly specific with a variable sensitivity.[79] The reliability of PCR testing for TB depends on the sensitivity and specificity of a particular assay being used. These measures are difficult to establish in tuberculosis as the culture that is the gold standard for comparison itself has a poor yield from intraocular specimens, and histopathology is mostly not available. Use of nPCR for MTB may substantially improve sensitivity.[80] In addition, real time PCR for M. tuberculosis has also helped in the differentiation of Mycobacterium from other contaminants.[80] At present there is however no proven effective and highly sensitive PCR technique for identifying ocular MTB infection. Better PCR tests for MTB are needed. A recent proposal from Gupta suggests that nested PCR may increase the sensitivity but this is not proven in any study with significant numbers.[80]

The diagnosis of presumed ocular tuberculosis remains a clinical challenge with currently available diagnostic modalities. Continued improvement in the currently available molecular diagnostic techniques including quantitative PCR may be valuable in our ability to establish an earlier etiologic diagnosis and institute appropriate antimycobacterial therapy.[78]

<u>Ocular Syphilis</u>

Patients with ocular syphilis may present with episcleritis, scleritis, dacryoadenitis, anterior uveitis, intermediate uveitis, papillitis, retinal vasculitis, neuroretinitis and chorioretinitis. In the past, ocular syphilis has been described as manifesting most commonly as an isolated anterior uveitis. In recent literature, more cases of posterior

segment inflammation have been described.[81-84] Syphilis is a 'great imitator' and so can present with many phenotypic disease expressions. Retinitis was recently reported as a common presentation in HIV-infected individuals, suggesting that HIV infection may somehow modulate the disease.[83]

Peripheral blood tests for detecting syphilitic infection include the treponemal and the non-treponemal tests. The treponemal tests are the fluorescent treponemal antibody-absorption (FTA-ABS) test and the two indirect agglutination treponemal tests, the Treponema Pallidum Haem Agglutination (TPHA) and the Treponema Pallidum Particle Agglutination (TPPA) tests. FTA-ABS has a sensitivity of 84% for detecting primary syphilis infection and almost 100% sensitivity for detecting syphilis infection in other stages. Its specificity is 96%.[85] For primary syphilis, TPPA has a sensitivity of 85% to 100%, and a specificity of 98% to 100%. In secondary and late-latent syphilis, TPPA has a sensitivity of 98% to 100%. The treponemal tests are however incapable of distinguishing past from present infection.[86-87]

In order to determine disease activity including antibody quantitation the non-treponemal Venereal Disease Research Laboratory (VDRL) test can be used.[88]

The VDRL test is commonly used to assess response to therapy and to detect CNS involvement. The basis of the test is that an antibody produced by a patient with syphilis reacts with an extract of ox heart (diphosphatidyl glycerol). It therefore detects anti-cardiolipin antibodies (IgG, IgM or IgA), visualized through foaming of the test tube fluid, or "flocculation". The Rapid Plasma Reagin (RPR) test uses the same antigen as the VDRL, but in that test it has been bound to several other molecules including a carbon particle to allow visualization of the flocculation reaction without the need of a microscope.

Many other medical conditions can produce false positive VDRL / RPR results, including autoimmune disorders, viruses, drugs and pregnancy. It is therefore important to perform

a treponemal test (TPHA or TPPA) in order to confirm the diagnosis, when non-treponemal tests are being used as screening tests.

The non-treponemal tests are very useful as the trend of titres is correlated to disease activity (i.e. falling titres indicate successful treatment).

If any of these tests (TPPA, TPHA or VDRL) are positive in the CSF, this indicates neurosyphilis.[89] In cases of suspected ocular syphilis, Treponemal tests are the most appropriate. The non-treponemal tests, VDRL or RPR, are insufficiently sensitive in late-stage syphilis, when ocular disease most often occurs. While the sensitivity and specificity of treponemal tests are higher, false negative and false positive results are also observed. The serologic diagnosis of syphilis is far from perfect.[90]

Ocular fluid antigen and antibody tests have not been proven to be useful.[91-93] PCR on ocular fluids has been used to detect syphilitic uveitis, but no large studies have been done. [94-95]

INTRAOCULAR FLUID SAMPLING SITES AND TECHNIQUE AFFECTING ACCURACY

Obtaining ocular fluid for diagnostic testing involves either an aqueous tap or a vitreous tap.[28,96] An aqueous tap can be performed in an outpatient setting, providing approximately 0.1 to 0.2 mL of fluid.[21] Anterior chamber paracentesis is generally safe (complication rate 0.7%) when performed at the slit lamp following adequate aseptic precaution, and appropriate counselling.[97]

A vitreous tap can be performed in the outpatient setting, or in theatre with the aid of an operating microscope. Vitreous fluid obtained during vitrectomy surgery can also be used for analysis. This fluid may however be diluted with balanced salt solution entering the vitreous cavity through the irrigating port. Obtaining a preinfusion aspirate can avoid this dilution effect. In younger patients where vitreous syneresis has not taken place, a larger bore needle is often required in order to obtain a sample; alternatively a diagnostic vitrectomy may have to be performed.

Both aqueous and vitreous biopsies for PCR diagnosis have been shown to be useful. [20,60] Harper and colleagues found aqueous samples to have a higher sensitivity, but the difference wasn't statistically significant and their study was subject to selection bias.

There is inadequate evidence in the literature to evaluate the relative sensitivity of aqueous versus vitreous fluid samples in PCR analysis in infectious posterior uveitis. A randomized trial to compare aqueous and vitreous samples sensitivity is required.

Local information and trends

Groote Schuur Hospital is located in Cape Town, which is the capital city of the Western Cape province in South Africa. Groote Schuur Hospital is a large tertiary care hospital that serves a significant proportion of the approximately 3 million population of the greater Cape Town Metropolitan area.

At Groote Schuur Hospital an average of about 50 patients per year present with suspected infectious posterior uveitis. There is also a significant HIV burden with an estimated HIV infection rate of 18% in the Cape Town Metropolitan area in 2008, which increased to 20% in 2010.[98]

In this study we set out to determine the pathogen distribution in infectious posterior uveitis in a representative population with a high HIV infection rate, as well as the value of performing routine PCR analysis in patients suspected of having infectious posterior uveitis.

426 References

427

428 1. London NJ, Rathinam SR, Cunningham ET Jr. The epidemiology of uveitis in
429 developing countries. Int Ophthalmol Clin. 2010;50:1-17.
430 2. McCannel, CA, Holland GN, Helm CJ, et al. Causes of uveitis in the general practice
431 of ophthalmology. Am. J. Ophthalmol. 1996;121:35–46.
432 3. Westeneng AC, Rothova A, de Boer JH, de Groot-Mijnes JD. Infectious uveitis in
433 immunocompromised patients and the diagnostic value of polymerase chain reaction and
434 Goldmann-Witmer coefficient in aqueous analysis. Am J Ophthalmol 2007;144:781-5.
435 4. Shafik SM, Foster CS. Definition, classification, etiology, and epidemiology. In: Foster
436 C, Vitale AT, editors. Diagnosis and treatment of uveitis. Philadelphia, Pennsylvania:
437 W.B. Saunders Company, 2002:17-26.
438 5. Gagliuso DJ, Teich SA, Friedman AH, Orellana J. Ocular toxoplasmosis in AIDS
439 patients. Trans Am Ophthalmol Soc 1990;88:63-88.
440 6. de Boer JH, Verhagen C, Bruinenberg M, et al. Serologic and polymerase chain
441 reaction analysis of intraocular fluids in the diagnosis of infectious uveitis. Am J
442 Ophthalmol. 1996;121:650-8.
443 7. Fox GM, Crouse CA, Chuang EL, et al. Detection of Herpesvirus DNA in Vitreous and
444 Aqueous Specimens by the Polymerase Chain Reaction. Arch Ophthalmol.
445 1991;109:266-71.
446 8. Pepose JS, Hilborne LH, Cancilla PA, Foos RY. Concurrent herpes and
447 cytomegalovirus retinitis and encephalitis in the acquired im- mune deficiency syndrome
448 (AIDS). Ophthalmology. 1984;91:1669-1677.
449 9. Tran THC, Rozenberg F, Cassoux N, et al. Polymerase chain reaction analysis of
450 aqueous humour samples in necrotising retinitis. Br J Ophthalmol 2003;87:79–83.

10. Dussaix E, Cerqueti PM, Pontet F, Bloch-Michel E. New approaches to the detection of locally produced antiviral antibodies in the aqueous of patients with endogenous uveitis. Ophthalmologica 1987;194:145-9.

11. Baer JC. Borreliosis. In: Foster CS, Vitale AT, editors. Diagnosis and treatment of uveitis. Philadelphia, Pennsylvania: W.B. Saunders Company, 2002:245-259.

12. Samson CM, Foster CS. Masquerade syndromes. In: Foster CS, Vitale AT, editors. Diagnosis and treatment of uveitis. Philadelphia, Pennsylvania: W.B. Saunders Company, 2002:528-536.

13. Manku H, McCluskey P. Diagnostic vitreous biopsy in patients with uveitis: a useful investigation? Clin Experiment Ophthalmol 2005;33:604-10.

14. Marangon FB, Miller D, Alfonso E. Laboratory results in ocular viral diseases: implications in clinical-laboratory correlation. Arq Bras Oftalmol 2007;70:189-94

15. Drancourt M, Berger P, Terrada C, et al. High prevalence of fastidious bacteria in 1520 cases of uveitis of unknown etiology. Medicine (Baltimore) 2008;87:167-76.

16. Goldmann H, Witmer R. [Antibodies in the aqueous humor.]. Ophthalmologica 1954;127:323-30.

17. Witmer R. Clinical implications of aqueous humor studies in uveitis. Am J Ophthalmol 1987;86:39-44.

18. Kijlstra A, Luyendijk L, Baarsma GS, et al. Aqueous humor analysis as a diagnostic tool in toxoplasma uveitis. Int Ophthalmol 1989;13:383-6.

19. Davis JL, Feuer W, Culbertson WW, Pflugfelder SC. Interpretation of intraocular and serum antibody levels in necrotizing retinitis. Retina 1995;15:233-40.

20. De Groot-Mijnes JD, Rothova A, Van Loon AM, et al. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. Am J Ophthalmol 2006;141:313-8.

21. Rothova A, de Boer JH, Ten Dam-van Loon NH, et al. Usefulness of aqueous humor analysis for the diagnosis of posterior uveitis. Ophthalmology 2008;115:306-11.

22. Espy MJ, Uhl JR, Sloan LM, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev 2006;19:165-256.

23. Arimura E, Deai T, Maruyama K, et al. Herpes simplex virus-2 quantification by realtime polymerase chain reaction in acute retinal necrosis. Jpn J Ophthalmol 2005;49:64–5.

24. Asano S, Yoshikawa T, Kimura H, et al. Monitoring herpesvirus DNA in three cases of acute retinal necrosis by real-time PCR. J Clin Virol 2004;29:206–9.

25. Smith IL, Macdonald JC, Freeman WR, Shapiro AM, Spector SA. Cytomegalovirus (CMV) retinitis activity is accurately reflected by the presence and level of CMV DNA in aqueous humor and vitreous. J Infect Dis. 1999 May;179(5):1249-53.

26. Chichili GR, Athmanathan S, Farhatullah S, et al. Multiplex polymerase chain reaction for the detection of herpes simplex virus, varicella-zoster virus and cytomegalovirus in ocular specimens. Curr Eye Res 2003;27:85–90

27. Ortega-Larrocea G, Bobadilla-del-Valle M, Ponce-de-León A, Sifuentes-Osornio J. Nested polymerase chain reaction for Mycobacterium tuberculosis DNA detection in aqueous and vitreous of patients with uveitis. Arch Med Res. 2003 Mar-Apr;34(2):116-9.

28. Bodaghi B, LeHoang P. Testing ocular fluids in uveitis. Ophthalmol Clin North Am 2002;15:271-9.

29. Van Gelder R. Polymerase Chain Reaction diagnostics for posterior segment disease. Retina 2003;23:445-452

30. McCann JD, Margolis TP, Wong MG, Kuppermann BD, Luckie AP, Schwartz DM, Irvine AR, Ai E. A sensitive and specific polymerase chain reaction-based assay for the diagnosis of cytomegalovirus retinitis. Am J Ophthalmol. 1995 Aug;120(2):219-26.

31. Ganatra JB, Chandler D, Santos C, et al. Viral causes of acute retinal necrosis syndrome. Am J Ophthalmol 2000;129:166–172.

503 32. Sugita S, Shimizu N, Watanabe K, Mizukami M, Morio T, Sugamoto Y, Mochizuki
504 M. Use of multiplex PCR and real-time PCR to detect human herpes virus genome in
505 ocular fluids of patients with uveitis. Br J Ophthalmol 2008;92:928–932.
506 33. Abe T, Tsuchida K, Tamai M. A comparative study of the polymerase chain
507 reaction and local antibody production in acute retinal necrosis syndrome and
508 cytomegalovirus retinitis. Graefes Arch Clin Exp Ophthalmol 1996;234:419–424.
509 34. Short GA, Margolis TP, Kuppermann BD, Irvine AR, Martin DF, Chandler D. A
510 polymerase chain reaction– based assay for the diagnosis of varicella-zoster virus
511 retinitis in patients with AIDS. Am J Ophthalmol 1997;123:157–164.19
512 35. Fekkar A, Bodaghi B, Touafek F, Le Hoang P, Mazler D, Paris L. Comparison of
513 Immunoblotting, Calculation of the Goldmann-Witmer Coefficient, and Real-Time PCR
514 Using Aqueous Humor Samples for Diagnosis of Ocular Toxoplasmosis. J Clin Microbiol.
515 2008, 46(6):1965
516 36. Montoya JG, Parmley S, Liesenfeld O, Jaffe GJ, Remington JS. Use of the
517 polymerase chain reaction for diagnosis of ocular toxoplasmosis. Ophthalmology. 1999
518 Aug;106(8):1554-63.
519 37. Labalette P, Delhaes L, Margaron F, Fortier B, Rouland JF. Ocular Toxoplasmosis
520 After the Fifth Decade. Am J Ophthalmol. 2002 Apr;133(4):506-15.
521 38. Errera M, Goldschmidt P, Batellier L, Degorge S, Héron E, Laroche L, Sahel J,
522 Westcott M, Chaumeil C. Real-time polymerase chain reaction and intraocular antibody
523 production for the diagnosis of viral versus toxoplasmic infectious posterior uveitis.
524 Graefes Arch Clin Exp Ophthalmol (2011) 249:1837–1846.
525 39. Sugita S, Ogawa M, Inoue S, Shimizu N, Mochizuki M. Diagnosis of ocular
526 toxoplasmosis by two polymerase chain reaction (PCR) examinations: qualitative
527 multiplex and quantitative real-time. Jpn J Ophthalmol (2011) 55:495–501
528 40. Yoser LS, Forster DJ, Rao NA. Systemic viral infections and their retinal end
529 choroidal manifestations. Surv Ophthalmol 1993; 37: 313-52.

41. Murray HW, Knox DL, Green WR, Susel RM. Cytomegalovirus retintis in adults. A manifestation of disseminated viral infection. Am J Med. Oct 1977;63(4):574-84

42. Friedman AH, Orellana J, Freeman WR, et al. Cytomegalovirus retinitis: a manifestation of the acquired immune deficiency syndrome (AIDS). Br J Ophthalmol 1983;67:372-80.

43. Mitchell S. Diagnostic assays in cytomegalovirus retinitis. British Journal of Ophthalmology 1996; 80: 195-196

44. Doornenbal P, Seerp Baarsma G, Quint WG, Kijlstra A, Rothbarth PH, Niesters HG. Diagnostic assays in cytomegalovirus retinitis: detection of herpesvirus by simultaneous application of the polymerase chain reaction and local antibody analysis on ocular fluid. Br J Ophthalmol 1996;80:235-40.

45. Guex-Crosier Y, Rochat C, Herbort CP. Necrotizing herpetic retinopathies. A spectrum of herpes virus-induced diseases determined by the immune state of the host. Ocul Immunol Inflam 1997;5:259–65.

46. Urayama A, Yamada N, Sasaki T: Unilateral acute uveitis with retinal periarteritis and detachment. Jpn J Clin Ophthalmol 1971; 25: 607.

47. Duker JS, Blemenkranz MS. Diagnosis and management of the acute retinal necrosis (ARN) syndrome. Survey of Ophthalmology Volume 35, Issue 5, March-April 1991, Pages 327-343

48. Forster DJ, Dugel PU, Frangieh GT, Liggett PE, Rao NARapidly progressive outer retinal necrosis in the acquired immunodeficiency syndrome. American Journal of Ophthalmology [1990, 110(4):341-8]

49. Engstrom RE Jr, Holland GN, Margolis TP, Muccioli C, Lindley JI, Belfort R Jr, Holland SP, Johnston WH, Wolitz RA, Kreiger AE. The progressive outer retinal necrosis syndrome. A variant of necrotizing herpetic retinopathy in patients with AIDS. Ophthalmology [1994, 101(9):1488-502]

556 50. Gaynor BD, Margolis TP, Cunningham ET, Jr. Advances in diagnosis and
557 management of herpetic uveitis. Int Ophthalmol Clin 2000;40:85-109.
558 51. Knox CM, Chandler D, Short GA, Margolis TP. Polymerase chain reaction– based
559 assays of vitreous samples for the diagnosis of viral retinitis. Ophthalmology
560 1998;105:37– 44, 44–45.
561 52. Mendelsohn AD, Jampol LM. Syphilitic retinitis. A cause of necrotizing retinitis.
562 Retina 1984;4:221-4.
563 53. Balansard b, Bodaghi B, Cassoux N, Fardeau C, Romand S, Rozenberg F, Rao N
564 A, LeHoang P. Necrotising retinopathies simulating acute retinal necrosis syndrome. Br J
565 Ophthalmol 2005;89:96–101.
566 54. Cunningham ET, Short GA, Irvine AR, Duker JS, Margolis TP. Acquired
567 immunodeficiency syndrome–associated herpes simplex virus retinitis. Clinical
568 description and use of a polymerase chain reaction– based assay as a diagnostic tool.
569 Arch Ophthalmol 1996;114:834–840.17
570 55. Yamamoto S, Pavan-Langston D, Kinoshita S, Nishida K, Shimomura Y, Tano Y.
571 Detecting herpes virus DNA in uveitis using the polymerase chain reaction. Br J
572 Ophthalmol 1996;80:465– 468.18
573 56. Jyotsom B Ganatra MPHa, Diane Chandler BSa, Carmen Santos MDb,
574 Baruch Kuppermann MD, PhDc, Todd P Margolis MD, PhD. Viral causes of the acute
575 retinal necrosis syndrome. Am J Ophthalmol. 2000 Feb;129(2):166-72.
576 57. Abe T, Sato M, Tamai M. Correlation of varicella-zoster virus copies and final
577 visual acuities of acute retinal necrosis syndrome. Graefes Arch Clin Exp Ophthalmol
578 1998;236:747–752.
579 58. Pendergast SD, Werner J, Drevon A, Wiedbrauk DL. Absence of herpes virus
580 DNA by polymerase chain reaction in ocular fluids obtained from immunocompetent
581 patients. Retina 2000;20:389 –393.

582 59. Harper TW, Miller D, Schiffman JC, Davis JL. Polymerase Chain Reaction
583 Analysis of Aqueous and Vitreous Specimens in the Diagnosis of Posterior Segment
584 Infectious Uveitis. Am J Ophthalmol. 2009 Jan;147(1):140-147.
585 60. Holland GN. Ocular toxoplasmosis: a global reassessment. Part I: epidemiology
586 and course of disease. Am J Ophthalmol 2003;136:973-88.
587 61. Holland GN. Ocular toxoplasmosis: a global reassessment. Part II: disease
588 manifestations and management. Am J Ophthalmol 2004;137:1-17.
589 62. Fardeau C, Romand S, Rao NA, et al. Diagnosis of toxoplasmic retinochoroiditis
590 with atypical clinical features. Am J Ophthalmol 2002;134:196-203.
591 63. Smith JR, Cunningham ET, Jr. Atypical presentations of ocular toxoplasmosis.
592 Curr Opin Ophthalmol 2002;13:387-92.
593 64. Jacobs MR, Mason PR. Prevalence of Toxoplasma antibodies in Southern Africa.
594 S Afr Med J. 1978 Apr 22;53(16):619-21
595 65. Global Tuberculosis Control Surveillance, planning, financing. WHO Report 2007.
596 Geneva, World Health Organization; 2007. Report no. WHO/HTM/TB/2007.376.
597 66. Kestelyn PG, Cunningham ET Jr. HIV/AIDS and blindness [review]. Bull World
598 Health Organ 2001; 79:208–213.
599 67. Mehta S, Gilada IS. Ocular tuberculosis in acquired immune deficiency syndrome
600 (AIDS). Ocul Immunol Inflamm 2005; 13:87–89.
601 68. Donahue HC. Ophthalmologic experience in a tuberculosis sanatorium. Am J
602 Ophthalmol 1967; 64:742–748.14
603 69. Bouza E, Merino P, Munoz P, et al. Ocular tuberculosis: a prospective study in a
604 general hospital. Medicine (Baltimore) 1997; 76:53–61.
605 70. Beare NA, Kublin JG, Lewis DK, et al. Ocular disease in patients with tuberculosis
606 and HIV presenting with fever in Africa. Br J Ophthalmol 2002; 86:1076–1079.
607 71. Tabbara KF. Tuberculosis. Curr Opin Ophthalmol 2007;18:493-501

608 72. Vasconcelos-Santos DV, Rao PK, Davies JB, Sohn EH, Rao NA. Clinical features
609 of tuberculous serpiginouslike choroiditis in contrast to classic serpiginous
610 choroiditis.Arch Ophthalmol. 2010 Jul;128(7):853-8
611 73. Gupta V, Gupta A, Arora S, et al. Presumed tubercular serpiginouslike choroiditis:
612 clinical presentations and management. Ophthalmology 2003; 110:1744–1749.
613 74. Detjen AK, Keil T, Roll S, Hauer B, et al. Interferon-gamma release assays
614 improve the diagnosis of tuberculosis and nontuberculous mycobacterial disease in
615 children in a country with a low incidence of tuberculosis. Clin Infect Dis 2007; 45:322–
616 328.
617 75. Dheda K, van Zyl Smit R, Badri M, Pai M.T-cell interferon-gamma release assays
618 for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-
619 burden settings.Curr Opin Pulm Med. 2009 May;15(3):188-200.
620 76. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, Hatherill M, Moyo S,
621 Hanekom W, Mahomed H.The utility of an interferon gamma release assay for diagnosis
622 of latent tuberculosis infection and disease in children: a systematic review and meta-
623 analysis. Pediatr Infect Dis J. 2011 Aug;30(8):694-700.
624 77. Luetkemeyer AF, Charlebois ED, Flores LL, et al. Comparison of an interferon-
625 gamma release assay with tuberculin skin testing in HIV-infected individuals. Am J Respir
626 Crit Care Med 2007;175:737-42.
627 78. Yeh S, Sen HN, Colyer M, Zapor M, Wroblewski K. Update on ocular
628 tuberculosis.Curr Opin Ophthalmol. 2012 Nov;23(6):551-6
629 79. Sharma P, Bansal R, Gupta V, Gupta A. Diagnosis of tubercular uveitis by
630 quantitative polymerase chain reaction. J Ophthalmic Inflamm Infect. 2011 March; 1(1):
631 23–27.
632 80. Gupta V, Gupta A, Rao NA.intraocular tuberculosis an update. Surv Ophthalmol.
633 2007 Nov-Dec;52(6):561-87

634 81. Butler NJ, Smith JR. Ocular syphilis in HIV-positive individuals Clinical and
635 Experimental Ophthalmology 2010; 38: 829–830
636 82. Tran THC, Cassoux N, Bodaghi B, Fardeau C, Caumes E, Lehoang P. Syphilitic
637 uveitis in patients infected with human immunodeficiency virus. Graefes Arch Clin Exp
638 Ophthalmol 2005; 243: 863–69.
639 83. Hughes EH, Guzowski M, Simunovic MP, Hunyor AP, McCluskey P. Syphilitic
640 retinitis and uveitis in HIVpositive adults. Clin Experiment Ophthalmol 2010; 38: 851–6.
641 84. Amaratunge BC, Camuglia JE, Hall AJ. Syphilitic uveitis: a review of clinical
642 manifestations and treatment outcomes of syphilitic uveitis in human immunodeficiency
643 virus-positive and negative patients. Clin Experiment Ophthalmol 2010; 38: 68–74.
644 85. [Guideline] U.S. Preventive Services Task Force. Screening for Syphilis Infection.
645 Recommendation Statement. 2004.
646 86. Creegan L, Bauer HM, Samuel MC, et al. An evaluation of the relative sensitivities
647 of the venereal disease research laboratory test and the Treponema Pallidum particle
648 agglutination test among patients diagnosed with primary syphilis. Sex Transm Dis.
649 2007;34:1016-1108.
650 87. Manavi K, Young H, McMillan A. The sensitivity of syphilis assays in detecting
651 different stages of early syphilis. Int J STD AIDS. 2006;17:768-771.
652 88. Aldave AJ, King JA, Cunningham ET, Jr. Ocular syphilis. Curr Opin Ophthalmol
653 2001;12:433-41.
654 89. Luger AF, Schmidt BL, Kaulich M. Significance of laboratory findings for the
655 diagnosis of neurosyphilis. Int J STD AIDS 2000;11:224-34.
656 90. Tamesis RR, Foster CS. Ocular syphilis. Ophthalmology 1990; 97:1281–1287.
657 Gaudio P. Update on ocular syphilis. Curr Opin Ophthalmol 17:562–566
658 91. Christman EH, Hamilton RW, Heaton CL, Hoffmeyer IM. Intraocular treponemes.
659 Arch Ophthalmol 1968;80:303-7.

92. Whitfield R, Wirostko E. Uveitis and intraocular treponemes. Arch Ophthalmol 1970;84:12-5.

93. Ryan SJ, Nell EE, Hardy PH. A study of aqueous humor for the presence of spirochetes. Am J Ophthalmol 1972;73:250-7.

94. Rajan MS, Pantelidis P, Tong CY, French GL, Graham EM, Stanford MR. Diagnosis of Treponema pallidum in vitreous samples using real time polymerase chain reaction. Br J Ophthalmol 2006;90:647-8.

95. Muller M, Ewert I, Hansmann F, et al. Detection of Treponema pallidum in the vitreous by PCR. Br J Ophthalmol 2007;91:592-5.

96. Van der Lelij A, Rothova A. Diagnostic anterior chamber paracentesis in uveitis: a safe procedure? Br J Ophthalmol 1997;81:976-9.

97. Trivedi d, Denniston AKO , Murray PI. Safety profile of anterior chamber paracentesis performed at the slit lamp. Clin & Exp Ophthalmol 2011;39: 725 - 728

98. The 2010 National antenatal sentinel HIV & Syphilis prevalence survey in South Africa. http://www.doh.gov.za/docs/reports/2011/hiv_aids_survey.pdf

Appendix – named journal and author contributions

Named peer reviewed journal:

Ophthalmology

Instructions for authors web address:

http://cdn.elsevier.com/promis_misc/ophtha_guideforauthors2012.pdf

Co-author contributions:

Lecuona KA – MMed supervisor and study advisor.

Rogers G – Assisted in clinical data capture and data entry.

Bunce C – Lead in statistical analysis

Corcoran C – Assisted in laboratory data capture and interpretation as well as the write-up of the laboratory methods section in the manuscript

Michaelides M – Assisted in final manuscript write up and grammatical correction / editing.

Title page

Title: Diagnostic and clinical value of routine polymerase chain reaction analysis of intraocular fluid specimens in the diagnosis of suspected infectious posterior uveitis

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ABSTRACT

Diagnostic and clinical value of routine polymerase chain reaction analysis of intraocular fluid specimens in the diagnosis of suspected infectious posterior uveitis

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Objective: To assess the diagnostic and clinical value of routinely performing polymerase chain reaction (PCR) analysis on intraocular fluid samples in patients with suspected infectious posterior uveitis in a population with a high prevalence of human immunodeficiency virus infection.

Design: Retrospective, interventional case series.

Participants: 159 consecutive patients presenting with suspected active infectious posterior uveitis.

Methods: Patients presenting with a first episode of suspected infectious posterior uveitis underwent PCR testing of ocular fluid samples in a tertiary care hospital over a five year period. PCR analysis was performed for cytomegalovirus (CMV), varicella zoster virus (VZV), herpes simplex virus type 1 and 2 (HSV), toxoplasma gondii (TG) and mycobacterium tuberculosis (MTB).

Results: The prevalence of the commonest causes of infectious posterior uveitis based on PCR studies was CMV in 47% of patients, VZV in 11% and TG in 10%. HSV was not

identified. PCR analysis confirmed the initial clinical diagnosis in 55 patients (35%) and altered the initial clinical diagnosis in 36 patients (23%). The clinical diagnosis prior to PCR testing was non specific (uncertain) in 51 patients (32%), with PCR providing a definitive final diagnosis in 20 of these patients (39%); necrotizing herpetic retinopathy and ocular toxoplasmosis were particularly difficult to diagnose correctly without the use of PCR analysis. The overall PCR sensitivity was 84%, specificity was 99%, positive predictive value was 97% and negative predictive value was 95%.

Conclusion: The clinical phenotype alone was unreliable in diagnosing the underlying infectious cause in a quarter of patients in this study. Since the outcome of incorrectly treated infective uveitis can be blinding, PCR analysis of ocular fluids is recommended early in the disease even in resource poor settings.

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MANUSCRIPT

INTRODUCTION

In developed countries, uveitis affects approximately 200 per 100,000 in the population, and uveitis and its complications accounts for up to 35% of severe visual impairment.[1] In less developed countries, uveitis and its complications are even more common, affecting an estimated 714 per 100,000 and contributing to 25% of blindness.[1] Posterior uveitis is thought to comprise approximately 5% of all uveitis entities, with the commonest pathogens responsible for *infectious* posterior uveitis and panuveitis being Herpes Simplex Virus (HSV) type 1 and 2, Varicella-Zoster Virus (VZV), Cytomegalovirus (CMV), Toxoplasma Gondii (TG), Treponema Pallidum and Mycobacterium Tuberculosis (MTB).[2] In developed countries, the most common infectious aetiologies are TG, HSV, and VZV, whereas CMV is a common pathogen in countries with a high prevalence of human immunodeficiency virus (HIV) / acquired immune deficiency syndrome (AIDS).[3-5]

Blindness and visual impairment caused by infectious uveitis can be prevented by early identification of the responsible pathogen, and the subsequent prompt administration of appropriate antimicrobial therapy.[1] This is particularly critical in immunocompromised patients.[3,6] The aetiological diagnosis of infectious uveitis is initially made on the basis of the associated clinical features, but there is often significant overlap between the phenotypic expressions of these different pathogens, thereby limiting the ability to accurately identify the causative organism by clinical examination.[7] Moreover, simultaneous infection of the retina with multiple different organisms in patients with

AIDS has been reported, making it almost impossible to make a correct complete diagnosis on clinical grounds alone.[8] Furthermore, establishing a diagnosis based on clinical findings is also difficult in cases where media opacity or poor pupil dilation may mask clinical features. Under these circumstances an incorrect diagnostic decision not only causes a delay of appropriate treatment and prevention of loss of vision, but also exposes the patients to side effects of an unnecessary medication.[9]

PCR of intra-ocular fluids is a reliable investigation that can identify most of the common causes of infective posterior uveitis.[10] It is a technique whereby theoretically a single, or a few copies of a piece of DNA, are amplified across several orders of magnitude, generating millions of copies of a particular nucleic acid sequence. PCR analysis of ocular fluid samples allows accurate and rapid detection of small quantities of DNA or RNA from potential pathogens infecting the eye. It has been shown to be highly sensitive and specific for CMV, HSV and VZV. [2,6,9,11-17] By comparison, PCR analysis in cases of TG posterior uveitis has a variable sensitivity and a combination of PCR & Goldman Witmer coefficient analysis improves diagnostic sensitivity. [3,6,18-25] PCR diagnosis of ocular MTB has been shown to be highly specific with a variable sensitivity.[26] PCR also offers significantly improved time to diagnosis compared to traditional techniques.

PCR testing is however not readily available in low income countries and clinicians have to rely on clinical findings to decide on initial treatment. The prevalence of PCR proven causes of infectious uveitis in a population with a high prevalence of HIV / AIDS has not

yet been described. This study was therefore performed to determine the prevalence of the commonest causes of infectious uveitis based on PCR studies in a population with a high prevalence of HIV, and to document the correlation between the clinical appearance and the laboratory findings in a general ophthalmology clinic to aid the development of suitable treatment protocols.

METHODS

Patients and Clinical Methods

Patients who underwent PCR testing of ocular fluids (vitreous and aqueous) for suspected infectious uveitis at the Ophthalmology Unit at Groote Schuur Hospital between 1 May 2004 and 30 June 2009 were identified. The Ophthalmology Unit at Groote Schuur Hospital in Cape Town, South Africa, is one of 2 tertiary institutions that serve a population of approximately 3 million. The estimated HIV prevalence in this area was 18% in 2009.[27] Ethics approval for this study was obtained from the University of Cape Town Health Sciences Human Research Ethics Committee.

Patients presenting to the Ophthalmology Triage Division with suspected infectious uveitis underwent routine PCR testing of ocular fluids. Other investigations included syphilis serology, a full blood count and differential, chest X-ray, and HIV testing if status was unknown. Skin tests for tuberculosis were not performed as this is of limited value in a population where tuberculosis is endemic.

Based on the phenotypic appearance, the appropriate treatment was commenced pending the results of the PCR testing. When indicated by the subsequent PCR result, treatment was changed. The initial diagnosis and management was made by ophthalmology residents in the Triage Division, after which patients were followed up in the Uveitis Clinic.

The study population consisted of the laboratory sample logs of all patients who underwent PCR testing of ocular fluids between 1 May 2004 and 30 June 2009. Patient charts were reviewed to determine clinical history and course, as well as patient characteristics. Patients who had a known previous episode of posterior uveitis were excluded from the study. PCR testing was performed for the commonest causative organisms namely CMV, HSV type 1 and 2, VZV, TG and MTB.

Ocular fluid samples were obtained by Ophthalmology residents and consultants. Vitreous samples were obtained by passing a 23 gauge needle through the pars plana and withdrawing 0.2 to 0.3 mls of core vitreous cavity fluid. A small number of vitreous samples were also obtained at the time of pars plana vitrectomy. If vitreous could not be aspirated, anterior chamber aqueous samples were obtained using a 28 to 30 gauge needle on a 1 ml syringe using tetracaine topical anaesthesia and one drop of topical 5% Povidone Iodine solution. Samples were then transported urgently to the diagnostic laboratory that was located on the hospital grounds.

LABORATORY METHODS for PCR analysis

a. Nucleic Acid Isolation

Total nucleic acid was extracted from the aqueous and vitreous fluid using the Nuclisens EasyMAG platform (bioMérieux, Boxtel, Netherlands) according to the manufacturer's instructions. Nucleic acid was eluted in 50µl elution buffer and stored at -4°C.

b. Nested PCR for the detection of CMV, HSV 1 & 2, VZV, TG and MTB.

In-house nested PCRs were used to screen the samples in this study for CMV, HSV 1 & 2, VZV, TG and MTB using previously published primer sequences.[28-32] The first round PCR was performed with a 50µl reaction mixture containing 10µl extracted DNA, 15mM Tris-HCL (pH 8), 50mM KCl, 1.5mM $MgCl_2$, 0.2mM deoxynucleotide triphosphates (ABgene, Epsom, UK), 20pmol of each forward and reverse primer and 1.5U Supertherm Taq polymerase (JMR Holdings, Kent, UK). Amplification was performed on a Thermo Hybaid PxE 0.2 thermal cycler (Thermo Scientific, Waltham, MA, USA), with the following conditions: 1 cycle of 94°C for 2 minutes, 40 cycles of 94 °C for 20s, 55°C for 30s, and 72°C for 45s, and a final elongation step at 72°C for 7 minutes. The second round PCR was performed using the same basic master mix ingredients containing 50pmol of each inner forward and reverse primer and 2µl of first round PCR product. Cycling conditions were as for the first round PCR, although the annealing temperature was increased to 58°C. Amplified products were separated by electrophoresis in 2% agarose gel, and visualized under UV irradiation after staining with ethidium bromide. The expected sizes of the inner PCR products were 160bp (CMV), 179bp (HSV 1 & 2), 251bp (VZV), 96bp (TG) and 194bp (MTB). All work was performed in an ISO-15189

accredited molecular laboratory which employs strict precautions to prevent contamination.

PCR results were reported as detected or not detected within 48 to 72 hours.

OUTCOME MEASURES

Initial pre-PCR diagnoses were made based on history and clinical findings on ocular and systemic examination. Final diagnoses were made based on investigation results, clinical behaviour and response to treatment. PCR test results were considered to confirm the initial diagnosis if PCR analysis was positive for the pathogen which was considered the inciting cause at presentation. PCR test results were considered to have changed the initial diagnosis if PCR analysis was positive for a different pathogen *and* the clinical course and response to treatment was consistent with the PCR positive result. If the PCR test results were positive for more than one pathogen and the clinical course and treatment response was consistent with possible co-infection then the PCR test result was also considered to alter the diagnosis. In all other cases the PCR test result was considered to have an undetermined effect on the final diagnosis.

There is no gold standard test against which to measure the sensitivity and specificity of PCR analysis in the diagnosis of infectious posterior uveitis.[33] The final diagnoses which were based on the clinical course, response to treatment and results of investigations were therefore used as the gold standard in order to calculate PCR sensitivity and specificity. The clinical sensitivity and specificity was calculated rather than the nominal

sensitivity of the test itself.[33] Positive predictive value (PPV) and negative predictive value (NPV) for PCR testing were also calculated.

Statistical analysis was performed by Dr. C Bunce from the Moorfields Eye Hospital Medical Statistics department. A Chi-square test was used to compare sensitivity values for PCR testing of vitreous compared to anterior chamber samples.

RESULTS

Of the 187 consecutive patients who underwent PCR ocular fluid testing, 159 patients were included in the study. There were 28 patients excluded from the study; 11 case notes were damaged or lost, 4 patients had a recurrent episode, 12 patients did not have any active posterior uveitis at the time of sampling, and 1 patient defaulted follow up within 1 week of presentation, preventing observation of clinical course and confirmation of the final diagnosis.

There were no documented complications due to aqueous or vitreous fluid aspiration procedures.

Patient characteristics and average visual acuities pre- and post treatment are shown in tables 1 and 2 respectively. The duration of follow up ranged from 1 week to 5 years. The number of PCR tests performed for each pathogen tested and the results are shown in table 3. There were 643 PCR tests performed, with a mean of 4 tests per patient. CMV, VZV, HSV and TG PCR tests were performed on most patients, whereas MTB PCR was performed less frequently (n=43) as local PCR testing for MTB was only

available for the last 18 months of the study (from January 2008). Forty one patients were tested for all 5 pathogens.

Initial Clinical Diagnoses

The pre-PCR clinical diagnoses compared with PCR positive findings are shown in table 4. The most common pre-PCR diagnoses were cytomegalovirus retinitis (CMVR) (n=70), necrotizing herpetic retinopathy (NHR) (n=14), and ocular toxoplasmosis (OT) (n=10). There were 51 patients whose diagnoses were uncertain because their clinical presentations were not characteristic for a particular pathogen. In 17 patients the view of the fundus was so poor due to a combination of severe vitritis and posterior synechiae, that it precluded accurate clinical diagnosis.

PCR Results

Of the 159 patients tested by PCR analysis, 94 patients had a positive PCR result (59%). PCR confirmed the suspected diagnosis in 55 patients (34.6%), altered the diagnosis in 36 patients (22.6%) and had an undetermined effect in 68 patients (42.8%). In the 51 patients who had an uncertain clinical diagnosis, PCR identified 20 patients (39%) with a PCR positive diagnosis consistent with the final diagnosis. CMV PCR tests were the most frequently positive (47%), followed by VZV (11%). There were no positive HSV PCR results.

Five patients tested PCR positive for more than one pathogen. Four patients were CMV and VZV positive, and one patient was CMV and TG positive. In the patients who tested

CMV and VZV positive, two were considered to have true active co-infections with CMV and VZV, one had a final diagnosis of CMVR alone and one had a final diagnosis of NHR due to VZV alone. The patient who tested positive for both CMV & TG had a final diagnosis of OT alone. Three of the five co-infections were therefore considered to be 'false positive' results.

Final diagnoses are shown in table 5. CMV retinitis was bilateral in 36 cases (49%), NHR was bilateral in 6 cases (35%), and OT was bilateral in 2 cases (13%). Using the final diagnoses as the gold standard, PCR sensitivities for the sampling sites, specificity, positive predictive value and negative predictive values were calculated and the results are shown in tables 6 and 7. The overall PCR sensitivity for pathogens tested was 84%.

Despite 148 samples being tested for HSV by PCR analysis, none were HSV positive. Simultaneous vitreous and aqueous specimens were obtained from 4 patients, solitary vitreous samples were taken from 105 patients, and solitary anterior chamber fluid samples from 47 patients. Sampling site information was omitted in 3 case notes (all 3 solitary samples). There were 2 patients who had repeat sampling during their treatment course (both vitreous repeat samples). Overall, vitreous samples had higher sensitivity than aqueous (P = 0.027).

Seventeen patients presented with a very poor view of the fundus. Seven cases were due to OT (4 of these were PCR confirmed) and 2 cases were due to CMVR (both PCR confirmed). Seven cases were idiopathic and one case was due to ocular syphilis.

Bilateral v Unilateral Disease

Of the 67 patients who presented with bilateral disease, 36 (54%) were due to CMV, 6 (9%) were due to NHR and 2 (3%) were due to OT. Of the 92 patients who presented with unilateral disease, 38 (41%) were due to CMV, 11 (12%) were due to NHR and 14 (15%) were due to OT.

DISCUSSION

This is the first study to describe the pathogen distribution based on PCR testing of patients with infectious posterior uveitis, in a population with a high prevalence of HIV / AIDS. The most frequent final diagnosis was CMVR, followed by NHR and OT (47%, 11% and 10% respectively). This is in direct contrast to Harper et al's study in a population with a lower HIV prevalence where NHR was the most common diagnosis followed by CMVR and OT.[33]

The initial pre-PCR clinical diagnosis was uncertain in 51 cases (32%). This was due to a number of factors. Many patients presented late with significant vitritis and posterior synechiae leading to an obscured fundus view. A significant number of cases presented with atypical findings making it difficult to make a definitive diagnosis. In addition, the initial clinical diagnosis was usually made by general ophthalmologists, not uveitis subspecialists; although this makes the findings of this study more generally clinically

relevant, especially in the developing world, but also potentially in developed countries. PCR analysis provided the correct final diagnosis in 20 of these patients (39%).

The initial clinical diagnosis changed in approximately a quarter of cases as a result of PCR testing. This is likely due to the significant overlap between the phenotypic expressions of these different pathogens.[7] Having an early definitive laboratory proven diagnosis is advantageous in instituting appropriate effective treatment in a timely fashion; this was not the case in a quarter of our patient population. In our study the clinical diagnosis prior to PCR testing was particularly challenging in patients who were subsequently confirmed to have NHR and OT, often due to a poor view of the fundus. Patients with NHR from VZV infection had findings that overlapped with CMV retinitis; whilst patients with OT were found to overlap with CMV retinitis and NHR phenotypes.

PCR was able to provide a final definitive clinical diagnosis in approximately 60% of our patients. Previous studies have shown PCR analysis of intraocular fluids to detect viral infection in posterior uveitis to be a sensitive and highly specific test. For CMV retinitis sensitivity ranges from 91% to 95% and for NHR sensitivity ranges from 79% to 100%.[6,9,11-13,16-17] Our study supports these findings with a viral sensitivity of 91% for CMV and 75% for NHR.

PCR analysis in patients with ocular toxoplasmosis is generally less sensitive than viral retinitis. Studies have shown variable sensitivity ranging from 27% to 85%.[6,18,21-25] It has been suggested that in immune compromised patients PCR analysis may have greater

sensitivity.[6] This is supported in our study where we found the sensitivity to be 75%. The diagnostic yield for PCR for toxoplasmosis chorioretinitis in this series was comparable with that for viral retinitis (12 of 16 cases, or 75%) and is higher than the 9 of 25 cases (36%) reported by De Groot-Mijnes et al, or by Fardeau et al for 34 patients with a final diagnosis of toxoplasmosis chorioretinitis, of whom 79% had positive intraocular antibodies and only 27% demonstrated positive PCR results.[6,18] Fardeau et al concluded that large lesions in immunocompromised individuals were more likely to have positive results. In the series reported by Groot-Mijnes et al, results for intraocular antibody production were positive in 92% of patients, and, in contrast to viral retinitis, delayed testing by more than three weeks after onset of symptoms was more likely to lead to positive PCR results for toxoplasmosis. In our study improved sensitivity would most likely have been achieved with the addition of Goldman Witmer Coefficient antibody testing.

The PCR sensitivity for MTB in our study was low. Better PCR tests for MTB are needed. A recent proposal from Gupta suggests that nested PCR may increase the sensitivity but this is not proven in any study with significant numbers.[34]

The overall sensitivity of PCR testing in our study was 84% and the specificity was very high at 99%, which are comparable to Harper's study (sensitivity of 81%; specificity of 97%.)[33] The timing of PCR testing may have played a role in the high sensitivity identified in our study - PCR was routinely performed at presentation (when the viral

sensitivity is thought to be maximal), as opposed to being used later in the disease if there is no response to initial treatment.

There were 3 false positive results in our study. Both CMV and VZV were isolated in 2 of these cases, but clinical presentation and course suggested CMV infection only. Both CMV and TG were isolated in the third case, CMV was considered falsely positive as the clinical presentation and course suggested TG infection only. These false positives may have been due to previous resolved infection with a small number of 'old' viruses still being present in the eye, or it may be 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. The false negative rate in our study was relatively low. This is in part attributable to the fast transport time to the on site laboratory, and may also be due to the high number of immunocompromised patients in our study.

The positive predictive value, defined as the likelihood of having disease related to the tested infectious agent given positive PCR results, was very high at 97%. The negative predictive value (NPV), defined as the likelihood of not having the specified disease given negative PCR results, was also very high at 95%. This is a high negative predictive value compared to Harper who found their NPV to be 68%.[33] The negative predictive value is a function of both the sensitivity of the test and the prevalence of the disease being tested for. Since PCR testing is particularly sensitive and almost 60% of the study population had infective uveitis, the high negative predictive value is to be

expected. This was of particular value in the 51 cases (32%) with uncertain diagnosis, where infectious uveitis could be excluded with confidence following a negative result.

In our study vitreous samples were more likely to provide a positive diagnosis (P = 0.027). There may however be selection bias and as a result it is difficult to draw any definite conclusion from this finding. Harper's study showed better sensitivity for aqueous compared to vitreous samples but their findings were not statistically significant.[33] No randomized trials exist at present to prove which is better.

No HSV was detected in any of our patients. Laboratory error was thought to be unlikely as external investigators confirmed the validity of the local laboratory HSV PCR detection method. Also, the local laboratory cerebrospinal fluid HSV PCR detection rate in patients with suspected HSV encephalitis is similar to published studies from around the world. This points to the possibility of a different epidemiology of necrotizing herpetic retinitis in our local population.

A large proportion of eyes presented with very poor vision. Although there was no improvement in the mean visual acuities of affected eyes, we believe that by instituting the appropriate treatment we may have prevented more eyes from going blind.

There are a number of inherent limitations to our study. It was retrospective and criteria for performing PCR were not specified – although all patients with presumed infectious uveitis would have had PCR testing undertaken. Some patients had short follow up and

there was a relatively high drop out rate to follow-up. Also, this was not a population based study but a referral centre study resulting in possible selection bias.

In summary, the prevalence of the commonest causes of infectious posterior uveitis based on PCR studies in a population with a high prevalence of HIV was CMV in 47%, VZV in 11% and OT in 10%. Tuberculosis was rare and HSV was not identified. On the basis of these findings, in the absence of the availability of PCR testing, the treatment of infectious posterior uveitis with intra-vitreal ganciclovir and systemic acyclovir, would be appropriate in 58% of cases. PCR testing changed the diagnosis in a quarter of cases, and confirmed the presence of infective uveitis in another third of cases. Since the outcome of incorrectly treated infective uveitis can result in irreversible blindness, PCR analysis of ocular fluids is recommended early in the disease process even in resource poor settings.

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391 References

392 1. London NJ, Rathinam SR, Cunningham ET Jr. The epidemiology of uveitis in
393 developing countries. Int Ophthalmol Clin. 2010;50:1-17.
394 2. McCannel, CA, Holland GN, Helm CJ, et al. Causes of uveitis in the general practice
395 of ophthalmology. Am. J. Ophthalmol. 1996;121:35–46.
396 3. Westeneng AC, Rothova A, de Boer JH, de Groot-Mijnes JD. Infectious uveitis in
397 immunocompromised patients and the diagnostic value of polymerase chain reaction
398 and Goldmann-Witmer coefficient in aqueous analysis. Am J Ophthalmol 2007;144:781-
399 5.
400 4. Shafik SM, Foster CS. Definition, classification, etiology, and epidemiology. In: Foster
401 C, Vitale AT, editors. Diagnosis and treatment of uveitis. Philadelphia, Pennsylvania:
402 W.B. Saunders Company, 2002:17-26.
403 5. Gagliuso DJ, Teich SA, Friedman AH, Orellana J. Ocular toxoplasmosis in AIDS
404 patients. Trans Am Ophthalmol Soc 1990;88:63-88.
405 6. de Boer JH, Verhagen C, Bruinenberg M, et al. Serologic and polymerase chain
406 reaction analysis of intraocular fluids in the diagnosis of infectious uveitis. Am J
407 Ophthalmol. 1996;121:650-8.
408 7. Fox GM, Crouse CA, Chuang EL, et al. Detection of Herpesvirus DNA in Vitreous and
409 Aqueous Specimens by the Polymerase Chain Reaction. Arch Ophthalmol.
410 1991;109:266-71.

411 8. Pepose JS, Hilborne LH, Cancilla PA, Foos RY. Concurrent herpes and
412 cytomegalovirus retinitis and encephalitis in the acquired im- mune deficiency syndrome
413 (AIDS). Ophthalmology. 1984;91:1669-1677.
414 9. Tran THC, Rozenberg F, Cassoux N, et al. Polymerase chain reaction analysis of
415 aqueous humour samples in necrotising retinitis. Br J Ophthalmol 2003;87:79–83.
416 10. Matos K, Muccioli C, Belfort R Jr, Rizzo LV. Correlation between clinical
417 diagnosis and PCR analysis of serum, aqueous, and vitreous samples in patients with
418 inflammatory eye disease. Arq Bras Oftalmol 2007;70:109-14.
419 11. McCann JD, Margolis TP, Wong MG, et al. A sensitive and specific polymerase
420 chain reaction-based assay for the diagnosis of cytomegalovirus retinitis. Am J
421 Ophthalmol. 1995;120:219-26.
422 12. Ganatra JB, Chandler D, Santos C, et al. Viral causes of acute retinal necrosis
423 syndrome. Am J Ophthalmol 2000;129:166–72.
424 13. Abe T, Tsuchida K, Tamai M. A comparative study of the polymerase chain
425 reaction and local antibody production in acute retinal necrosis syndrome and
426 cytomegalovirus retinitis. Graefes Arch Clin Exp Ophthalmol 1996;234:419–24.
427 14. Abe T, Sato M, Tamai M. Correlation of varicella-zoster virus copies and final
428 visual acuities of acute retinal necrosis syndrome. Graefes Arch Clin Exp Ophthalmol
429 1998;236:747–52.
430 15. Van Gelder R. Polymerase Chain Reaction diagnostics for posterior segment
431 disease. Retina 2003;23:445-52

16. Short GA, Margolis TP, Kuppermann BD, et al. A polymerase chain reaction–based assay for the diagnosis of varicella-zoster virus retinitis in patients with AIDS. Am J Ophthalmol 1997;123:157–64.

17. Sugita S, Shimizu N, Watanabe K, 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 2008;92:928–32.

18. De Groot-Mijnes JD, Rothova A, Van Loon AM, et al. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. Am J Ophthalmol 2006;141:313-8.

19. Rothova A, de Boer JH, Ten Dam-van Loon NH, et al. Usefulness of aqueous humor analysis for the diagnosis of posterior uveitis. Ophthalmology 2008;115:306-11.

20. Fardeau C, Romand S, Rao NA, et al. Diagnosis of toxoplasmic retinochoroiditis with atypical clinical features. Am J Ophthalmol 2002;134:196-203.

21. Fekkar A, Bodaghi B, Touafek F, et al. Comparison of Immunoblotting, Calculation of the Goldmann-Witmer Coefficient, and Real-Time PCR Using Aqueous Humor Samples for Diagnosis of Ocular Toxoplasmosis. J Clin Microbiol. 2008;46:1965

22. Montoya JG, Parmley S, Liesenfeld O, et al. Use of the polymerase chain reaction for diagnosis of ocular toxoplasmosis. Ophthalmology. 1999;106:1554-63.

23. Labalette P, Delhaes L, Margaron F, et al. Ocular Toxoplasmosis After the Fifth Decade. Am J Ophthalmol. 2002;133:506-15.

24. Errera M, Goldschmidt P, Batellier L, et al. Real-time polymerase chain reaction and intraocular antibody production for the diagnosis of viral versus toxoplasmic infectious posterior uveitis. Graefes Arch Clin Exp Ophthalmol 2011;249:1837–46.

455 25. Sugita S, Ogawa M, Inoue S, et al. Diagnosis of ocular toxoplasmosis by two
456 polymerase chain reaction (PCR) examinations: qualitative multiplex and quantitative
457 real-time. Jpn J Ophthalmol 2011;55:495–501
458 26. Sharma P, Bansal R, Gupta V, Gupta A. Diagnosis of tubercular uveitis by
459 quantitative polymerase chain reaction. J Ophthalmic Inflamm Infect. 2011;1:23-7.
460 27. The 2010 National antenatal sentinel HIV & Syphilis prevalence survey in South
461 Africa. http://www.doh.gov.za/docs/reports/2011/hiv_aids_survey.pdf
462 28. Lakeman FD, Whitley RJ. Diagnosis of Herpes simplex encephalitis: application
463 of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and
464 correlation with disease. J. Infectious Diseases 1995;171:857.
465 29. Gilden DH, Dueland AN, Devlin ME, et al. Varicella-Zoster virus reactivation
466 without rash. J. Infectious Diseases 1992;166:(Suppl): S30.
467 30. Ishigaki S, Takeda M, Kura T, Ban N, et al. Cytomegalovirus DNA in the sera of
468 patients with cytomegalovirus pneumonia. Br. J. Haematol. 1991;79:198.
469 31. Jones CD, Okhravi N, Adamson P, et al. Comparison of PCR Detection Methods
470 for B1, P30 and 18S rDNA Genes of T.Gondii in Aqueous Humor. Investigative
471 Ophthalmology & Visual Science 2000;41:634-44.
472 32. Takahashi T, Nakayama T, Tamura M, et al. Nested Polymerase Chain Reaction
473 for assessing the clinical course of tuberculous meningitis. Neurology 2005;64:1789-93.
474 33. Harper TW, Miller D, Schiffman JC, Davis JL. Polymerase Chain Reaction
475 Analysis of Aqueous and Vitreous Specimens in the Diagnosis of Posterior Segment
476 Infectious Uveitis. Am J Ophthalmol. 2009;147:140-147.

34. Gupta V, Gupta A, Rao NA. Intraocular tuberculosis an update. Surv Ophthalmol. 2007;52:561-87.

Tables for manuscript – TABLES 1 to 7.

TABLE 1. Patient characteristics

Mean age	34 (Range 14 - 53)
Gender	
Male	58
Female	101
Laterality	
Bilateral	67
Right eye	42
Left eye	50
HIV +ve	142
HAART treatment @ presentation	65
MTB treatment @ presentation	67

HIV = human immunodeficiency virus
HAART = highly active antiretroviral treatment

TABLE 2. Average visual acuities (VA) pre- and post treatment

	Pre treatment	Post treatment
VA ≥ 6/18	42%	33%
VA 6/24 - 4/60	16%	14%
VA ≤ 3/60	42%	53%
Eyes assessed	226	191

Thirty five eyes were lost to follow up (due to patients defaulting follow up before completion of treatment)

TABLE 3. PCR tests performed

Infectious agent	No. tests perf.	No. +ve	% +ve
CMV	154	72	47%
TG	150	12	8%
VZV	148	17	11%
HSV	148	0	0%
MTB	43	1	2%
TOTAL TESTS	643	102	16%

TABLE 4. Pre-PCR clinical diagnoses correlated with PCR positive results

Pretest diagnoses	No.	CMV +ve	VZV +ve	HSV +ve	TG +ve	MTB +ve	CMV & VZV +ve	CMV & TG +ve	PCR +ve rate No.	%
CMV	70	51	4		4		1		60	86%
NHR	14	3	2		3		2		10	71%
TG	10	1			1				2	20%
MTB	7					1			1	14%
Syphilis	5								0	0%
IRU	2								0	0%
Unsure	51	11	5		3		1	1	21	41%
TOTAL	159	66	11	0	11	1	4	1	94	59%

IRU = Immune reconstitution uveitis

TABLE 5. Final diagnoses

CMVR	74
NHR	17
OT	16
MTB	5
Syphilis	4
CMV/VZV coinfection	2
HIV associated retinopathy	2
Toxocara Canis	1
Immune reconstitution uveitis	1
Blood dyscrasia	2
Idiopathic or end stage late presentation	35

TABLE 6. Sensitivity, specificity, positive predictive value & negative predictive value for each pathogen tested and broken down into AC & vitreous samples

	Sample no.	True +ve		False +ve		True -ve		False -ve		Sens	Spec	PPV	NPV
CMV	154	69	45%	1	1%	74	48%	7	5%	91%	99%	99%	91%
AC	45	13	29%	0	0%	31	69%	1	2%	93%	100%	100%	97%
V	106	56	53%	1	1%	43	41%	6	6%	90%	98%	98%	88%
NS	3	2	67%	0	0%	1	33%	0	0%	-	-	-	-
VZV	148	15	10%	2	1%	126	85%	5	3%	75%	98%	88%	96%
AC	43	1	2%	0	0%	39	91%	3	7%	25%	100%	100%	93%
V	102	14	14%	2	2%	84	82%	2	2%	88%	98%	88%	98%
NS	3	0	0%	0	0%	3	100%	0	0%	-	-	-	-
TOXO	150	12	8%	0	0%	134	89%	4	3%	75%	100%	100%	97%
AC	45	1	2%	0	0%	43	96%	1	2%	50%	100%	100%	98%
V	102	11	11%	0	0%	88	86%	3	3%	79%	100%	100%	97%
NS	3	0	0%	0	0%	3	100%	0	0%	-	-	-	-
MTB	43	1	2%	0	0%	39	91%	3	7%	25%	100%	100%	93%
AC	20	1	5%	0	0%	16	80%	3	15%	25%	100%	100%	84%
V	22	0	0%	0	0%	22	100%	0	0%	0%	-	-	100%
NS	1	0	0%	0	0%	1	100%	0	0%	-	-	-	-
TOTAL	495	97	20%	3	1%	373	75%	19	4%	84%	99%	97%	95%

AC = Anterior chamber V = Vitreous NS = Not specified Sens = sensitivity Spec = Specificity
PPV = positive predictive value NPV = negative predictive value

1.Three patients had samples taken from an unknown site (not specified in the clinical notes)
2. Simultaneous vitreous and aqueous sampling was performed on 4 patients
3. Two patients had repeat sampling (both were vitreous sample repeats)

TABLE 7. Comparison of sensitivity, specificity, positive predictive value & negative predictive value for specimen sites

	Sample no.	True +ve		False +ve		True -ve		False -ve		Sens	Spec	PPV	NPV
AC	153	16	10%	0	0%	129	84%	8	5%	67%	100%	100%	94%
V	332	81	24%	3	1%	237	71%	11	3%	88%	99%	96%	96%
NS	10	2	20%	0	0%	8	80%	0	0%	-	-	-	-
TOTAL	495	97	20%	3	1%	373	75%	19	4%	84%	99%	97%	95%

AC = Anterior chamber V = Vitreous NS = Not specified Sens = sensitivity Spec = Specificity
PPV = positive predictive value NPV = negative predictive value

1.Three patients had samples taken from an unknown site (not specified in the clinical notes)
2. Simultaneous vitreous and aqueous sampling was performed on 4 patients
3. Two patients had repeat sampling (both were vitreous sample repeats)

OPHTHALMOLOGY

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A. INTRODUCTION – Basic Information

B. DETAILED SPECIFICS

C. DOWNLOADABLE FORMS

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CNS	central nervous system
DNA	deoxyribonucleic acid
HLA	human leukocyte antigen
IM	intramuscular(ly)
LASIK	laser in situ keratomileusis
mRNA	messenger ribonucleic acid
RNA	ribonucleic acid

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Registration of Clinical Trials, Leonard A. Levin; Justin L. Gottlieb; Roy W. Beck; Daniel M. Albert; Thomas J. Liesegang; Creig S. Hoyt; Andrew Dick; Robert Bhisitkul; Andrew P. Schachat, Arch Ophthalmo 2005;123:1263 -4

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1. Drazen JM, de Leeuw PW, Laine C, et al. Toward More Uniform Conflict Disclosures. The Updated ICMJE Conflict of Interest Reporting Form. N Engl J Med 2010;363(2):188-9.

2. DeAngelis CD, Fontanarosa PB. Resolving unreported conflicts of interest. JAMA, 2009;302(2):198-9

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Submission: The text of the editorial, a signed copyright(s) and ICMJE conflict of interest form(s) need to be submitted – you can add anything you wish the editor to know in the "enter comments" section of the submission process. Figures are generally not included or encouraged in these types of submissions. If figures are used please submit following the same criteria for manuscripts outlined above. Most likely they will be online only supplemental materials. Copyright form(s) and ICMJE conflict of interest form(s) should be uploaded with initial submission but must be uploaded no later than first revision.

Process: Editorials undergo peer review regardless of whether they are solicited or unsolicited submissions. Once received, an Editorial is assigned a number of which the author is advised. The paper will go through the usual review process, often with some specific insight or guidelines offered to reviewers by the Editor. The author is then advised of any changes which need to be made and references are checked. Upon return of the revised paper, the editor gives his approval and it goes to the publisher.

ENGLISH EDITING ASSISTANCE

Members of the (United States) Council of Biology Editors (and others) have expressed interest in helping authors of manuscripts submitted to Ophthalmology with English editing. Authors may contact these individuals or services directly by mail, phone, fax, or e-mail. All financial arrangements are strictly between the two parties. Ophthalmology neither endorses nor recommends any specific individual or service. The Journal office may return a submission and recommend professional editing prior to review. Professional editing, while often recommended by the editors or reviewers, does not ensure acceptance or publication of a manuscript.

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EVIDENCE BASED STUDIES – ADDITIONAL GUIDELINES

The journal is eager to receive evidence-based studies. These papers incorporate a systematic review of the literature and summarize clinical recommendations using the structured format outlined below. Authors interested in submitting these manuscripts are encouraged to correspond with the Editor-in-Chief in advance to be sure that the topic is of interest. The main text of these articles will conclude with summary recommendations for testing or therapy of the clinical problem discussed. Each recommendation will include author-designated and peer reviewed ratings displayed in superscripts (see definitions below) indicating the importance of recommendations to clinical outcome (A, B, C) and the overall strength of evidence of supporting literature (I, II, II I). The strength of evidence ratings will be based on author judgment as to the quality and validity of the existing fund of peer-reviewed or other published literature. Authors and co-author methodologists with special expertise in the topic may be recruited by the Journal Editor to write these summary updates.

Authors will be expected to conduct thorough literature searches (systematic reviews) of national and international peer-reviewed publications utilizing available databases and other sources as necessary. In many topic areas no recent high-quality studies may be available, in which case the discussion should emphasize to clinicians what studies are needed and the inadequacy of the evidence that justifies current management.

Completed articles will be reviewed using the usual Journal peer-review process, including author-assigned ratings for the importance of clinical recommendations and the strength of supporting evidence. Publication may be scheduled, after revisions as indicated through peer-review, and articles will be placed in regular forthcoming issues at the discretion of the Editor-in-Chief.

Definitions of Superscript Ratings:

Superscript ratings for clinical recommendations:

"A" indicates that the recommendation is considered very important or crucial to a good clinical outcome

"B" that the recommendation is considered moderately important to clinical outcome

"C" that the recommendation may be relevant but cannot be definitely related to clinical outcome.

Superscript ratings for peer reviewed or other cited evidence:
"I" indicates strong evidence in support of the statement. In general, the study or studies cited used designs which allowed the issue to be addressed, were performed in the population of interest, were executed in a manner to produce reliable and accurate data, and were analyzed using appropriate statistical methods. The study or studies produced either statistically significant differences between control and experimental groups or showed no statistically significant differences, despite a design, which had high statistical power to detect differences and/or narrow confidence limits on the parameters of interest.

Strong evidence includes well-done randomized controlled clinical trials designed to address the issue in question, especially regarding the efficacy of treatment or the superiority of one treatment over another. Well-done meta-analyses (retrospective reviews of previously published randomized controlled trials) may also constitute level "I" supporting evidence.

"II" indicates there is substantial evidence in support of the statement but the evidence lacks some qualities, thereby preventing its justifying the statement without qualification. Deficiencies might include unavailability of well-done randomized trials, or studies lacking other elements of high-quality evidence such as adequate control groups, sufficiently long follow up, good compliance with therapy, or acceptable loss to follow up.

Nonrandomized comparative trials involving sufficient subjects to demonstrate statistically significant differences between study and control groups might provide strong evidence for the efficacy of a therapy. Noncomparative case series or case reports might be justifiably included as strong evidence for linking complications or adverse events to a specific therapy without stating the probability of their occurrence.

Observational studies, including control groups such as Cohort studies and Case-control studies, might provide strong evidence for or against therapy in terms of longitudinal data about disease natural history, outcome of therapy, adverse events, or specific anatomical or functional outcomes. Well-done cross-sectional studies might provide strong evidence for the importance of the clinical problem. Well-done systematic literature reviews or meta-analyses might also provide moderately strong evidence for or against a test or therapy.

Even an otherwise well-done randomized controlled trial dealing with the issue of interest might have been performed using too select a population and may not be clearly applicable to a broader population of interest, or it might have produced only marginally statistically significant differences between control and experimental groups. A large consecutive case series might also fit in to this category if it compares outcome only to a historical control group from the same clinical setting.

"III" indicates a weak body of evidence insufficient to provide support for or against the efficacy of a test or therapy and would generally apply to panel consensus or individual opinions, small noncomparative case series, and individual case reports. Non-comparative studies (without controls), cohort studies with variable follow up across the patient population studied, retrospective chart reviews with missing data, or even randomized controlled trials evaluating highly subjective outcome data would be examples of weak forms of evidence.

Authors of evidence-based manuscripts should follow the guidelines outlined in the Instructions for authors unless specifically stated below:

Title Page - The title should clearly describe the main topic and indicate the manuscript is an evidence-based summary. (Example: Management of nonsymptomatic retinal tears and lattice degeneration: an evidence-based summary.) The title should include the phrase: evidence-based review or evidence-based update.

Précis - The précis should indicate what new insight the article offers or what principal controversy persists.

Structured Abstract Abstracts for evidence-based manuscripts must be limited to 250 words and include the following five sections:
1. Topic: identify the specific clinical problem and therapy to be evaluated.
2. Clinical relevance: characterize the magnitude/importance of the problem/disorder and define the current standard of care.
3. Methods/literature reviewed: describe the sources of peer-reviewed materials utilized and dates of publication.
4. Results: summarize the materials identified and obvious contrasts with prior and current standards of care.
5. Conclusion: summarize the strength of evidence for the recommended therapy or test.

Text - The text should utilize standard Journal formatting as described in *Ophthalmology's* Instructions for Authors and be divided into five distinct sections:

1. The introduction/background (unlabeled) should clarify the magnitude of the clinical problem, (prevalence or incidence) and provide perspectives on the importance of its management to patient well-being and quality of life.

2. The Sources and Methods of Literature Search (titled) should identify the databases and/or specific journals searched and the dates of publication. The methodology of the literature search, including criteria utilized for selection and inclusion, should be listed insufficient detail to permit duplication of the effort. If only poor quality supporting evidence exists, author comments should emphasize this in the discussion, in addition to assigning appropriate overall ratings for the strength of supporting literature.

> Suggested sources for literature searches include, for example, PubMed (http://www.pubmed.com) and Medical Matrix (http://www. medmatrix.org).
>
> The Cochrane Library is an additional excellent source of high quality reviews of general medical information, systematic reviews, and meta-analyses, including some eye topics (http://www.cochranelibrary.com).

3. Summary of Evidence (titled) should summarize the findings in text or tables.

4. The Clinical Recommendation(s) (titled) should be listed in order of importance, and each separate recommendation accompanied by bracketed superscripts "A,""B," or "C," indicating the author's impression as to its importance to clinical outcome. Superscripts "I,""II," or "III" will also be used to indicate the author's judgment about the overall (average) veracity of supporting literature. When appropriate, recommendations should include typical clinical scenarios. (Example of clinical recommendation and author-designated superscripts: A symptomatic superior horseshoe retinal tear with a cuff of surrounding subretinal fluid should be promptly encircled by several rows of laser burns. [A, I]). Please indicate appropriate

crosschecking with AAO products (PPPs, Pro-Vision Series, Focal Points, Basic and Clinical Science Course Books) to avoid or acknowledge inconsistencies in clinical recommendations.

5. References should be limited to the highest quality studies available, regardless of the study type. One set of complete copies of all cited references should be included. Duplicates will be sent to peer reviewers upon request. For reference formatting examples, please go to References and Reference Style Guide

FIGURES (illustrations, graphs, photos for all submission item types)
Whether submitting individual images or a composite, please note the artwork guidelines that follow. Figures will be included in the final PDF but the figure file names will not be visible to reviewers. Figures, that are not a composite, should be loaded to individual files and clearly identified. For all figures the figure number must be entered in the file description field before the figure is uploaded. This can be done on the "attach files page" by choosing "figure" in the pull down menu. Below it there is the “Description” box; enter the figure number to the right of the word "Figure" before opening and attaching each figure file. Do not enter legends here, just the figure number. For linear art created by MSOffice or similar type software, the figure number should also be typed on the figure page.

The Journal may provide one page of color illustrations per calendar year for each first author without charge, at the discretion of the Editor-in-Chief. The criterion generally used is whether the color illustration best conveys the information being illustrated. Additional color pages may be published at the author’s expense. Formatting requirements may lead to illustration placement on more than one page, although we try to avoid this as much as possible. The cost varies from $650 to $1200 per additional page and you will be advised of the cost when you receive your proofs.

If a manuscript has been reviewed and accepted with color photos, it must be published with color photos. The author is responsible for page charges for color photos that occupy more than one page, and cannot opt to have them printed in black and white without the permission of the journal office. Please check with the Journal office or the publisher for information.

Clinical photographs (including those generated electronically from machines such as MRIs, fluorescein angiography, visual fields, etc.) must be masked to prevent identification of the patient. Clinical photographs that permit identification of an individual (those exposing anything more than just the eyes) must be accompanied by a signed statement by the patient or guardian granting permission for publication of the pictures for educational purposes. All graphics, including composites (such as clinical photographs, fluorescein angiography, CT, MRI, x-ray, photomicrographs, etc.) should be submitted at the actual size that they would be presented in the journal, 100 % of their print dimensions so that no scaling is necessary, but remember that very few pictures are full page pictures. The width should be no more than 7 inches.

The publisher will not re-draw or rework your photographs or illustrations. Submit all figures in the order they appear in the legends. If there are six or more color pictures, a composite maybe preferred so they fit on a standard journal page and potentially decrease your color figure costs. However, be sure to do this only if the quality of what you are attempting to portray with the figures is not compromised. The completed composite must meet the guidelines for artwork submission. Composites must also be labeled using typed text in a corner of the each image. Composite are encouraged for multipanel figures (e.g., Fig 1A, 1B, 1C, 1D, 1E).

	BLACK & WHITE LINE ART*	COLOR LINE ART*	LINE ART/PHOTO COMBINATION	BLACK & WHITE PHOTO	COLOR PHOTO
TIFF	YES YES		YES	YES	YES
WORD FILE	YES YES		NO	NO	NO
PDF FILE	YES YES		NO	NO	NO
COL OR MODE IN PHOTOSHOP	BITMAP RGB			GRAYSCALE	RGB
RESOLUTION (PIXELS/INCH)	150 300**		600 (will be large file size)	300 300	
TYPICAL FILE SIZE	Less than 2MB	No larger than 10 MB	Can be as large as 60 MB	More than 10 MB	5 to 15 MB

***** Line art can be submitted in the original file format that it was created (e.g., Word, Excel, PowerPoint, etc.)
****** If very little or no text – otherwise, print to a PDF

General

- The physical dimensions of any artwork must fit within the dimensions of the pages within the Journal. (i.e., width no more than 7 inches)
- Be consistent in the font type and size used in the artwork.
- Artwork must use recomm ended nam ing conventions. Som e exa mples include fig1.tif (figure 1 in TIFF format). Always ensure that the file extension is present to ensure quick and easy format identification.

We have upgraded our electronic submission system. You may now choose to load each figure file individually or to take all the individual figures files and zip them into a single zip file, which will reduce the size of your upload (and hence the time) it takes to upload your files and complete your submission. This does not mean you can load everything in one file – each piece needs to be in a separate file and those individual files can then be zipped and uploaded. The system will unzip them for you.

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FINANCIAL SUPPORT

Identify all funding sources, public and private. On the title page please state "Financial Support: None" or provide the agency name and city, company name and city, fellowship name, and grant number. If there is financial support, please provide also one of the two following statements: "The sponsor or funding organization had no role in the design or conduct of this research."
OR "The sponsor or funding organization participated in (list those that are appropriate: the design of the study, conducting the study, data collection, data management, data analysis, interpretation of the data, preparation, review or approval) of the manuscript."

IN PRESS/ONLINE RELEASE

As of September 1, 2007, manuscripts are automatically available on line as "in press" articles after completing the proofing process. This early online release is not a draft version since it is

produced after all editorial and author corrections are made; however there is a disclaimer in case a critical error is found. No routine editing will occur once this is online. The “in press" version is not meant to be a last editing opportunity for authors, however if a major, critical error is found we may be able to make corrections prior to publication or an erratum will be published in a future issue. This "in press" version is removed as soon as the monthly issue is available online.

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INSTITUTIONAL REVIEW BOARD/ETHICS COMMITTEE APPROVAL (IRB)

If the study being reported involved human subjects, human derived materials, or human medical records, please include one of the two following statements in the Materials/ Patients and Methods section: Institutional Review Board (IRB)/Ethics Committee approval was obtained OR IRB/Ethics Committee ruled that approval was not required for this study.

LEGENDS

Figure legends (photos, drawings, graphs) should follow figures. Figures must be numbered consecutively as they appear in text. Histological figures, stains and magnifications should be noted in the legends. Any figure that has been published elsewhere should have an acknowledgment to the original source; a copy of the release to publish the figure, signed by the copyright holder, must also be submitted. Legends must identify all symbols, abbreviations, acronyms or letters that appear on the prints. Table legends should be within the table. All abbreviations in each table must be defined even when repetitive to each other.

LETTERS TO THE EDITOR AND ASSOCIATED REPLIES

General: Letters to the Editor should be concise comments focusing on an article published in the Journal within the last six months. The letter should offer alternative perspective, elucidate a flaw in methodology or a perceived misinterpretation of data, addressing no more than two major points. The letters should start with “Dear Editor” and the article being commented on should be referenced in the first paragraph of the letter. Gratuitous comments such as “… I commend the author for their fine study” or overly critical remarks are not necessary or appropriate. Letters should end with the name, degree and location(city, state or city, country) for each author. For example Andrew P. Schachat, MD, Cleveland, Ohio.

Format: Letters should be limited to 700 words, double-spaced and no more than five references. Please note that letters do not have tables or figures published but they are put up as online only supplemental material. The figures or tables will not appear in the printed version but will be archived with the online version on the publisher's website www.ophsource.com/periodicals/ophtha and accessible through Medline and other online databases. Therefore, in the appropriate location where you mention your table, graph, figure or chart please insert "(available at http://aaojournal.org).” Although figures (photos, charts, graphs, tables) are not included in publication, the online version needs to conform to the same requirements regarding legends and identifying all abbreviations in each figure.

Submission: The text of the letter, a signed copyright(s) and ICMJE conflict of interest form(s) need to be submitted. These should be uploaded into the system with your initial submission. You can add information you wish the editor to know in the “enter comments”

section of the submission process. The title should be limited to 40 characters.

Process: Upon receipt, a letter to the editor is reviewed by the Editor in Chief, and, in some instances, by outside reviewers. If the letter is to be accepted for publication, it is forwarded to the corresponding author of the article which it addresses for the opportunity to respond. If the invitation is accepted, both letter and reply are edited and reference checked and published together. If the invitation to reply is declined the original letter will be processed and published by itself. The titles of all letters are limited to 40 characters. If needed, the Editor will create titles to fit this limit.

When the journal office receives a Letter to the Editor addressing an article, the corresponding author of the article being discussed usually will receive an email entitled "Invitation to Reply to a Letter to Editor". It is imperative that you log onto the system as an author and accept this invite immediately and then upload and submit your reply letter within 21 days to the Editorial Office.

Occasionally, you may be told by the Editorial Office that a manuscript is rejected but the option to reformat and resubmit it as a letter is suggested. This can only be done at the Editor's discretion. If you decide to reformat your paper as a letter, you should send it as a new, separate submission. In these scenarios only, WHEN UPLOADING, USE THE "MS TO LTR" SELECTION AS THE TYPE OF SUBMISSION. Also be sure in the "Additional Comments" section to advise us of the manuscript number of this original paper you are reformatting so we can make reference to it if necessary. All other Letter to the Editor guidelines (700 words, double-spaced, etc) apply including the need for a copyright and ICMJE conflict of interest forms to be uploaded.

LITERATURE REVIEWS

Literature reviews have great teaching value, but the focus of Ophthalmology is on "new" material. Reviewing the past literature tends not to add "new" information to the current literature. But, if you incorporate new knowledge into the review by aggregating past information to create new knowledge, such reviews are considered. For example, a metanalysis combines old data in a way that teaches new knowledge. Better literature reviews tend to be highly structured with inclusion and exclusion criteria for which papers will be included and they involve more than, e.g., "we searched PubMed on 'cataract'." There is excellent information on metanalyses and structured and methodical literature reviews available at the Cochrane Collaboration website (cochrane.org). In addition, the Journal will consider and may accept so called "evidence-based" reviews. There are detailed instructions in this Guide for "evidence based studies – additional guidelines."

MANUSCRIPT TEXT FORMAT

Double space the entire manuscript after the title page. Line numbering will be automatically inserted into your manuscript text file by the system when it builds the PDF. The average published manuscript in Ophthalmology, including references, is up to printed 6 pages in length. This corresponds, depending on font size and printing, to between 16-20 pages of double-spaced draft.

1. Title Page

The title page should include the following information.

a) Title: The title should be meaningful and as brief as possible. No longer than 135 characters, including spaces. Declarative titles should not be used. Do not use abbreviation in titles other than

those approved in Abbreviations. Please do not include any lecture titles or award titles in the manuscript title. Recognition of such can be made with an asterisk at the end of the title and the award/lecture noted in the footnotes.

b) Authors: Provide first name, middle initial, last name and no more than two advanced degrees or professional certifications. The Journal does not print society affiliations. Also indicate each author's affiliation during the course of the study in footnotes on the title page using superscript numbers, not symbols (e.g., Ronald Smith[1]). Specifically identify the corresponding author.

Please carefully review the very extensive "Authorship" section of this guide. It carefully addresses authorship criteria, group/writing committee authorship, guest authors, ghost authors, corresponding authors and related responsibilities, numbers of authors, and entering authors into the system.

c) Meeting Presentation: If the material is under consideration for presentation or has been previously presented, supply the name, place, and date of the meeting. (e.g., the American Academy of Ophthalmology Annual Meeting, November, 2003). This is especially important for AAO Meeting papers as we have first right of refusal on these papers.

d) Financial Support: - Identify all sources, public and private. On the title page please state "Financial Support: None" or Provide the agency name and city, company name and city, fellowship name, and grant number. If there is financial support, please provide also one of the two following statements: "The sponsor or funding organization had no role in the design or conduct of this research."

OR

"The sponsor or funding organization participated in (list those that are appropriate: the design of the study, conducting the study, data collection, data management, data analysis, interpretation of the data, preparation, review or approval of) the manuscript."

e) Conflict of Interest: - A blanket statement that "no conflicting relationship exists for any author" is requested on the title page, if appropriate. Otherwise, the corresponding author should summarize the disclosures sent to him by each author and upload the ICMJE form of each author as well. (See detailed conflict of interest section above.) Either way ICMJE conflict of interest forms must be uploaded from every author.

f) Running head: The running head, also known as the short title, which appears on the top of each right hand published page of your manuscript, should be no longer than 60 characters.

g) Address for reprints

2. <u>Abstract</u> – see separate "Abstract" section

3. <u>Text</u>

a. Introduction: Without a heading, the introduction should refer only to the most pertinent past publications and should not be an extensive review of the literature.

b. Intervention or Methods or Testing: This section should be written with sufficient detail to permit others to duplicate the work. Also required are the following, as appropriate within the methods section:

FOR HUMAN SUBJECTS:

- Informed Consent - Manuscripts reporting the results of experimental investigation on human subjects must include a statement to the effect that informed consent was obtained.
- HIPAA - For studies conducted in the United States a statement that the work is HIPAA-compliant is required (See Ophthalmology 2003; 110:1074-5.)
- IRB/Ethics Committee - Human subjects/materials/medical records - If the study being reported involved human subjects, human derived materials, or human medical records, please include one of the two following statements in the Materials/ Patients and Methods section:

Institutional Review Board (IRB)/Ethics Committee approval was obtained
OR
IRB/Ethics Committee ruled that approval was not required for this study.

- Declaration of Helsinki - A statement is required that described research adhered to the tenets of the Declaration of Helsinki
- Clinical Trial Registration – A statement should be provided in the methods section of the manuscript that this was done and where the registration information is publicly available. (see Clinical Trial Registration for more detailed information)
- We encourage authors to use the American Joint Commission on Cancer TNM Classification scheme when describing patients with ophthalmic malignancies (American Joint commission on Cancer. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer; 2009.) This classification scheme can also be found at http://www.cancerstaging.org/mission/whatis.html

FOR ANIMAL SUBJECTS:

If animals were used in a study, the notice of approval by the appropriate Institutional Animal Care and Use Committee should be included in the methods section of the manuscript.

c. Results: Results must be concise.

d. Discussion: The discussion should be restricted to the significant findings presented. Digressions and theorizing are not appropriate. NOTE: Discussion is the final section of a manuscript. Please do not insert a conclusion section; only the abstract has a conclusion section.

ONLINE ONLY PUBLICATIONS

Some manuscripts are not accepted due to lack of space rather than lack of science and in some cases an author may be given an option of having their entire manuscript printed "online only." If the Editor deems it appropriate, you will be given the option of having your manuscript published online only. There will be no printed version of this manuscript BUT it will appear in the table of contents under a new section called "Online Only Publications"[1] and it shall be citable just like any other online resource. We are told that Pub Med and other similar databases will pick it up as an online citation. Submission guidelines are the same as they would be for acceptance in the print edition. Color figures in an online only publication will be at no cost to the author.

ONLINE SUPPLEMENTAL MATERIALS

Space in Ophthalmology is highly competitive and sometimes good manuscripts or data cannot be published due to space limitations. For articles that ARE ACCEPTED for publication in the journal but whose authors have agreed to cut back on the amount of material provided due to space considerations, we now offer online only supplements to printed articles. Such supplements will generally include tables, charts, figures, etc. that would further enhance a published article but for which there is insufficient room in a given issue to print it. The availability of this additional information will be noted in the Table of Contents by an icon. The information will not appear in the printed version but will be archived with the online version on the publisher's website http://www.ophsource.com/periodicals/ophtha and accessible through Medline and other online databases. In the printed manuscript, on the cover page and in the appropriate, corresponding section of your text, there will be a notation that "Supplemental materials are provided at the end of the online version of this manuscript".

If you opt for an online supplement, add a reference to it in parenthesis after the mention of the information to appear online: For example, "…as shown in Table N (available at http://aaojournal.org)." Online tables or figures should be numbered consecutively as they appear in the text, in the same sequence as printed figures or tables. Also, add a statement to the title page that should read similar to: "This article contains additional online-only material. The following should appear online-only: Figures X, Y, Z and Table N." The materials will not appear in the printed version but will be archived with the online version on the publisher's website http://www.ophsource.com/periodicals/ophtha and accessible through Medline and other online databases.
In some cases, when the Editor decides there are too many figures, tables or other supplemental information (e.g. study group listings) to publish in print, an author may be given the option of providing a PDF of the item(s) for online only release versus removing them completely from the submission.. These are not proofed or edited in any way by the publisher thus eliminating cost and not counting to a limited budget of online only supplemental pages. These figures need to have their legends included in the figure file along with the figure number.

All supplemental materials must follow the same rules and regulations as if they were to appear in print. For example, tables must be able to stand alone with all abbreviations, references, etc. identified. Table legends would include definitions for the abbreviations, if any. Color figures that might appear online only are at no cost to the author.

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PRÉCIS

All manuscripts must include a précis of 35 words or less summarizing the main finding/outcome of the study. The précis should not duplicate the abstract conclusion. Please respect the 35-word limit as formatting requirements lead to strict application of the word limit. If the paper is published, the précis will appear under the title in the Table of Contents. The précis is submitted as a separate file and should not be included the manuscript file. Try not to use abbreviations/acronyms in the précis so that the words are not used up in defining them; remember the précis has a 35 word limit.

PRIOR AND REPETITIVE PUBLICATION:

The Journal will not consider manuscripts that have appeared, in part or in total, in other publications, except in special circumstances approved by the Editor-in-Chief. Likewise, updates of previously published studies that add little data to an existing publication will not be considered. Overlap between patient groups described in serial manuscripts must be acknowledged, and references to previous publications that include the same patients must be provided. Authors uncertain as to whether or not specific data represent prior or repetitive publication should alert the Editor-in-Chief in the author/additional comments section of the submission process and reference copies of the publications in question.

PRECEDENCE

Authors who claim precedence for an idea, observation, or therapy are well advised to review existing literature thoroughly and also to include an appropriate disclaimer on the cover page of the manuscript --"We were unable to find any previous report of this in the literature" or "As far as we are aware, this has not been previously reported." Some widely read journals might not currently be included in databases, such as that of the National Library of Medicine.

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With no volume #:
Taulbee P. Maryland Quality Project puts new focus on processes of care. Rep Med Guideline Outcomes Res. June 1994;10-1.

Supplements:
Davis JT, Allen HD, Powers JD, et al. Population requirements for capitation planning in pediatric cardiac surgery. Arch Pediatr Adolesc Med 1996;150(suppl):257-9.

In Press (accepted by a journal):
Davis JT, Allen HD, Powers JD, et al. Population requirements for capitation planning in pediatric cardiac surgery. Arch Pediatr Adolesc Med. In press.

A discussion:
Allo MD. In discussion of: McKindley DS, Antibiotic pharmacokinectics following fluid resuscitation from traumatic shock. Arch Surg 1994;272:1825-31.

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Journal available only online:
Hussain N, Clive J, Bhandari V. Current incidence of retinopathy of prematurity, 1989-1997. Pediatrics [serial online] 1999;104:e26. Available at http://www.pediatrics.org/cgi/content/full/104/3/e26. Accessed July 12, 2002.

Letter:
Davis JT, Allen HD, Powers JD, et al. Population requirements for capitation planning in pediatric cardiac surgery [letter]. Arch Pediatr Adolesc Med 1996;150:257-9.

Study Groups:
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Crist WM, Garnsey L, Beltangady MS, the Intergroup Rhabdomyosarcoma Committee. Prognosis in children with rhabdomyosarcoma: a report of the intergroup rhabdomyosarcoma studies I and II. J Clin Oncol 1990;8:443-52.

No authors listed other than the study group:
Fluorouracil Filtering Surgery Study Group. Fluorouracil filtering surgery study: one-year follow-up. Am J Ophthalmol 1990;109:613-6.

BOOKS

Book:
Miller NR. Walsh and Hoyts Clinical Neuro-Ophthalmology. Baltimore, MD: Williams & Wilkins; 1991:xx-xx. (include specific inclusive pagination for material being referenced)

Article or chapter in book:
Hollis S, Rozakis GW. Complications, special cases and management. In: Rozakis GW, ed. Refractive Lamellar Keratoplasty. Thorofare, NJ: SLACK Inc.; 1994:111-22.

Edited book:
Letheridge S, Cannon CR, eds. Bilingual Education: Teaching English as a Second Language. Vol. 1. 3rd ed. New York: Praeger; 1980:xx-xx.

Article in edited book, reprint from another source:
Sluzki CE, Beavin J. Symmetry and complementarity. In: Watzlawick P, Weakland JH, eds. The Interactional View. New York: Norton; 1977:711-30. Reprint from: Acta Psiquiatr Psicol Am Lat 1965;11:321-30.

Proceedings published as a book:
Chaddock TE. Gastric emptying of a nutritionally balanced liquid diet. In: Daniel EE, ed. Proceedings of the Fourth International Symposium on Gastrointestinal Motility. Ames, IA: Mitchell Press; 1974:83-92.

Book without authors or editors:

College Bound Seniors. Princeton, NJ: College Board Publications; 1979:xx-xx.

Several volumes in a multi-volume edited work:
Wilson JG, Fraser FC, eds. Handbook of Teratology. Vol. 1-4. New York: Plenum Press; 1977-88.

English translation of a book:
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Health Care Financing Administration. 1996 statistics at a glance. Available at: http://www.hcfa.gov/stats/stathili.htm. Accessed December 2, 1996.

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Software Manual cite in references: Dean AG, Jean JA. Epi Info, Version 6: A Word-processing Database. Atlanta, GA: Centers for Disease Control and Prevention; 1994:xx-xx.

GOVERNMENT DOCUMENTS

Klein R, Klein BE. Beaver Dam Eye Study. Manual of Operations (Revised). Report for 16 Jun 87 - 31 May 92. Springfield, VA: US Dept of Commerce; 1991:xx-xx. NTIS Publication PB91-149823.

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Comparing previous exposure(s) between a group of patients with a given disease or outcome and a group without the given disease or outcome?	*CASE-CONTROL STUDY	
Determining the prevalence of a symptom, sign, or disease in a group of individuals or examining associations between factors at one point in time?	CROSS-SECTIONAL STUDY	Clinic-based, hospital-based, community-based, population-based
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We do not have video editing software, but a website with useful tips on reducing file size can be found at http://www.deskshare.com/Resources/articles/dmc_ReduceFileSize.aspx

1. Maximum: 8 minutes total. We recommend several smaller clips that total no more than 8 minutes.
2. Size: no larger than 10 MB for each file
3. File extension types: .MPG (MPEG-1 or 2), .AVI, .MOV
4. Audio commentary, describing what is being shown is highly recommended. Do not use copyrighted music.
5. Within the submission, there must be a brief legend describing the contents of the video and the indicating the viewing order.
6. Video files should be loaded with your submission into the Electronic Submission System. File names should correspond to video legends.
7. On the title page add: "This manuscript contains (number) video clips.
8. Load them into your submission using the "multimedia" file type

C. DOWNLOADABLE FORMS

All forms, except for the Study Design Worksheet, allow you to type in the required information and save as files to your desktop. Copyrights can be filled out online but will need to be printed out
for original signatures. Signatures must be original, electronic signatures are not acceptable. ICMJE and copyrights should be uploaded at the time of your submission.

The copyright and conflict of interest disclosure forms WILL NOT appear as full text but rather only as a link in the PDF that you approve after you've uploaded them. This is so the transmitted file will be as small as possible for transmittal to reviewers and editors.

AUTHORS
Authorship Criteria Statement
Copyright Assignment Form
ICMJE Conflict of Interest Form (COI form)

REVIEWERS
CME Credit Request for Manuscript Review

OTHER
Consort Agreement is mandatory for a Randomized Controlled Trial
Cover Art Copyright Form

D. MISCELLANEOUS INFORMATION

1. Developing a Manuscript

Authors are well advised to plan for eventual publication early in the conduct of their research, including the choice of journal and the order of authorship. The most current Guide/Instructions for Authors for the intended journal should be obtained and read carefully in preparation for eventual manuscript submission. The order of authorship, assuming more than one individual is involved, should be established by mutual consent early in the manuscript preparation process to avoid subsequent conflicts. In rare instances, authors ask for changes in authorship after submission and do not agree themselves what they want. In such cases, the Editor will withdraw the manuscript from consideration and allow the authors to resubmit once they agree, with new and correct copyright transfer forms. For *Ophthalmology*, a listing as an author implies a substantial intellectual contribution to the conduct of research and preparation of the manuscript (see Guide for Authors regarding authorship, group authorship, and acknowledgments).

Clinical or basic science investigations must be designed (planned) properly and executed rigorously to permit meaningful analysis of resulting data. Appropriate study design experts, biostatisticians, or other advisors as indicated should be incorporated in both the initial planning and/or the authorship for all research publications.

It is strongly recommended that you plan the research, obtain appropriate IRB and or regulatory approval, do the research and then write the manuscript. In other words, prospective research is favored.

A. *Ophthalmology's* Study Design Scheme

As part of the Structured Abstract, authors are required to describe the design of their study. The specific designation of a "study design" serves several purposes. It forces authors to give careful thought to what they have actually done, it provides an important shortcut for editors and reviewers to use in categorizing the submission, and it provides the busy reader with a useful capsule of the type of study that was performed.

The worksheet (modified CONSORT agreement) for randomized controlled trials has been required since 1996 and is available online. The chart below provides basic information regarding the direction we are heading with the new designs.

STUDY	DESIGN	OPTIONAL MODIFIERS
Reporting observation on a single patient?	CASE REPORT	
Reporting observations on multiple patients, with similar findings, or treated in a similar way, but without a comparison group?	CASE SERIES	
Comparing observations or results on similar patients who have been treated in more than one way? Comparing a treated and untreated group?	COMPARATIVE CASE SERIES	

Comparing previous exposure(s) between a group of patients with a given disease or outcome and a group without the given disease or outcome?	*CASE-CONTROL STUDY	
Determining the prevalence of a symptom, sign, or disease in a group of individuals or examining associations between factors at one point in time?	CROSS-SECTIONAL STUDY	Clinic-based, hospital-based, community-based, population-based
Reporting on a group of individuals with defined characteristics before developing a condition or undergoing a procedure, and then observing them over time for the appearance of a disease or surgical result or complication.	COHORT STUDY	
Reporting the results of a clinical experiment that you have registered with clinicaltrials.gov or a similar database, in which defined groups of subjects receive different treatments, placebo, or no treatment?	CLINICAL TRIAL	Randomized, non-randomized, masked, multicenter
Evaluating a diagnostic test or comparing more than one diagnostic test?	EVALUATION OF DIAGNOSTIC TEST OR TECHNOLOGY	
Developing a questionnaire or interviewing instrument?	QUESTIONNAIRE DEVELOPMENT	
No human subjects studied (only tissue, biopsies, and animals)?	EXPERIMENTAL STUDY	
Reporting the available data addressing a specific clinical question?	EVIDENCE-BASED MANUSCRIPT	Systematic review, meta-analysis
Reporting on a phase 4 open-label study, a registry or surveillance system, or an administrative database?	DATABASE STUDY	

*Case-control study design must meet these criteria. If you have simply compared a group of cases and selected a control group, the design is most likely "Comparative case series".

B. Literature Review

A thorough review of available literature with appropriate data bases (Index Medicus, PubMed, MEDLINE, Cochrane Central Register (Cochrane Library), EMBASE, LILACS, etc.) is mandatory during the planning phases of a research project to avoid unnecessary duplication of effort and errors in acknowledging credit due others. When you allude to your interpretation of the previous literature, e.g., "we report the first case of …" in the methods section or discussion section be sure to explain the depth and breadth of your search strategy – where you searched, on what search terms,

when the search was undertaken, and whether any more than a basic computer search was conducted. Non-English literature should be included with help from library resources as necessary. *Ophthalmology* requests that authors include only essential references that relate directly to the work being reported and that they verify their accuracy. Refer to references for formatting of various types of references.

To expedite processing, if you are asked to revise your manuscript, you will also be asked to provide a photocopy of the title page (that include publication information—journal name, vol. year, page numbers) of any work cited that was published prior to 1970 in the United States. You will also be asked to submit the title page for all work cited that was published outside of the United States regardless of year. Also include for any books referenced, the book's copyright page and the first page of any chapters referenced. Although not required upon first submission, it is strongly suggested that you make copies of these items during the researching of your manuscript so they are readily available if needed.

C. Organizing Research Data

The Study Design should be defined clearly before data collection is carried out with pre-designed forms/methodology to enable proper preservation and eventual analysis of data collected, regardless of whether data collection is retrospective or prospective.

D. Epidemiological and Statistical Considerations

Definitions of relevant terms are provided in the Glossary of Terms.

Generally, statistical tests should be applied appropriately with consideration for potentially confounding variables. P-value and/or confidence intervals should be provided as appropriate.

Two key questions should be answered prior to submission of the manuscript:

1. Is the information adequate to permit interpretation of the results?
2. Are the conclusions justified?

Cautionary notes about terminology:

1. Ensure proper use of "procedures" vs. "eyes" vs. "patients" vs. "subjects".
2. Clarify whether or not the "last" follow-up information or a summary of "interval" information is presented. Interval follow up is preferred. (DiLoreto DA Jr, Bressler NM, Bressler SB, Schachat AP. Use of best and final visual acuity outcomes in ophthalmological research. Arch Ophthalmol. 2003;121:1586-90.)
3. Univariate and multivariate analyses are frequently misused in current literature. Their appropriateness should be verified by expert consultation as necessary.
4. P-values are frequently misused.
5. "Incidence" describes new cases over some interval of time
6. "Prevalence" describes cases at one defined interval in time.
7. Remember to distinguish accurately between "standards" and "standardized" and "computed" and "computerized"
8. The terms "safety" and "efficacy" are hackneyed and often misused. Please review a pertinent editorial on this: Schachat AP, Chambers WA, Liesegang TJ, Albert DA. Safe

and Effective. Ophthalmology.2003;110-2073-4.

2. EQUIVALENT VISUAL ACUITY CONVERSION CHART

The *Journal* publishes articles from around the world, where standards for measuring visual acuity vary. This table will help readers interpret visual acuity findings in familiar units.

Table of Equivalent Visual Acuity Measurements

Snellen Visual Acuity				
4 Meters	6 Meters	20 Feet	Decimal Fraction	LogMAR
4/40	6/60	20/200	0.10	+1.0
4/32	6/48	20/160	0.125	+0.9
4/25	6/38	20/125	0.16	+0.8
4/20	6/30	20/100	0.20	+0.7
4/16	6/24	20/80	0.25	+0.6
4/12.6	6/20	20/63	0.32	+0.5
4/10	6/15	20/50	0.40	+0.4
4/8	6/12	20/40	0.50	+0.3
4/6.3	6/10	20/32	0.63	+0.2
4/5	6/7.5	20/25	0.80	+0.1
4/4	6/6	20/20	1.00	0.0
4/3.2	6/5	20/16	1.25	-0.1
4/2.5	6/3.75	20/12.5	1.60	-0.2
4/2	6/3	20/10	2.00	-0.3

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3. GLOSSARY OF TERMS

- **adverse event** Complication of therapy or disease occurring during a study.
- **analysis** Comparison of study and control groups or examination of outcomes in non-controlled studies. Assessment of data, including primary and secondary comparisons of interest.
- **assignment** Designation of individuals as study or control subjects.
- **assessment** Determination of the results of the investigation.
- **bias** A non-chance event arising from faults in study design or measurement or data collection. Bias may prejudice results in that traditional statistical analysis may be precluded or unreliable. Bias may be introduced into a study by many factors including subject selection, follow-up, study factor choice, unmasked data collection, temporal trends in disease, co-management of disease if not concurrent in time, ecological fallacy, retrieval methods, play of chance, publication choice or prejudice of investigators.
- **case-control study** An observational (non-interventional, usually retrospective) study that begins by identifying individuals with a disease (cases) for comparison to individuals without a disease (controls or reference group), in which analysis proceeds from effect to cause.

- **case series** Case series include those studies describing more than one consecutive or non-consecutive case, studied retrospectively or prospectively, usually with regard to the outcome of an intervention for its efficacy, safety, and complications. Non-comparative case series generally have no control group included but outcome may be compared to that in the literature.

- **case report** Usually a retrospective report of a single interventional or observational case experience, often with clinical-pathological correlation.

- **clinic-based** Term used to define the population studied derived from a single clinic population or set of populations

- **cohort** A group of individuals (subjects) who share a common experience or condition.

- **cohort study** An observational (usually prospective) study that begins by identifying individuals with (study group) and without (control group) a factor being investigated to observe over time with regard to disease outcome; study and control groups may be concurrent or non-concurrent but must be derived from the same well defined cohort; almost always prospective with regard to data collection. Almost always longitudinal in that a particular group of patients is followed forward from a point in time. May or may not be population-based.

- **comparative study** Study including two or more defined groups, compared one to another, to make a judgment about the influence of some factor or treatment.

- **confounding variables** Risk factors that may affect the relationship between a risk factor and an outcome.

- **control group** Reference group or group of individuals similar to treatment group except for exposure to study intervention.

- **crossover design** This type of study compares two or more treatments or interventions in which the subjects or patients, upon completion of one therapy, are switched to the alternative(s).

- **cross-sectional study** An observational study that identifies individuals with and without the condition or exposure being studied at the same time (synonymous with prevalence study). May or may not be population-based.

- **double-masked study** At the times of data collection and analysis, neither evaluators nor subjects know which intervention or test is applied.

- **ecological fallacy** This term applies to summary data which misrepresent a relationship within a larger group. Risk cannot be inferred for an individual based on group results.

- **epidemiology study** Prospective or retrospective observational investigation of disease or characteristics; ideally according to pre-determined protocol; includes prevalence, incidence, and cross-sectional studies.

- **experimental study** No human subjects involved.

- **extrapolation** Drawing conclusions about the meaning of the study for individuals or situations not included in the study.

- **external validity** A study's conclusions may be valid only for a specified external population; (how general are the findings?).

- **frequency** The number of occurrences of an event or the proportion of members of a population or statistical sample falling into a particular class; the number of occurrences of a periodic or recurrent process per unit time or per sample.

- **genetic terminology** Terminology used in genetics manuscripts should conform to Human Gene Nomenclature (HGNC) Guidelines. Please visit the HGNC website for the most current draft version of the guidelines http://www.gene.ucl.ac.uk/nomenclature. Do not submit scrambled pedigrees. If a scrambled pedigree is required, please correspond with the Editor-in-Chief at the time of manuscript submission for a waiver of this policy. Base sequences, such as for PCR primers, should not be included in the text of a manuscript. Authors may opt for an online supplement or provide a URL where the primers can be found or an email address for interested readers. Human or animal tissue examination employing traditional morphologic methods including light, scanning, and transmission electron microscopy.

- **historical controls** A collection of patients used as a comparison group, who were identified and treated or observed in the past in a period that predates the time covered by other study groups.

- **historical manuscript** A manuscript describing prior events, usually in chronological order, or the history of individuals or organizations.

- **incidence** The rate of event or disease occurrence in those at risk in a defined population per unit time.

- internal validity The observed differences between index and comparison groups are attributable to the independent variables under study.

- **interpretation** Drawing conclusions about the meaning of similarities and differences found between study and control groups or between studies.

- **intervention** Manipulation(s), treatment(s), test(s), or observation(s) employed to generate data for purposes of achieving the study goals.

- **interventional study** A study that includes an attempt to alter the course of disease by medical or surgical or other therapy.

- **matched controls** Subjects who have specific characteristics similar to cases (study subjects). Commonly used matching characteristics include age, gender, race, and socioeconomic status.

- **meta-analysis** Data gathered entirely from existing literature using statistical methodology to integrate and summarize results of several studies. The data from individual studies may be weighted by the degree of variance or other study characteristics to arrive at a pooled estimate of the relation between a factor and an outcome. Usually now applied only to analysis of previously published randomized controlled trials.

- **modifiers** Terms used to specify details about a study: (comparative, prospective, retrospective, interventional, non-interventional, observational, randomized, non-randomized, controlled, non-controlled, histopathologic, experimental, human, non-human, primate, etc.)

- **multicenter clinical trial** A clinical (human) trial involving two or more clinical centers, a common study protocol, a data center, and a data coordinating center, or coordinating centers to receive, process and analyze study data.

- **observational study** No intervention or attempt to alter the natural course of disease or physical condition.

- **ocular trauma terminology** Terminology used in descriptions of ocular trauma should conform to the recommendations of the United States Eye Injury Registry and the International Society of Ocular Trauma. (See: Kuhn F, Morris R, Witherspoon CD, et al. A standardized classification of ocular trauma. Ophthalmology 1996; 103:240- 3).

- **odds (of an event)** Odds = # of patients fulfilling endpoint criterion / # of patients not fulfilling endpoint criterion

- **odds ratio (relative odds, cross product)** = ad/bc where:

	Exposed	Unexposed
Disease	a	b
No Disease	c	d

- **phase I, II, III, IV (FDA)** [US FDA Classifications: (modifiers) applicable to new human therapies, including drugs and devices, under consideration for marketing approval]

- **Phase I**: Safety and dose testing in humans (usually without controls) (Studies a small number of patients to determine tolerated doses [dose escalation] and side effects for risks of new agents, devices)

- **Phase II**: Testing of safety (with or without controls) and efficacy (requires controls) in affected subjects,

- **Phase III**: Testing of efficacy and safety (with controls) (randomized controlled trial)

- **Phase IV**: Post-market surveillance (with or without controls)]

- [retrospective, comparative studies of interventions, drugs, devices]

- **placebo** An inert (pharmacologically inactive) medication, which lacks a therapeutically active ingredient.

- **population-based** A study including all individuals in a defined geographical area or otherwise clearly defined subgroup of the population. A study conducted on a randomly selected representative group (10%, 20% etc.) of the population at risk.

- **prevalence** The proportion of subjects with a particular disease or condition at a point in time (best estimate of the probability of disease before performing the test or intervention).

- **prevalent** This term implies a characteristic which is widespread.

- **prospective study** Data are collected before and/or after interventions, measurements or events by using previously defined protocols.

- **protocol deviation** Departure from the planned sequence of testing, interventions follow-up, or analysis during a study.

- **publication bias** Negative studies are unlikely to be published and are less likely than positive studies to be available for detailed literature reviews or meta-analyses. Studies which duplicate previous studies are also less likely to be published.

- **randomized (controlled) trial** A trial (human or non-human) that involves at least one experimental treatment group and one control group, concurrent enrollment, and follow-up of the test and control groups, and in which the assignment to experimental and control groups is by a randomization process. Neither the subjects nor the persons responsible for treatment can influence the assignments, and the assignments remain unknown to the subjects and staff until eligibility has been determined.

- **referral based** The subjects studied are accumulated through an intermediary (referred).

- relative frequency The average rate of occurrences of a particular event in a large number of repeated trials.

- **relative risk** The Relative Risk (RR) = $\frac{\text{risk of disease in treatment group}}{\text{risk of disease in control group}}$

- **retrieval bias** Retrieval bias may occur when data is not obtained from all relevant cases or studies.

- **retrospective study** Data collected and analyzed after all measurements, interventions, or events have taken place.

- **review** A manuscript which summarizes the scientific history and current understanding of a topic, procedure, or disease.

- **risk** The risk in a defined population and time equals:

$$\frac{\text{\# patients fulfilling endpoint criterion}}{\text{total \# patients}}$$

- **sham procedure** A deliberately ineffective intervention.

- **single masked study** The subjects or the evaluators, but not both, know which intervention is applied.

- **study size**: (for *Ophthalmology* Data base Coding)
- (Total number of study subjects)

  small series = $n \leq 10$
  medium series = $10 > n \leq 30$
  large series = $n > 31$

- **systematic review** A detailed review and analysis of previously published literature.

- **triple masked study** All participants are masked to the intervention. None of the investigators, the subjects, the data and safety monitoring committee, nor the biostatisticians know which intervention or analysis is applied.

4. Grammar/Language Guide

Good writing supports and augments good research. Clear, concise language is highly desirable in scientific communications and consistent with good scholarship. Sentence structure should be grammatically correct and language use should incorporate a reasonable breadth of vocabulary. Obfuscation, circuitous verbiage, and poor logic devalue the communication and only increase the risk of confusing the reader. Redundancy of text or duplication of text points in tables wastes precious space and unnecessarily complicate a manuscript. Authors should plan to do several revisions before submission to shorten and to focus an article. Clear writing itself greatly enhances the impact of research findings. If the following does not answer your basic issues, you may wish to submit your paper to an English Editor.

Examples of specific flaws in language use to avoid include:

a. Passive Voice

Active voice is much preferred to passive voice, which should be used sparingly. Passive voice tends to "depersonalize" the subject and remove the author(s) from active responsibility (or bias?) for his/her work. Active voice is generally more concise than passive voice and saves space and time. Passive voice may force the reader to stop and think about whom is doing the action. It does not relieve the author of direct responsibility for observations, opinions, or conclusions (e.g., "The problem of blood flow was investigated..." vs. "We investigated the problem of blood flow..."; "A slow gradual subsidence of the swelling and normalization of visual acuity was

found." vs. "The swelling subsided gradually and visual acuity returned to normal.")

b. Impersonal Passive

Many authors "cheat" the passive voice with weak sentence openers that are literally active but functionally passive. Avoid phrases such as: "It is...", "There is...", "It is important to note that...", "It is essential that...". Removing such phrases permits more succinct and clear thought. (e.g., "Although there is evidence suggesting involvement of genetic factors, the exact role of such factors and mode of inheritance remain to be elucidated fully." The same point is stated more clearly as: "The role of genetic factors is unknown.")

c. Subject/Verb Separation

Remember that a reader can hold the subject of a sentence in his consciousness only so long. Sentences in which the subject sits many words away from its verb may force the reader to reread the entire paragraph to understand the thought. For example: "The smallest of the URFs (URFA6L), a 207-nucleotide (nt) reading frame overlapping out of phase the NH2-terminal portion of the adenosinetriphosphatase (ATPase) subunit 6 gene, has been identified as the animal equivalent of the recently discovered yeast H+ - ATPase subunit 8 gene."
In this 41-word sentence, 23 words separate the subject "smallest" from its verb "has been identified." A possible revision would appear: "The smallest of the URFs (URFA6L) has been identified as the animal equivalent of..."

Keep subjects and verbs reasonably close together.

d. Abstruse, Obtuse, Arcane, or Numerous Abbreviations/Acronyms

A reasonable balance must exist between the introduction of an unconventional abbreviation and the use of the full term. Many authors tend to use abbreviations/acronyms for any phrase that has two or three words in it, in titles, captions, and text. When these abbreviations/acronyms are multiple and repetitive, reading becomes analysis of shorthand. In general, minimize use of abbreviations. Tables and figures need to make sense on their own so readers should not need to click back to the main text and search out definitions of abbreviations/acronyms. Abbreviations/acronyms need to be defined parenthetically in each figure and in a legend for each table. Similarly, they need to be defined in the précis and abstract since there things also need to make sense on a "stand alone" basis. Abbreviations should defined again at first use in the main text. There is a brief list of abbreviations/acronyms that have become "accepted" overtime and these are the only ones that do not need defining and the only ones that can be used in titles.

e. Improper Subject-Verb Agreement

Rules of prescriptive grammar require the agreement of subject(s) and verb(s) in person and number and the agreement of pronouns and antecedents in number, person, and gender. Subjects and verbs must agree. "Data" is always plural.

- "My own experience and that of my colleagues argue that..."
- "This datum from this study suggests that 1000 cGy of ext ernal beam photon therapy is not beneficial in treating CNV."

- "The linkage data and haplotype data are presented."
- "The majority of cases is considered to be multifactorial in origin."

f. Avoid split infinitives

"My mother told me to never split an infinitive." should be "My mother told me never to split an infinitive."

g. Non-Agreement of Verb Tenses

The use of both past (or imperfect) and present tenses in the same sentence or paragraph can be awkward. (e.g., "On last examination, her visual acuity is 20/40 and further surgery was refused.")

Harmonize tenses in a paragraph or presentation.

h. Redundancies

Repetition weakens a thought or presentation and sometimes can lead to amusing results

- "[Glaucoma] is caused by alterations in the sieve-like trabecular meshwork."
- "The entire tumor was excised completely."
- "For more information, communicate with the Director by writing him at..."
- "An area encompassing a 2 disc diameter radius centered on the foveal center was graded for each eye."
- "We examined a large number of patients after a fairly long, and standardized, follow-up period."
- "The family studied has twice previously been reported in the literature."

i. Human Characteristics Attributed to Disease Processes

Insensitivity and jargon often cause us to attribute human senses to a disease (e.g., "We have no explanation for the tumor's predilection for younger females.".)

j. Circumlocution and Compression (too many words vs too few)

Sometimes, in an attempt to be brief, a compressed thought will yield a bizarre statement.

- "Sudden death from heart block may require early cardiac pacing."
- "Blood shortages in Houston hit dangerously low levels."
- "The eye with the more severe pathology was used in patients with bilateral clinically significant macular edema."

k. Misplaced Modifiers

When an adjective or adverb directly precedes or follows the word that it modifies, the connection cannot be mistaken. But a modifier in an unusual position may fall into the wrong company and f orm an unsuitable attachm ent. The m omentary m isreading distracts from the substance of wha t you are saying. (e.g., "Forty-five patients were entered into the linkage analysis twenty-four of whom were affected.")

Read each sentence and thought carefully and place the modifiers precisely.

l. Hyperbole of Emphasis

An author can make a point with a powerful word alone. Adding an emphatic modifier, an intensive adverb (e.g., very, really, truly, actually, etc.), attenuates the phrase and defeats the purpose. It reduces the adjective to conversational pablum, depriving it of force. The repeated superlative or modified adjective indicates extreme positions (e.g., "absolutely no justification", "much more frequently").

m. Hyperbole of Thought

Don't use big words! Keep it simple versus

> "When promulgating your esoteric cogitative or articulating your superficial sentimentalities and amicable philosophical and psychological observations, beware of platitudinous ponderosity. Let your verbal evaporations have lucidity, intelligibility, and veracious vivacity without rodomontade or thespian bombast. Sedulously avoid all polysyllabic profundity, pompous propensity, and sophomore vacuity."

n**.** The Dangling Participle

Participles, verb forms functioning as adjectives, may detach themselves from the formal subject that they should qualify. In other words, they dangle. (e.g., "Having expressed a direct interest in our institution, we have enclosed the materials that you requested with an application form.")

The most common and misused dangling participle in medical and scientific literature is "using." Inexplicably, reviewers and editors have tolerated the admission of the dangling participle "using" in text and title. In these examples, who or what is "using"?

- "Genotyping was performed using a semi-automated fluorescence scanning system."
- "Linkage analysis was performed using both genetic model-dependent and model-independent methods."
- "The present study measured vision using the ETDRS protocol with standardized refraction."
- "Patients with useful vision in the fellow eye were treated using a lateral field, entering at a 45-degree angle, using a 45-degree couch rotation to achieve this."

Substitute a preposition as appropriate, or rewrite the phrase.

o. Stating the Obvious

"The development of this tumor probably precedes its clinical appearance." Do we really need to be so informed?

p. Slang, Jargon, and Colloquialism

"This gene probably plays some role in "run-of-the-mill" glaucoma..."

Avoid wordy and colloquial expressions such as:

- a majority of (= most)
- at the present time (= now)
- due to the fact that (= because)
- in the event that (=If)
- it is clear that (= clearly)
- it is suggested that (= I think)
- prior to (= before)
- take into consideration (= consider)
- with respect to (= about)

q. Run-on Sentences

Sentences should be reasonable in length and convey one primary thought or relationship. Not presenting several thoughts or relationships in one sentence often is confusing and create questionably inter-related concepts. While brief is better, avoid one sentence paragraphs except in rare circumstances. Usually, the thought can be appended to the preceding or following paragraph.

r. Spelling Errors

In the modern era of electronic spell checkers, typographical and spelling errors should be less frequent. Remember that spell checkers and grammar checkers have their limits and nothing replaces a good, careful final read of the manuscript. Read the manuscript (again!). Private editing is a good investment. Even ask a colleague or spouse to read the manuscript before it is submitted to the Journal.

s. Its, It's, and Its'

Its conveys possession. *It's* is a contraction of it is. *Its'* is not in use.