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Title page

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

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.

M.A. Scheepers
ABSTRACT

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

Scheepers MA, Lecuona KA, Rogers G, Bunce C, Corcoran C, Michaelides M
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


Appendix to protocol - Data capture spreadsheet
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- **Race**: B=Black, W=White, M=Mixed, I=Indian
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- **CD4 COUNT**: U=Unknown
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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

[Signature]

PROFESSOR M BLOCKMAN  
CHAIRPERSON, HSE HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.  
Institutional Review Board (IRB) number: IRB00001938
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.
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 its 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. Negative serological results make the chances of an infection less likely, but cannot rule out the possibility of that infection. 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. 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.) 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.\textsuperscript{10,19} False negatives may occur when high serum antibodies combined with extensive blood aqueous barrier breakdown may mask a positive coefficient.\textsuperscript{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.\textsuperscript{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.\textsuperscript{22} Real time PCR has been used to measure viral loads and disease activity in patients with herpetic uveitis as well as CMV retinitis.\textsuperscript{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.\textsuperscript{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. 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. False negative results may occur in cases of low specimen antigenic load or polymorphism (genetic variability or mutation). 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%. PCR analysis in patients with ocular toxoplasmosis is generally less sensitive than viral retinitis. Studies have shown variable sensitivity ranging from 27% to 85%. 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)\textsuperscript{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.\textsuperscript{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).\textsuperscript{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.\textsuperscript{41} CMV retinitis may also present as an indolent type CMV retinitis that presents with atrophic central lesions with granular whitish active borders.\textsuperscript{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/mm³. 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.\textsuperscript{3,20-21,62}

PCR appears to be particularly valuable in cases of atypical OT in immunocompromised patients.\textsuperscript{6,62} Improved PCR techniques such as qualitative multiplex PCR used by Sugita and colleagues have also improved sensitivities.\textsuperscript{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.\textsuperscript{29} Fekkar noted the combination of GWC & PCR to increase diagnostic sensitivity to 93% in a study of 34 patients with OT.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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. Serpiginous choroiditis like chorioretinal lesions may also be observed. Tuberculoceraspiginous-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. Optic nerve papillitis and juxtapapillary chorioretinitis may occur as well. 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. 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.

Although IGRA’s can distinguish exposure to M. tuberculosis from the Bacillus Calmette-Guerin vaccine strain, they currently lack the specificity to distinguish between latent tuberculosis infection and active tuberculosis. 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{80} In addition, real time PCR for M. tuberculosis has also helped in the differentiation of Mycobacterium from other contaminants.\textsuperscript{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.\textsuperscript{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.\textsuperscript{78}

Ocular Syphilis

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. 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.

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

INTRAOCULAR FLUID SAMPLING SITES AND TECHNIQUE AFFECTING ACCURACY

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

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. 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. 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.
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Appendix – named journal and author contributions

Named peer reviewed journal:

Ophthalmology

Instructions for authors web address:


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: 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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Conflict of Interest: No conflicting relationship exists for any author

Running head: Scheepers et al – The value of PCR in the diagnosis of infectious posterior uveitis

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Medical School, Observatory 7925
ABSTRACT

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

Scheepers MA, Lecuona KA, Rogers G, Bunce C, Corcoran C, Michaelides M
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 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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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. 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. 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). 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. This is particularly critical in immunocompromised patients. 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. 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. 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.

PCR of intra-ocular fluids is a reliable investigation that can identify most of the common
causes of infective posterior uveitis. 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. 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. PCR
diagnosis of ocular MTB has been shown to be highly specific with a variable
sensitivity. 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 MgCl₂, 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.\textsuperscript{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.\textsuperscript{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. 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%. 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%. It has been suggested that in immune compromised patients PCR analysis may have greater
sensitivity. 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. 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.

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%). 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%. 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.
References


### TABLE 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>34 (Range 14 - 53)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58</td>
</tr>
<tr>
<td>Female</td>
<td>101</td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>67</td>
</tr>
<tr>
<td>Right eye</td>
<td>42</td>
</tr>
<tr>
<td>Left eye</td>
<td>50</td>
</tr>
<tr>
<td>HIV +ve</td>
<td>142</td>
</tr>
<tr>
<td>HAART treatment @ presentation</td>
<td>65</td>
</tr>
<tr>
<td>MTB treatment @ presentation</td>
<td>67</td>
</tr>
</tbody>
</table>

HIV = human immunodeficiency virus  
HAART = highly active antiretroviral treatment

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

<table>
<thead>
<tr>
<th>VA</th>
<th>Pre treatment</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 6/18</td>
<td>42%</td>
<td>33%</td>
</tr>
<tr>
<td>6/24 - 4/60</td>
<td>16%</td>
<td>14%</td>
</tr>
<tr>
<td>≤ 3/60</td>
<td>42%</td>
<td>53%</td>
</tr>
<tr>
<td>Eyes assessed</td>
<td>226</td>
<td>191</td>
</tr>
</tbody>
</table>

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

### TABLE 3. PCR tests performed

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>No. tests perf.</th>
<th>No. +ve</th>
<th>% +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>154</td>
<td>72</td>
<td>47%</td>
</tr>
<tr>
<td>TG</td>
<td>150</td>
<td>12</td>
<td>8%</td>
</tr>
<tr>
<td>VZV</td>
<td>148</td>
<td>17</td>
<td>11%</td>
</tr>
<tr>
<td>HSV</td>
<td>148</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>MTB</td>
<td>43</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>TOTAL TESTS</td>
<td>643</td>
<td>102</td>
<td>16%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Pretest diagnoses</th>
<th>No. CMV +ve</th>
<th>No. VZV +ve</th>
<th>No. HSV +ve</th>
<th>No. TG +ve</th>
<th>No. MTB +ve</th>
<th>No. CMV &amp; VZV +ve</th>
<th>No. CMV &amp; TG +ve</th>
<th>PCR +ve rate No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>70</td>
<td>51</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>60</td>
<td></td>
<td>86%</td>
<td></td>
</tr>
<tr>
<td>NHR</td>
<td>14</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td></td>
<td>71%</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>10</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>MTB</td>
<td>7</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>IRU</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Unsure</td>
<td>51</td>
<td>11</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td>21</td>
<td>41%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>159</td>
<td>66</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>4</td>
<td></td>
<td>94</td>
<td>59%</td>
</tr>
</tbody>
</table>

IRU = Immune reconstitution uveitis
### TABLE 5. Final diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMVR</td>
<td>74</td>
</tr>
<tr>
<td>NHR</td>
<td>17</td>
</tr>
<tr>
<td>OT</td>
<td>16</td>
</tr>
<tr>
<td>MTB</td>
<td>5</td>
</tr>
<tr>
<td>Syphilis</td>
<td>4</td>
</tr>
<tr>
<td>CMV/VZV coinfection</td>
<td>2</td>
</tr>
<tr>
<td>HIV associated retinopathy</td>
<td>2</td>
</tr>
<tr>
<td>Toxocara Canis</td>
<td>1</td>
</tr>
<tr>
<td>Immune reconstitution uveitis</td>
<td>1</td>
</tr>
<tr>
<td>Blood dyscrasia</td>
<td>2</td>
</tr>
<tr>
<td>Idiopathic or end stage late presentation</td>
<td>35</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Sample no.</th>
<th>True +ve</th>
<th>False +ve</th>
<th>True -ve</th>
<th>False -ve</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>154</td>
<td>69</td>
<td>1</td>
<td>1</td>
<td>74</td>
<td>48</td>
<td>7</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>AC</td>
<td>45</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>69</td>
<td>1</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td>V</td>
<td>106</td>
<td>56</td>
<td>1</td>
<td>1</td>
<td>43</td>
<td>41</td>
<td>6</td>
<td>6</td>
<td>90</td>
</tr>
<tr>
<td>NS</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>VZV</td>
<td>148</td>
<td>15</td>
<td>2</td>
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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

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OPHTHALMOLOGY

GUIDE FOR AUTHORS

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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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7. **Conclusions**: states the conclusion(s) derived from the data analysis.

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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.
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   Debra L Hanson, MS; Susan Y. Chu, PhD; Karen M. Farizo, MD; John W. Ward, MD; and the Adult and Adolescent Spectrum of HIV Disease Project Group

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   Debra L Hanson, MS; Susan Y. Chu, PhD; Karen M. Farizo, MD; John W. Ward, MD for the Adult and Adolescent Spectrum of HIV Disease Project Group
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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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Authors can download the form from either previously mentioned location, add the requested information, and save the completed form on their computer. The completed form can then be sent to the corresponding author to be uploaded during the submission process. Over time, more journals may request the identical document, which will simply need to be updated by the authors in relation to the current manuscript prior to uploading. The corresponding author will list any disclosures on the cover page of the submission as well as financial support for the work, if any.

Every published manuscript will have a blanket statement, inserted by the publisher, within the abstract box.; either "None of the authors have any conflicts of interest to disclose." OR "Authors with financial interests or relationships to disclose are listed after the references." Corresponding authors will be asked to confirm or update conflict of interest statements as part of the final steps of manuscript acceptance with the journal office, prior to transmittal to the publisher.
Ophthalmology will be vigilant in the quest to ensure that the public continues to trust that the medical literature and our authors are not inappropriately influenced by their financial relationships with industry or other prejudices. If allegations arise, the journals must and will react.2


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

COPYRIGHT ASSIGNMENT FORM

Start circulating copyright forms among authors early so they are completed in time for submission. As of January 2012, copyright(s) must be uploaded into the system preferably at first submission but no later than first revision.

The method of submitting your copyright form(s) is to upload it with your manuscript. We suggest the corresponding author collect all signed copyrights and submit them with the initial manuscript submission or, if absolutely necessary, when submitting a first revision to the editorial office. We ask that the corresponding author coordinate this effort to be sure each form is done correctly prior to submission to the editorial office. Type in the agreed upon title and author order on the top of the copyright form(s), print out the form. **Every copyright submitted for a given manuscript must have identical and complete information at the top of the form where the title and author lines are.** You can then circulate for signature one or more copies of this form for all authors to sign. Once original signatures are obtained from all authors, scan the form(s) (preferably to PDF format) and upload them at submission time.

The copyright form signed by each author states that you either own the copyright, or have written permission to use all the material in your article. If you are submitting any material to which you do not own copyright, please secure permission to use the copyrighted materials.

**NOTE:** Once a manuscript has been submitted, the order of authorship (including adding or removing authors) CAN NOT be changed without a written request to the Editorial Office from the corresponding author. This request must include a statement signed by all authors that they are in agreement with the change along with a new copyright form, both signed by all authors. Specifically, if an author is removed, a letter from that author agreeing to his/her removal is required. The new copyright form must show the title and authors’ names in the order they should appear in print on the top of the form and include original signatures from each; signature order does not matter. If the original authors are not able to agree among themselves on authorship changes, please withdraw the paper. The Editor and Editorial Office do not choose to arbitrate such debates. AUTHORSHIP CHANGES CAN NOT BE SUBMITTED WITH PROOF CHANGES. The publisher can not approve such changes and it will delay the publication of your manuscript.

COVER FIGURES

Ophthalmology publishes color photographs and images on the cover of the printed journal. The Cover Page Editor for the journal is James D. Brandt, M.D. of the University of California, Davis.

Our cover pages are usually generated from figures in articles appearing in a given issue, but our criteria are that images considered for the cover be visually striking and technically excellent (and fit on the cover layout). In case there are no appropriate images among the articles slated to appear in a given issue, we then turn to photographs submitted by ophthalmic photographers and
clinicians for consideration. These pictures don’t need to be something rare – our goal is to find technically excellent and striking images that make the reader look at the cover and say ‘wow’. So a gorgeous image of a common ophthalmic finding is just as welcome as a photo of something rare. Square or portrait (vertical) format images work best, as they can be laid out with space for the text box announcing issue highlights along with room for the mailing label along the bottom. Composites of several photographs (e.g., a sequence over time or a comparison of color photography with angiography, pathology, etc.) also work well and provide flexibility in layout.

To submit an image for consideration as a future cover, Dr. Brandt is happy to take a look at images sent to him by e-mail (jdbrandt@ucdavis.edu); please use the subject header “Cover Image for Ophthalmology” so that your e-mail is appropriately flagged. Send Dr. Brandt a JPEG version of your image along with a brief description of the case (a one sentence description is all that is run with the photo in the Table of Contents) and the names and institution of the clinician(s) and photographer(s) responsible for the image (limit of two each). If it is determined that the photograph is appropriate, he will work with you to generate appropriate file(s) for publication (see technical considerations below).

If your image is selected for use as a potential cover image, Ophthalmology will need a completed copyright transfer form (see downloadable forms.) Once the form is received, the Editorial Office will put the image in queue for a future issue. Cover images submitted by photographers and clinicians in this manner are used for covers only two to four times a year, so even if we determine that your image is appropriate for a future cover, it may take a year or more before it would appear in print.

Technical Considerations
The four color printing process used in producing the journal cover requires the highest resolution files to achieve the best quality. Should your image be chosen for the cover, the file(s) should be available as minimally compressed JPEG or ideally uncompressed (e.g., TIFF or PSD) high resolution files of at least 8”x8” at 300 dpi. Screen grabs from video (even high definition video) do not upscale adequately for print and look quite blurred in print; similarly, output from most diagnostic instruments do not upscale well and can look very pixelated with ‘jaggies’ on a cover. The only exception to this is when images from video or diagnostic instruments are reproduced as part of a composite – smaller images can reproduce well, and Dr. Brandt will work with you to see if adequate quality can be achieved in this manner.

Please do not perform any post-processing of the digital image other than light dusting and spot removal. sRGB colorspace is fine; do not convert to CMYK, as this will be done by the publisher during pre-press processing. The high resolution files for final publication are usually too big to send by e-mail. You can use a free web-based large file transfer service (e.g., www.yousendit.com) or mail a CD to Dr. Brandt.

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Copyright for the image(s) must be transferred to the American Academy of Ophthalmology. The copyright transfer form must be signed by all the listed authors. Please note that if the image has already appeared as part of an article in another journal or in a textbook, you probably do not have the right to transfer the copyright to the AAO. If the image has appeared as part of a photography contest (and especially if it won a prize), please check the conditions of your contest participation – you may have signed away the right to submit the image to Ophthalmology.

The copyright transfer form should be scanned and sent to Dr. Brandt as an e-mail attachment.
**DRUG and EQUIPMENT MANUFACTURER NAMES**

**Drug names**
Do not use drug trade names in titles. In the abstract use the generic name, but include the trade name once, in parentheses, after the first use of the generic name. In the text, use the generic name, but include the trade name once, in parentheses, after the first use of the generic name.

**Device/Equipment Names**
The device name is permitted in the title, abstract and text. However after the device has been identified at first use in the abstract and text, thereafter refer to it generically. In the case of equipment, include the manufacturer’s name, city, state and/or country parenthetically at the first use in the text.

**EDITORIALS**
General: A two-page editorial is usually published in each issue of *Ophthalmology*. Editorials are generally solicited by the Editor-in-Chief, although unsolicited submissions will also be considered.

Editorials may deal with clinical or non-clinical topics in summary form and must not exceed 1400 words, including references. Often editorials are linked with a particular manuscript awaiting publication and, therefore, adherence to deadlines is critical and mandatory. Although discouraged, if a figure is absolutely necessary, decrease the word count by approximately 200.

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.
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). 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 in dicated 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 each image. Composite are encouraged for multipanel figures (e.g., Fig 1A, 1B, 1C, 1D, 1E).
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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 recommended naming conventions. Some examples 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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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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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.

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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.

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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.

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**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 Smith¹). 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.

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g) Address for reprints

2. Abstract – see separate “Abstract” section

3. Text

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:

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  IRB/Ethics Committee ruled that approval was not required for this study.

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- 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.

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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 study designs.

<table>
<thead>
<tr>
<th>Study Design Description</th>
<th>Study Design</th>
<th>Optional Modifiers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reporting observation on a single patient?</td>
<td>Case Report</td>
<td></td>
</tr>
<tr>
<td>Reporting observations on multiple patients, with similar findings, or treated in a similar way, but without a comparison group?</td>
<td>Case Series</td>
<td></td>
</tr>
<tr>
<td>Comparing observations or results on similar patients who have been treated in more than one way? Comparing a treated and untreated group?</td>
<td>Comparative Case Series</td>
<td></td>
</tr>
<tr>
<td>Comparing previous exposure(s) between a group of patients with a given disease or outcome and a group without the given disease or outcome?</td>
<td>Case-control study</td>
<td></td>
</tr>
<tr>
<td>Determining the prevalence of a symptom, sign, or disease in a group of individuals or examining associations between factors at one point in time?</td>
<td>Cross-sectional study</td>
<td>Clinic-based, hospital-based, community-based, population-based</td>
</tr>
<tr>
<td>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.</td>
<td>Cohort study</td>
<td></td>
</tr>
<tr>
<td>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?</td>
<td>Clinical trial</td>
<td>Randomized, non-randomized, masked, multicenter</td>
</tr>
<tr>
<td>Evaluating a diagnostic test or comparing more than one diagnostic test?</td>
<td>Evaluation of Diagnostic test or technology</td>
<td></td>
</tr>
<tr>
<td>Developing a questionnaire or interviewing instrument?</td>
<td>Questionnaire development</td>
<td></td>
</tr>
<tr>
<td>No human subjects studied (only tissue, biopsies, animals)?</td>
<td>Experimental study</td>
<td></td>
</tr>
<tr>
<td>Reporting the available data addressing a specific clinical question?</td>
<td>Evidence-based manuscript</td>
<td>Systematic review, meta-analysis</td>
</tr>
<tr>
<td>Reporting on a phase 4 open-label study, a registry or surveillance system, or an administrative database?</td>
<td>Database study</td>
<td></td>
</tr>
</tbody>
</table>

*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”.*
TABLES
Tables require substantial space; please give careful consideration to the number of tables submitted. The information should not be extensively reiterated in the text. Place the information in the text or in a table but not both.

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- **Translational Science Reviews** – submissions about translational advances that are on the cusp of widespread clinical application to the readers; this is primarily “by invitation only”.

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a) stream **line reviewer queries** by sending you only relevant requests to review which likewise reduces the turnaround time and gets timely decisions back to authors.
b) to **maintain non-biased, quality reviews** by knowing who is at which institution/organization (we avoid using reviewers from the author's institution/organization.)
c) **with updated emails, we can contact you in a timely fashion** regardless of your role as author, reviewer or editor.

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6. If you have moved within the past year, we suggest you also try putting in your previous e-mail address so that you do not generate duplicate registrations within the system. If your old e-mail is in the system (and it is still accessible to you) click on “register” and follow the steps in #5 above.

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**If for any reason you cannot access** your information or are not sure if you are in the system, please send an e-mail to vdoyle@jhmi.edu with your first name, last name, city and state or city and country as appropriate and your new e-mail address. The Editorial Office will update your information and then send you an e-mail with your user name and password so you can log in and access your contact data and personal classifications and update as needed.

**VIDEO CLIPS**

If you opt for to submit a video as an online supplement, add a reference to it in parenthesis at an appropriate place within the text of the manuscript. Also, add a statement to the title page that should read similar to: “This article contains a video as additional online-only material. The following should appear online-only: Clip 1, Clip 2 and Clip 3” Obviously, the materials can 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](http://www.ophsource.com/periodicals/ophtha) and accessible through Medline and other online databases.

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](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.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>DESIGN</th>
<th>OPTIONAL MODIFIERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reporting observation on a single patient?</td>
<td>CASE REPORT</td>
<td></td>
</tr>
<tr>
<td>Reporting observations on multiple patients, with similar findings, or treated in a similar way, but without a comparison group?</td>
<td>CASE SERIES</td>
<td></td>
</tr>
<tr>
<td>Comparing observations or results on similar patients who have been treated in more than one way? Comparing a treated and untreated group?</td>
<td>COMPARATIVE CASE SERIES</td>
<td></td>
</tr>
<tr>
<td>Research Question</td>
<td>Study Design</td>
<td>Description</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Comparing previous exposure(s) between a group of patients with a given disease or outcome and a group without the given disease or outcome?</td>
<td><strong>CASE-CONTROL STUDY</strong></td>
<td></td>
</tr>
<tr>
<td>Determining the prevalence of a symptom, sign, or disease in a group of individuals or examining associations between factors at one point in time?</td>
<td><strong>CROSS-SECTIONAL STUDY</strong></td>
<td>Clinic-based, hospital-based, community-based, population-based</td>
</tr>
<tr>
<td>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.</td>
<td><strong>COHORT STUDY</strong></td>
<td></td>
</tr>
<tr>
<td>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?</td>
<td><strong>CLINICAL TRIAL</strong></td>
<td>Randomized, non-randomized, masked, multicenter</td>
</tr>
<tr>
<td>Evaluating a diagnostic test or comparing more than one diagnostic test?</td>
<td><strong>EVALUATION OF DIAGNOSTIC TEST OR TECHNOLOGY</strong></td>
<td></td>
</tr>
<tr>
<td>Developing a questionnaire or interviewing instrument?</td>
<td><strong>QUESTIONNAIRE DEVELOPMENT</strong></td>
<td></td>
</tr>
<tr>
<td>No human subjects studied (only tissue, biopsies, and animals)?</td>
<td><strong>EXPERIMENTAL STUDY</strong></td>
<td></td>
</tr>
<tr>
<td>Reporting the available data addressing a specific clinical question?</td>
<td><strong>EVIDENCE-BASED MANUSCRIPT</strong></td>
<td>Systematic review, meta-analysis</td>
</tr>
<tr>
<td>Reporting on a phase 4 open-label study, a registry or surveillance system, or an administrative database?</td>
<td><strong>DATABASE STUDY</strong></td>
<td></td>
</tr>
</tbody>
</table>

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


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](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.


• **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:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Exposed</th>
<th>Unexposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>No Disease</td>
<td>c</td>
<td>d</td>
</tr>
</tbody>
</table>

• **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:

\[
\text{risk} = \frac{\text{# patients fulfilling endpoint criterion}}{\text{total # patients}}
\]

• **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...” vs. “We observed a slow gradual subsidence of the swelling and normalization of visual acuity...”.)
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 external beam photon therapy is not beneficial in treating CNV.”
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 form an unsuitable attachment. The momentary misreading distacts from the substance of what 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 sophomoric 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.