An Investigation into the Causes of Intraocular Inflammation in HIV-positive and HIV-negative Patients in the Western Cape Province, South Africa

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Declaration

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This dissertation includes 5 original papers published in peer reviewed journals or books and 3 unpublished publications. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and for each of the cases where this is not the case a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

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Summary

The causes of intraocular inflammation are divided into 3 large groups namely infectious, non-infectious and idiopathic. This research project set out to establish the prevalence of these 3 large groups and their different subgroups in an effort to determine whether HIV infection plays an important role in how frequently they occur in the Western Cape Province.

Out of a total of 106 participants with uveitis enrolled in this study, 66 cases (62.3%) were HIV- and 40 (37.7%) HIV+ with a median CD4+ cell count of 242 x 10^6/l. The majority of participants were black (n=52; 49.1%) or of mixed ethnicity (n=49; 46.2%) and 59.6% of blacks were HIV+ versus 16.3% of mixed ethnicity participants. Anatomically, most cases were either anterior uveitis (58.5%) or panuveitis (32.1%) while infectious uveitis (n=70; 66.0%) was more common than non-infectious (n=18; 17.0%) or idiopathic (n=18; 17.0%) uveitis. An infectious cause was found in 80.0% of HIV+ cases versus 57.6% in HIV- cases.

Intraocular tuberculosis (IOTB) was the most common cause of infectious uveitis in this study (n=35; 33.0%) where possible IOTB (n=23; 21.7%) was more common than probable IOTB (n=12; 11.3%). Tuberculin skin testing alone was more sensitive (90.3% vs 85.7%) and had a higher negative predictive value (92.1% vs 81.5%) than QuantiFERON alone and the latter therefore does not warrant the extra expense in our highly endemic setting. Herpetic uveitis formed the second largest group (n=13; 12.2%) with VZV (53.8%) responsible for more cases than CMV (38.5%) and HSV (7.7%). Syphilis was the third most common cause of infectious uveitis (n=11; 10.4%). Using a novel immunoblot approach the study investigated the relationship between ocular and neurosyphilis and demonstrated that these 2 conditions do not always co-exist. HIV infection was present in 31.4% of IOTB cases, 61.5% of herpetic cases and 81.8% of syphilitic cases. Toxoplasma (n=4; 3.8%), Rubella virus and poststreptococcal uveitis (n=3; 2.8% each) as well as HIV-induced uveitis (n=1; 0.9%) were responsible for the remainder of the infectious uveitis cases. EBV was often identified on multiplex PCR (n=11; 10.4%) but no evidence of active intraocular replication or antibody production was

found to prove that EBV caused uveitis in these cases. In most cases an alternative treatable cause of uveitis was identified (n=9; 81.8%).

Sarcoidosis and HLA-B27 associated anterior uveitis (n=8; 7.5% each) were the most common causes of non-infectious uveitis. All patients with ocular sarcoid and 75% of patients with HLA-B27 uveitis were HIV-.

The percentage of idiopathic cases in this study was lower than in many similar studies (n=18; 17.0%). This is likely due to the high percentage of cases of possible IOTB diagnosed using a recently proposed classification as many of those cases would have been labelled as idiopathic in other studies. The majority of idiopathic uveitis cases were HIV- (n=12; 66.7%).

This study revealed that infectious uveitis is the commonest form of uveitis in both HIV+ and HIV- patients but that the specific pathogens differ between patients with and without HIV infection.

Opsomming

Die oorsake van intraokulêre inflammasie word verdeel in 3 groot groepe naamlik infektief, non-infektief en idiopaties. Die doel van hierdie navorsingsprojek was om die prevalensie van hierdie 3 groepe asook hulle onderskeie subgroepe te bereken om te bepaal of HIV infeksie ‘n belangrike rol speel in hoe dikwels hulle in die Wes-Kaap provinsie voorkom.

Uit ‘n totaal van 106 deelnemers aan hierdie studie was 66 gevalle (62.3%) HIV+ and 40 (37.7%) HIV- met ‘n mediane CD4+ seltelling van 242 x 10^6/l. Die meerderheid deelnemers was swart (n=52; 49.1%) of van gemengde etniese afkoms (n=49; 46.2%) en 59.6% van swart deelnemers was HIV+ teenoor 16.3% van deelnemers van gemengde afkoms. Anatomies was die meeste gevalle anterior uveitis (58.5%) of panuveitis (32.1%) terwyl infektiewe uveitis (n=70; 66.0%) meer algemeen was as non-infektiewe (n=18; 17.0%) of idiopatiese (n=18; 17.0%) uveitis. ‘n Infektiewe oorsaak is gevind in 80.0% van HIV+ gevalle teenoor 57.6% in HIV- gevalle.

Intraokulêre tuberkulose (IOTB) was die algemeenste oorsaak van infektiewe uveitis in hierdie studie (n=35; 33.0%) waar moontlike IOTB (n=23; 21.7%) meer algemeen was as waarskynlike IOTB (n=12; 11.3%). ‘n Tuberkulien veltoets alleen was meer sensitief (90.3% vs 85.7%) en het ook ‘n hoër negatiewe voorspellende waarde (92.1% vs 81.5%) gehad as QuantiFERON alleen en laasgenoemde regverdig dus nie die addisionele finansiële uitgawe in hierdie hoogs endemiese gebied nie. Herpetiese uveitis was die tweede grootste groep (n=13; 12.2%) met VZV (53.8%) verantwoordelik vir meer gevalle as CMV (38.5%) en HSV (7.7%). Sifilis was die derde algemeenste oorsaak van infektiewe uveitis (n=11; 10.4%). Met behulp van ‘n nuwe immunoblot benadering is daar ondersoek ingestel na die verwantskap tussen okulêre sifilis en neurosifilis en is bewys dat dié 2 toestande nie altyd saam voorkom nie. HIV infeksie was teenwoordig in 31.4% van IOTB gevalle, 61.5% van herpetiese gevalle en 81.8% van sifilis gevalle. Toksoplasma (n=4; 3.8%), rubella-virus en poststreptokokkale uveitis (n=3; 2.8% elk) asook HIV-geinduseerde uveitis (n=1; 0.9%) was verantwoordelik vir die oorblywende infektiewe uveitis gevalle. EBV was dikwels teenwoordig op multipleks PKR (n=11; 10.4%) maar ons kon geen bewyse vind van aktiewe intraokulêre replikasie of teenliggaam produksie nie wat sou bewys dat EBV uveitis in hierdie gevalle veroorsaak het nie. In meeste gevalle is ‘n alternatiewe behandelbare oorsaak gevind (n=9; 81.8%).

Sarkoiedose en HLA-B27 geassosieerde anterior uveitis (n=8; 7.5% elk) was die algemeenste oorsake van non-infektiewe uveitis. Al die pasiënte met okulêre sarkoiedose en 75% van pasiënte met HLA-B27 uveitis was HIV-.

Die persentasie idiopatiese gevalle in hierdie studie was laer as in baie soortgelyke studies (n=18; 17.0%). Dit is waarskynlik as gevolg van die hoë persentasie gevalle met moontlike IOTB wat gediagnoseer is met ‘n onlangs gepubliseerde klassifikasie aangesien baie van daardie gevalle in ander studies as idiopaties beskou sou word. Die meerderheid idiopatiese gevalle was HIV- (n=12; 66.7%).

Hierdie studie toon dat infektiewe uveitis algemeenste vorm van uveitis is in beide HIV+ en HIV- pasiënte maar dat die spesifieke patogene verskil tussen pasiënte met en sonder HIV infeksie.

Dedication

I dedicate this dissertation to my late parents, Derrick and Carina Smit, for their love and for always believing in me. Thank you, Lord, for all our blessings.

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Chapter 1: Introduction and literature review

The term “uveitis” refers to inflammation of the uvea which is the vascular pigmented layer of the eyeball. From anterior to posterior, this layer consists of the iris, the ciliary body and the choroid. Conditions causing inflammation of the uvea may also affect other intraocular structures such as the retina, the vitreous humor and the optic nerve which are anatomically not part of the uvea.[1] In the ophthalmic literature, inflammation of the retina (retinitis), vitreous humor (vitritis) and optic nerve (optic neuritis) are often included under the umbrella term of uveitis although strictly speaking this is not accurate. The term “intraocular inflammation” would be preferable since it encompasses inflammation of any intraocular structures. It is, however, important to note that the terms “uveitis” and “intraocular inflammation” are often used interchangeably.

Uveitis is classified according to the anatomical site(s) of inflammation inside the eye using the Standardization of Uveitis Nomenclature working group’s system from 2005.[2] Uveitis may thus be classified as anterior, intermediate or posterior depending on where most of the inflammation is located. The term panuveitis is used when inflammation occurs throughout the eyeball. Based on clinical appearance, uveitis may also be subdivided into granulomatous or non-granulomatous.[3] Even though the clinical picture does not always correlate perfectly with histopathological findings, this subdivision often provides a useful clue as to where to start searching for a cause of the condition.

Uveitis is an important cause of ocular morbidity throughout the world. In Western countries, uveitis occurs in approximately 200 persons per 100 000[4] with up to 35 % of patients suffering severe visual disability as a result.[5] Between 5 and 10% of all cases of legal blindness in the United States and Europe are caused by uveitis.[6] In developing countries, uveitis is even more common and occurred in 1 out of every 140 persons (equivalent to 714 persons per 100 000) in a study from southern India.[7] Uveitis is also responsible for up to 25% of all blindness in these countries.[8-10]

The identifiable causes of uveitis may be divided into two main groups namely infectious and non-infectious. However, a definite cause was previously only found in 65% of cases with the remainder being considered idiopathic.[11] Due to recent improvements in the quality, quantity and availability of diagnostic modalities, the percentage of idiopathic cases is steadily decreasing. Infectious causes of uveitis such as tuberculosis and toxoplasmosis occur more commonly in developing countries while non-infectious causes such as sarcoidosis and HLA-B27 uveitis predominate in developed countries.[12] The prevalence of infectious and non-infectious causes of uveitis has to date not been comprehensively researched in South Africa with only 1 other research paper that appeared in 2016 before the results of this study were published.[13] It showed that uveitis was predominantly infectious in origin in the rural north-eastern corner of South Africa.

A pilot study was conducted at Tygerberg Academic Hospital to review the role of polymerase chain reaction (PCR) testing of ocular fluid in identifying different herpes viruses as probable infectious causes of uveitis. The study also sought to determine whether HIV status affects PCR findings. Out of 72 participants, 45.8% were HIV negative and 54.2% were HIV positive. PCR testing provided a positive result in 47.2% of cases and a significant correlation was found between a positive PCR yield and a positive HIV status (p=0.0018). Patients with posterior uveitis were also found to have a significantly increased PCR yield (p=0.014).[14] This study laid part of the foundation for a much larger and more detailed research project to further investigate the different causes of intraocular inflammation in patients with and without HIV infection in the Western Cape.

Non-infectious causes of uveitis

Non-infectious causes of uveitis include the following conditions: sarcoidosis [15-17], HLA-B27 uveitis [18-20], tubulo-interstitial nephritis and uveitis syndrome (TINU)[21-24], sympathetic ophthalmia [25-28] , Vogt-Koyanagi Harada disease [29-31], Behcet's syndrome [32-34], birdshot chorioretinopathy [35-37], serpiginous choroiditis [38,39], traumatic uveitis and lens-induced uveitis.[40] These conditions have been studied

extensively in other parts of the world but the prevalence of these conditions in South Africa is unknown.

Infectious causes of uveitis

The infectious causes of uveitis may be subdivided into four large groups namely bacterial, viral, parasitic and fungal. An infectious aetiology is suspected in many idiopathic conditions but has not yet been proven conclusively. The prevalence of infectious causes of uveitis in South Africa was unknown until very recently.

a) Bacterial causes

Bacterial causes of uveitis include mycobacteria and spirochetes as well as gram-positive and gram-negative bacteria. *Mycobacterium tuberculosis*[41-43] and *M leprae* are examples of mycobacteria that may cause uveitis while *Treponema pallidum*[44-47] and *Borrelia burgdorferi*[48,49] are spirochetes known to cause uveitis. Uveitis due to gram-positive bacteria may form part of a poststreptococcal syndrome[50,51] and gram-negative bacteria such as *Tropheryma whippelii* [52]and *Bartonella henselae* [53]have also been proven to cause intraocular inflammation. Bacterial endophthalmitis may be caused by various gram-positive and gram-negative organisms.

b) Viral causes

Several different viruses are known to cause intraocular inflammation in humans. The majority of these viruses are herpesviridae which include herpes simplex virus 1 and 2 (HSV-1 and HSV-2)[54-56], varicella-zoster virus (VZV)[54,55], Epstein-Barr virus (EBV)[57,58], cytomegalovirus (CMV)[59-61] and human herpes virus 6 (HHV6)[62,63]. Rubella virus[64-66] and HIV[67,68] may both cause uveitis while lesser known viruses such as human parechovirus[69] have also been proposed as possible causes of intraocular inflammation.

c) Parasitic causes

Parasitic causes of intraocular inflammation in humans include *Toxoplasma gondii*[70,71], *Toxocara cati, Toxocara canis*[72]and *Oncocerca volvulus*.[73] Ocular toxoplasmosis is one of the commonest causes of retinochoroiditis worldwide but whether this is also the case in South Africa still needs to be determined.

d) Fungal causes

Many species of fungi are known to cause intraocular inflammation, especially endophthalmitis. The three main fungi implicated in this setting are *Candida*[74-76], *Aspergillus* [75,76] and *Cryptococcus*[75]. Fungal endophthalmitis is a rare cause of intraocular inflammation but remains an important diagnosis to make since early treatment with specific anti-fungal agents can prevent extensive loss of vision.

Finding the cause of intraocular inflammation

First-line investigations

Historically, once a clinical diagnosis of uveitis was made, a standard battery of first-line screening investigations was requested to start looking for a specific cause. These investigations included a full blood count (FBC) and erythrocyte sedimenatation rate (ESR), serum creatinine, syphilis serology, HIV testing, serum angiotensin converting enzyme levels (sACE) and a chest X-ray (CXR). If the investigations all returned negative results the cause of the uveitis would be listed as “idiopathic” and the patient would be treated empirically with corticosteroids and other immunosuppressive agents as needed.

Today, however, if these tests are negative, a whole new array of investigations is being employed to enhance the search for an underlying cause. Many of these newer investigations are aimed at identifying infectious causes of uveitis. In some countries, these newer investigations have been in

routine clinical use for well over a decade but in South Africa these investigations have only recently become available and are not yet used routinely.

The role of PCR and GWC

In the 1990's, publications started appearing that reported the use of PCR and local antibody analysis on ocular fluids (both aqueous and vitreous humor) to look for the presence of herpes viruses.[77-79] One study found that PCR had a sensitivity of 95% for the diagnosis of untreated CMV retinitis in patients with AIDS [78] while another found that the sensitivity of local antibody analysis was much lower at 44%.[79] Subsequent studies started using qualitative multiplex PCR which enabled the investigators to test for more than one herpes virus at the same time and eventually quantitative real-time PCR was employed to not only determine the presence of a specific virus but also to quantify the viral load.[80,81] In a study from Thailand, real-time PCR was performed on ocular fluid samples from 100 HIV-negative patients and 47 HIV-positive patients with uveitis. Positive PCR results were found in 33% of HIV-negative patients and 70% of HIV-positive patients.[56] Other authors found that calculation of the Goldmann-Witmer coefficient which reflects local antibody production provides additional information to that obtained by PCR alone.[82,83] In their one study, GWC and PCR were both positive in 43% of cases while in 48% only GWC was positive and in 9% only PCR was positive. It was found that PCR detecting viral DNA tended to be positive early in infection while the GWC only became positive after a few weeks.[82] In their other study, GWC was found to be more informative in immunocompetent patients while PCR was useful in immunocompromised patients.[83] By combining the results of PCR and GWC testing one therefore increases the likelihood of obtaining a positive result over a longer period of time in both immune-competent and immunocompromised patients. GWC determination has also been shown to have a higher sensitivity than PCR for the diagnosis of both ocular toxoplasmosis[83] and rubella infection.[65,66] A recent literature search revealed only three articles originating from South Africa regarding the use of anterior chamber (AC) taps and aqueous humor (AH) analysis to diagnose the cause of uveitis, one of which was our own

pilot study.[14,84,85] This study is the first from South Africa to investigate the role of combined PCR and GWC testing for herpes viruses, rubella *and* toxoplasmosis in the diagnosis of infectious uveitis as the study by Schaftenaar et al used PCR and GWC to look for herpes viruses only. It is also one of the first studies worldwide to prospectively evaluate the role of PCR and GWC testing to diagnose infectious uveitis in HIV-positive and HIV-negative patients.

The role of Epstein-Barr virus (EBV) as a cause of uveitis remains unclear. The first three cases of presumed EBV-associated uveitis were described in 1990[57] but EBV has only ever been demonstrated histologically in the retina on one occasion.[86] More recent studies have reported finding high copy numbers of EBV DNA by quantitative PCR on ocular fluids although the significance of these findings remains uncertain.[87,88] In our study, we aimed to explore this role of EBV further by measuring the EBV viral load on specimens that tested positive by multiplex PCR as well as performing an EBV GWC to look for antibody production against the virus. If a high viral load is found in combination with significant antibody production it could indicate that EBV does act as a pathogen and is not merely present as an incidental commensal.

Where does ocular syphilis fit in?

Syphilis is caused by the spirochete *Treponema pallidum* and may cause ocular involvement during any of the four stages of disease namely primary, secondary, latent and tertiary syphilis.[89,90] During the secondary and tertiary stages of the disease, uveitis is the most common ocular manifestation of syphilis.[44] Embryologically, the optic nerve and retina are extensions of the brain and many authors contend that syphilitic retinitis and optic neuritis represent a form of neurosyphilis and should therefore be treated as such.[89,91,92] Whether syphilitic anterior uveitis should be considered in the same light is the subject of an ongoing debate. Many experts suggest that all cases of syphilitic uveitis (SU) should be considered identical to neurosyphilis while others are not yet convinced.[93] When co-infection with HIV enters the picture, the diagnosis of SU and neurosyphilis becomes even more complicated.

The diagnosis of SU is made, after exclusion of other possible causes, if a patient has ocular inflammation compatible with syphilis and positive syphilis serology which should include both a non-treponemal test and a treponemal test. Non-treponemal tests include the Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagin (RPR) which detect antibodies directed against membrane phospholipids such as cardiolipin. These tests are used to screen for active disease and to quantify antibodies but may give false positive results in diseases other than syphilis such as collagen vascular diseases.[44] A treponemal test such as the fluorescent treponemal antibody absorption (FTA-ABS) test is used to confirm current or previous infection. Treponemal test reversion may occur in 5 – 17% of patients who were treated for early syphilis. This contradicts the common misconception that these tests always remain positive after infection by T pallidum – the so-called serological scar.[44]

According to the Centres for Disease Control (CDC), confirmed neurosyphilis is diagnosed when VDRL testing is positive on cerebrospinal fluid (CSF) and probable neurosyphilis is diagnosed when CSF VDRL is negative but CSF protein and/or white cell count is elevated in the presence of clinical signs or symptoms which may include ocular findings.[94] However, CSF abnormalities such as higher mean white cell counts and protein levels are common in HIV-infected patients, even in the absence of syphilis. Diagnosing probable neurosyphilis in HIV-infected patients is therefore problematic.

The advent of techniques such as PCR has brought about interesting new diagnostic possibilities in both SU and neurosyphilis. PCR has been used to detect the presence of treponemal DNA in both aqueous and vitreous humor from eyes with suspected SU, thus confirming the diagnosis.[91,95-97] It has also been used to detect treponemal DNA in CSF from patients with neurosyphilis.[98] However, what still needed to be determined was whether performing PCR on both intraocular fluid and CSF from patients with suspected SU and neurosyphilis would enable us to develop a better understanding of how these two conditions relate to one another.

Western blotting is another technique that is able to confirm the diagnosis of syphilis by detecting antibodies to specific treponemal antigens. In a study that compared a Western blot to the FTA-ABS as a confirmatory test for syphilis both tests had sensitivities of 100% while the specificities were 100% and 94.5% for the Western blot and FTA-ABS respectively.[99] In another study, the Western blot had 93.8% sensitivity and 100% specificity compared to the 91.7% sensitivity and 92.0% specificity of the FTA-ABS.[100] Western blotting has previously been used to detect antibodies against *T pallidum* antigens in the CSF of patients with neurosyphilis[101] but there are no reports in the literature of it having been used for the detection of treponemal antibodies in aqueous or vitreous humor. The use of PCR to detect treponemal DNA and Western blotting to detect antibodies against *T pallidum* in the CSF and aqueous humor of patients suspected of having SU and neurosyphilis could potentially improve diagnostic accuracy and increase our insight into how these conditions relate to each other.

Could it be TB?

Intraocular TB (IOTB), also called TB-associated uveitis (TAU), is caused by *Mycobacterium tuberculosis* and represents a form of extrapulmonary tuberculosis (EPTB). In the United States, the proportion of EPTB has increased from 13.5 % in 1975 to 21.0% in 2006 and this phenomenon has been attributed to the rising prevalence of immune compromise.[42] HIV co-infection plays an important role in this setting since EPTB may occur in up to 70% of patients who suffer from concomitant TB and HIV infection.[42] Due to a combined lack of standardized diagnostic criteria as well as difficulty in making a laboratory diagnosis, the exact prevalence of IOTB is uncertain. In reports from India, a country where pulmonary TB (PTB) is endemic, uveitis was caused by TB in between 5.6 – 10.1% of cases.[43] The majority of patients with IOTB have no history of PTB or TB in any other organ while about 60% of patients with EPTB do not have evidence of PTB.[43]

IOTB has a multitude of clinical manifestations making diagnosis based on clinical findings alone extremely difficult. It is one of four intraocular inflammatory conditions that are collectively referred to as “the great mimickers in uveitis” and should therefore be considered in all patients with uveitis

– especially in areas where TB is endemic.[3,43] Also, depending on the clinical manifestation, the inflammation may either be the result of direct tissue invasion by the organism or a hypersensitivity reaction to tubercular antigens.[43,102]

By the time that this project started, diagnostic criteria had been proposed which allowed the clinician to make a diagnosis of either definitive ocular TB or presumed ocular TB.[41] Definitive (or confirmed) ocular TB was diagnosed when *M tuberculosis*, or its DNA, could be demonstrated in ocular fluids by microscopy, culture or PCR. Presumed ocular TB, on the other hand, was diagnosed when a suggestive clinical picture was combined with indirect evidence of TB infection provided other uveitis entities have been excluded. Examples of this indirect evidence include a positive tuberculin skin test (TST), signs of active or healed pulmonary PTB on chest X-ray (CXR) or chest CT, a positive interferon gamma release assay (IGRA) or a response to empirical anti-tuberculosis therapy (ATT).[3,41,42]

During the course of this project a revised classification was proposed by Gupta et al which now made provision for a diagnosis of confirmed, probable or possible IOTB.[103] They identified six clinical signs commonly found in IOTB and in their classification one or more of these signs had to be present before a diagnosis of IOTB could be entertained. If one or more of the signs were present along with PCR, culture or microscopic evidence of TB on a sample taken from the eye then IOTB was confirmed. If one or more of the signs were present *and* there was evidence of TB elsewhere in the body *and* there was a positive TST or IGRA then probable IOTB could be diagnosed. Lastly, if one or more signs were present and there was *either* evidence of TB elsewhere in the body *or* there was a positive TST and/or IGRA then possible IOTB could be possible. The caveat in diagnosing probable or possible IOTB was that all other possible causes of IOTB needed to be excluded first before making a diagnosis of IOTB. We made use of this second classification during our study and present our published findings later.

Direct microscopy of a smear does not often aid the diagnosis of IOTB since intraocular fluids do not yield many acid-fast bacilli (AFB). For the same reason, attempts to culture *M tuberculosis* from ocular fluids often lead to false-negative results. Older culture media such as Lowenstein-Jensen also require a protracted incubation period of up to 8 weeks which cause a significant delay in diagnosis. Newer culture media, such as Middlebrook 7H9 broth used in the Mycobacteria Growth Indicator Tube (MGIT), are able to provide a positive result in a shorter period of time (median 14 days) but they also only need to be incubated for 5 weeks before a negative result can be recorded.[104] The detectable limit of the MGIT test is stated as 10 organisms per ml which is usually adequate when analysing large volumes of fluid. However, the average AH sample size obtained during an AC tap varies between 0.1 – 0.2 ml which makes it easier to understand why the MGIT test has little chance of successfully culturing MTB from the eye. PCR to detect DNA from *M tuberculosis* in ocular fluids appears more useful. It can be performed on a very small amount of intraocular fluid since it amplifies the DNA in the specimen several times for easier detection. With early reported sensitivities between 37 – 47% and high specificity it has the potential to increase the number of confirmed diagnoses of IOTB.[43] More recent reports have shown that the sensitivity of PCR testing for IOTB is on the increase. In 2013, Sharma et al described a multi-targeted PCR using 3 targets specific for MTB namely IS6110, MPB64 and protein b. They reported a sensitivity of 77.77% and specificity of 100% respectively.[105] Later, the same researchers compared the sensitivity and specificity of devR PCR and MPB64 PCR for the diagnosis of IOTB. They found the sensitivity and specificity of devR PCR to be 64% and 100% respectively while that of MPB64 PCR was 72% and 100% respectively.[106] More recently, vitrectomy samples from 11 eyes were subjected to multi-targeted PCR, GeneXpert MTB/RIF assays and a line probe assay (GenoType MTBDRplus) to detect the MTB genome in cases of multifocal serpiginoid choroiditis.[107] The multi-targeted PCR was positive in 10 of 11 eyes while the line probe assay was positive in 6 of 11 eyes and the GeneXpert in 4 of 11 eyes. At present, no studies have been published that evaluate the use of a combination of MGIT culture and TB-specific PCR to make a diagnosis of confirmed IOTB.

A TST or Mantoux test uses an intradermal injection of 5 units of purified protein derivative (PPD) which is then read 48-72 hours later. In parts of the world where TB is non-endemic, a positive TST can aid the diagnosis of IOTB.[108-111] In the Western Cape, with its high prevalence of PTB, TST is not performed routinely in adults to screen for TB due to various reasons. These include the high prevalence of latent TB, the inability of the test to distinguish between active and latent TB and the fact that most adults in the region received Bacillus Calmette-Guérin (BCG) vaccinations during childhood. Interpretation of the test is said to be problematic in patients with HIV infection and false-positive results may be obtained in patients infected by non-TB mycobacteria (NTM).

IGRAs are blood tests that measure in vitro release of interferon-gamma (IFN-γ) from peripheral blood cells in response to stimulation by specific antigens derived from *M tuberculosis.* The one test, called T-SPOT.TB (Oxford Immunotec, Oxford, UK), is an enzyme-linked immunospot (ELIspot) assay which quantifies IFN-γ secreting T-cells whereas the other, QuantiFERON®-TB Gold (QFT) test (Cellestis Inc., Chadstone, Victoria, Australia), is an enzyme-linked immunosorbent assay (ELISA) that measures IFN-γ concentration in supernatant.[43] At the beginning of our study, the role of IGRAs in the diagnosis of IOTB was not yet completely understood. As is the case with TST, an IGRA cannot distinguish between active and latent infection.[112] An IGRA result is, however, not influenced by prior BCG vaccination since the mycobacterial antigens used differ from those in BCG nor is it affected by NTM.[110] Some studies, especially those from Singapore, have shown that TST was more sensitive in diagnosing IOTB than both T-SPOT.TB and QFT but that both the IGRAs were more specific in diagnosing IOTB.[111,113,114] These studies also showed that the likelihood of having ITOB is significantly increased if both the TST and IGRA are positive.[111,113] Another study has suggested that an IGRA should be considered instead of TST in immune-compromised patients as well as patients who had previously been vaccinated with BCG.[112] In South Africa, the majority of patients with suspected IOTB would fall into one or both of these categories. A study from France showed that patients with presumed IOTB who had a higher QFT value where more likely to respond to ATT than those with lower values.[115] In India, where TB is also highly prevalent, authors found the sensitivity

and specificity of QFT in detecting intraocular TB to be 82% and 76% respectively [116] while others reported favourable clinical outcomes in all QFT positive patients presumed to have IOTB who received ATT.[117] Only one of these studies mentioned the HIV-status of the participants and, of note, none of the patients tested in that study had HIV infection. The role of IGRAs in diagnosing IOTB in an area with a high prevalence of both TB and HIV infections therefore still needed to be determined and we present our findings later in this dissertation.

In most cases of TB, the lungs are the site of primary infection and imaging of the chest remains an important investigation in the workup of a patient with possible IOTB. A chest radiograph (CXR) is routinely requested as the first imaging modality since it is inexpensive and widely available. Unfortunately, the sensitivity of a CXR to detect PTB is relatively low and a normal CXR result does not exclude IOTB.[41,110] Computed tomography (CT) scanning of the chest is a more sensitive imaging modality which is superior to CXR in diagnosing hilar lymphadenopathy and subtle parenchymal changes.[110] Case series have been reported where both normal resolution chest CT and high resolution chest CT (HRCT) have enabled clinicians to diagnose IOTB in patients with normal CXRs.[118,119] In instances where both the CXR and chest CT are negative but a strong suspicion of IOTB remains based on a positive IGRA result, combined positron emission tomography (PET) and CT scans have been performed.[120,121] In one of these studies, evidence of metabolic activity in mediastinal or hilar lymph nodes was found in 9 of 20 (45%) patients with a positive QFT test while no abnormalities were only found in 4 of 20 (20%).[120] Metabolic activity alone is however not enough to support a diagnosis of TB and microbiological evidence of TB was eventually only obtained in 2 cases. In the Western Cape, a prospective study to evaluate the role of HRCT in diagnosing IOTB is feasible and should be explored further. Despite the availability of PET/CT at our institution the high cost of this investigation initially precluded it from further investigation in our setting. However, during the course of this study a collaboration between die Divisions of Ophthalmology and Nuclear Medicine was initiated and the resulting findings about the role of

PET/CT in diagnosing the underlying cause of uveitis will be submitted for publication as a Master of Medicine dissertation by a registrar in the Division of Nuclear Medicine.

Conclusion

There are many unanswered questions when it comes to finding a specific underlying cause in a patient with uveitis anywhere in the world. Recent advances in diagnostic modalities have increased our chances of finding a cause but there have not been many significant reports from South Africa about the value of these modalities in a local context. HIV infection alters the susceptibility to infection throughout the human body and it is unlikely that the situation in the eye will be any different. Syphilis and TB commonly occur in HIV-infected patients but, as yet, it is not known whether these conditions cause uveitis more frequently in HIV positive patients than in HIV negative patients. Also, the relationship between ocular syphilis and neurosyphilis remains uncertain – especially against a background of HIV infection. Our study aimed to start providing answers to these important questions.

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Chapter 2: Research question, aims and objectives

Research question:

Do the causes of intraocular inflammation differ between patients who are HIV-positive and HIV-negative in the Western Cape Province, South Africa?

Aim:

To compare the causes of intraocular inflammation in HIV-positive patients and HIV-negative patients to determine whether significant differences exist

Secondary aim:

1. To determine whether ocular syphilis may occur in the absence of neurosyphilis
2. To evaluate the contribution of different special investigations in making the diagnosis of ocular tuberculosis in a endemic area

Study design:

Cross-sectional analytical study

Objectives:

In HIV-positive and HIV-negative patients in the Western Cape:

1. To determine and compare the prevalence of *non-infectious* causes of intraocular inflammation
2. To determine and compare the prevalence of *viral* causes (herpes viruses, rubella, HIV) of intraocular inflammation by analysis of aqueous humor and blood samples
3. To determine and compare the prevalence of *parasitic* causes (toxoplasma) of intraocular inflammation by analysis of aqueous humor and blood samples

4. To determine and compare the prevalence of *ocular syphilis* and to study the relationship between ocular syphilis and neurosyphilis

5. To determine and compare the prevalence of *ocular tuberculosis* and to ascertain the value of different special investigations in making this diagnosis

6. To determine and compare the prevalence of *idiopathic* causes of intraocular inflammation

Secondary objectives:

1. To test aqueous humor and CSF samples of patients with positive syphilis serology to look for treponemal DNA and/or anti-treponemal antibodies

2. To perform radiological, endoscopic, serological and microbiological investigations in patients with suspected ocular tuberculosis to determine which tests are most useful in our setting

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Abstract

Purpose: To report the prevalence of intraocular tuberculosis in South Africa using a revised classification system

Methods: A prospective study to determine the underlying etiology in patients presenting with uveitis to a tertiary Eye Clinic.

Results: Thirty-five of 106 patients (33.0%) were diagnosed with intraocular tuberculosis of which 11 (31.4%) had HIV infection. Twenty-three patients (65.7%) had possible intraocular tuberculosis and 12 (34.3%) probable intraocular tuberculosis. Patients with probable intraocular tuberculosis were younger than those with possible intraocular tuberculosis (p=0.003). More males (66.7%) had probable intraocular tuberculosis and more females (73.9%) had possible intraocular tuberculosis (p=0.031). More HIV positive patients had probable intraocular tuberculosis and more HIV negative patients had possible intraocular tuberculosis (p=0.002).

Conclusions: South Africa has a high prevalence of intraocular tuberculosis. Younger, male, HIV positive patients more likely have probable intraocular tuberculosis while older, female, HIV negative patients more likely have possible intraocular tuberculosis.

Keywords: intraocular tuberculosis; HIV; prevalence; South Africa; classification

Introduction

Mycobacterium tuberculosis (MTB) infection in humans is common worldwide but nowhere more so than in Africa. In 2014, 9.6 million people were estimated to have fallen ill with TB worldwide and Africa accounted for 28% of these cases. The incidence on the African continent is 281 cases per 100 000 people as compared to the global average of 133 cases per 100 000 people.[1]

Of the 9.6 million people who contracted TB worldwide in 2014, an estimated 1.2 million (12%) were HIV positive and Africa accounted for 74% of cases. In 2014 the prevalence of TB in South Africa was 696 cases per 100 000 population and the incidence 834 cases per 100 000 – almost three times more than on the rest of the continent and by far the highest of the 22 high-burden countries highlighted in the WHO annual report. In South African TB patients, 61% were reported to have HIV co-infection which is five times higher than the global figure. [1] In patients with active TB there is wide variation in the reported prevalence of intraocular TB (IOTB). IOTB occurs more commonly in patients with extrapulmonary TB (>20%) than in those with pulmonary infection (±1%).[2] Furthermore, the reported rates of IOTB also vary by region with less than 1% occurring in North America compared to more than 10% in highly endemic areas.[3-5]

The difficulty of confidently diagnosing IOTB due to the absence of gold standard tests is well documented and this has led to the proposal of classification systems that make provision for different levels of certainty with which IOTB can be diagnosed.[6] In 2007, Gupta et al proposed a classification which enabled clinicians to diagnose either “confirmed IOTB” or “presumed IOTB” based on the amount of supporting evidence available to them.[4] More recently, in 2014, an updated classification was proposed which now provides criteria to diagnose IOTB as either “confirmed”, “probable” or “possible”.[7] The rationale for adding the “possible IOTB” category was to enable

clinically ambiguous cases to also be diagnosed as IOTB which was not possible when using the earlier classification.

A recent study by Schaftenaar et al reported that TB was found to be the cause of uveitis in 18 of 103 cases (17.5%) in the Limpopo Province of South Africa.[8] These findings were based on Xpert MTB/RIF assays in patients with productive cough and chest X-rays taken in patients with suspected TB. In this report we shall describe our findings regarding the prevalence of IOTB in uveitis patients with and without HIV infection in South Africa using the most recently proposed classification mentioned above.

Materials and methods

Study participants and overview of management

A prospective study was conducted where 106 consecutive patients presenting with either a new diagnosis of uveitis or chronic uveitis of unknown cause were enrolled between February 2014 and July 2015. They presented to the Eye Clinic at Tygerberg Academic Hospital, a tertiary referral hospital in the northern suburbs of Cape Town, South Africa. Ethics approval was obtained from the Health Research Ethics Committee of Stellenbosch University (Ref no N13/10/146). Participants were excluded if they: 1) were under 18 years of age, 2) had uveitis with known or clinically obvious cause and 3) were not willing to consent to HIV testing after appropriate counselling. After enrolment patients completed a detailed systemic review questionnaire followed by a full ocular examination and a standardised panel of investigations as set out below.

Investigations

All of the participants underwent blood tests to determine their HIV status, full blood count, erythrocyte sedimentation rate, creatinine, venereal diseases research laboratory test and Treponema pallidum antibodies for syphilis as well as serum angiotensin converting enzyme levels. Chest X-rays and dipstick urinalysis were also requested in all cases. A tuberculin skin test (TST) could

only be done in 89 participants as there was an international shortage of purified protein derivative (PPD) during a part of the study. The participants received a 0.1ml intradermal injection of 5 units of PPD-S 5TU to the volar aspect of the forearm and the reaction was measured with a ruler 48 – 72 hours later. The TST was considered positive if it was >10 mm in HIV negative participants and >5 mm in HIV positive participants.[4]

A QuantiFERON®-TB Gold (QFT) test (Cellestis Inc., Chadstone, Victoria, Australia) was performed according to the manufacturer's instructions in 105 participants with blood being taken before intradermal injection of PPD for the TST in all cases. The QFT result was considered positive if the TB Antigen minus Nil value was ≥ 0.35 IU/mL and >25% of the Nil value. An HLA-B27 test was only requested in cases with severe fibrinous anterior uveitis and Anti-Streptolysin O titers were only requested in participants under 40 years of age. Anterior chamber taps were subsequently performed in cases where baseline testing was normal and aqueous humor (AH) samples were tested for toxoplasma, herpes viruses and rubella. A multiplex polymerase chain reaction (PCR) test was used to test AH samples for herpes viruses 1 to 6 as previously described.[9] Goldmann-Witmer Coefficient determinations were performed on AH and serum for toxoplasma, herpes simplex virus, varicella-zoster virus and cytomegalovirus at the University Medical Centre Utrecht, Netherlands.[10,11]

In cases where a high index of suspicion for IOTB existed after all other potential causes had already been excluded AH samples were also collected for Mycobacteria Growth Indicator Tube (MGIT) culture and IS6110-targeted TB PCR testing.[12]

Statistical analysis

IBM SPSS version 23 was used to analyse the data. A p value <0.05 was considered as statistically significant. Data were summarised using mean, standard deviation and range in the case of quantitative normally distributed variables, and median and interquartile range for ordinal or skewed variables. Nominal and binary data were represented in frequency tables. Associations

between categorical variables were represented in contingency tables with Pearson's chi square or Fisher's exact tests as appropriate. Means were compared between groups using t-tests or ANOVA as appropriate and medians were compared using Mann Whitney or Kruskal-Wallis tests according to the number of groups being compared.

Results

Demographics and clinical findings

A total of 106 consecutive participants with uveitis were enrolled during the course of our study. After consideration of the clinical findings and the results of the special investigations, 71 participants (67.0%) were either diagnosed with an underlying etiology other than TB or no specific cause was found. Figure 1 illustrates that in the remaining 35 participants (33.0%), 23 fulfilled the criteria to be classified as possible IOTB (65.7%) while 12 were classified as probable IOTB (34.3%). The criteria used to diagnose possible or probable IOTB in each of these cases are tabulated in Table 1.

No cases of confirmed IOTB were recorded since all TB cultures and TB PCR tests were negative. The mean age of patients diagnosed with possible IOTB was 42.7 ± 14.3 years while the mean age of patients diagnosed with probable IOTB was 31.1 ± 7.2 years (Table 2). Patients diagnosed with probable IOTB were therefore younger than those diagnosed with possible IOTB (P=0.003). A total of 21 females (60.0%) and 14 males (40.0%) were diagnosed with IOTB. More males (66.7%) than females (33.3%) were diagnosed with probable IOTB while more females (73.9%) than males (26.1%) were diagnosed with possible IOTB (P=0.031). These differences could however not be ascribed to differences in HIV status between the two genders. Seventeen cases (48.6%) had bilateral and 18 cases (51.4%) unilateral involvement. No association existed between laterality and a diagnosis of possible or probable IOTB (P=0.11). Anatomically, 19 cases had anterior uveitis (54.3%), 15 panuveitis (42.9%) and 1 posterior uveitis (2.9%). Of the 19 cases with anterior uveitis, 13 were HIV-

and 6 were HIV+ while of the 15 cases with panuveitis 10 were HIV- and 5 were HIV+. No significant relationship between anatomical involvement of uveitis and HIV status could be demonstrated (P=0.48). A diagnosis of possible IOTB was made in 15 of the 19 anterior uveitis cases while in the other 4 cases a diagnosis of probable IOTB was made. In 8 of the 15 panuveitis cases possible IOTB was diagnosed while probable IOTB was diagnosed in 7 cases. Anatomical distribution of uveitis was not related to a diagnosis of possible or probable IOTB in this study (P=0.11). Clinical features suggestive of a granulomatous uveitis were present in 22 cases (62.9%) and absent in 13 cases (37.1%). In patients with granulomatous uveitis, 12 cases (54.5%) had anterior uveitis, 9 cases (40.9%) had panuveitis and 1 case (4.5%) had posterior uveitis.

HIV status

IOTB was diagnosed in 35 cases of which 11 were HIV+ (31.4%) and 24 were HIV- (68.6%). In the HIV+ patients the median CD4+ cell count was 249 x 10^6/L (range 809 x 10^6/L). Of the 11 HIV+ cases, 8 had probable IOTB and 3 had possible IOTB while of the 24 HIV- cases 4 had probable IOTB and 20 possible IOTB (Figure 1). HIV+ patients were therefore more likely to have a diagnosis of probable IOTB than possible IOTB (RR=4.36; 95% CI 1.66 - 11.45) (P=0.002). Conversely, HIV- patients had a higher chance of being diagnosed with possible IOTB than probable IOTB (RR=3.06; 95% CI 1.15 - 8.15) (P=0.002). As stated previously, participants with possible IOTB were older than participants with probable IOTB but this did not take HIV status into account. In HIV- cases, participants with probable IOTB were younger (Mean age 28.0 ± 9.5 years) than those with possible IOTB (Mean age 43.0 ± 14.4 years) while in HIV+ cases participants with probable IOTB were also younger (Mean age 32.6 ± 5.8 years) than those with possible IOTB (40.7 ± 16.3 years) thus demonstrating a trend for participants with possible IOTB to be older than participants with probable IOTB regardless of HIV status (P=0.06). In 3 cases (numbers 2, 4 and 7) a diagnosis of probable IOTB was made despite the participants not having immunological evidence of TB infection. In all 3 cases the QFT test was negative, two had a negative TST and one did not have a TST result available. However, in all 3 cases

there was other evidence of extraocular TB as tabulated and all 3 cases had advanced HIV infection with mean CD4+ count of 68 x 10^6/L (range 9 x 10^6/L). In the group of 22 participants who had granulomatous uveitis 8 were HIV+ (36.4%) and 14 were HIV- (63.6%). The median CD4+ count in the HIV+ group with granulomatous uveitis was 220 x 10^6/L compared to 249 x 10^6/L in the HIV+ group with non-granulomatous uveitis (p=0.63). In the HIV+ group the median CD4+ count of participants with anterior uveitis was 464 x 10^6/L compared to 72 x 10^6/L in participants with panuveitis (p=0.068).

Discussion

The prevalence of IOTB has not been previously reported from South Africa which is the country with the highest prevalence and incidence of TB in the world.[1] In our study based in a tertiary referral hospital in the northern suburbs of Cape Town, 35 out of 106 participants (33.0%) had clinical and other findings meeting the revised criteria proposed by Gupta et al[7] for a diagnosis of either possible or probable IOTB after other possible causes had been rigorously excluded. At first glance, this prevalence of 33.0% appears high when compared to published numbers from other parts of the world. In the United States the prevalence has been reported as 1%, in China and Italy 4% and 6% respectively while in Saudi Arabia 16%.[3] Figures originating from India range from as low as 0.39 – 1.39%[13] to as high as 20.8%.[14,15] If one however compares the overall prevalence of TB in China (89 cases per 100 000) and in India (195 cases per 100 000) to that of South Africa (696 cases per 100 000) it becomes clear that TB in South Africa is 7 times more prevalent than in China and at least 3 times more prevalent than in India.[1] The same WHO report also shows that South Africa has 61% HIV+ TB patients compared to the 2% in China and 4% in India. It can therefore be expected that the prevalence of IOTB in South Africa should be higher than in other countries.

In this study we found that in all patients diagnosed with IOTB, those with probable IOTB were significantly younger than those with possible IOTB. This trend, whilst not reaching statistical significance, also persisted when the patients with IOTB were divided into HIV+ and HIV- subgroups

which seems to indicate that this phenomenon was not necessarily linked to a patient's HIV status. In order to diagnose probable IOTB when using the revised classification, the clinician needs some radiological or microbiological evidence of TB infection elsewhere in the patient's body while to diagnose possible IOTB such evidence is not required. It therefore appears that in our study cohort younger patients were more likely to have evidence of active infection while older patients only had evidence of prior exposure to TB which most likely reflects their prolonged exposure to MTB in a highly endemic environment. The strong male preponderance of probable IOTB and the strong female preponderance of possible IOTB is more difficult to unravel. A difference in HIV status between the two genders was considered as a possible explanation but statistical analysis could not validate this theory.

This study demonstrated that HIV+ patients were more likely to have a diagnosis of probable IOTB while HIV- patients were more likely to have a diagnosis of possible IOTB. It stands to reason that HIV+ patients with a compromised immune system would be more susceptible to active TB infection while the converse should apply to HIV- patients. The 3 HIV+ cases who were diagnosed with probable IOTB despite not having immunological evidence of TB infection illustrate this point. All 3 cases suffered from severe immunosuppression with CD4+ counts between 63 x 10^6/L and 72 x 10^6/L which most likely explains why they were unable to mount a positive immunological response in the first instance. They all did however have evidence of extraocular TB including a pleural effusion, positive sputum culture and chest CT suggestive of pulmonary TB. In our opinion this highlights the fact that in HIV+ patients with low CD4+ counts (<100 x 10^6/L) one cannot rely too heavily on immunological evidence of TB infection as a diagnostic criterion as these tests will often produce false negative results.

A clinical picture suggestive of granulomatous uveitis was more common in HIV- participants. However, in the HIV+ group the median CD4 count was lower in participants with granulomatous uveitis which shows that a granulomatous picture does not require a high CD4+ count. In fact,

granulomatous uveitis was seen in participants with CD4+ counts as low as 54 x 10^6/L. In the HIV+ group the median CD4+ count of participants with anterior uveitis (464 x 10^6/L) was higher than those with panuveitis (72 x 10^6/L) and, although we could only demonstrate a trend due to the small numbers involved, this might support the theory that panuveitis results from direct mycobacterial invasion while anterior uveitis is more likely an immune response against circulating antigens.[3]

The revised classification we used in this study enabled us to assign a diagnosis of IOTB to patients who we previously would have had to label as idiopathic using earlier classifications. However, despite the improvements incorporated into this classification, we still encountered cases during the course of the study where we could not diagnose patients as having IOTB despite having a high index of clinical suspicion as they did not meet all of the required criteria. We acknowledge that the protean manifestations of ocular TB make it virtually impossible to include an exhaustive list of clinical signs in the classification that could be indicative of TB.[16]

For this reason, and based on our experience, we are of the opinion that a positive response to a trial of anti-TB treatment (ATT) remains valuable in cases where ocular TB is suspected but diagnostic criteria cannot be met. . In immunocompetent patients with a positive QFT and/or TST and compatible clinical signs that *aren't* contained in the current criteria a positive trial of therapy could support a diagnosis of possible IOTB. In the setting of advanced HIV infection this becomes even more important as we have demonstrated that these patients often have false negative immunological tests.

A potential limitation of our study is that we were only able to perform PCR testing targeting IS6110 at the time the study was conducted. Since then it has been reported that multi-targeted PCR is more sensitive in the diagnosis of IOTB. In one study where IS6110, MPB64 and protein b were targeted the sensitivity and specificity were 77% and 100% respectively.[17] Another study showed that devR PCR alone was not as sensitive as MPB64 PCR alone but a combination of the two targets

increased the sensitivity to 80%.[18] Future studies in our highly endemic setting should therefore consider using multi-targeted PCR to help diagnose IOTB.

In a recent development from India, new guidelines for the management of extrapulmonary TB have been introduced (INDEX-TB Guidelines: http://icmr.nic.in/guidelines/TB/Index-TB%20Guidelines%20-%20green%20colour%202594164.pdf). These guidelines contain an updated classification of IOTB which now consists of three new diagnostic categories namely possible, clinically diagnosed and bacteriologically confirmed ocular TB. It also lists molecular evidence of MTB infection as one of the diagnostic criteria for possible ocular TB which could prove useful in HIV+ patients with a low CD4+ count who are prone to false negative TST and/or IGRA results. Further research is required to determine how useful this new classification will be in a clinical setting.

Conclusion

South Africa has the highest incidence and prevalence of TB in the world and the prevalence of IOTB calculated in this study is also higher than those reported from other countries. The revised classification proposed for the diagnosis of IOTB succeeded in making it possible to diagnose clinically ambiguous cases as IOTB which would not have been possible if the earlier classification system was used. The newly introduced INDEX-TB guidelines provide an updated classification of IOTB which could be used in future research especially in settings with a high prevalence of both TB and HIV.

Figure 1 Diagnoses and HIV status

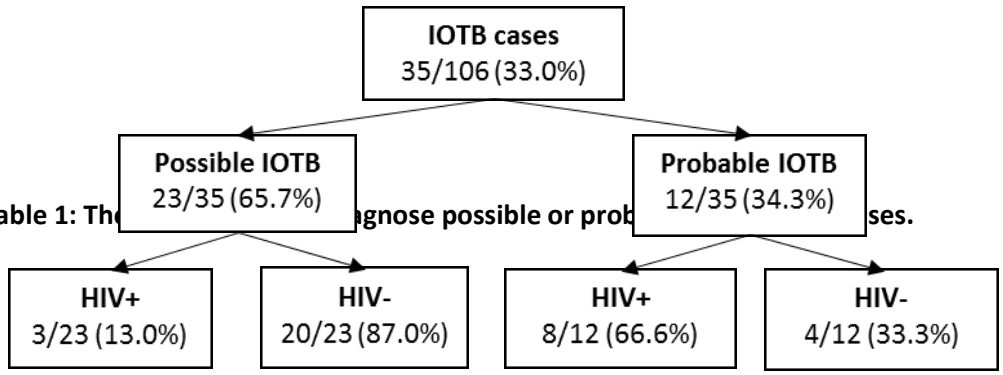

Table 1: Th ...gnose possible or prob... ...ses.

TABLE 1. The criteria used to diagnose possible or probable IOTB in our cases.

No.	Age[a]	Gender	Eye(s)	Anat. dist.	Granulomatous?	VA OD	VA OS	HIV-	CD4 ($\times 10^6$/L)	QFT (IU/mL)	TST (mm)	TST result	IOTB	Ocular findings	Systemic findings
1	45	F	OU	Panuveitis	N	CF near	CF 1 m	Neg	N/R	>10	20	Pos	Possible	Broad PS, multifocal choroiditis	Nil
2	35	M	OS	Panuveitis	N	1.2	CF 3 m	Pos	72	N	N/A	N/A	Probable	Multifocal choroiditis	Right pleural effusion, on TB Rx at presentation
3	20	M	OU	Posterior	Y	1.2	0.15	Neg	N/R	5.67	>20	Pos	Probable	Multifocal serpiginoid choroiditis, choroidal granuloma	Excellent response to TB Rx
4	30	M	OU	Panuveitis	Y	0.3	PL+	Pos	63	N	0	Neg	Probable	Broad PS, retinal perivasculitis and granulomas	Previous positive sputum culture
5	50	F	OU	Anterior	Y	CF 3 m	0.7	Neg	N/R	N	20	Pos	Possible	Mutton-fat KP, broad PS	Nil
6	54	F	OU	Panuveitis	Y	0.15	0.2	Neg	N/R	>10	20	Pos	Possible	Broad PS, Busacca nodules, choroidal scars	Nil
7	30	F	OS	Panuveitis	Y	1	PL+	Pos	69	N	0	Neg	Probable	Choroidal granuloma	Chest CT suggestive of PTB, better on Rx
8	29	M	OU	Panuveitis	N	0.1	HM	Neg	N/R	>10	18	Pos	Probable	Broad PS, disc swelling	CXR suggestive of PTB
9	35	F	OU	Panuveitis	Y	0.15	NPL	Neg	N/R	>10	>20	Pos	Possible	Broad PS, evidence of previous retinal perivasculitis	Granulomatous inflammation on histology. No AFBs
10	28	F	OS	Anterior	Y	1	1	Pos	54	>10	0	Neg	Probable	Broad PS, Busacca nodule	On TB Rx at presentation
11	39	M	OS	Anterior	N	1	0.4	Neg	N/R	>10	16	Pos	Possible	Broad PS	Pulmonary nodules on chest CT not typical of TB
12	56	F	OS	Anterior	Y	0.8	0.6	Neg	N/R	>10	17	Pos	Possible	Broad PS, mutton-fat KP	Previous Rx for pulmonary and cutaneous TB
13	41	M	OU	Panuveitis	Y	HM	CF 1m	Neg	N/R	>10	>20	Pos	Probable	Broad PS, Busacca nodules	Lymph node fine-needle aspiration positive for TB
14	40	F	OD	Anterior	Y	0.7	1	Neg	N/R	>10	>20	Pos	Possible	Broad PS, mutton-fat KP	Nil
15	40	F	OS	Anterior	N	1	0.15	Neg	N/R	>10	20	Pos	Possible	Broad PS, optic neuropathy	Nil
16	22	F	OS	Panuveitis	Y	1	CF near	Neg	N/R	>10	>20	Pos	Probable	Choroidal granuloma, optic neuropathy	Active lymph nodes on PET/CT

Table 1 continued

17	44	M	OU	Anterior	Y	CF 2 m	HM	Pos	513	0.87	N/A	N/A	Probable	Broad PS, mutton-fat KP	PET/CT suggestive of active pulmonary TB
18	50	M	OU	Anterior	N	0.7	1	Neg	N/R	4.45	N/A	N/A	Possible	Broad PS	Previous PTB
19	66	F	OU	Anterior	Y	0.15	0.2	Neg	N/R	>10	N/A	N/A	Possible	Broad PS, Koeppe and Busacca nodules	Nil
20	35	M	OU	Anterior	Y	1	0.5	Pos	415	6.48	>20	Pos	Probable	Broad PS, mutton-fat KP, Koeppe nodules	AFBs on bronchoalveolar lavage
21	28	F	OD	Panuveitis	N	NPL	0.8	Pos	249	0.51	18	Pos	Possible	Broad PS, retinal detachment on B scan ultrasound	Nil
22	50	F	OD	Anterior	Y	PL+	1	Neg	N/R	0.91	19	Pos	Possible	Broad PS, Koeppe nodules	Nil
23	32	F	OU	Panuveitis	N	0.6	CF 2m	Neg	N/R	8.72	20	Pos	Possible	Optic neuropathy	Nil
24	21	M	OU	Anterior	N	1	1	Neg	N/R	3.36	18	Pos	Possible	Broad PS	Nil
25	70	F	OU	Anterior	Y	HM	HM	Neg	N/R	>10	20	Pos	Possible	Broad PS, Berlin nodule	Nil
26	36	F	OS	Anterior	Y	1	0.3	Neg	N/R	N	20	Pos	Possible	Broad PS, Koeppe nodules	Nil
27	48	M	OU	Anterior	N	0.6	NPL	Neg	N/R	>10	>20	Pos	Possible	Broad PS	Nil
28	25	M	OS	Anterior	Y	1	0.8	Pos	338	0.65	18	Pos	Probable	Broad PS, Busacca nodule	On TB Rx at presentation
29	17	F	OU	Panuveitis	N	0.7	NPL	Neg	N/R	>10	>20	Pos	Possible	Broad PS	Nil
30	28	M	OD	Panuveitis	Y	HM	1	Neg	N/R	9.73	20	Pos	Possible	Optic nerve granuloma, discrete choroiditis	Nil
31	58	F	OS	Anterior	N	0.4	CF near	Neg	N/R	7.3	20	Pos	Possible	Broad PS	Nil
32	34	F	OS	Panuveitis	Y	1	NPL	Pos	102	2.28	20	Pos	Probable	Broad PS, mutton-fat KP	On TB Rx at presentation
33	59	M	OD	Anterior	N	0.7	1	Pos	863	>10	>20	Pos	Possible	Broad PS	Nil
34	25	F	OS	Panuveitis	Y	1	0.4	Neg	N/R	>10	17	Pos	Possible	Retinal perivasculitis, multifocal serpiginoid choroiditis	Excellent response to TB Rx
35	35	F	OD	Anterior	Y	0.2	1.5	Pos	610	0.45	12	Pos	Possible	Broad PS, Koeppe and Busacca nodules	Nil

Anat. dist., anatomic distribution; F, female; M, male; CF, counting fingers; PL+, perception of light with projection; HM, hand movements; NPL, no perception of light; N/R, not requested; N/A, not available; PS, posterior synechiae; KP, keratic precipitates; Rx, treatment; AFBs, acid-fast bacilli.

[a]Age in years.

Table 2: Demographics and anatomical classification of study cohort

Characteristics	All IOTB (n=35)	Possible IOTB (n=23)	Probable IOTB (n=12)	p-value
Age, years (±SD)	38.7 (13.4)	42.7 (14.3)	31.1 (7.2)	0.003
HIV+		40.7 (16.3)	32.6 (5.8)	
HIV-		43.0 (14.4)	28.0 (9.5)	0.06
Gender (%)				
Male	14 (40.0)	6 (26.1)	8 (66.7)	
Female	21 (60.0)	17 (73.9%)	4 (33.3)	0.031
Anatomical distribution[a] (%)				
Anterior	19 (54.3)	15 (65.2)	4 (33.3)	
Intermediate	0 (0)	0 (0)	0 (0)	
Posterior	1 (2.9)	0(0)	1 (8.3)	
Panuveitis	15 (42.9)	8 (34.8)	7 (58.3)	0.11
Laterality (%)				
Right eye	6 (17.1)	6 (26.1)	0 (0)	
Left eye	12 (34.3)	6 (26.1)	6 (50.0)	
Both eyes	17 (48.6)	11 (47.8)	6 (50.0)	0.11
HIV (%)				
Positive	11 (31.4)	3 (13.0)	8 (66.7)	
Negative	24 (68.6)	20 (87.0)	4 (33.3)	0.002

[a] Standardized Uveitis Nomenclature (SUN) Working Group criteria

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Classification of Intraocular Tuberculosis: A South African Perspective

Dear Editor

We read the paper about the new classification of intraocular tuberculosis (IOTB) proposed by Gupta et al[1] with great interest as we work in the Eye clinic of a tertiary hospital in suburban Cape Town, South Africa. According to the 2015 WHO Global tuberculosis report, South Africa has both the highest incidence (834 cases per 100 000) and prevalence (696 cases per 100 000) of TB of any country worldwide.[2] We used the new classification in a prospective study that included 106 consecutive patients, with and without HIV infection, presenting to our clinic with uveitis over a period of 17 months and found that 35/106 (33.0%) patients could be classified as having either possible or probable IOTB. (unpublished data)

The new classification enabled us to diagnose IOTB in clinically ambiguous cases where such a diagnosis would not have been possible using earlier classifications.[3] We did however discover that we would have been able to diagnose more cases of IOTB if the new classification included a few minor modifications. We present two cases, one HIV negative and the other HIV positive, to support this view.

Case 1

A 34-year-old lady presented with a 4 year history of left ocular discomfort and redness. She was referred to us by an endocrinologist to whom she had presented with a Cushingoid appearance

secondary to prolonged oral corticosteroid use. She had previously consulted several other ophthalmologists but a definitive cause for her eye problem had not been found. On examination she had an uncorrected visual acuity (VA) of 0.7^{-1} in the affected eye which corrected to 1.0 with a pinhole. Both the episcleral and scleral blood vessels were diffusely injected and the sclera had a bluish tinge superiorly (Figure a). Her cornea showed areas of stromal scarring with deep stromal blood vessels at the 3 o'clock position. Corneal sensation was intact. A trace of flare and cells was present in the anterior chamber and the rest of the examination was normal. A clinical diagnosis of sclerokeratouveitis was made and investigations were requested to search for an underlying cause. HIV testing, syphilis serology and HLAB27 were negative. The full blood count (FBC), erythrocyte sedimentation rate (ESR), serum angiotensin converting enzyme (sACE), dipstick urinalysis, chest X-ray (CXR) and high resolution chest CT (HRCCT) were all normal. Aqueous humour PCR testing for herpesviruses 1 – 6 was negative. Both her Quantiferon (QFT) interferon gamma release assay (7.25 IU/mL) and tuberculin skin test (TST = 20mm) showed strong positive results. Given the absence of other positive findings as well as the chronicity and side effects of corticosteroid treatment it was decided to start a trial of ATT with rifampicin, isoniazid, pyrazinamide and ethambutol. Three weeks later a significant improvement was noted in her left eye and she reported that she had discontinued all other medication one week after commencing ATT due to the dramatic positive response to treatment (Figure b).

Case 2

A 30-year-old lady, on antiretroviral (ARV) treatment for 9 years, presented with a 3 week history of reduced vision, pain and redness in her left eye. Her right eye was unaffected. She had been treated for pulmonary TB twice before in 2001 and 2005. The VA in her left eye was light perception with poor projection in all four quadrants. She was found to have scanty keratic precipitates and 2+ cells with some fibrin in the anterior chamber. The vitreous humor also contained 2+ cells and fundus examination revealed multiple subretinal masses with elevation of the sensory retina (Figure c). A

left-sided panuveitis was diagnosed clinically and investigations were requested to look for an underlying cause. Despite ARV treatment her CD4+ count was 69 x 10^6/L. The FBC and sACE were normal, syphilis serology was negative but ESR was raised at 104 mm/hr. Both QFT (0.01 IU/mL) and TST (0 mm) were negative and CXR was normal. HRCCT showed a 16 x 15 x 23mm mass in the right lower lobe as well as small nodules with "tree in bud" configuration in the right middle lobe but sputum microscopy, TB culture and GeneXpert testing were negative. Broncho-alveolar lavage (BAL) was considered but given her previous history of PTB, her low CD4+ count, her chest CT findings and the high clinical index of suspicion for IOTB a trial of four-drug ATT (as above) was prescribed after consultation with our Infectious Diseases specialists, despite the fact that she did not meet the criteria for a diagnosis of IOTB according to the new classification. A repeat HRCCT 3 months after initiation of treatment showed complete resolution of the lung pathology and 4 months after commencing ATT her left eye no longer showed any signs of inflammation (Figure d).

These case reports illustrate that the proposed classification as it currently stands will under certain circumstances not lead to a diagnosis of IOTB even if it is strongly suspected clinically and there are various reasons for this. Firstly, the classification does not include the full spectrum of clinical signs compatible with a diagnosis of ocular TB as this would presumably make it too cumbersome.[4] Although the emphasis here is on *intraocular* TB it is well known that TB may cause scleritis with or without associated corneal or intraocular involvement and therefore including scleritis in the classification as one of the suggestive clinical signs would allow it to identify more cases of ocular TB. Secondly, the classification does not make provision for unusual presentations of IOTB in immunocompromised patients. Babu et al[5] found that in HIV/AIDS cases choroidal granulomas were still the most common manifestation of IOTB but that subretinal masses and panophthalmitis could also occur. Thirdly, the classification does not address the effect of severe immunosuppression on QFT and TST results which often become false negative when CD4+ counts drop below 100 x 10^6/L. We would therefore suggest that immunological evidence of TB infection not be considered essential to diagnose IOTB in severely immunocompromised patients provided the rest of the

criteria are met. Lastly, in contrast to the earlier classification,[3] the new classification does not contain a positive trial of ATT as a diagnostic criterion. As illustrated in both cases, a positive therapeutic trial can be very valuable in cases where the clinical presentation does not include the clinical signs considered suggestive of IOTB and/or immunological evidence of TB infection cannot be obtained in severely immunocompromised individuals.

In our experience, the most recently proposed classification of IOTB by Gupta et al has succeeded in its goal of increasing the number of cases of intraocular inflammation that can correctly be attributed to TB and has proven valuable in our clinical practice. However, in our highly endemic setting we have found that by including a few more suggestive clinical signs, making certain exceptions in severely immunocompromised cases and reintroducing a positive trial of ATT as a diagnostic criterion we could potentially further improve the accuracy with which this classification identifies cases of IOTB.

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David Meyer FCOphth(SA), PhD

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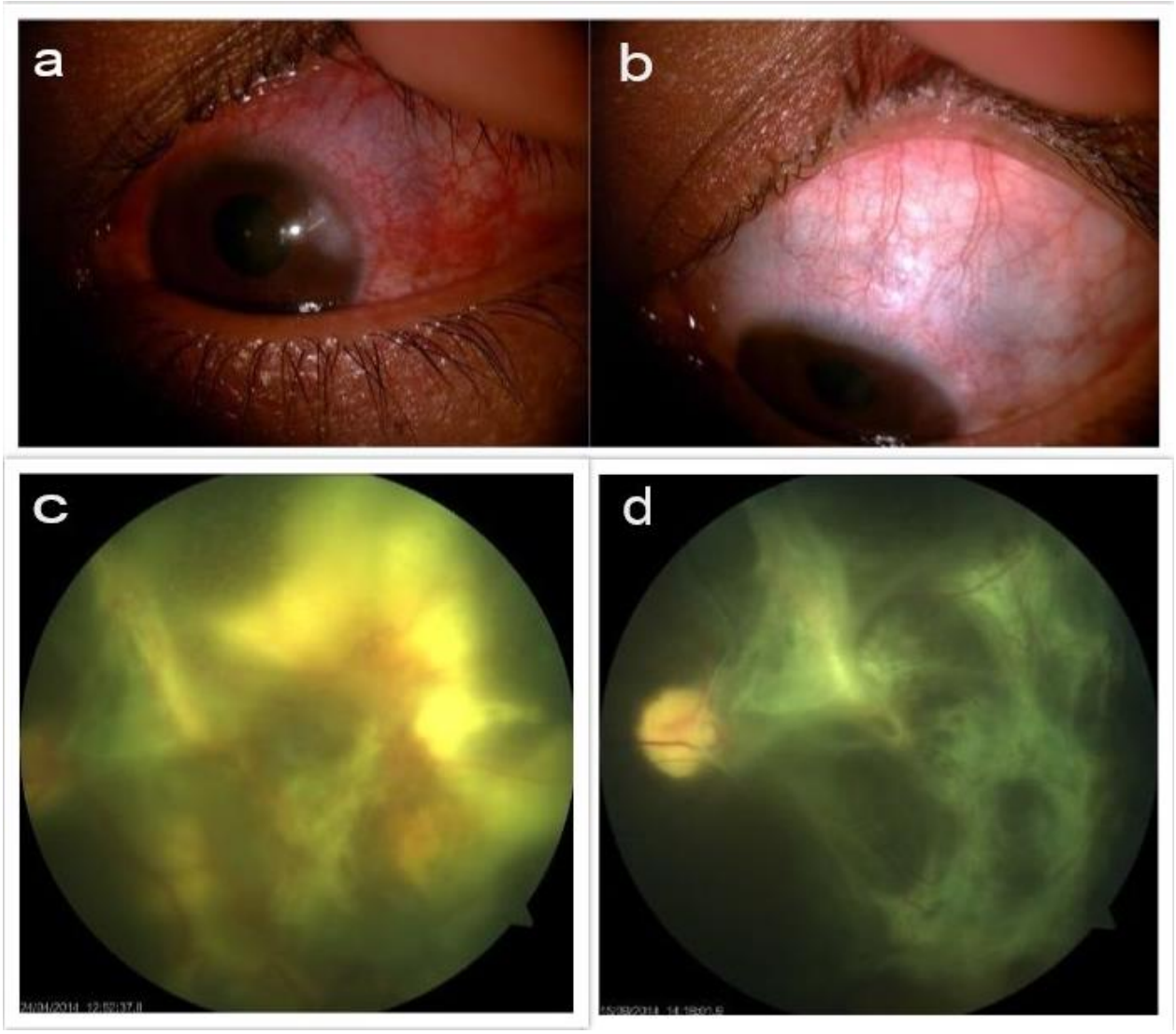

Figure 1 caption

Figure 1 (a) Chronic left sclerokeratouveitis on presentation, **(b)** left eye 3 weeks after starting four drug anti-TB treatment, **(c)** subretinal masses and elevation of the sensory retina of the left eye on presentation and **(d)** no residual activity 4 months after starting anti-TB treatment

Chapter 5: Original article – Published in Ocular Immunology and Inflammation

Citation:

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Abstract

Purpose: To compare QuantiFERON®-TB Gold and tuberculin skin testing as diagnostic tests for intraocular tuberculosis in HIV positive and negative patients.

Methods: A prospective study evaluating two different tests to help diagnose intraocular tuberculosis

Results: Thirty-five of 106 patients (33.0%) were diagnosed with intraocular tuberculosis including 11 (31.4%) with HIV infection. Patients were 6.95 times more likely to have intraocular tuberculosis if TST alone was positive ($p<0.001$) versus 2.19 times more likely if Quantiferon alone was positive ($p=0.04$). Tuberculin skin testing showed superior specificity (60.3% vs 33.3%)($p=0.001$) but similar sensitivity (90.3% vs 85.7%), positive (54.9% vs 40.5%) and negative predictive values (92.1% vs 81.5%) compared to Quantiferon. Specificity did not increase significantly if both skin testing and Quantiferon were positive.

Conclusions: In South Africa with its high HIV burden and limited public health resources Quantiferon testing should not replace tuberculin skin testing as it provides little additional diagnostic information.

Keywords: intraocular tuberculosis; diagnosis; HIV; Quantiferon; tuberculin skin test; South Africa

Introduction

Mycobacterium tuberculosis (MTB) infection in humans is a worldwide occurrence but the burden of disease is much higher in certain areas. In 2014, 9.6 million people contracted TB worldwide and 1.2 million (12%) of these were HIV positive with 74% of those cases coming from Africa.[1]

In South Africa the prevalence of TB in 2014 was 696 cases per 100 000 population and the incidence 834 cases per 100 000 – higher than any other country mentioned in the WHO annual report.[1] Furthermore, 61% of South African TB patients had HIV co-infection which is five times higher than the global average.

For many years the tuberculin skin test (TST) formed the cornerstone of immunological testing for MTB infection and, more recently, the interferon-gamma release assays (IGRAs) were developed to attempt to improve the accuracy with which different forms of MTB infection could be diagnosed. Many authors have reported and commented on the clinical value of both TST and IGRAs in the diagnosis of intraocular tuberculosis (IOTB).[2-11] It has been reported that false-negative TST results can exceed 50% in patients with decreased cellular immunity including those with HIV infection.[12] It has also been suggested that IGRAs may be more specific than TST in patients with prior BCG vaccination[13] and more sensitive than TST in HIV-infected patients.[12] In this study we compared an IGRA (QuantiFERON®-TB Gold) with TST as a diagnostic test for IOTB in HIV positive and HIV negative patients in Cape Town, South Africa.

Materials and methods

Study participants and management

We conducted a prospective study including consecutive patients with either a new diagnosis of uveitis or chronic uveitis of unknown cause. Between February 2014 and July 2015 a total of 106 patients presenting to the Eye Clinic at a tertiary referral hospital in the northern suburbs of Cape Town, South Africa were enrolled after obtaining informed consent. The study was approved by the

Health Research Ethics Committee of Stellenbosch University (Ref no N13/10/146). Patients were not included if they: 1) were under 18 years of age, 2) had uveitis with known or clinically obvious cause and 3) would not consent to HIV testing after appropriate counselling. A detailed systemic questionnaire was answered verbally by all patients. This was followed by a comprehensive ocular examination as well as a standardised panel of special investigations.

Investigations

All participants underwent an extensive battery of special investigations to exclude possible causes of uveitis other than TB (Table 1).[14]

Classification of IOTB

In our study we used the revised classification system for IOTB proposed by Gupta et al.[15] According to this classification patients with IOTB may fall into one of three clinical diagnostic groups namely confirmed, probable and possible IOTB based on the presence of clinical signs suggestive of IOTB and the amount of microbiological, radiological and immunological evidence available to the clinician.

Statistical analysis

IBM SPSS version 23 was used to analyse the data. A p value <0.05 was considered as statistically significant. Continuous data were compared between two groups using t tests or Mann Whitney tests as appropriate. Categorical data were compared using chi square tests, and relative risks and 95% confidence intervals were reported as effect measures. Measures of diagnostic accuracy including AUC and likelihood ratios were computed using the “diagti” command in Stata version 14. McNemar’s chi square tests for paired proportions were used to compare sensitivity and specificity between the two tests.

Results

Demographics and clinical findings

A total of 106 consecutive patients with uveitis were enrolled during the study period of which 66 (62.3%) were HIV- and 40 (37.7%) HIV+. Seventy-one cases (67.0%) either had an underlying etiology other than TB or were considered idiopathic. The remaining 35 participants (33.0%) were diagnosed with IOTB where 23 patients (65.7%) had possible IOTB and the remaining 12 probable IOTB (34.3%). No patients were diagnosed with confirmed IOTB as all TB cultures and PCRs were negative.

The clinical characteristics of the participants are shown in Table 2. The mean age of patients with IOTB was 38.7 ± 13.4 years and most of the patients were female (n=21, 60.0%). Both eyes were involved in 17 cases (48.6%) and 18 cases (51.4%) were unilateral. Nineteen cases (54.3%) had anterior uveitis, 15 cases (42.9%) panuveitis and 1 case (2.8%) posterior uveitis. HIV testing was positive in 11 cases (31.4%) and negative in 24 cases (68.6%). HIV+ cases had a median CD4+ cell count of 249 x 10^6/L (range 809 x 10^6/L).

QFT and TST results in the total study cohort

QFT tests were performed in 105 of 106 cases of which 4 (3.8%) had indeterminate results. Of the remaining 101 QFT results, 74 (73.3%) were positive and 27 (26.7%) negative (Figure 1). Fifty-two cases (70.3%) with a positive QFT result were HIV- and the remaining 22 cases (29.7%) HIV+. In the group with negative QFT results, 13 cases (48.2%) were HIV- and 14 cases (51.8%) HIV+. HIV- cases were therefore more likely than HIV+ cases to have a positive QFT result (p=0.04). TST results were obtained in 89 cases of which 38 (42.7%) were negative and 51 (57.3%) positive (Figure 1). In the group with positive TST results, 37 cases (72.5%) were HIV- and 14 cases (27.5%) HIV+ while in the group with negative results, 21 cases (55.3%) were HIV- and 17 cases (44.7%) HIV+. This demonstrated a trend for HIV+ cases to have negative TST results (p=0.09). In the HIV+ group (n=40, 37.7%), the median CD4+ count of cases with negative QFT results was noticeably lower at 93 x 10^6/L

[interquartile range 69 to 184] compared to the 415 x 10^6/L [interquartile range 234 to 610] of cases with positive QFT results (P = 0.005)(Figure 2). Also in the HIV+ group, the median CD4+ count of cases with negative TST results was again noticeably lower at 156 x 10^6/L [interquartile range 75 to 414] as opposed to 443 x 10^6/L [interquartile range 121 to 716] in cases with positive TST results (p=0.04)(Figure 3). In the HIV- group, 33 of 66 cases (50.0%) had positive results for both QFT and TST while in the HIV+ group only 12 of 40 cases (30.0%) had positive results for both tests. Both tests were therefore more likely to have positive results in HIV- cases (p=0.043). In the HIV- group, 10 of 66 cases (15.2%) tested negative for both QFT and TST while in the HIV+ group 16 of 40 cases (40.0%) had negative results for both tests. Patients were therefore 2.05 times more likely to have negative results for both tests if they were HIV+ (RR=2.05; 95% CI 1.31 – 3.22) (P=0.004).

Discussion

In our study, a diagnosis of IOTB (possible or probable) was made in 35 of 106 cases (33.0%). When analysing each test individually, the TST alone (71.0%; 95% CI 0.60 – 0.80) had a higher diagnostic accuracy than the QFT test alone (51.0%; 95% CI 0.41 – 0.61) (Table 3). Patients were 6.95 times more likely to have a diagnosis of possible or probable IOTB if the TST test alone was positive than if it was negative (RR=6.95; 95% CI 2.28 – 21.19) (p<0.001) but were only 2.19 times more likely to have a diagnosis of possible or probable IOTB if the QFT test alone was positive than if it was negative (RR=2.19; 95% CI 0.95 – 5.06) (p=0.04). Compared to a positive QFT alone, a positive TST alone had a similar sensitivity (90.3%; 95% CI 0.73 – 0.97 vs 85.7%; 95% CI 0.69 – 0.95; p=1.000), significantly higher specificity (60.3%; 95% CI 0.47 – 0.73 vs 33.3%; 95% CI 0.23 – 0.46; p=0.001), and similar positive and negative predictive values (PPV) (54.9%; 95% CI 0.40 – 0.69 vs 40.5%; 0.29 – 0.53), (NPV) (92.1%; 95% CI 0.78 – 0.98 vs 81.5%; 0.61 – 0.93). Area under the receiver operator characteristic curve (AUC) was borderline non significantly different between the two tests (0.75; 95% CI 0.67 – 0.84 vs 0.60; 95% CI 0.51 – 0.68).

If either the QFT or TST was positive, patients were 3.47 times more likely to have a diagnosis of possible or probable IOTB than if both were negative (RR=3.47; 95% CI 1.16 – 10.39) (p=0.007). If either the QFT or TST was positive the diagnostic accuracy was 52.0% (95 % CI 0.42 – 0.62) which is lower than that of TST alone although the sensitivity (91.4%; 95% CI 0.76 – 0.98) was slightly higher than TST alone. Specificity (32.4%; 95% CI 0.22 – 0.45), PPV (40.0%; 95% CI 0.29 – 0.52), NPV (88.5%; 95% CI 0.69 – 0.97) and AUC (0.62; 95% CI 0.55 – 0.69) were all lower than for TST alone. Lastly, if both the QFT and TST were positive, patients were 3.92 times more likely to have a diagnosis of possible or probable IOTB than if both were negative (RR=3.29; 95% CI 2.04 – 7.52) (p<0.001). If both tests were positive the sensitivity (74.3%; 95% CI 0.56 – 0.87), NPV (85.2%; 95% CI 0.73 – 0.93) and AUC (0.74; 95% CI 0.65 – 0.83) were lower than for TST alone whereas the specificity (73.2%; 95% CI 0.61 – 0.83), PPV (57.8%; 95% CI 0.42 – 0.72) and diagnostic accuracy (74.0%; 95% CI 0.64 – 0.81) were higher than for TST alone although none of these differences were statistically significant.

Several studies have compared TST and an IGRA as diagnostic tools for the diagnosis of IOTB. In some instances TST was compared to T-SPOT.TB[2,3] while in others it was compared to QFT.[4,16] However, none of these studies specifically considered the role of QFT and TST in diagnosing IOTB in HIV+ and HIV- patients.

In our total study cohort, we demonstrated that HIV- patients were more likely to have a positive QFT result than HIV+ patients. Conversely we also demonstrated a trend for patients with a negative TST to be HIV+. Patients were more likely to test positive for both QFT and TST if they were HIV- and also more likely to test negative for both QFT and TST if they were HIV+. However, in the HIV+ group, the median CD4+ count was found to be significantly lower in patients with a negative TST than in those with a positive TST (156 vs 443). Similarly, also in the HIV+ group, the median CD4+ count was again lower in patients with a negative QFT than in those with a positive QFT (93 vs 415). This indicates that HIV+ status *per se* might not account for an increased likelihood of having a negative QFT and/or TST result but rather that it is linked to patients with lower CD4+ counts.Table 4

compares our results to those published from India[7], Singapore[4], Korea[17] and Spain[16]. The largest study comparing the role of TST and QFT came from Singapore and found that TST alone demonstrated higher sensitivity (95.5%) for diagnosing IOTB than QFT alone (90.9%) while QFT alone had higher specificity (81.8%) compared to TST alone (72.7%).[4] The study from Spain however found that QFT alone had a higher sensitivity and specificity than TST alone while the study from India also showed that TST alone (92.0%) had a higher sensitivity than QFT alone (82.0%). In our study we also found that the sensitivity of TST alone (90.3%) was higher than that of QFT alone (85.7%) but, in contrast to the other studies, the specificity of TST alone (60.3%) was also significantly superior to that of QFT alone (33.3%). Both South Africa and India are developing countries endemic for TB while the other 3 countries are not and it is interesting to note that the TST demonstrated superior sensitivity to that of QFT in both endemic countries. Furthermore, TST alone had a higher PPV and NPV (54.9% and 92.1% respectively) than QFT alone (40.5% and 81.5% respectively). If both the TST and QFT were positive the specificity (73.2%) was higher than if the TST alone was positive but this difference was insignificant.

In our study, the subgroup diagnosed with IOTB consisted of 24 HIV- patients (68.4%) and 11 HIV+ patients (31.4%) with the latter having a median CD4+ count of 249 x 10^6/L (range 809 x 10^6/L). Four of the 11 HIV+ patients had a CD4+ count of <75 x 10^6/L which in most instances would lead to a false negative TST and QFT result. In our opinion these HIV+ patients with very low CD4+ counts could account for the lower sensitivity of both TST and QFT in our study as compared to those reported by Ang et al.[4] We also found that TST alone had a higher specificity than QFT alone which indicates that in our highly endemic setting the QFT test produced too many false positive results. Positive predictive values for TST and QFT alone were lower than those reported from areas with lower prevalence of TB but the NPV of TST and QFT were much higher than those reported from elsewhere. The 92.1% NPV of TST alone shows that, in our setting, a negative TST result allows the clinician to exclude IOTB in HIV+ and HIV- patients with a high degree of certainty although we must bear in mind that a negative TST result in a patient with a CD4+ count <100 x 10^6/L should be

considered with caution. The NPV of QFT alone was more than 10% lower than that of TST alone and therefore it seems that the extra cost of QFT testing to exclude IOTB cannot be justified in a limited resource environment as found in many developing countries. According to our data the only possible advantage of doing a QFT in addition to a TST is that if both tests are positive the specificity increases to 73.2% compared to 60.3% of TST alone and 33.3% of QFT alone but given that this difference is not statistically significant it remains uncertain whether this questionable benefit warrants the extra cost.

One potential limitation of our study is that the number of participants in our IOTB subgroup was quite small and subsequently we could not demonstrate statistically significant differences between sensitivity and specificity of TST and QFT in HIV+ and HIV- patients for detection of possible or probable IOTB. Further studies should be undertaken to determine how these tests perform in HIV+ and HIV- patients respectively.

Conclusion

Our study demonstrated that both TST, and to a lesser extent QFT, play a valuable role in the diagnosis of IOTB, even if a patient has HIV infection. However, once the CD4+ count drops to <100 x 10^6/L a negative result for either test should be considered with caution. In limited resource settings encountered in many developing countries it is doubtful whether the slight benefit of QFT testing in addition to TST justifies the extra expense.

Figure 1 QuantiFERON and tuberculin skin test results

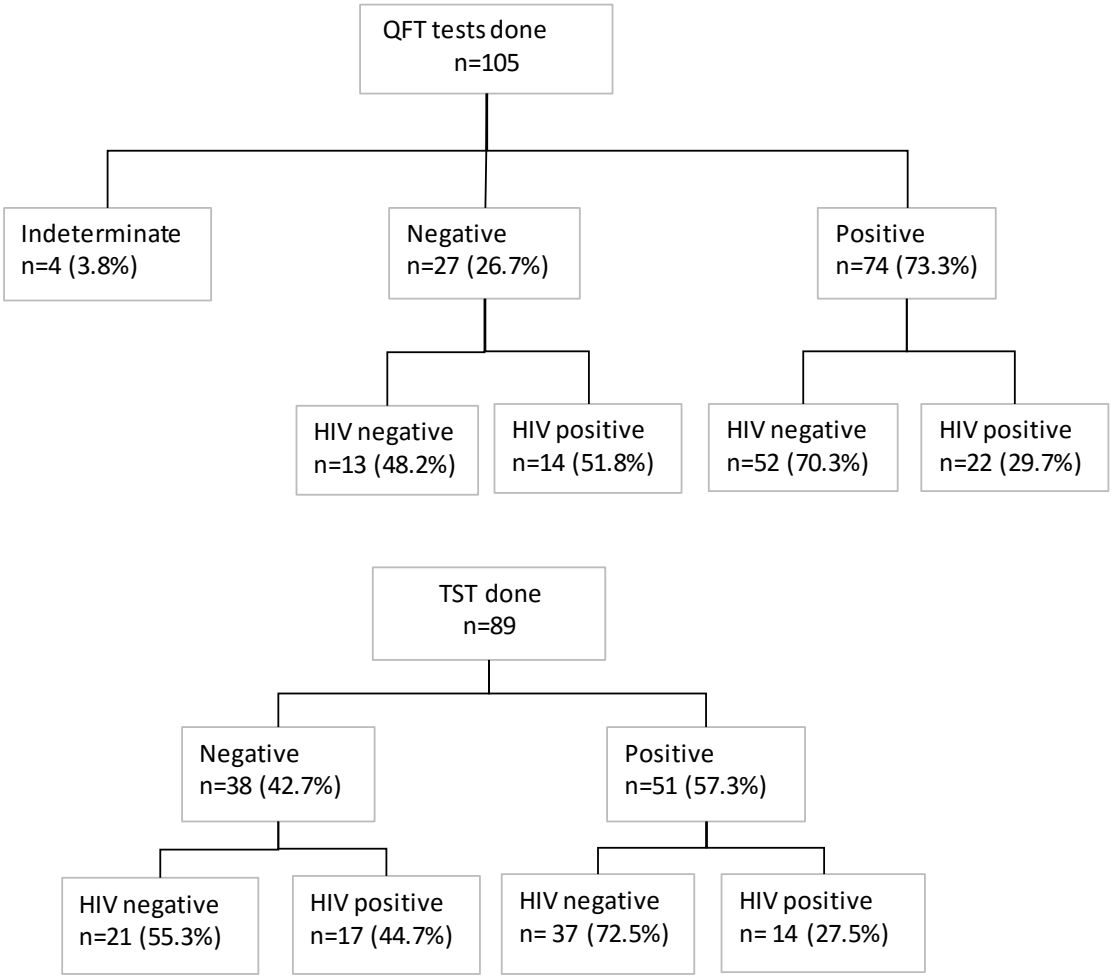

Figure 2: CD4 counts of HIV+ patients with positive and negative QFT results

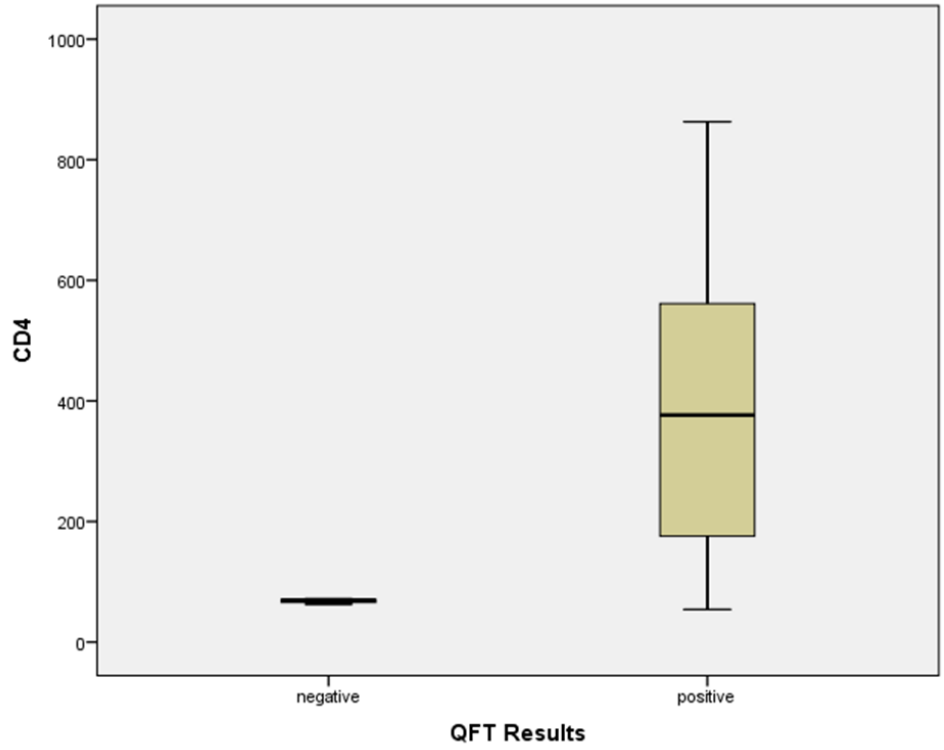

Figure 3: CD4 counts of HIV+ patients with positive and negative TST results

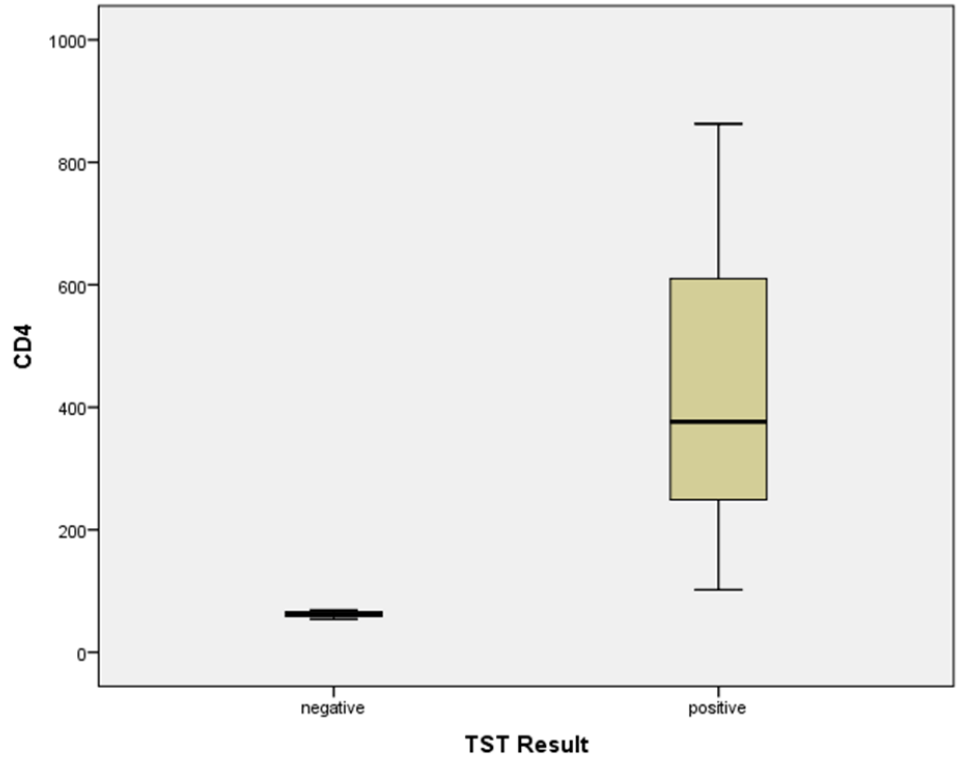

Table 1: Summary of special investigations performed

Baseline investigations in all cases

- HIV (& CD4 count if indicated)
- Full blood count (FBC)
- Erythrocyte sedimentation rate (ESR)
- Rapid plasma reagin (RPR) and *Treponema pallidum* antibodies (TPAbs)
- Creatinine
- Serum angiotensin converting enzyme (sACE)
- Dipstick urinalysis
- Chest X-ray (CXR)
- Tuberculin skin test (TST)
- Quantiferon-TB Gold (QFT)

Other investigations if indicated

- Chest CT (standard or high resolution)
- PET/CT
- HLA-B27
- Anti-Streptolysin O titer (ASOT)

Second-line investigations (if baseline tests negative)

- Anterior chamber tap for:
    - PCR for herpesvirus 1 – 6, rubella, toxoplasma
    - GWC for HSV, VZV, CMV and toxoplasma
    - Mycobacteria Growth Indicator Tube (MGIT) and IS6110 TB PCR (if ocular TB suspected)

Table 2 Demographics and anatomical classification of uveitis in study participants

Characteristics	All (n=35)	QFT result		
		Positive (n=30)	Negative (n=5)	p-value
Age, years (±SD)	38.7 (13.4)	33.5 (19.1)	36.2 (8.2)	0.66
HIV+		36.0 (11.0)	31.7 (2.9)	0.53
HIV-		40.3 (15.2)	43.0 (9.9)	0.81
Gender (%)				1
Male	14 (40.0)	12 (40.0)	2 (40.0)	
Female	21 (60.0)	18 (60.0)	3 (60.0)	
Laterality (%)				1
Unilateral	18 (51.4%)	15 (50.0)	3 (60.0)	
Bilateral	17 (48.6%)	15 (50.0)	2 (40.0)	
Anatomic distribution (%)				0.68
Anterior	19 (54.3)	17 (56.7)	2 (40.0)	
Posterior	1 (2.8)	1 (3.3)	0 (0)	
Panuveitis	15 (42.9)	12 (40.0)	3 (60.0)	
HIV status (%)				0.14
Positive	11 (31.4%)	8 (26.7)	3 (60.0)	
Negative	24 (68.6%)	22 (73.3)	2 (40.0)	

Table 3: Comparison of accuracy between QFT, TST and combinations thereof

Features of diagnostic tests	***Positive test result, (95%CI)***			
	QFT	***TST***	***QFT or TST***	***QFT & TST***
Sensitivity	85.7% (0.69, 0.95)	90.3% (0.73, 0.97	91,4% (0.76, 0.98)	74.3% (0.56, 0.87)
Specificity	33.3% (0.23, 0.46)	60.3% (0.47, 0.73)	32.4% (0.22, 0.45)	73.2% (0.61, 0.83)
Positive predictive value	40.5% (0.29, 0.53)	54.9% (0.40, 0.69)	40.0% (0.29, 0.52)	57.8% (0.42, 0.72)
Negative predictive value	81.5% (0.61, 0.93)	92.1% (0.78, 0.98)	88.5% (0.69, 0.97)	85.2% (0.73, 0.93)
Positive likelihood ratio	1.29 (1.03, 1.6)	2.28 (1.62, 3.19)	1.35 (1.12, 1.64)	2.78 (1.80, 4.27)
Negative likelihood ratio	0.43 (0.18, 1.03)	0.16 (0.05, 0.48)	0.26 (0.08, 0.82)	0.35 (0.20, 0.63)
Risk ratio	2.19 (0.95, 5.06)	6.95 (2.28, 21.19)	3.47 (1.16, 10.39)	3.92 (2.04, 7.52)
Accuracy	51.0% (0.41, 0.61)	71.0% (0.60, 0.80)	52.0% (0.42, 0.62)	74.0% (0.64, 0.81)
AUC	0.60 (0.51, 0.68)	0.75 (0.67, 0.84)	0.62 (0.55, 0.69)	0.74 (0.65, 0.83)

Abbreviations : CI - confidence intervals; QFT - Quantiferon; TST - Tuberculin skin test; AUC - area under curve

Table 4: Combination of sensitivity and specificity of Quantiferon and TST in different countries

	South Africa	India	Singapore	Korea	Spain
TB incidence/100 000	834	217	44	80	12
Sensitivity Q+	85.70%	82.00%	90.90%	100.00%	90.90%
Sensitivity T+	90.30%	92.00%	95.50%	N/A	87.80%
Sensitivity Q+T+	74.30%	74.00%	73.30%	N/A	78.70%
Specificity Q+	33.30%	76.00%	81.80%	72.00%	82.80%
Specificity T+	60.30%	N/A	72.70%	N/A	78.50%
Specificity Q+T+	73.20%	95%	88.20%	N/A	78.50%
PPV Q+	40.50%	N/A	90.90%	16.7 - 51.7%	71.40%

Abbreviations: Q+ = Quantiferon positive; T+ = TST positive; Q+T+ = Quantiferon and TST positive; PPV = positive predictive value; N/A = not available

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Abstract

Purpose: To use polymerase chain reaction (PCR) and Goldmann-Witmer Coefficient (GWC) calculation to search for evidence that Epstein-Barr virus (EBV) causes uveitis.

Methods: A prospective cross-sectional study where participants with positive multiplex EBV PCR results were further investigated by: 1) real-time PCR for EBV viral loads (VL) and 2) EBV GWC.

Results: Eleven of 106 consecutive uveitis patients (10.4%) had positive multiplex PCR for EBV on aqueous humor sampling and 7/11 (63.6%) were HIV-positive. Only 4/10 (40%) cases had detectable intraocular EBV VLs which were always lower than the blood or plasma VL. EBV GWC was negative in all 10 cases tested. In 9/11 (81.8%) of these cases an alternative, more plausible cause of uveitis was identified.

Conclusion: We found no evidence of active intraocular replication or antibody production to prove that EBV caused uveitis in these cases. In most cases an alternative treatable cause of uveitis was identified.

Keywords: polymerase chain reaction; Goldmann-Witmer coefficient; Epstein-Barr virus; uveitis

Introduction

Epstein-Barr virus (EBV), also known as human herpes virus 4 (HHV-4), forms part of the group of human herpes viruses. EBV is a ubiquitous virus and the vast majority of adults will show evidence of prior infection. EBV has been strongly linked to a variety of diseases including nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's lymphoma and infectious mononucleosis.[1,2] In the past, several attempts have been made to implicate EBV as a cause of uveitis but the diagnosis was often only substantiated by the demonstration of antibodies against EBV in serum and/or aqueous humor (AH).[3,4] To date, evidence to support a possible role for EBV in the pathogenesis of uveitis is limited. Yamamoto and co-workers, using multiplex polymerase chain reaction (PCR) for HHV 1 - 8, detected EBV DNA in 17 out of 60 ocular fluid samples.[5] Only 3 of these samples showed high copy numbers (6.6×10^3; 2.4×10^8 and 7.3×10^3 copies/ml respectively) of EBV DNA when using real-time PCR. Unfortunately they did not report the EBV viral loads (VL) in the blood at the time of taking the ocular samples, so one cannot determine whether the viral load in the blood was higher or lower than in the eye. In another report, in-situ hybridization studies of a retinal biopsy specimen were positive for EBV but the virus was located in atypical lymphoid cells infiltrating the retina and not the retinal cells themselves.[6] Only one report was able to demonstrate cells reacting with antibodies against cytomegalovirus (CMV) and EBV in the ganglion cell layer and inner granular layer of the retina using double immunostaining techniques.[7] The authors could however not prove whether EBV was causing the retinitis or whether it just happened to be present in the retinal tissue. On the other hand, Ongkosuwito and co-workers provided evidence showing that EBV does not play an important role in the pathogenesis of intraocular inflammation.[1] They found only 3 patients with evidence of local antibody production by Goldmann-Witmer coefficient (GWC) determination in the eye out of 82 patients tested. Two of these patients had borderline positive GWCs and the other had uveitis caused by varicella-zoster virus (VZV). Furthermore, none of these 3 patients had detectable intraocular EBV DNA. They found detectable EBV DNA in 6 out of 11 HIV negative immunocompromised patients but 4 of these 6 patients had another more plausible cause for their

uveitis (two patients had CMV retinitis, one had toxoplasma chorioretinitis and one had VZV acute retinal necrosis). Similarly, in a more recent report EBV PCR was positive in 3 patients of which 2 were known to have *Toxoplasma* chorioretinitis and the other had uveitis of unknown cause. All 3 patients had negative GWC for EBV and the authors concluded that more studies using a combination of PCR and GWC were required.[8] Lau and co-workers detected EBV in 3 of 18 eyes (16.7%) with acute retinal necrosis but in all 3 cases VZV was also present in the same eye and assumed to ultimately be the cause of the retinal necrosis.[9] Other authors have discussed various ocular conditions that may be associated with EBV but conclude that, apart from a dendritic keratitis from which EBV was cultured, there is little evidence that EBV causes intraocular inflammation.[10,11]

In an earlier paper we reported that in our setting HIV positive patients with uveitis were more likely to test positive for EBV by multiplex PCR than HIV negative patients.[12] We were however unable to measure intraocular viral loads of EBV at that time and could not determine whether significant intraocular viral replication was responsible for the uveitis. We subsequently designed a prospective study whereby we were able to determine intraocular viral loads of EBV by real-time PCR after we obtained a positive multiplex PCR for EBV as a screening test. In addition, GWC analysis to demonstrate intraocular antibody production against EBV was performed.

Materials and methods

Study participants

Between February 2014 and July 2015 we conducted a prospective study including consecutive patients with either a new diagnosis of uveitis or a diagnosis of chronic uveitis with unknown cause. Patients were excluded if they: 1) were under 18 years old, 2) had uveitis with known or clinically obvious cause and 3) declined consent to HIV testing after appropriate counselling. A total of 106 patients who presented to the Eye Clinic at Tygerberg Hospital, a tertiary referral hospital serving the eastern Metropole of Cape Town, South Africa were enrolled after informed consent was obtained.

The study was approved by Stellenbosch University Health Research Ethics Committee (Ref no N13/10/146) and adhered to the tenets of the Declaration of Helsinki.

Investigations

All participants underwent extensive investigation to look for the underlying cause of the ocular inflammation as previously described.[13] Blood tests included HIV status (plus CD4+ lymphocyte count if positive), rapid plasma reagin (RPR), *Treponema pallidum* antibodies, serum angiotensin converting enzyme levels, full blood count, erythrocyte sedimentation rate, creatinine levels as well as HLA-B27 testing and anti-Streptolysin O titers if clinically indicated. Dipstick urinalysis and chest radiograms were obtained in all cases while chest CT scans (standard or high-resolution) and PET/CT scans were ordered if considered necessary after consultation with a specialist pulmonologist.

In cases where baseline tests were negative, anterior chamber (AC) taps were performed as second-line investigations. AH samples underwent qualitative multiplex PCR testing for human herpes viruses 1 – 6, rubella virus and *Toxoplasma* at the National Health Laboratory Services (NHLS) Medical Virology laboratory, Tygerberg Hospital, Cape Town, South Africa as previously described.[12] In addition, GWC for herpes simplex virus, varicella-zoster virus, CMV, rubella virus and *Toxoplasma* were performed at the University Medical Center Utrecht, the Netherlands.[14,15] If a positive multiplex PCR result for EBV was recorded then a second AH sample was taken from the affected eye for quantitative PCR to determine the EBV VL in the eye. At the time of performing the second AC tap we also obtained an anticoagulated peripheral blood sample to measure the EBV VL in the plasma and/or whole blood. EBV VL measurements on both AH and plasma/blood were performed by the NHLS laboratory, Groote Schuur Hospital, Cape Town using EBV R-gene® Quantification assays (bioMérieux, Marcy l'Etoile, France). In cases with a positive EBV PCR, EBV GWC was determined if sufficient sample volumes were available. EBV IgG was measured in serum and aqueous humor by analysing four two-fold serial dilutions starting at 1:101 and 1:50.5, respectively, using the

Virion\Serion classic Epstein-Barr Virus VCA IgG kit (Würzburg, Germany). The GWC was calculated as described previously.[15]

Results

Demographics and clinical findings

A total of 106 participants were included in the prospective study and 11 (10.4%) of these tested positive for EBV by multiplex PCR on AH (Table 1). In this paper we only describe the demographics and clinical findings of the subgroup of 11 EBV positive cases. Seven of the 11 cases were of mixed ethnicity and the remaining 4 cases were black Africans. Seven of the 11 (63.6%) EBV positive cases also had HIV infection with a median CD4+ count of 181 x 10^6/L. In our previous study we found an even higher proportion of EBV positive cases to also have HIV infection (10/12, 83.3%) (p=0.026).[12] The mean age of EBV positive cases was 37.0 ± 8.93 years and 6 (54.5%) were males. Unilateral involvement (7/11, 63.6%) was more common than bilateral involvement (4/11, 36.4%) and anterior uveitis (7/11, 63.6%) was the most common type of uveitis followed by panuveitis (3/11, 27.3%) and intermediate uveitis (1/11, 9.1%). Granulomatous uveitis was noted in 3 cases only.

Quantitative PCR and GWC results

Quantitative PCR was subsequently performed on aqueous humor from 10 eyes, plasma from 7 individuals and whole blood from 4 individuals (Table 1). In aqueous humor the EBV VL was above the detectable limit (180 copies/mL) in only 4 cases (40%). In plasma the EBV VL was above the detectable limit in only one case (14.3%) and in whole blood in all 4 cases (100%). In 3 of the 4 cases where EBV VL was detectable in aqueous humor the EBV VL in the eye was lower than that measured in whole blood and in the fourth case the EBV VL in whole blood was not measured. EBV GWC was calculated in 10 of the EBV PCR positive patients (90.9%). All 10 patients were seropositive for EBV while EBV IgG antibodies were detected in 8 of 10 AH samples. However, none of these cases had a positive GWC for EBV (Table 2).

Furthermore, in 9 of the 11 cases (81.8%) another more plausible cause of intraocular inflammation was identified (Table 1). Syphilitic uveitis was diagnosed in two cases, possible ocular tuberculosis (TB) was diagnosed in two cases and there was one case each of post-streptococcal uveitis, CMV retinitis, VZV/HSV co-infection and HLAB27 positive acute anterior uveitis. One case was diagnosed with HIV-associated uveitis and here, in contrast to the EBV VL in the eye, the HIV VL of the AH was more than 150 times higher than the HIV VL in the blood which implies HIV replication inside the eye.[16] The two remaining cases were labelled as idiopathic despite having positive EBV PCR as both had a low EBV VL and negative EBV GWC.

Discussion

To date there is very little convincing evidence in the ophthalmic literature to support an active role for EBV in the pathogenesis of uveitis. Only one case could demonstrate EBV in retinal cells but CMV was also present in the same specimen.[7] In our prospective study using a combination of multiplex and real-time PCR for EBV we did not find a single case where EBV VL was significantly higher in the eye than in the blood or plasma which would appear to indicate that EBV replication was not actively taking place inside the eye. In the cases where EBV VL could be compared between the eye and the blood it was found that the VL in the blood was considerably higher than in the eye. This appears to support the leakage theory whereby it is assumed that EBV cell free or present in lymphocytes in the bloodstream enters the eye when the blood-eye barriers break down as a result of ocular inflammation caused by some other trigger. Alternatively, but not mutually exclusive, in the course of uveitis specific lymphocytes latently infected with EBV may be attracted to the inflamed eye, which could give rise to positive PCR results on AH.

In our case series an alternative and more plausible cause of uveitis was found in over 80% of cases. Furthermore, the finding that in our setting almost two thirds of EBV positive cases had concomitant HIV infection also raises questions. One possible explanation could be that HIV infection *per se* increases vascular permeability and allows EBV and EBV-infected lymphocytes to leak into the eye

although further research is required to establish whether this is indeed the case. It has been postulated that EBV may influence the course of intraocular inflammation due to other causes such as toxoplasma or the other herpes viruses by producing an active homologue of interleukin-10 and therefore playing a secondary role in the pathogenesis of uveitis.[1]

Measurement of EBV IgG levels in serum and AH produced two interesting findings. Firstly, serum EBV IgG levels were much higher than those in the AH in all 10 cases and secondly, no local antibody production could be demonstrated inside the eye as the GWC was negative in all cases. A concentration gradient was therefore present between high IgG levels in the blood and lower (or non-detectable) levels inside the eye. We hypothesize that once the permeability of the blood-aqueous barrier separating the two compartments increased due to inflammation then diffusion of the IgG could take place along that gradient.

A possible limitation of this study is the small sample size and future studies should therefore seek to include a larger sample size. However, to the best of our knowledge, this is the first prospective report that utilized both qualitative and quantitative PCR as well as GWC to study the role of EBV in uveitis in HIV+ and HIV- patients.

Conclusion

Given that we found no evidence of either EBV replication or increased local antibody production in the eye and that in most cases a more plausible cause of inflammation was found we currently conclude that EBV is unlikely to be the perpetrator. EBV may still turn out to be an accomplice or even the cause in some cases of uveitis, but is probably just an innocent bystander in the majority of cases. Qualitative PCR on intraocular fluid and/or blood serology alone are not sufficient to conclusively establish a diagnosis of an intraocular infection with EBV. Additional diagnostic tools, such as EBV VL on AH and plasma as well as EBV GWC, are required to determine whether EBV plays

a significant role in any given case of uveitis and we therefore propose a stepwise approach to making or excluding a diagnosis of EBV-associated uveitis (Table 3).

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Table 1: Demographics, clinical finding and final diagnoses in patients with positive EBV PCR

Pt no	HIV	CD4	Final diagnosis	Explanation
1	Neg	N/R	Idiopathic	EBV VL not significant
2	Pos	483	Idiopathic	EBV VL not significant
3	Neg	N/R	Possible ocular TB	Meets criteria & TST positive
4	Pos	414	Poststreptococcal	Raised Anti Dnase
5	Pos	184	VZV/HSV coinfection	VZV GWC = 48.45 & HSV GWC = 6.71
6	Pos	138	Syphilis	RPR = 1:512
7	Pos	121	HIV uveitis	HIV VL $_{eye}$ >> HIV VL $_{blood}$
8	Neg	N/R	Possible ocular TB	Meets criteria and TST and QFT both positive
9	Pos	181	Syphilis	RPR = 1:16
10	Neg	N/R	HLAB27	HLAB27+ with typical phenotype
11	Pos	86	CMV	Frosted branch angiitis and CMV PCR+

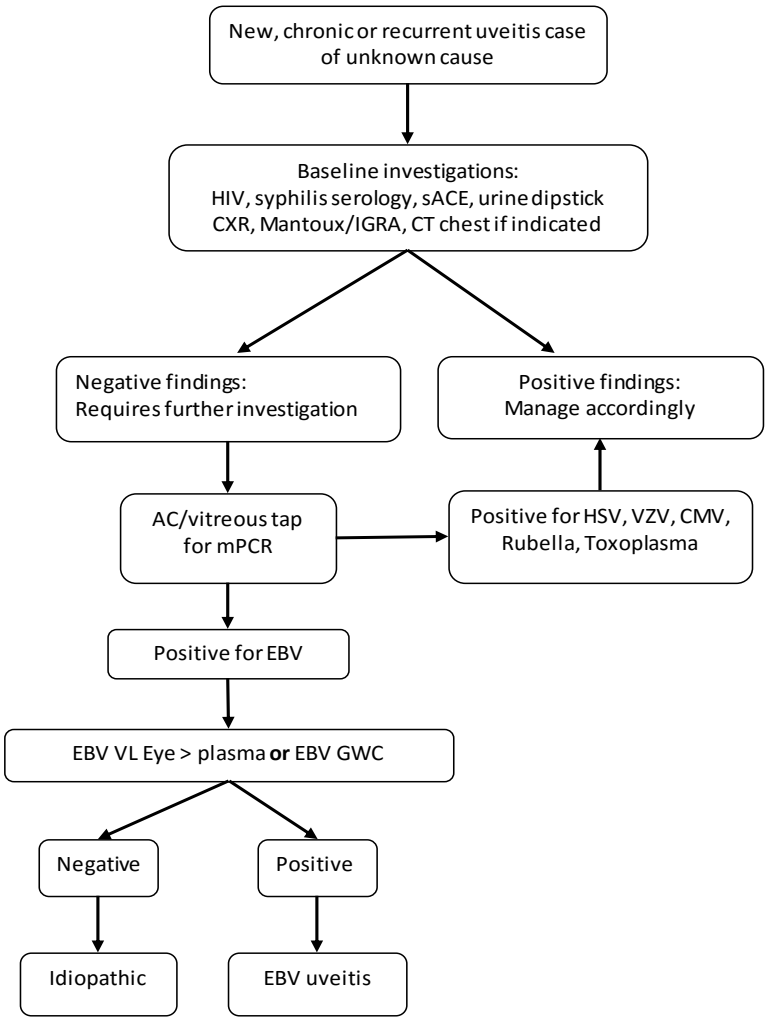
Abbreviations: Anat distrib = anatomical distribution; Granul = Granulomatous; VL = viral load; N/R = not relevant; N/A = not available; LDL = lower than detectable limit; TST = tuberculin skin test; QFT = Quantiferon TB Gold; RPR = rapid plasma reagin

Table 2: EBV viral loads and EBV GWC results

Pt no	EBV VL eye (cps/ml)	EBV VL plasma (cps/ml)	EBV VL blood (cps/ml)	Se EBV IgG (IU/ml)	AH EBV IgG (IU/ml)	EBV GWC
1	692	LDL	N/A	68.4	<4	<3
2	LDL	LDL	N/A	339.2	179.5	1.31
3	LDL	LDL	N/A	1172.8	97.8	0.86
4	LDL	LDL	N/A	2256.8	28.4	0.35
5	LDL	LDL	N/A	211	<4	<3
6	8258	LDL	25650	866.6	42	0.28
7	LDL	15797	N/A	3148	6.1	0.28
8	LDL	N/A	82	100.8	13.8	<3
9	678	N/A	85133	3273.6	698.8	0.43
10	N/A	N/A	N/A	125.7	66.6	0.87
11	186	N/A	91424	N/A	N/A	N/A

Abbreviations: VL = viral load; cps/ml = copies per milliliter; IU/ml = international units per milliliter; Se = serum; AH = aqueous humor
LDL = lower than detectable limit; N/A = not available

Table 3: Stepwise approach to diagnose EBV uveitis



Abbreviations: IGRA = Interferon Gamma Release Assay; AC = anterior chamber; mPCR = multiplex PCR; VL = viral load

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Chapter 7: Correspondence – published in the journal "AIDS"

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Introduction

According to the World Health Organization, the number of people of all ages living with HIV infection in South Africa was 7 million in 2015 which translates to a prevalence of 19.2% amongst individuals aged 15 years and older. The number of new infections reported in South Africa during 2015 was 380 000 with 180 000 deaths attributed to AIDS during the same period.[1] More than 3.3 million (48.0%) people living with HIV received highly active antiretroviral therapy (HAART) during 2015 and this appears to be slowly turning the tide against the HIV pandemic in the country.

Despite recent gains in the battle against HIV/AIDS, South Africa remains one of the countries with the highest prevalence of this disease in the world and intermittently an unusual clinical presentation is encountered that must be shared with clinicians who work with patients living with HIV. HIV-induced uveitis is such a condition.

Case report

A 44 year old male presented to the Eye Clinic at Tygerberg Academic Hospital in Cape Town with a 3 week history of redness and progressive vision loss in his right eye. He had no previous ocular or medical history of note. On examination his uncorrected visual acuity was decreased in both eyes. The right eye read 0.6 and the left eye 0.5 on a decimal Snellen chart. Both eyes showed mild circumcorneal injection and large keratic precipitates on the endothelium (Figure 1a). Inflammatory activity was noted in the anterior chambers and the anterior vitreous humor of both eyes. In both

eyes small fluffy nodules were prominent all along the pupil margin (Figure 1b). The rest of the eye examination was normal. Topical corticosteroid therapy was commenced to address the inflammation while special investigations were being performed.

Routine first-line investigations were requested to search for the underlying cause of the uveitis. These included a full blood count, erythrocyte sedimentation rate, creatinine, syphilis serology as well as serum angiotensin converting enzyme level and all had negative results. An HIV test was requested after obtaining informed consent. The patient was newly diagnosed with HIV infection with a CD4+ cell count of 121 x 10^6/L. Chest radiography was normal and dipstick urinalysis revealed 1+ protein only. A tuberculin skin test (17mm) and QuantiFERON-TB Gold test (1.59) were both positive but subsequent high-resolution chest computed tomography (CT) scan was normal making a diagnosis of intraocular tuberculosis unlikely. A 0.1mL sample of aqueous humor (AH) was obtained from the right anterior chamber and tested by multiplex PCR for herpes viruses 1 -6. The qualitative multiplex PCR was positive for Epstein-Barr virus (EBV) although a quantitative PCR showed that the EBV viral load (VL) was lower than the detectable limit and therefore also unlikely to be the cause of the inflammation.

Since the intraocular inflammation was not improving on topical corticosteroid treatment, a second paired AH and blood sample was obtained to determine the HIV VL in the ocular fluid and blood. The HIV VL in the blood was 215 810 copies/mL while the HIV VL in the AH was 35 724 280 copies/mL thereby demonstrating that the virus had been replicating inside the eye. This was regarded as convincing evidence for the diagnosis of HIV-induced uveitis. The patient was commenced on first-line HAART and the inflammation subsided within three weeks without any further corticosteroid treatment and has remained asymptomatic for more than 30 months.

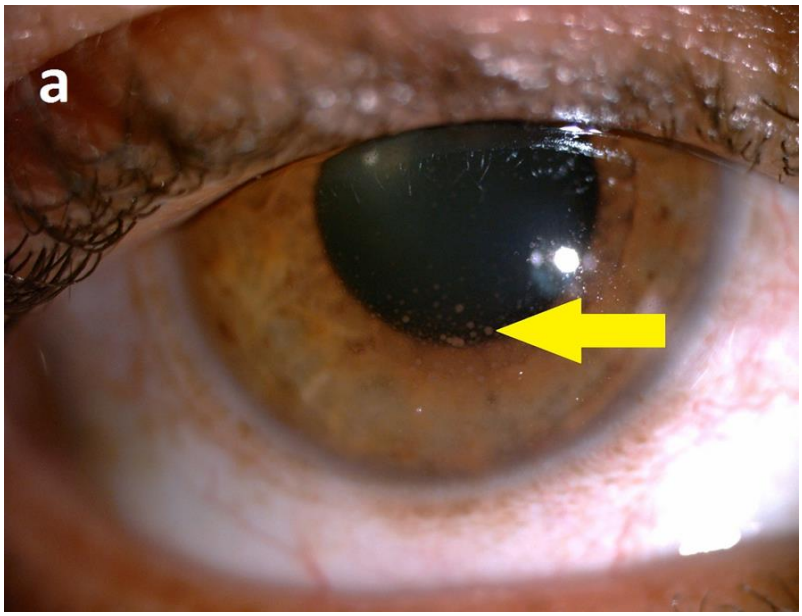
Discussion

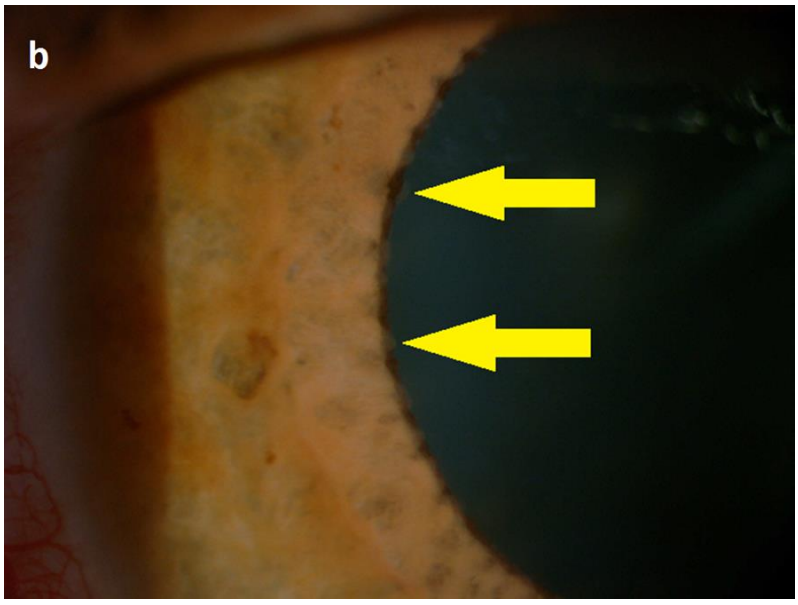
It is well known that HIV infection predisposes patients to a wide range of opportunistic infections that may also involve the eye and cause intraocular inflammation.[2,3] The notion that HIV infection *per se* may also cause intraocular inflammation has been entertained since the late 1980's but laboratory evidence to support this hypothesis was lacking for many years and the diagnosis was initially based on a positive response to zidovudine monotherapy.[4] In 1998, Rosberger *et al* cultured HIV from the AH of 3 eyes and vitreous humor of 1 eye suspected of having HIV-induced uveitis.[5] In 2008, Rothova *et al* demonstrated that a patient with HIV-induced uveitis had an intraocular HIV-1 RNA viral load which was several times higher than that in the plasma thus indicating that HIV can locally replicate inside the eye and cause inflammation.[6]

Subsequent reports from Thailand helped to elucidate the clinical manifestations characteristic of HIV-induced uveitis.[2,7] Kunavisarut *et al* found that all patients presented with decreased visual acuity and that none were receiving HAART at the time of diagnosis.[2] On clinical examination none of the patients had conjunctival hyperemia despite all having anterior uveitis with characteristic keratic precipitates on the corneal endothelium. Furthermore, no retinal lesions or scars were noted and no clinical evidence suggestive of opportunistic infections was found. Laboratory investigations also did not provide any evidence of opportunistic infections and in all cases the intraocular HIV load was found to be much higher than the plasma HIV load although in some instances the inflammation was present for 2 years before the diagnosis was confirmed. None of the patients responded to topical and/or systemic corticosteroid therapy but in all cases complete resolution of the intraocular inflammation occurred after administration of HAART. The case described in this paper therefore matches every aspect of this description but also includes a previously unreported finding of small fluffy nodules along the pupil margin. Interestingly, the patients described in both 1988 and 1998 also show significant similarities to these cases.[4,5] The concept of HIV-induced uveitis has therefore existed for almost 30 years and yet many health care practitioners, including ophthalmologists, who

work with patients living with HIV are unaware of this condition. In contrast, most health workers are well aware of the opportunistic ocular infections and inflammation associated with HIV infection. It is therefore important to bring HIV-induced uveitis to the attention of everyone working in the field of HIV medicine in order to ensure that the condition is suspected, diagnosed and treated correctly before any permanent ocular damage occurs.

Legends: Figure 1a: Large keratic precipitates on the corneal endothelium (arrow). Figure 1b: Small fluffy nodules all along the pupil margin (arrows)





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Polymerase Chain Reaction and Goldmann-Witmer Coefficient Testing in the Diagnosis of Infectious Uveitis in HIV-positive and HIV-negative Patients in South Africa

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Abstract

Purpose: To use polymerase chain reaction (PCR) and Goldmann-Witmer coefficient (GWC) calculation to diagnose infectious uveitis

Methods: Prospective cross-sectional study

Results: Twenty-seven of 106 patients (25.5%) had positive PCR and/or GWC results on aqueous humor (AH) sampling and 15 of 27 (55.6%) were HIV-positive. Patients with non-anterior uveitis (NAU) were more likely to be HIV+ (p=0.005). More than 1 possible pathogen was identified in 9 of 27 patients (33.3%) of whom 7 (77.7%) were HIV+. The final clinical diagnosis was discordant with AH findings in 9 of 27 cases (33.3%). A positive EBV PCR result was associated with a discordant diagnosis (p=0.001). All cases of herpetic anterior uveitis (42.9% HIV+) tested PCR-/GWC+ while all cases of herpetic NAU tested PCR+/GWC- (83.3% HIV+). All rubella virus cases were PCR+/GWC+.

Conclusion: PCR is useful to diagnose herpetic NAU in HIV+ patients while GWC is useful to diagnose herpetic anterior uveitis.

Keywords:

Polymerase chain reaction; Goldmann-Witmer coefficient; diagnosis; infectious uveitis; HIV; South Africa

Declaration of Interest:

The authors report no conflicts of interest

Introduction

South Africa is in the unique position of having the highest prevalence of both HIV infection and tuberculosis (TB) of any country in the world.[1] It is therefore not surprising that the prevalence of intraocular TB in South Africa has recently been reported to be as high as 33.0% in the Western Cape Province.[2] As in other developing countries, South Africa also has a high burden of other infectious causes of intraocular inflammation. Ocular syphilis, for example, is well known to occur more frequently in areas with a high prevalence of HIV infection.[3]

In some instances, the laboratory diagnosis of infectious uveitis is largely based on positive blood serology with examples including syphilis (*Treponema pallidum*) and cat scratch disease (*Bartonella henselae*). In other cases however positive blood serology is neither sensitive nor specific enough to confidently confirm an infectious cause of uveitis. Examples here would include herpes viruses such as Herpes Simplex virus 1 & 2 (HSV), Varicella-Zoster virus (VZV) and cytomegalovirus (CMV) as well as protozoa such as *Toxoplasma gondii* . Over the past decade, the emphasis in the latter cases has shifted towards the use of molecular biology techniques to examine ocular samples either in isolation or in combination with blood samples. Polymerase chain reaction (PCR) was the first molecular technique that came into widespread use during the previous decade.[4-12] However, it later became apparent that PCR alone could yield false negative results under certain conditions and that a combination of PCR and Goldmann-Witmer coefficient (GWC) testing to detect intraocular antibody production was superior to either test alone.[13-15]

The first molecular test to become readily available at our institution was a multiplex PCR that analysed for the presence of herpes viruses 1 – 6. In an earlier paper we reported a 47.2% positive yield with multiplex PCR in patients presenting with undifferentiated uveitis and in this group a positive PCR yield correlated significantly with HIV infection, posterior uveitis and duration of symptoms less than 30 days.[16] We hypothesized that if GWCs are determined in addition to PCR

testing one would then be able to diagnose infectious causes of uveitis in a wider spectrum of cases. We present the findings of the ensuing study in this report.

Materials and methods

Study participants and management

A prospective study was conducted at the Eye Clinic of Tygerberg Hospital, a tertiary referral hospital serving the Eastern Metropole of Cape Town, South Africa. Between February 2014 and July 2015 a total of 106 consecutive patients presenting with either a new diagnosis of uveitis or chronic uveitis of unknown cause were enrolled. Informed consent was obtained from all participants and the study was approved by the Health Research Ethics Committee of Stellenbosch University (Ref no N13/10/146). The study adhered to the tenets of the Declaration of Helsinki. Exclusion criteria were: 1) age under 18 years, 2) uveitis of known cause and 3) declined HIV testing after appropriate counselling. All participants verbally completed a detailed systemic uveitis questionnaire followed by a comprehensive ocular examination and a tailored panel of special investigations.

Investigations

All participants underwent extensive special investigations to search for underlying causes of their intraocular inflammation as previously reported.[2] Blood samples were obtained for HIV status (plus CD4+ count if positive), full blood count, erythrocyte sedimentation rate, rapid plasma reagin (RPR) and *Treponema pallidum* antibodies for syphilis, creatinine and serum angiotensin converting enzyme (sACE) levels. Tuberculin skin testing (TST) and QuantiFERON®-TB Gold (QFT) tests (Cellestis Inc., Chadstone, Victoria, Australia) were performed in 89 and 105 participants, respectively. Dipstick urinalysis and chest X-rays were requested in all cases. In selected cases, after consultation with a specialist pulmonologist, standard or high-resolution chest CT scans were requested while in other cases PET/CT scans were obtained if considered necessary. HLA-B27 testing was done if patients

presented with severe fibrinous anterior uveitis and Anti-Streptolysin O titers were performed in patients under the age of 40 years.

Anterior chamber taps were performed as second-line investigations using the method described previously.[16] Aqueous humor (AH) samples from 100 patients were subjected to PCR testing for herpes viruses 1 to 6, rubella virus (RV) and *Toxoplasma* at the National Health Laboratory Services (NHLS) Medical Virology laboratory, Tygerberg Hospital, Cape Town, South Africa. Paired serum and AH samples from 82 patients in the same cohort were sent to the University Medical Center Utrecht, Netherlands where Goldmann-Witmer Coefficients were determined for HSV, VZV, CMV, RV and *Toxoplasma* as previously described.[13,17]

Statistical analysis

IBM SPSS version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) was used to analyse the data, using a significance level of 0.05. Categorical factors were compared between groups using Pearson's chi square test if assumptions were met, otherwise Fisher's exact 2-sided tests were used. Continuous variables were tested for normality and if plausibly normally distributed, means were compared between two groups using independent samples t-tests, and if not, non-parametric equivalent tests for instance, Mann Whitney tests were used.

Results

Demographics and clinical findings

During the study period a total of 106 consecutive participants with uveitis were enrolled of which 66 were HIV- and 40 HIV+ with a median CD4+ cell count of 242 x 10^6/l [interquartile range 100 - 501].

The clinical characteristics of the AH test-positive and test-negative patients with reference to their HIV status are summarised in Table 1. The mean age of patients with a positive PCR and/or GWC was 39.6 ± 8.8 years and the majority were female (n=16). Both eyes were involved in 8 cases (29.6%). HIV testing was positive in 15 cases (55.6%) and HIV+ cases had a median CD4+ cell count of 181 x 10^6/l [interquartile range 88 – 483] but showed no differences regarding age and laterality when compared to the HIV- group. Sixteen cases had anterior uveitis, 6 cases panuveitis and 2 cases each intermediate and posterior uveitis. Only 5 of 16 patients with anterior uveitis were HIV+ whilst 6 of 7 patients with panuveitis were HIV+ and all cases with intermediate and posterior uveitis were HIV+. Patients with any distribution of uveitis other than anterior were therefore more likely to have HIV infection (p=0.005). There were no significant differences between males and females regarding age (p=0.59), laterality (p=0.67), HIV status (p=0.93) or distribution of inflammation (p=0.43). Eight cases presented with a granulomatous and 19 with a non-granulomatous appearance.

PCR and GWC results

The 27 patients in our study with positive ocular fluid tests produced 37 positive PCR and/or GWC results (Table 2). Nineteen of the 37 positive PCR and/or GWC results were from HIV+ patients. In the HIV+ group, 13 samples were only PCR+ and 6 samples only GWC+, while in the HIV- group 10 samples were PCR+ and 8 samples GWC+. Four samples in the HIV- group were however PCR+ and GWC+ for RV.

More than 1 possible pathogen was identified in 9 of 27 patients of whom 7 were HIV+ with a median CD4+ cell count of 181 x 10^6/l [interquartile range 130 – 292] (Table 3). In 9 of the 27 patients with positive PCR and/or GWC results the final clinical diagnosis was either only partially supported by the positive result or in some cases not at all. In most instances where the final diagnosis was discordant with the PCR and/or GWC results the potential pathogen identified by PCR was EBV. In 9 of 11 EBV PCR+ cases an alternative, more plausible cause for the inflammation was found and 2 cases were considered to be idiopathic as EBV viral loads were lower than the

detectable limit. A positive EBV PCR result was therefore associated with a high likelihood of a discordant final diagnosis (p=0.001). In 10 of the 11 EBV PCR+ cases where sufficient sample volume was available EBV GWCs were calculated and these were also all negative thus providing further evidence that EBV was unlikely to be the cause of inflammation in these cases (in press).

However, in 3 other cases we identified two different pathogens that are each known to cause uveitis on its own. In the first case (Table 3, number 14) a 44-year-old female presented with recurrent granulomatous anterior uveitis OU. At first presentation 4 years prior to enrolment she was noted to have mutton-fat keratic precipitates (KP) and Bussaca nodules OU and 2+ cells in both anterior chambers (ACs). Upon enrolment she had no iris nodules but again had bilateral mutton-fat KPs on the central cornea only which later formed large ghost KPs (Figure 1). Both eyes had IOP = 17mm Hg and early cataracts. Her GWC for CMV was 13.72 but she also tested PCR+ for RV with a RV GWC = 333.07. Interestingly, despite the much lower GWC for CMV her clinical picture was much more in keeping with chronic anterior uveitis secondary to CMV than Fuchs' uveitis syndrome (FUS). However, despite not attending follow-up visits for 2 years, her clinical picture changed over time to more closely resemble FUS albeit in both eyes with diffusely spread white KPs as well as small transparent iris nodules at both the pupillary margin (Koeppe) and on the surface of the iris (Busacca) as previously described (Figure 2).[19] In the second case (Table 3, number 23) a 29-year-old female presented with mutton-fat keratic precipitates, Bussaca nodules, cells in the anterior chamber and vitreous humor as well as a superotemporal retinal granuloma OD. She had been on anti-retroviral treatment for 7 years and had a CD4+ count of 789 x 10^6/L. Her GWCs for Toxo and VZV were 5.83 and 4.80 respectively but she was lost to follow-up before definitive treatment could be commenced. We decided that Toxo was most likely the primary pathogen for three reasons. Firstly, the clinical picture was more compatible with Toxo than VZV. Secondly, the GWC was higher for Toxo than for VZV and thirdly, low positive VZV GWCs without any clinical characteristics of VZV uveitis may occur (personal observation of JDF de Groot-Mijnes); with respect to the latter, it is

interesting to note that subclinical reactivation of VZV may occur intrathecally particularly in HIV+ individuals. [20]

In the third case (Table 3, number 26) a 50-year-old female presented with a dense vitritis and anterior spillover OS. On further investigation we made a new diagnosis of HIV infection with CD4+ count of 400 x 10^6/L and found her serum RPR titer to be 1:128. CSF analysis revealed a positive VDRL of 1:4 and she was subsequently treated with intravenous Penicillin G for 14 days. She did however also have a positive AH multiplex PCR result for HSV-1 and was treated with oral acyclovir 400mg 5 times a day for the same period after which the uveitis resolved. One therefore cannot determine with certainty whether or not the HSV-1 contributed significantly to her clinical picture.

Thirteen of 27 patients had a final diagnosis of uveitis caused by one of the herpes viruses other than EBV (Figure 3). Seven of 13 cases had anterior uveitis and all of these cases tested GWC+ only (1 HSV, 3 VZV and 3CMV). Conversely, 6 of 13 cases had non-anterior uveitis and here all cases tested PCR+ only (4 VZV and 2 CMV). In the PCR-/GWC+ anterior uveitis group only 3 of 7 cases were HIV+ while in the PCR+/GWC- non-anterior uveitis group 5 of 6 cases were HIV+. A final diagnosis of infectious uveitis was made in 14 of 15 HIV+ patients as follows: 1 HSV, 4 VZV, 3 CMV, 1 HIV-induced, 3 ocular syphilis, 1 poststreptococcal and 1 *Toxoplasma*. On the other hand, 10 of 12 HIV- patients had a final diagnosis of infectious uveitis: 3 VZV, 2 CMV, 3 RV and 2 possible ocular TB. No cases of rubella virus were therefore seen among HIV+ patients while no cases of ocular syphilis or *Toxoplasma* were seen among HIV- patients in this subgroup.

Discussion

Very little is known about the aetiology of infectious uveitis in South Africa and to the best of our knowledge there is only one other report of the use of PCR and GWC to determine the underlying cause of uveitis in South African patients although that study was conducted in a rural area 2000 km from our metropole.[18] In our area we found the prevalence of HIV infection among all patients

presenting with uveitis to be 37.7%. In the subgroup with a positive PCR and/or GWC result studied in this paper the prevalence was 55.6%. When looking at the actual samples tested it is interesting to note that in the HIV+ group 68.4% of samples were PCR+ while only 31.6% were GWC+. In contrast, in the HIV- group 55.6% of samples were PCR+ compared to 44.4% that were GWC+. This suggests that in HIV+ patients the pathogen detection frequency of PCR is more than twice as high as that of GWC whereas in HIV- patients this is not the case. To us, this implies that HIV+ cases more frequently present early and have actual infection whereas in HIV- patients presentation is may sometimes be slightly more delayed, thereby allowing time for antibody production to take place. Even though this finding did not reach statistical significance due to small sample size it does suggest that PCR may be more useful than GWC in HIV+ uveitis cases with low CD4+ cell counts. Not surprisingly, the majority of patients from whom >1 pathogen was identified were HIV+ with a median CD4+ cell count of 181 x 10^6/l, whereas HIV+ patients from whom ≤1 pathogen was identified had a median CD4+ cell count of 249 x 10^6/l [interquartile range 94 – 501], illustrating that patients become more susceptible to multiple infections as their CD4+ cell counts drop. .

A noteworthy finding is that only 31.3% of patients with anterior uveitis were HIV+ whereas 90.9% with any non-anterior uveitis were HIV+ (p=0.005). This underlines the importance of HIV testing in patients presenting with any form of uveitis in our area but even more so in cases presenting with a form other than anterior uveitis. Furthermore, in a study from the Netherlands, 43 of 51 immunocompromised patients with posterior or panuveitis were found to have an infectious aetiology by using a combination of PCR and GWC.[21] The vast majority (49%) of those cases were caused by CMV followed by *Toxoplasma* (26%), *Treponema pallidum* (14%) and VZV (7%). In our study, VZV accounted for more cases of posterior or panuveitis in HIV+ individuals than CMV and we also found viral causes of anterior uveitis in HIV+ individuals with HSV, VZV, CMV and HIV itself accounting for one case each. It would therefore appear as though the patterns of infectious uveitis in immunocompromised patients differ between the two countries. In Thailand, CMV was also found to be the most common cause of posterior and/or panuveitis in HIV-patients whereas in our study

the only cause of panuveitis in an HIV+ individual was VZV while CMV was only detected in HIV- patients with anterior uveitis.[17] The seroprevalence of CMV in The Netherlands is 45.6% while in Thailand it has been reported to vary from 50.0 – 93.3%.[22-24] The seroprevalence of both CMV and VZV in the Western Cape is unknown although it has been reported to be as high as 100% in rural South Africans with HIV infection.[25] Future research should therefore be aimed at measuring the seroprevalence of the different human herpes virus in the Western Cape to determine whether this might explain why VZV was more commonly found to cause uveitis than CMV in our study. The ongoing development of newer PCR techniques should in future also allow us to diagnose infectious uveitis more accurately. The development of multiplex PCR strip kits targeting a host of viruses, bacteria, fungi and parasites known to cause ocular infections is cause for optimism that our ability to accurately diagnose intraocular infections will continue to improve in future.[26]

Possible limitations in our study include relatively small sample sizes and the fact that due to practical reasons we were not able to perform GWCs in as many participants as compared to PCR. Positive considerations are the prospective and structured nature of the study which is the first ever of its kind to study the causes of infectious uveitis in such detail in South Africa.

Conclusions

In the area we serve there are differences in the clinical presentation and underlying causes of infectious uveitis in patients with and without HIV infection. Infections caused by more than one pathogen occur more frequently in HIV+ patients with low CD4+ cell counts and in these patients PCR is positive more than twice as many times as GWC. In HIV- patients both PCR and GWC make substantial contributions to determining the underlying cause of infectious ocular inflammation.

Table 1 Demographic and clinical data of HIV+ and HIV- patients in ocular fluid test-positive subgroup

Characteristics	All (n=27)	HIV+ (n=15)	HIV- (n=12)	p-value
Age, years (±SD)	39.6 (8.8)	38.5 (9.4)	41.0 (8.2)	0.47
PCR+	37.8 (9.9)			
GWC+	38.5 (10.1)			
Gender				
Male	11	6	5	0.93
Female	16	9	7	
Laterality				0.23
Unilateral	19	9	10	
Bilateral	8	6	2	
Anatomic distribution				0.005
Anterior	16	5	11	
Intermediate	2	2	0	
Posterior	2	2	0	
Panuveitis	7	6	1	
Appearance				0.09
Granulomatous	8	5	3	
Non-granulomatous	19	10	9	

Table 2: PCR and GWC results per HIV status.

	HIV+			HIV-		
	PCR+/GWC-	**PCR+/GWC+**	**PCR-/GWC+**	**PCR+/GWC-**	**PCR+/GWC+**	**PCR-/GWC+**
CMV	2	0	1	0	0	2
HSV	1	0	2	0	0	0
VZV	3	0	2	1	0	2
EBV	7	0	0	4	0	0
RV	0	0	0	0	4	0
Toxo	0	0	1	0	0	0
HHV6				1		
Total PCR	13			10		
Total GWC		6			8	

Footnote: PCR+ = polymerase chain reaction positive; GWC- = Goldmann-Witmer coefficient negative; GWC+ = Goldmann-Witmer coefficient positive; PCR- = polymerase chain reaction negative; HHV6 = human herpesvirus 6

Table 3 Clinical characteristics, test results and final diagnoses highlighting discordant AC tap results

No	Age	Gender	Eye	Anat	Gran	HIV	CD4	Positive AC tap result	Final diagnosis	Explanation
1	35	M	OD	Ant	N	N	N/A	EBV PCR	*Idiopathic**	Insignificant EBV VL
2	28	F	OD	Pan	N	Y	483	EBV PCR	*Idiopathic**	Insignificant EBV VL
3	41	F	OS	Ant	N	Y	754	CMV GWC = 7.40	CMV	CMV GWC+
4	34	M	OD	Ant	N	N	N/A	RV PCR & GWC = 102.07	FUS due to RV	RV PCR+ & GWC+
5	43	M	OD	Ant	N	N	N/A	VZV GWC = 48.37	VZV	VZV GWC+
6	44	F	OD	Post	N	Y	88	CMV PCR	CMV	CMV PCR+
7	50	F	OU	Ant	N	N	N/A	EBV PCR	*Possible ocular TB**	TST+, meets criteria
8	57	F	OD	Ant	Y	N	N/A	VZV GWC = 25.21	VZV	VZV GWC+
9	34	F	OU	Post	N	Y	32	VZV PCR	VZV	VZV PCR+
10	32	M	OU	I/med	Y	Y	414	EBV PCR	*Poststreptococcal**	Raised anti-DNase B
11	25	F	OD	Ant	Y	Y	184	VZV GWC = 48.45, HSV GWC = 6.71, EBV PCR	VZV	VZV GWC > HSV GWC
12	26	F	OD	Pan	Y	Y	138	EBV PCR	*Syphilis**	RPR = 1 : 512
13	43	M	OU	Ant	N	Y	121	EBV PCR, HIV VL > 35 x 10^6 copies/ml	HIV-induced uveitis	HIV VL eye >> HIV VL blood
14	44	F	OU	Ant	Y	N	N/A	CMV GWC = 13.72, RV PCR & GWC = 333.07	CMV	Picture *not* compatible with FUS
15	40	F	OS	Ant	N	N	N/A	EBV PCR	*Possible ocular TB**	TST+, QFT+, meets criteria
16	35	M	OS	Ant	N	Y	181	EBV PCR	*Syphilis**	RPR = 1 : 16
17	50	M	OD	Ant	N	Y	714	HSV GWC = 19.71	HSV	HSV GWC+
18	51	M	OU	Pan	N	Y	35	VZV PCR	VZV	VZV PCR+
19	39	F	OU	Pan	N	Y	99	VZV PCR	VZV	VZV PCR+
20	27	F	OS	Ant	N	N	N/A	RV PCR & GWC = 48.14	FUS due to RV	RV PCR+ & GWC+
21	32	M	OD	Ant	N	N	N/A	HHV6 PCR, RV PCR & GWC = 32.65	FUS due to RV	RV PCR+ & GWC+
22	45	F	OS	Pan	N	N	N/A	VZV PCR	VZV	VZV PCR+
23	29	F	OD	Pan	Y	Y	789	VZV GWC = 4.80, Toxo GWC = 5.83	Toxoplasma	Retinal granuloma, better on Toxo Rx
24	43	M	OD	Ant	N	N	N/A	EBV PCR	*HLA-B27**	Typical phenotype, HLA-B27+
25	42	F	OS	Ant	Y	N	N/A	CMV GWC = 3.98	CMV	CMV GWC+
26	50	F	OS	I/med	Y	Y	400	HSV-1 PCR	*Syphilis**	RPR = 1 : 128
27	50	M	OU	Pan	N	Y	86	CMV PCR, EBV PCR	CMV	CMV PCR+

Key: Anat = anatomical distribution; Gran = Granulomatous; AC = anterior chamber; Ant = anterior; Pan = panuveitis; Post = posterio; I/med = intermediate; N/A = not applicable; VL = viral load; RV = rubella virus; FUS = Fuchs' uveitis syndrome; TST = tuberculin skin test; QFT = Quantiferon; RPR = rapid plasma reagin
*Italics** = cases where final diagnosis is discordant with positive AC tap result

Figure 1 Large central ghost KP (white arrow) and new KP (yellow arrow)

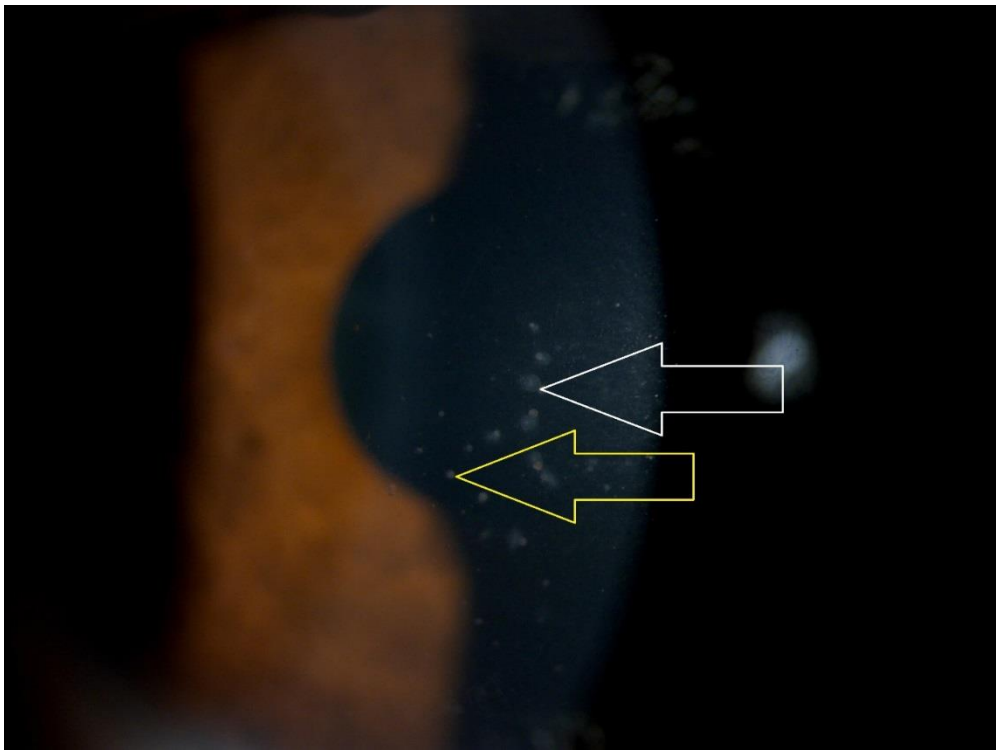


Figure 2 Koeppe nodules (white arrow) and Busacca nodules (yellow arrow) OS

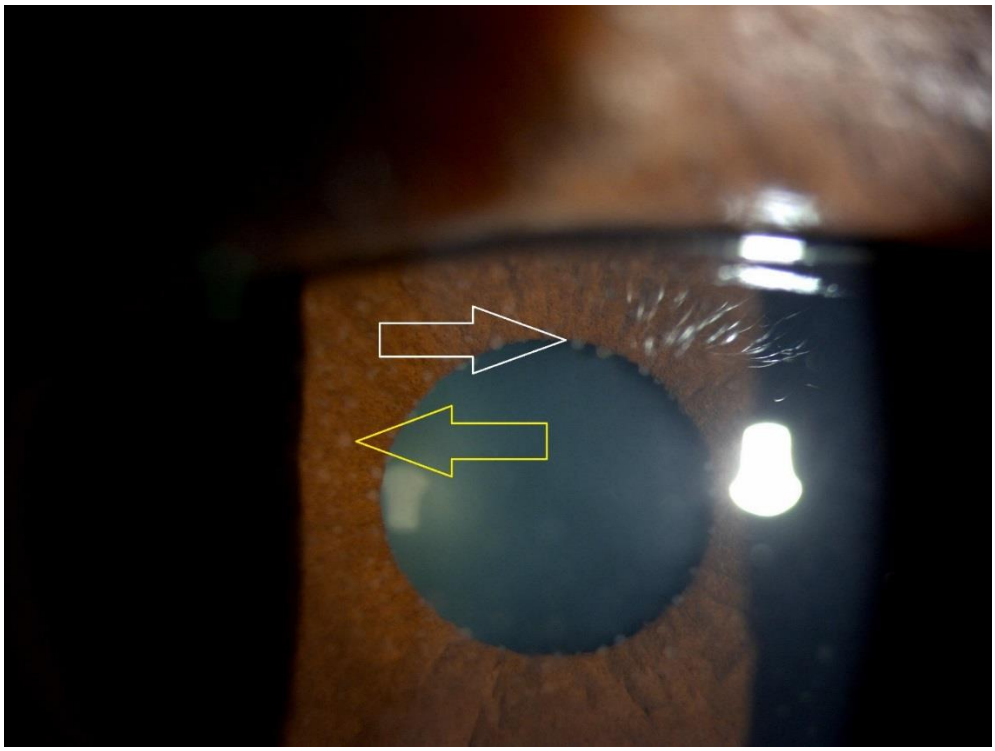
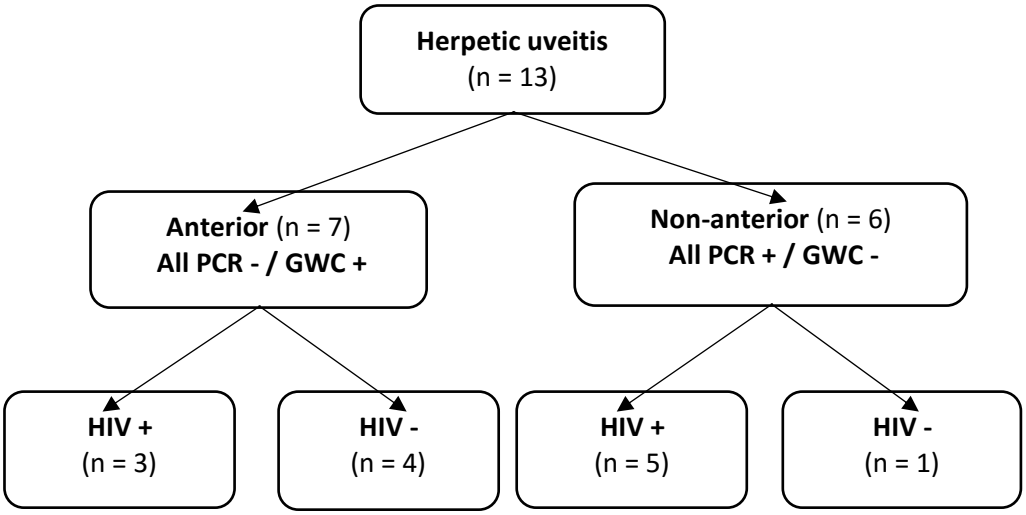


Figure 3 Herpetic uveitis stratified by anatomical distribution and HIV status



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The Aetiology of Intraocular Inflammation in HIV positive and HIV negative Patients at a Tertiary Hospital in Cape Town, South Africa

Abstract

Purpose: To describe the prevalence of different causes of uveitis in South Africa

Methods: Prospective cross-sectional study

Results: One-hundred-and-six patients were enrolled and 37.7% were HIV+. Anterior and panuveitis were most frequently seen. Infectious, non-infectious and idiopathic uveitis were diagnosed in 66.0%, 17.0% and 17.0% of all cases, respectively. Eighty percent of HIV+ cases had infectious uveitis. Overall, intraocular tuberculosis (IOTB), herpetic and syphilitic uveitis were the commonest infectious causes. Sarcoidosis and HLA-B27-associated uveitis were the commonest non-infectious causes. In anterior uveitis, HIV+ cases most frequently had probable IOTB, syphilitic or idiopathic uveitis while HIV- cases had possible IOTB, idiopathic or HLA-B27-associated uveitis. In panuveitis, HIV+ cases mostly had syphilis, probable IOTB, Toxoplasma and varicella-zoster-virus whereas HIV- cases mostly had possible IOTB, sarcoidosis and idiopathic uveitis.

Conclusion: Infectious uveitis is common in South Africa, especially amongst HIV+ patients. Causes of anterior and panuveitis differ between HIV+ and HIV- patients.

Keywords:

Etiology; causes; uveitis; infectious; non-infectious; idiopathic; HIV; South Africa; Africa

Declaration of Interest:

The authors report no conflicts of interest

Introduction

South Africa has the highest prevalence of both tuberculosis (TB) and Human immune deficiency virus HIV infection of any country in the world and 61% of South African TB patients are reported to have HIV co-infection.[1] Furthermore, Sub-Saharan Africa has the highest antenatal syphilis prevalence of all 6 WHO world regions with South Africa and Zimbabwe featuring prominently in the sub-region which is not surprising given the high prevalence of HIV in these countries.[2,3] To date there has been a disconcerting shortage of literature describing the patterns of uveitis in both Sub-Saharan Africa as a region and South Africa as a country. In the 1970's Freedman described the incidence, clinical findings and possible causes of uveitis in South African blacks but very little data followed till after the turn of the century.[4,5]

Some recent publications have addressed specific clinical entities as well as diagnostic special investigations but epidemiological data from South Africa has remained sparse.[6-11]

In 2016 Schaftenaar et al. described their findings in a rural setting in the North-Eastern corner of South Africa.[12] They reported that 64% of participants in their study were HIV positive (HIV+) and that the cause of uveitis was infectious in 72%, idiopathic in 16% and autoimmune in 12%. Interestingly, the majority of their infectious cases (51%) was attributed to herpes viruses, followed by TB (24%) and *Treponema pallidum* infection (7%). In this paper we report our findings from a tertiary hospital in Cape Town, South Africa which serves a culturally diverse population living in an area that differs markedly with regard to climate, geography and socio-economic factors from the rural setting described previously.

Materials and methods

Study participants and overview of management

A prospective, cross-sectional study was conducted which enrolled 106 consecutive patients with either a first episode of uveitis or pre-existing chronic or relapsing uveitis of unknown cause

between February 2014 and July 2015. The patients were all seen and included in the study by a single clinician (DPS) at the Eye Clinic of Tygerberg Academic Hospital, a tertiary hospital serving the Eastern Metropole of Cape Town as well as the rural West Coast and interior regions of the Western Cape Province. Despite being located in a tertiary hospital, most of the patients seen in our Eye Clinic were referred from primary level health care practitioners due to the paucity of secondary level eye care for patients without medical insurance in our drainage area. To be included in the study, patients had to: 1) be 18 years or older, 2) have uveitis of unknown cause and 3) consent to HIV testing after appropriate counselling. All participants completed a detailed verbal systemic uveitis questionnaire which was followed by a thorough eye examination and a panel of special investigations tailored to each patient. The study was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University (Ref no N13/10/146) and adhered to the tenets of the Declaration of Helsinki.

Investigations

a) Baseline

All participants underwent comprehensive special investigations to search for the underlying cause of intraocular inflammation. Blood samples were taken to determine HIV status, CD4+ count if indicated, full blood count, erythrocyte sedimentation rate (ESR), rapid plasma reagin (RPR) and *Treponema pallidum* antibodies (TPAbs) for syphilis, creatinine and serum angiotensin converting enzyme (sACE) levels. Urine dipstick analysis and chest radiographs were requested in all cases. Tuberculin skin tests (TST) and QuantiFERON®-TB Gold (QFT) tests (Cellestis Inc., Chadstone, Victoria, Australia) were performed in 89 and 105 participants, respectively. In selected cases, standard or high-resolution chest computerized tomography (CT) scans were obtained after consultation with a specialist pulmonologist while in some cases positron emission tomography (PET) PET/CT scans were requested if considered necessary. An HLA-B27 test was only requested if patients presented with severe unilateral fibrinous acute anterior uveitis while anti-Streptolysin O (ASOT) and anti-DNase B

titers were only performed in patients under the age of 40 years for possible poststreptococcal uveitis(PSU).[9]

Second-line

Anterior chamber (AC) taps were performed as second-line investigations using the method described previously.[6] Aqueous humor (AH) samples from 100 patients were analysed by polymerase chain reaction (PCR) testing for herpes viruses 1 – 6, rubella virus (RV) and *Toxoplasma gondii* (*Toxo*) by the National Health Laboratory Services Medical Virology laboratory at Tygerberg Academic Hospital. Furthermore, paired serum and AH samples from 82 of the participants were sent for determination of Goldmann-Witmer coefficients (GWC) for herpes simplex virus (HSV), Varicella-Zoster virus (VZV), Cytomegalovirus (CMV), RV and Toxoplasmosis as described previously at the department of Medical Microbiology, University Medical Center in Utrecht, The Netherlands.[13]

How diagnoses were made

In this study, intraocular TB (IOTB) was diagnosed as confirmed, probable or possible using the proposed classification by Gupta et al.[14] Syphilitic uveitis was diagnosed if patients had a compatible clinical picture and both a positive TPAbs test as well as a RPR titer ≥ 1:16. Patients with only a positive TPAbs test were therefore not diagnosed as having syphilitic uveitis. Poststreptococcal uveitis was diagnosed in patients with elevated anti-Streptolysin O and/or anti-DNase but only after all other potential causes had been excluded.[8]

Herpetic and rubella-virus associated uveitis was identified in patients with a suggestive clinical picture as well as a positive PCR and/or GWC result for the specific virus. All 3 cases of rubella-virus associated uveitis had a clinical picture compatible with Fuchs' uveitis syndrome (FUS). HIV-induced uveitis was diagnosed in the presence of a typical clinical picture with the HIV viral load (VL) in the eye at least 2 log units higher than the HIV VL in the serum.[11] For a diagnosis of *Toxoplasma* uveitis we required a compatible clinical picture with either positive serology or a positive PCR and/or GWC

for *Toxoplasma*. Sarcoidosis and Vogt-Koyanagi-Harada (VKH) disease were diagnosed according to the International Workshop on Ocular Sarcoidosis (IWOS) criteria and revised diagnostic criteria for VKH disease, respectively.[15,16] HLAB27-associated acute anterior uveitis was diagnosed in patients with a severe unilateral fibrinous anterior uveitis and a positive HLAB27 blood test while Granuloma annulare-associated uveitis was diagnosed when histological findings on a skin biopsy confirmed the condition in the absence of any other positive findings.[17,18] In those cases where the intraocular inflammation could not be attributed to any ocular or systemic disease the uveitis was considered to be idiopathic.

Statistical analysis

IBM SPSS version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) was used to analyse the data, using a significance level of 0.05. Categorical factors were compared between groups using Pearson's chi square test. Continuous variables were tested for normality and if plausibly normally distributed, means were compared between more than two independent groups using one-way ANOVA tests with Bonferroni adjusted post hoc tests, and if not, non-parametric equivalent tests, for instance the Kruskal-Wallis test, were used, with Mann Whitney tests to compare relevant two-way associations.

Results

Demographics and clinical findings

A total of 106 consecutive patients with uveitis were enrolled of which 52 (49.1%) were black African, 49 (46.2%) of mixed ethnicity, 3 (2.8%) Caucasian and 2 (1.9%) of Asian origin (Table 1). The mean age of all participants was 38.6 ± 12.5 years and most were female (n=62; 58.5%). Sixty-six participants (62.3%) were HIV negative (HIV-) and 40 (37.7%) HIV+ with a median CD4+ cell count of 242 x 10^6/l [interquartile range 99 - 507]. Seventeen of the 40 HIV+ cases (42.5%) were already using highly active anti-retroviral therapy (HAART) when they were enrolled in the study while 12 of 40

HIV+ cases (30%) were newly diagnosed with HIV infection upon presentation to our Eye clinic. Thirty-one of 52 black patients (59.6%) were HIV+ whereas 8 of 49 patients (16.3%) of mixed ethnicity were HIV+. In our study population, black African patients were much more frequently HIV+ compared to patients of mixed ethnicity ($p<0.001$). There was however no meaningful difference in median CD4+ cell count between HIV+ patients in the black (338.0) and mixed ethnicity (168.5) groups, respectively ($p=0.720$, Mann-Whitney U test). Bilateral involvement occurred in 47 cases (44.3%) and unilateral involvement in 59 cases (55.7%). Anatomically, 62 cases (58.5%) had anterior uveitis, 34 cases (32.1%) panuveitis and 6 cases (5.7%) posterior uveitis while 4 cases (3.8%) presented with intermediate uveitis. Granulomatous uveitis was seen in 45 cases (42.5%).

At presentation, 48 patients (45.3%) had a decimal Snellen visual acuity (VA) of worse than 6/60 in the affected or worse affected eye while 27 patients (25.5%) had a VA between 6/60 – 6/15 and 31 patients (29.2%) had a VA ≥ 6/12. In patients with infectious uveitis 38 cases (54.3%) had a VA < 6/60, 17 cases (24.3%) had a VA between 6/60 – 6/15 and 15 cases had a VA ≥ 6/12 while in patients with non-infectious uveitis only 2 cases (11.1%) had a VA < 6/60, 7 (38.9%) had a VA between 6/60 – 6/15 and 9 (50.0%) had a VA ≥ 6/12 (Table 2). Patients with infectious uveitis therefore had poorer VA than those with non-infectious uveitis ($p=0.014$, Pearson Chi-Square test). Twenty-eight of 31 patients (90.3%) with VA ≥ 6/12 had anterior or intermediate uveitis while only 3 of 31 patients (9.7%) with posterior or panuveitis presented with VA ≥ 6/12. In contrast, 27 of 48 patients (56.3%) with VA < 6/60 had posterior or panuveitis. Patients with anterior or intermediate uveitis therefore were more likely to have VA ≥ 6/12 than patients with posterior or panuveitis ($p<0.001$, Pearson Chi-Square test).

Causes of uveitis

An infectious cause was identified in 70 cases (66.0%) whereas 18 cases (17.0%) either had a non-infectious cause or were considered to be idiopathic, respectively (Table 1). In 40 HIV+ patients an infectious cause was found in 32 cases (80.0%) while a non-infectious cause was found in only 2

cases (5.0%) and 6 cases (15.0%) were idiopathic (p=0.024, Pearson Chi-Square test). In 66 HIV- cases an infectious cause was found in 38 cases (57.6%) while a non-infectious cause was identified in 16 cases (24.2%) and 12 cases (18.2%) were considered to be idiopathic.

a) Infectious uveitis

IOTB was the most common infectious cause identified in our study. In total, 35 of 106 cases (33.0%) were diagnosed as having probable or possible IOTB representing 35 of 70 (50.0%) of all infectious cases (Table 3). Twelve of 35 cases (34.3%) fulfilled the criteria for probable IOTB and 23 of 35 cases (65.7%) were labelled as possible IOTB. Eight of the 12 probable IOTB cases (66.6%) and only 3 of the 23 possible IOTB cases (13.0%) were HIV+.[9] The median CD4+ cell count of all HIV+ cases with IOTB was 249 x 10^6/l [interquartile range 70 – 464].

Herpetic uveitis accounted for 13 of 106 cases (12.2%) making it the second largest group with VZV being responsible for 7 of 13 cases (53.8%), CMV for 5 cases (38.5%) and HSV for 1 case (7.7%) in this subgroup. Eight of 13 cases (61.5%) of herpetic uveitis were HIV+ with a median CD4+ cell count of 94 x 10^6/l [interquartile range 61 – 449] (Table 4). Syphilis was the third most common cause of infectious uveitis with 11 of 106 cases (10.4%) testing positive for the disease. Nine of 11 (81.8%) cases with syphilitic uveitis were HIV+ with a median CD4+ cell count of 172 x 10^6/l [interquartile range 138 – 400]. Four cases (3.8%) of *Toxoplasma*, 3 cases (2.8%) each of rubella virus and PSU as well as 1 case of HIV-induced uveitis (0.9%) were responsible for the remainder of the infectious cases. Infectious causes were much more common than other causes in HIV+ cases (p=0.024, Pearson Chi-Square test). In HIV+ cases with CD4+ cell count <200 x 10^6/l, 18 of 19 cases (94.7%) had an infectious cause while in cases with CD4+ cell count ≥ 200 x 10^6/l only 14 of 21 cases (66.7%) had an infectious cause. Whilst this demonstrated a trend for infectious causes to be more common in HIV+ patients with lower CD4+ cell counts it did not reach statistical significance (p=0.079, Pearson Chi-Square test).

b) Non-infectious uveitis

Sarcoidosis was diagnosed in 8 of 106 participants (7.5%) all of whom were HIV-. According to the IWOS criteria there were 6 cases of presumed ocular sarcoidosis and 1 case each of probable and definite ocular sarcoidosis. HLAB27-associated acute anterior uveitis was also diagnosed in 8 of 106 cases (7.5%) of which 6 (75%) were HIV-. The remaining 2 cases of non-infectious uveitis were diagnosed with VKH and Granuloma annulare-associated uveitis, respectively.

c) Idiopathic uveitis

In a total of 18 of 106 cases (17.0%) we were not able to identify an underlying ocular or systemic cause for the intraocular inflammation. The median CD4+ cell count of HIV+ patients with idiopathic uveitis was 489 x 10^6/l [interquartile range 467 – 716] which is considerably higher than that seen in herpetic (94 x 10^6/l), syphilitic (172 x 10^6/l) and tuberculous (249 x 10^6/l) uveitis respectively (p=0.002, Mann-Whitney U test). The median CD4+ cell counts of HIV+ patients with different underlying diagnoses are summarised in Table 4. Immune recovery uveitis was considered in all cases where HAART had been recently initiated but none of the cases warranted such a diagnosis.

Anatomical distribution of uveitis

Table 3 shows a summary of all the causes of uveitis stratified by HIV status and anatomical distribution. The 3 most common diagnoses in HIV+ patients with anterior uveitis were probable IOTB (n=4; 20.0%), syphilis (n=4; 20.0%) and idiopathic (n=4; 20.0%) while in HIV- patients with anterior uveitis the 3 most common diagnoses were possible IOTB (n=13; 31.0%), idiopathic (n=9; 21.4%) and HLA-B27 associated anterior uveitis (n=6; 14.3%).

The most common diagnoses in HIV+ patients with panuveitis in descending order were syphilis (n=4; 26.7%), probable IOTB (n=4; 26.7%) and *Toxoplasma* as well as VZV (n=2 each; 13.3%) whereas in HIV- individuals with panuveitis the most common diagnoses were possible IOTB (n=7; 36.8%), presumed ocular sarcoidosis, probable IOTB and idiopathic uveitis (n=3 each; 15.8%).

In posterior uveitis, syphilis, *Toxoplasma* and probable IOTB were diagnosed in the HIV- patients while the HIV+ group had diagnoses of CMV, VZV and idiopathic PU. Both HIV- patients with intermediate uveitis had presumed ocular sarcoidosis while the 2 HIV+ patients with intermediate uveitis had poststreptococcal uveitis and syphilitic uveitis, respectively.

Interestingly, HIV+ cases were not more prone to having posterior or panuveitis and HIV- cases were not more prone to having anterior or intermediate uveitis (p=0.568). HIV+ cases with anterior and intermediate uveitis did however have much higher median CD4+ cell counts than those with posterior and panuveitis (p=0.006, Kruskal-Wallis test).

Discussion

This study provides prospectively collected data about the aetiology of uveitis in patients living in the South-western corner of South Africa, an area that is culturally, geographically and socio-economically diverse. The majority of patients seen at our facility are either black Africans or of mixed ethnicity and live under suboptimal socio-economic circumstances. In total, 37.7% of patients included in our study had HIV infection which is noticeably lower than the 64% reported from rural South Africa.[12] Closer inspection however reveals that only 16.3% of mixed ethnicity patients were HIV+ compared to 59.6% of black patients. Less than half of the HIV+ patients were receiving HAART when they were enrolled in the study for two main reasons. Firstly, the policy of the South African National Department of Health at the time when the study was conducted was to only provide anti-retroviral medication to HIV+ individuals with CD4+ counts < 350 x 10^6/l. This policy has subsequently been amended so that HAART is now available to all patients with newly diagnosed HIV infection in South Africa. Secondly, almost 1 in 3 of the HIV+ cases were newly diagnosed with HIV infection upon presenting to the Eye clinic which underlines the importance of testing the HIV status of patients presenting with uveitis in our area.

In the HIV+ group some interesting observations emerged regarding the CD4+ counts. In table 1 we indicated that, as expected, patients with infectious uveitis had much lower median CD4+ counts than those with non-infectious or idiopathic uveitis. In table 2 we explored the possible association between low CD4+ count and poor visual acuity (< 6/60) and although we could not demonstrate statistical significance due to the relatively small sample size we still found that half of all HIV+ cases had a VA < 6/60 and that this group had a median CD4+ count of well below 200 x 10^6/l whereas cases with better visual acuity had higher CD4+ counts. In table 3 we demonstrated that patients with posterior or panuveitis had significantly lower median CD4+ counts than those with anterior or intermediate uveitis while in table 4 we highlighted the fact that cases with a high likelihood of an actual infection such as probable IOTB, herpetic and syphilitic uveitis had much lower median CD4+ counts than idiopathic or possible IOTB cases where the inflammation is more likely to be due to an immune-mediated response against unknown antigens.

Overall, infectious uveitis was far more common than non-infectious and idiopathic uveitis respectively but even more so in the HIV+ group compared to the HIV- group. The vast majority of uveitis cases were either classified as anterior or panuveitis which is compatible with what has previously been reported from elsewhere in Africa.[19] Cases with anterior or intermediate uveitis were found to have better VA than cases with posterior or panuveitis while cases with infectious uveitis were found to have worse VA than cases with non-infectious uveitis. Given the high prevalence of both TB and HIV in our area and their known clinical association it is not surprising that possible or probable IOTB was the most common cause of infectious uveitis in our study. This once again raises the issue about the pathophysiology of IOTB and how much of the clinical picture in any given patient should be attributed to direct infection as opposed to an immunological reaction against as yet unspecified TB antigens. In an earlier paper we reported that HIV- patients are more likely to have possible IOTB and HIV+ patients are more likely to have probable IOTB and here we have shown that HIV+ cases with possible IOTB have a higher median CD4+ count than those with

probable IOTB.[9] It has recently been proposed that TB and sarcoidosis may actually lie on opposite ends of the same spectrum of disease.[20]

In keeping with this hypothesis, both possible and probable IOTB would then fit in somewhere between pure sarcoidosis and pure TB – most likely in the tuberculous sarcoid (TS) classification – as they have clinical and immunological features suggestive of TB and yet a definitive diagnosis of TB cannot be made. Herpetic uveitis was the second most common cause of infectious uveitis and, as in other reports from Sub-Saharan Africa, VZV was again responsible for more cases than CMV.[12,21] Syphilitic uveitis was the third most common infectious cause and was associated with HIV infection in over 80% of cases. This finding lends support to reports from elsewhere in the world that syphilis is still an important cause of uveitis, especially in HIV+ patients, and should be actively searched for in the workup of any patient with uveitis.[3] Other infectious causes identified included *Toxoplasma* and rubella virus even though neither of these infections were diagnosed in the rural setting. This may reflect regional differences in pathogens.

In patients with non-infectious uveitis, sarcoidosis and HLA-B27 associated AU were the most common causes and most patients were HIV-. Idiopathic uveitis was twice as prevalent in HIV- patients while HIV+ patients with idiopathic uveitis had significantly higher median CD4+ cell counts than those with ocular infections. This would appear to suggest that immunocompromised patients with lower CD4+ cell counts are less likely to have a final diagnosis of idiopathic uveitis.

A potential limitation of our study is that it was conducted at a tertiary hospital instead of a primary healthcare facility which may introduce selection bias due to the high likelihood that not all cases of uveitis would have been referred to our facility. Another limitation of our study is that patient follow-up was often erratic due to socio-economic challenges and that in certain cases the response to empiric treatment could not be monitored, definitive diagnoses could not be communicated to patients or imaging studies could not be performed. A potential strength of this study is that it provided patients with access to investigations such as QuantiFERON®, GWC and PET/CT that would

previously have been inaccessible due to financial constraints and logistical problems. It also afforded the investigators the opportunity to assess the value of these investigations in the South African context.

Conclusion

Uveitis is a multi-faceted clinical entity that often requires a considerable amount of effort to allow the correct diagnosis to be made. Despite ongoing advances in diagnostic modalities, a definitive diagnosis can still not be made in a significant proportion of patients. In developing countries such as South Africa where HIV, TB and syphilis are highly prevalent an infectious cause may be found in up to two-thirds of cases and must therefore be sought in earnest since properly targeted treatment may significantly reduce the burden of this potentially blinding entity on society.

Table 1: Demographic and clinical data stratified by infectious, non-infectious and idiopathic uveitis

Characteristics	All participants (n=106)	Infectious (n=70)	Non-infectious (n= 18)	Idiopathic (n=18)	p-value
Age, years (±SD)	38.6 (12.5)	39.0 (11.8)	38.2 (13.1)	37.7 (15.0)	0.919
Gender (%)					0.736
Male	44 (41.5)	30 (42.9)	6 (33.3)	8 (44.4)	
Female	62 (58.5)	40 (57.1)	12 (66.7)	10 (55.6)	
Ethnicity (%)					0.232*
Black	52 (49.1)	38 (54.3)	6 (33.3)	8 (44.4)	
Mixed	49 (38.9)	30 (42.9)	12 (66.7)	7 (38.9)	
White	3 (2.8)	1 (1.4)	0 (0)	2 (11.1)	
Asian	2 (1.9)	1 (1.4)	0 (0)	1 (5.6)	
Laterality (%)					0.55
Unilateral	59 (55.7)	40 (57.1)	11 (61.1)	8 (44.4)	
Bilateral	47 (44.3)	30 (42.9)	7 (38.9)	10 (55.6)	
Anatomic distribution (%)					0.276
Anterior	62 (58.5)	37 (52.9)	12 (66.7)	13 (72.2)	
Intermediate	4 (3.8)	2 (2.9)	2 (11.1)	0 (0)	
Posterior	6 (5.7)	5 (7.1)	0 (0)	1 (5.6)	
Panuveitis	34 (32.1)	26 (37.1)	4 (22.2)	4 (22.2)	
Appearance (%)					0.395
Granulomatous	45 (42.5)	33 (47.1)	6 (33.3)	6 (33.3)	
Non-granulomatous	61 (57.5)	37 (52.9)	12 (66.7)	12 (66.7)	
Snellen VA (%)					0.014
≥ 6/12	31 (29.2)	15 (21.4)	9 (50.0)	7 (38.9)	
6/60 - 6/15	27 (25.5)	17 (24.3)	7 (38.9)	3 (16.7)	
<6/60	48 (45.3)	38 (54.3)	2 (11.1)	8 (44.4)	
HIV-status (%)					0.024
Negative	66 (62.3)	38 (54.3)	16 (88.9)	12 (66.7)	
Positive	40 (37.7)	32 (45.7)	2 (11.1)	6 (33.3)	
Median CD4 [IQR]	242 [99 - 507]	172 [87 -415]	719 [552 - 886]	486 [467 - 716]	0.04**

Abbreviations: SD = standard deviation; IQR = interquartile range; VA = visual acuity

*p-value represents comparison between black and mixed ethnicity groups

** p-value represents comparison between infectious and non-infectious as well as

infectious and idiopathic

Table 2: Visual acuity according to anatomical distribution, type of uveitis and HIV status

		Snellen BCVA			
	Total cases	**VA <6/60 (n=48)**	**VA 6/60 - 6/15 (n=27)**	**VA ≥6/12 (n=31)**	**p-value**
Anatomical distribution					<0.001
Anterior uveitis	62	20 (41.7)	15 (55.6)	27 (87.1)	
Panuveitis	34	24 (50.0)	7 (25.9)	3 (9.7)	
Posterior uveitis	6	3 (6.3)	3 (11.1)	0	
Intermediate uveitis	4	1 (2.0)	2 (7.4)	1 (3.2)	
Type of uveitis					0.014
Infectious	70	38 (79.2)	17 (63.0)	15 (48.4)	
Non-infectious	18	2 (4.2)	7 (25.9)	9 (29.0)	
Idiopathic	18	8 (16.7)	3 (11.1)	7 (22.6)	
HIV status					0.699
Negative	66	28 (58.3)	17 (63.0)	21 (67.7)	
Positive	40	20 (41.7)	10 (37.0)	10 (32.3)	
Median CD4 count if HIV+ [IQR]	242 [99 - 507]	172 [86 - 486]	298 [88 - 467]	484 [121 - 863]	0.218

BCVA = best corrected visual acuity

Table 3: Causes of uveitis stratified by HIV status and anatomical distribution

	All cases (n=106)			Anterior uveitis (n=62)		Panuveitis (n=34)		Posterior uveitis (n=6)		Intermediate uveitis (n=4)		p-value
Classification of cause	**Total**	**HIV+ (n=40)**	**HIV- (n=66)**	**HIV+ (n=20)**	**HIV- (n=42)**	**HIV+ (n=15)**	**HIV- (n=19)**	**HIV+ (n=3)**	**HIV- (n=3)**	**HIV+ (n=2)**	**HIV- (n=2)**	
Infectious (%)												
Probable ocular TB	12 (11.3)	8 (20)	4 (6.1)	4 (20.0)		4 (26.7)	3 (15.8)		1 (33.3)			
Possible ocular TB	23 (21.7)	3 (7.5)	20 (30.3)	2 (10.0)	13 (31.0)	1 (6.7)	7 (36.8)					
Syphilis	11 (10.4)	9 (22.5)	2 (3.0)	4 (20.0)	1 (2.4)	4 (26.7)			1 (33.3)	1 (50.0)		
Poststreptococcal	3 (2.8)	1 (2.5)	2 (3.0)		2 (4.8)					1 (50.0)		
CMV	5 (4.7)	3 (7.5)	2 (3.0)	1 (5.0)	2 (4.8)	1 (6.7)		1 (33.3)				
VZV	7 (6.6)	4 (10.0)	3 (4.5)	1 (5.0)	2 (4.8)	2 (13.3)	1 (5.3)	1 (33.3)				
HSV	1 (0.9)	1 (2.5)	0 (0)	1 (5.0)								
Rubella	3 (2.8)	0(0)	3 (4.5)		3 (7.1)							
HIV	1 (0.9)	1 (2.5)	0 (0)	1 (5.0)								
Toxoplasma	4 (3.8)	2 (5.0)	2 (3.0)			2 (13.3)	1 (5.3)		1 (33.3)			
Subtotal	*70 (66.0)*	*32 (80.0)*	*38 (57.6)*	*14 (70.0)*	*23 (54.8)*	*14 (93.3)*	*12 (63.2)*	*2 (66.6)*	*3 (100.0)*	*2 (100.0)*		
Non-infectious (%)												
Sarcoidosis	8 (7.5)	0 (0)	8 (12.1)		2 (4.8)		4 (21.1)				2 (100.0)	
HLAB27	8 (7.5)	2 (5.0)	6 (9.1)	2 (10.0)	6 (14.3)							
VKH	1 (0.9)	0 (0)	1 (1.5)		1 (2.4)							
Granuloma annulare	1 (0.9)	0 (0)	1 (1.5)		1 (2.4)							
Subtotal	*18 (17.0)*	*2 (5.0)*	*16 (24.2)*	*2 (10.0)*	*10 (23.8)*		*4 (21.1)*				*2 (100.0)*	
Idiopathic (%)	18 (17.0)	6 (15.0)	12 (18.2)	4 (20.0)	9 (21.4)	1 (6.7)	3 (15.8)	1 (33.3)				
Median CD4+ count [IQR]		242 [99 - 507]		480 [209 - 715]		102 [72 - 163]		88 [32 - 467]		407 [400 - 414]		0.006

Table 4: Median CD4 counts of HIV+ patients with different diagnoses			
HIV+ patient group	**Median CD4+ count (x10^6/l)**	**[IQR]**	**p-value**
Aetiology			
All (n=40)	242	99 - 507	
All IOTB (n=11)	249	70 - 464	
Probable IOTB (n=8)	87	66 - 377	
Possible IOTB (n=3)	610	249 - 863	
Herpetic uveitis (n=8)	94	61 - 449	
Syphilitic uveitis (n=9)	172	138 - 400	
Idiopathic (n=6)	489	467 - 716	0.002*
IQR = interquartile range; IOTB = intraocular tuberculosis			
*median CD4+ count of idiopathic cases compared to the combined			
CD4+ counts of probable IOTB, herpetic and syphilitic uveitis			

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Chapter 10: Original article – NOT YET SUBMITTED FOR PUBLICATION

Ocular syphilis and neurosyphilis in HIV positive and HIV negative patients: Can immunoblotting shed new light?

Introduction

Syphilis is a sexually transmitted infection caused by the spirochete *Treponema pallidum*. The clinical manifestations of systemic syphilis are typically divided into 4 stages: primary, secondary, latent and tertiary although ocular involvement may occur in any of these stages.[1] Syphilis was an important cause of uveitis in the first half of the twentieth century but after the introduction of penicillin the prevalence dropped considerably. However, during the first decade of the twenty-first century there has been a resurgence with an increasing number of cases being reported by several authors.[2-5]

The majority of these cases were reported in males, especially those between 20 - 30 years of age, many of whom exhibited high-risk sexual behaviour.[6] The association between syphilis and HIV infection has also been well described and all HIV-infected patients with uveitis should be investigated for syphilis and *vice versa*.[7] Furthermore, it has been reported that the association between uveitis and neurosyphilis is greater in HIV+ patients than in immunocompetent patients and that cerebrospinal fluid (CSF) analysis should therefore be performed in all HIV+ patients with syphilitic uveitis.[1]

During the secondary and tertiary stages of the disease, uveitis is the most common ocular manifestation of syphilis.[8] Embryologically, the optic nerve and retina develop as extensions of the brain and many authors contend that syphilitic retinitis and optic neuritis represent a form of neurosyphilis and should therefore be treated as such.[3,9,10] Whether syphilitic anterior uveitis should be considered in the same light is the subject of an ongoing debate. Many experts suggest that all cases of ocular syphilis should be considered identical to neurosyphilis while others are not yet convinced.[11]

The diagnosis of ocular syphilis is made, after exclusion of other possible causes, if a patient has ocular inflammation compatible with syphilis and positive syphilis serology which should include both a treponemal and a non-treponemal test. The Centres for Disease Control (CDC) recommend a treponemal test such as an enzyme immunoassay (EIA) which detects antibodies to treponemal antigens as an initial screening test for syphilis.[4] If positive, this should be followed by a non-treponemal test such as the Venereal Disease Research Laboratory (VDRL) or the rapid plasma reagin (RPR) which detects antibodies directed against membrane phospholipids such as cardiolipin. These tests are used to screen for active disease and to quantify antibodies but may give false positive results in diseases other than syphilis such as collagen vascular diseases.[8] Specimens with discordant results (i.e. EIA positive and RPR negative) should be submitted for a confirmatory test such as treponema pallidum particle agglutination test (TP-PA) as a diagnosis of syphilis is confirmed if the latter test is positive. Treponemal test reversion may occur in 5 – 17% of patients who were treated for early syphilis. This contradicts the dogma that these tests always remain positive after infection by T pallidum, which is in fact a misconception.[8]

According to the CDC, confirmed neurosyphilis is diagnosed when VDRL testing is positive on cerebrospinal fluid (CSF) and probable neurosyphilis is diagnosed when CSF VDRL is negative but CSF protein and/or white cell count is elevated in the presence of clinical signs or symptoms which may include ocular findings.[12,13] However, CSF abnormalities such as higher mean white cell counts and protein levels are common in HIV-infected patients, even in the absence of syphilis. Diagnosing probable neurosyphilis in HIV-infected patients may therefore be problematic although recently published algorithms on diagnosing neurosyphilis, in HIV+ and HIV- patients respectively, are proving helpful.[14]

The advent of techniques such as PCR has brought about interesting new diagnostic possibilities in both ocular syphilis and neurosyphilis. PCR has been used to detect the presence of treponemal DNA in both aqueous and vitreous humor from eyes with suspected ocular syphilis, thus confirming the

diagnosis.[10,15-17] It has also been used to detect treponemal DNA in CSF from patients with neurosyphilis.[18] However, further investigation is required to determine whether performing PCR on both intraocular fluid and CSF from patients with suspected ocular syphilis and neurosyphilis will enable us to develop a better understanding of how these two conditions relate to one another.

Immunoblotting is another technique that is able to confirm the diagnosis of syphilis by detecting antibodies to specific treponemal antigens. In a study that compared a Western blot to the FTA-ABS as a confirmatory test for syphilis both tests had sensitivities of 100% while the specificities were 100% and 94.5% for the Western blot and FTA-ABS, respectively.[19] In another study, the Western blot had 93.8% sensitivity and 100% specificity compared to the 91.7% sensitivity and 92.0% specificity of the FTA-ABS.[20] Immunoblotting has previously been used to detect antibodies against *T pallidum* antigens in the CSF of patients with neurosyphilis[21] but there are no reports in the literature of it having been used for the detection of treponemal antibodies in aqueous or vitreous humor. The use of PCR to detect treponemal DNA and immunoblotting to detect antibodies against *T pallidum*[22] in the CSF and aqueous humor of patients suspected of having ocular syphilis and neurosyphilis could potentially improve diagnostic accuracy and increase our insight into how these conditions relate to each other.

Materials and methods

Study participants

Between February 2014 and July 2015 we enrolled 106 consecutive patients in a prospective, cross-sectional study who had either a first episode of uveitis or pre-existing chronic or relapsing uveitis of unknown cause. All patients were seen and included in the study by a single clinician (DPS) at the Eye Clinic of Tygerberg Academic Hospital on the outskirts of Cape Town. Inclusion criteria were: 1) age 18 years or older, 2) uveitis of unknown cause and 3) consent for HIV testing after appropriate

counselling. The study was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University (Ref N13/10/146) and adhered to the tenets of the Declaration of Helsinki.

Investigations

a) Baseline

All participants underwent comprehensive special investigations to look for the underlying cause of the intraocular inflammation as described previously.[23,24] Specifically, blood samples were taken to determine HIV status, and CD4+ count if indicated, as well as an EIA for Treponema pallidum antibodies (TPAbs) and RPR. Due to financial constraints we were not able to perform a confirmatory TP-PA test in patients with discordant results but patients with TPAbs+ RPR- results were included for further investigation as outlined below.

b) Second-line

If participants were found to have a positive serum RPR and/or TPAbs result they were investigated further to search for evidence of ocular and/or neurosyphilis (Figure 1). An aqueous humor (AH) sample was collected from all patients with positive serology, as previously described,[25] for syphilis PCR testing as well as a syphilis immunoblot. If either the syphilis PCR or immunoblot were positive the patient was considered to have confirmed ocular syphilis. If both tests were negative and no alternative, plausible underlying cause of uveitis was identified the patient was considered to have probable ocular syphilis. However, given the high prevalence of syphilis in developing countries and the high likelihood of previous or partial treatment of syphilis we only made a final diagnosis of ocular syphilis if the RPR titer was ≥ 1:16 in an attempt to exclude false positive cases.

Lumbar punctures were also performed to collect CSF samples to test for VDRL, FTA-ABS, syphilis PCR, syphilis immunoblot, CSF protein and CSF leukocytes. If any of the VDRL, syphilis PCR or syphilis immunoblot were positive the patient was considered to have confirmed neurosyphilis. If all 3 were negative but CSF proteins or leukocytes were raised a diagnosis of probable neurosyphilis was made

in HIV- patients whereas if all 3 were negative but CSF leukocytes were raised or CSF FTA-ABS was positive then probable neurosyphilis was diagnosed in HIV+ patients according to the most recent guidelines.[13]

c) Details of novel laboratory techniques and their interpretation – PCR and immunoblot

PCR analysis was performed essentially as described previously with minor modification.[26] Nucleic acids was extracted from 12.5 µl of ocular fluid and 250 µl of cerebrospinal fluid, respectively, using the MagnaPure 96 DNA and Viral NA LV extraction kit (Roche, Mannheim, Germany). Each samples was spiked with a fixed amount of Phocid herpes virus type 1 to monitor the extraction and amplification process. Samples were eluted in 100 µl of elution buffer of which 10 µl was used per amplification. Real-time PCR was performed on an ABI Taqman Fast 7500 (ABI, Foster City, CA, USA). Treponema pallidum-specific primers and probe were as described by Koek et al.; forward primer 5'GGT AGA AGG GAG GGC TAG TA 3', reverse primer 5'CTA AGA TCT CTA TTT TCT ATA GGT ATG G 3', probe 5' FAM-ACA CAG CAC TCG TCT TCA ACT CC-TAMRA 3'.[27]

Immunoblotting was performed using the INNO-LIA™ Syphilis Score (Fujirebio, Gent, Belgium) according to the instructions of the manufacturer. Samples were tested at a 1:100 dilution unless indicated otherwise. Determination of intraocular or intrathecal antibody production against treponemal proteins TpN47, TpN17, TpN15 and TmpA was done by visual assessment in the first instance (Figure 2). Reversed intensity of bands between ocular fluid and CSF versus serum was considered indicative for local antibody production. In addition, the intensity of the blot bands were quantitated in order to calculate a Goldmann-Witmer coefficient (GWC) for local antibody production. To this end, the immunoblot strips were scanned in the bio-imaging analyzer LAS 4000. The intensity of the individual antigen bands was quantified using the 1D gel analysis module of the ImageQuant TL software (IQTL version 8.1; GE Healthcare, Little Chalfont, UK). Measurement lanes were drawn manually across each scanned strip and the intensity of the bands was calculated automatically by the IQTL software. Band intensities were adjusted to the background and

normalized to the intensity of the +/- control band present on each strip, where the control band was set at 100%. Each strip was measured three times and the mean intensity was used for further calculation of the GWC for each TP antigen band. Prior to GWC calculation the measured band intensities were corrected for the dilution used. The GWC was calculated as follows: [mean intensity TP antigen OF or CSF/total IgG OF or CSF]/[mean intensity TP antigen serum/total IgG serum] were a value over 3 was considered positive.

Results for CSF and AH were summarised after both visual assessments and LAS/GWC calculations had been completed. Overall results were considered positive if in a specific sample both the visual assessment and at least one band of the LAS/GWC were positive. If visual assessment was negative a LAS/GWC result was considered positive when at least 2 bands on the strip registered positive result. Overall results were considered indeterminate when either the visual assessment or the LAS/GWC result was positive for a specific sample and a negative result was recorded if both the visual assessment and the LAS/GWC results were negative.

Results

Demographics and clinical findings of all TPAbs positive cases

In total, 21 of 106 cases tested positive for TPAbs by EIA. The mean age of this subgroup of patients was 42.6 ± 12.8 years and the male to female ratio was 1:2. Eleven patients were black Africans, 9 were of mixed ethnicity and 1 Caucasian. Bilateral involvement occurred in 10 cases and only 8 had a granulomatous appearance clinically. Twelve patients had anterior uveitis, 1 intermediate, 2 posterior and 6 panuveitis.

Routine serum tests

Of the 21 patients who tested positive for TPAbs by EIA, only 14 also had a positive RPR result with titers ranging from 1:1 to 1:512. Syphilis immunoblot tests that were carried out at a later stage at the University Medical Center in Utrecht, The Netherlands, revealed that 15 samples had positive

immunoblot results, 2 had indeterminate results and 1 was negative. Three samples were not tested by immunoblot. Furthermore, 15 of the 21 participants with positive TPAbs results were also HIV+ with a median CD4+ cell count of 181 x 10^6/L (Table 1).

Routine CSF tests

VDRL was positive in 3 cases, negative in 15 cases and not done in 3 cases. FTA-ABS was positive in 6 cases, equivocal in 2 cases and negative in 13 cases. All 3 cases that did not have a VDRL test had negative FTA-ABS test results which effectively ruled out a diagnosis of neurosyphilis. CSF protein levels and total white cell counts are also shown in Table 1. According to these results, 4 cases meet the CDC criteria for neurosyphilis as 3 cases have a reactive VDRL test on CSF (confirmed neurosyphilis) and the other has a positive FTA-ABS test as well as raised CSF white cell count (probable neurosyphilis). However, according to the UpToDate algorithms by Marra, 2 additional patients would have required treatment for probable neurosyphilis.[13] Both of these cases were HIV- and had negative results for both VDRL and FTA-ABS on CSF but one had significantly raised CSF protein levels and the other a raised CSF white cell count.

Additional CSF tests

The PCR and immunoblot results, including the GWC results for each antigen band, are given in Table 2. Five cases tested positive for treponemal proteins on CSF by immunoblot and it should be noted that 2 cases with neurosyphilis, 1 confirmed and 1 probable according to the CDC criteria, were not tested by immunoblot due to insufficient sample availability. Furthermore, 9 cases tested negative, 2 results were indeterminate and 5 cases in total were not tested. Of the five immunoblot CSF-positive cases, the first (Patient 2) would have been diagnosed as having probable neurosyphilis according to the CDC criteria based on a positive CSF FTA-ABS result and raised white cell count. The second positive case (Patient 11) did not have neurosyphilis according to either the CDC criteria or the UpToDate algorithms but had an equivocal FTA-ABS result and a lymphocyte count of 18/µL in the

CSF i.e. 2 more would have resulted in a diagnosis of probable neurosyphilis. The third immunoblot positive case (Patient 50) would have had probable neurosyphilis based on raised CSF protein while the fourth (Patient 52) would have been diagnosed as having probable neurosyphilis had the CSF white cell count been 6 instead of 5. The fifth case (Patient 85) was CSF VDRL- FTA-ABS + but did not fulfil the criteria for probable neurosyphilis based on a normal CSF white cell count. All cases were negative by syphilis PCR on CSF.

Additional aqueous humor tests

The PCR and immunoblot results, including the GWC results for each antigen band, are given in Table 2. Three cases tested positive for treponemal proteins by immunoblot and it should be noted that 5 cases with probable ocular syphilis were not tested by immunoblot for various reasons although some samples are due to be tested in the near future (Table 2). Eight cases tested negative and 8 cases in total were not tested. Also, in 2 cases the results were considered to be indeterminate when there were conflicting outcomes between the visual evaluation and the actual immunoblot results. The first 2 cases with positive AH immunoblot results also had positive CSF immunoblot results while the third case had a negative blot result for CSF even though the CSF VDRL test was reactive with a titer of 1:4. All cases tested negative for syphilis PCR on AH.

Summary

After reviewing all the available data for each patient, and then applying our proposed classification, we arrived at a final diagnosis as depicted in Table 2.

Discussion

The relationship between ocular syphilis and neurosyphilis is poorly understood and a lot of our current understanding is based on assumption rather than scientific fact. The first of these assumptions is that ocular syphilis may be accurately diagnosed based on positive blood serology, preferably consisting of a positive treponemal and non-treponemal test. While this holds true in

most cases of systemic syphilis it is not necessarily true in ocular syphilis. We would therefore like to propose that ocular syphilis also be classified into confirmed ocular syphilis and probable ocular syphilis in a similar fashion to neurosyphilis. Previously such an approach would not have been viable but as molecular diagnostic techniques are constantly evolving and sampling of intraocular fluid has become common practice it has now become feasible. It is our contention that a diagnosis of confirmed ocular syphilis would require molecular diagnostic evidence in the form of either a positive syphilis PCR on ocular fluid or at least evidence of intraocular antibody production against known treponemal antigens.[10,15,17] On the other hand, a diagnosis of probable ocular syphilis would require positive blood serology and the exclusion of other possible causes of ocular inflammation. Given the protean manifestations of ocular syphilis we would not include a suggestive clinical presentation as a diagnostic criterion.

Another of these assumptions is that ocular syphilis is a form of neurosyphilis because the eye is effectively, or at least embryologically, part of the brain. This paper describes 3 cases where immunoblotting of both the AH and CSF of the same patient has delivered positive results which, in our opinion, proves that these patients had both ocular and neurosyphilis However, 4 other cases were diagnosed as having probable ocular syphilis without any evidence of neurosyphilis and an additional 5 cases were diagnosed as having probable ocular syphilis and confirmed neurosyphilis according to our proposed classification. Of the 21 patients with positive serum TPAbs tests, only 12 were eventually diagnosed with ocular syphilis and treated with intravenous penicillin. Based on our proposed classification, 3 of these cases could be classified as confirmed ocular syphilis based on positive immunoblot results and the remaining 9 as probable ocular syphilis. In one additional case, Patient 65, it was uncertain whether there was enough evidence to diagnose ocular syphilis based on a positive TPAbs and serum RPR titer of 1:2. Interestingly enough, this patient did not have neurosyphilis according to the CDC criteria but did require treatment for neurosyphilis according to Marra's algorithms as she was HIV- and had a CSF lymphocyte count of >5/µL. Unfortunately there

was insufficient CSF and AH samples to perform immunoblotting in this case as it may have provided some much needed answers.

To us, it was interesting to note how immunoblotting could potentially improve the accuracy with which neurosyphilis is diagnosed. For example, in Patient 11, who was HIV+ with a CD4+ count of 234 x 10^6/L, neurosyphilis could not be diagnosed according to the CDC criteria and according to the UpToDate algorithms the patient also did not require treatment for neurosyphilis because the CSF lymphocyte count was 18/µL instead of the 20 required. However, one should bear in mind that these cut-off values are arbitrary and that in a borderline case such as this a positive immunoblot result provides supplementary evidence that the patient does indeed require treatment for neurosyphilis. In another case, Patient 52, neurosyphilis could not be diagnosed according to the CDC criteria and the patient would only have required treatment for neurosyphilis according to Marra's algorithms had the CSF lymphocyte count been >5/µL instead of exactly 5. Once again, the positive immunoblot result probably provides enough additional evidence to show that this patient requires treatment for neurosyphilis.

Given the previous papers reporting the use of PCR as a diagnostic tool for ocular syphilis it was disappointing to not find a single positive PCR result on either CSF or AH in this study. This may be partly to blame on the fact that AH was exclusively sampled and higher yield could possibly have been achieved if vitreous humor had been sampled instead. It may, however, also indicate that providing evidence of intraocular antibody production against treponemal proteins could possibly be a more sensitive diagnostic tool in certain settings although future research would be required to confirm or disprove this theory.

This study has certain limitations in that the sample sizes are quite small and that the authors were unable to test all the samples that they ideally would have liked to. However, on the positive side, this study is the first to describe the use of immunoblotting on CSF and AH in an attempt to better understand the relationship between ocular and neurosyphilis. Also, when considering the final

diagnoses in Table 2 it is worthwhile noting that no false positive results were recorded with the immunoblot. A much larger study will however be needed to accurately determine the sensitivity and specificity of this technique in diagnosing both ocular and neurosyphilis.

Conclusion

Given the availability of modern diagnostic techniques, the time has likely come to begin distinguishing between confirmed and probable ocular syphilis. Immunoblotting of CSF and AH samples of patients with positive blood syphilis serology may provide additional information to aid the diagnosis of both ocular and neurosyphilis especially in cases where other results are equivocal.

Figure 1: Algorithm to diagnose ocular syphilis and neurosyphilis

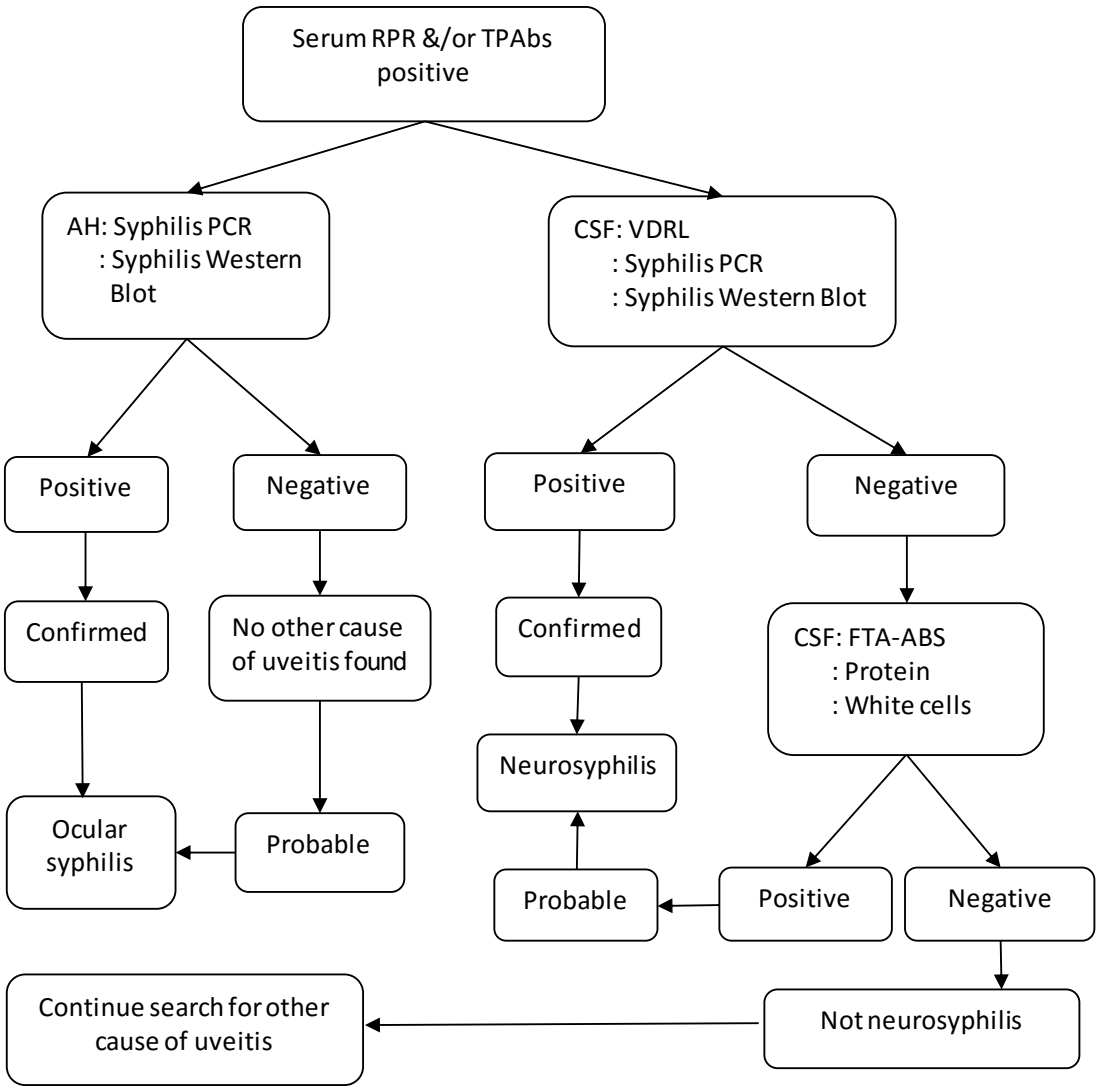


Table 1 Demographics, clinical findings, serum and CSF results pertaining to neurosyphilis

Demographic and clinical findings							Serum results				CSF results				CDC criteria for	Treat according
Pt	Age	Gender	Ethnic	Eye	Granul	Anat distrib	TP Abs	RPR	HIV	CD4	VDRL	FTA-ABS	Prot	WBC	Neurosyphilis	to UpToDate?
2	27	M	M	OU	N	Anterior	Pos	128	Pos	471	Neg	Pos	0.29	26	Yes, probable	Yes, WBC >20
6	50	F	B	OD	N	Anterior	Pos	512	Pos	411	Neg	Neg	0.24	2	No	No
11	21	F	B	OU	N	Anterior	Pos	256	Pos	234	Neg	Equivocal	0.18	18	No	No
16	44	F	B	OD	N	Posterior	Pos	Neg	Pos	88	N/D	Neg	N/D	0	No	No
17	50	F	M	OU	N	Anterior	Pos	Neg	Neg	N/A	N/D	Neg	N/D	0	No	No
28	34	M	B	OS	N	Panuveitis	Pos	16	Pos	75	Pos 1:1	Pos	0.56	3	Yes, confirmed	Yes, VDRL reactive
32	25	F	B	OD	Y	Anterior	Pos	Neg	Pos	184	Neg	Neg	1.22	9	No	No
33	26	F	B	OD	Y	Panuveitis	Pos	512	Pos	138	Neg	Neg	0.27	20	No	No
34	28	F	B	OS	Y	Anterior	Pos	Neg	Pos	54	Neg	Neg	0.23	0	No	No
42	52	F	B	OU	N	Anterior	Pos	Neg	Pos	1589	Neg	Neg	0.3	3	No	No
50	57	F	M	OD	N	Anterior	Pos	1	Neg	N/A	Neg	Neg	0.94	N/D	No	Yes, raised prot
52	59	M	M	OS	Y	Anterior	Pos	128	Neg	N/A	Neg	Equivocal	0.39	5	No	No
58	35	M	M	OS	N	Anterior	Pos	16	Pos	181	Neg	Neg	0.37	15	No	No
60	46	F	M	OU	N	Posterior	Pos	512	Neg	N/A	Pos 1:4	Pos	0.79	39	Yes, confirmed	Yes, VDRL reactive
61	50	M	B	OD	N	Anterior	Pos	8	Pos	714	Neg	Neg	0.38	16	No	No
64	51	M	C	OU	N	Panuveitis	Pos	Neg	Pos	35	N/D	Neg	1.16	3	No	No
65	37	F	M	OU	Y	Panuveitis	Pos	2	Neg	N/A	Neg	Neg	0.31	9	No	Yes, WBC >5
79	70	F	B	OU	Y	Anterior	Pos	Neg	Neg	N/A	Neg	Neg	0.27	1	No	No
85	42	F	M	OU	Y	Panuveitis	Pos	256	Pos	156	Neg	Pos	0.43	2	No	No
96	50	F	M	OS	Y	Intermed	Pos	128	Pos	400	Pos 1:4	Pos	0.67	2	Yes, confirmed	Yes, VDRL reactive
103	40	M	B	OU	N	Panuveitis	Pos	64	Pos	119	Neg	Pos	0.44	0	No	No

Key: Pt = patient; Granul = Granulomatous; Anat distr = anatomical distribution; WBC = white blood cells; N/D = not done; N/A = not applicable; Indet = indeterminate; Etnic = ethnicity; M = mixed; B = black African; C = Causasian; OU = both eyes; OD = right eye; OS = left eye

Table 2 Results of additional analysis of CSF and AH samples

Pt no	CSF PCR	AH PCR	Visual	LAS (GWC)				Visual	LAS (GWC)				Summary		Final diagnosis (based on all available data)	Rationale
			CSF vs serum	TpN47	TpN17	TpN15	TmpA	AH vs serum	TpN47	TpN17	TpN15	TmpA	CSF	AH		
2	-	-	**Pos**	-	**Pos**	-	-	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	Confirmed OS & NS	AH & CSF IB+
6	-	-	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	Probable OS*	RPR 1:512
11	-	-	**Pos**	**Pos**	**Pos**	-	-	nd	nd	nd	nd	nd	**Pos**	nd	Probable OS, confirmed NS	RPR 1:256, CSF IB+
16	-	-	-	-	-	-	-	nd	nd	nd	nd	nd	-	nd	CMV	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Possible TB	-
28	-	-	-	**Pos**	**Pos**	-	-	-	**Pos**	**Pos**	**Pos**	**Pos**	Indet	Indet	Probable OS, confirmed NS	RPR 1:16, CSF VDRL+
32	-	-	-	-	-	-	-	-	**Pos**	-	-	-	-	-	VZV	-
33	-	-	-	**Pos**	**Pos**	**Pos**	-	**Pos**	-	-	-	-	Indet	Indet	Probable OS	RPR 1:512
34	-	-	nd	nd	nd	nd	nd	-	-	**Pos**	-	-	nd	-	Probable TB	-
42	-	-	-	-	**Pos**	-	-	-	-	**Pos**	-	-	-	-	Idiopathic	-
50	-	-	**Pos**	-	**Pos**	-	-	**Pos**	-	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	Confirmed OS & NS	AH & CSF IB+
52	-	-	**Pos**	**Pos**	**Pos**	-	-	nd	nd	nd	nd	nd	**Pos**	nd	Probable OS, confirmed NS	RPR 1:128, CSF IB+
58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Probable OS	RPR 1:16
60	-	-	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*	Probable OS, confirmed NS*	RPR 1:512, CSF VDRL+
61	-	-	-	-	**Pos**	-	-	-	-	-	-	-	-	-	HSV	-
64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	VZV	-
65	-	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Uncertain OS, probable NS[2]	CSF WCC > 5/µL
79	-	-	-	-	-	-	-	nd	nd	nd	nd	nd	-	nd	Possible TB	-
85	-	-	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	-	-	-	-	-	**Pos**	-	Probable OS, confirmed NS	RPR 1:256, CSF IB+
96	-	-	-	-	-	-	-	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	-	**Pos**	Confirmed OS & NS	AH IB+, CSF VDRL+
103	-	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Probable OS	RPR 1:64

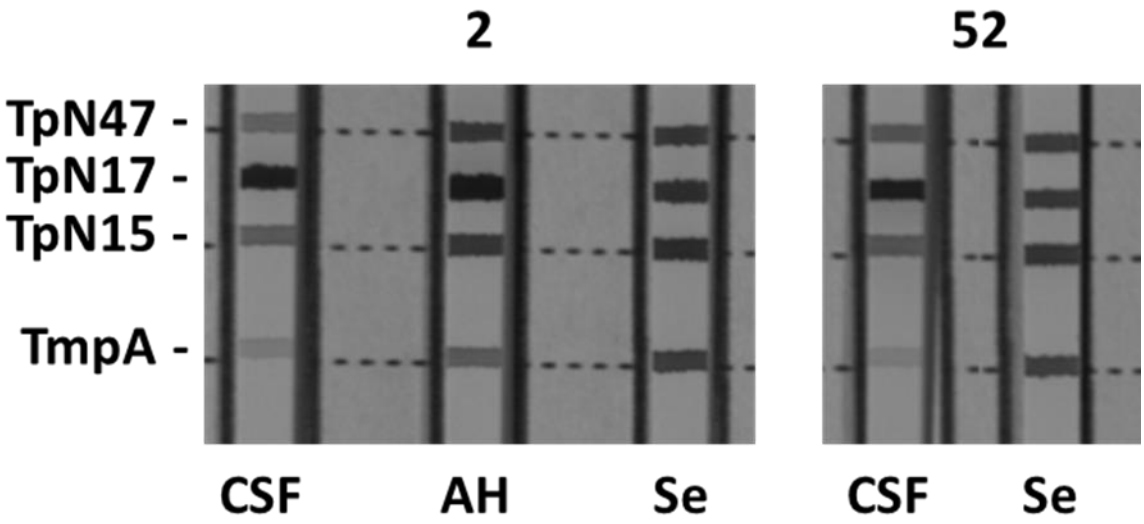
* = some results currently still outstanding

[1] = Provisional other diagnosis (HLA-B27 uveitis) changed to syphilis after obtaining immunoblot results

[2] = Provisional other diagnosis (Idiopathic) changed to syphilis after obtaining immunoblot results

Key: CSF = cerebrospinal fluid; AH = aqueous humor; OS = ocular syphilis; NS = neurosyphilis; IB = immunoblot; indet = indeterminate; nd = not done

Figure 2: Scanned images of immunoblot strips from two patients with clear differences in their pattern profiles. Above the patient number is shown. On the left the four Treponema pallidum antigens present on the immunoblot strips are indicated. Below the patient material is given. The immunoblot of patient 2 (left hand side) shows a difference in band intensity between the cerebrospinal fluid and aqueous humor on the one hand and serum on the other. Clearly, the intensity of band TpN17 is higher in CSF and AH than in serum compared to the other bands in the same material. The immunoblot of patient 52 (right hand side) shows a difference in intensity between CSF and serum, where in the serum the intensity of all band is similar, whereas in CSF the TpN17 band is more intense and the TmpA band is less intense than the other two.



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Chapter 11: Conclusion

Introduction

Very little data were available regarding the epidemiology of uveitis in South Africa prior to when this research project was undertaken. From conception to the final stages of this project more than four years passed and, as alluded to earlier, very few other publications have focused on this important topic over that period of time. During the conceptualization of this study the investigators therefore were, in a sense, both presented with and confronted by a blank canvas. The main challenge was to determine which questions needed to be prioritised and which ones could either wait or be referred to other researchers to be answered in the meantime. In the end it was decided that the overall epidemiology of intraocular inflammation in our area needed to be investigated as no such data existed and that, while doing so, we would pay close attention to the diagnosis of specific uveitis entities.

The pilot study for this project assessed the utility of multiplex PCR in the diagnosis of herpetic uveitis (Appendix A).[1] Not only was a 47.2% positive PCR yield recorded but it also described a significant correlation between being HIV+ and: 1) having a positive PCR yield, 2) having a positive EBV PCR result and 3) having a positive CMV PCR result. Furthermore patients with posterior uveitis and symptom duration <30 days were also more likely to have a positive PCR yield. These results enabled us to make a definitive diagnosis of herpetic uveitis in many cases where this would not previously have been possible. These findings also presented new questions. One specific question that kept recurring was how to interpret the finding of a positive EBV PCR result in our setting? It was therefore decided to examine this matter more closely.

Considering that South Africa has the highest prevalence of both TB and HIV infection in the world, and that the local patient profile provided a unique setting in which to study this, it was decided to focus a lot of attention on these two disease entities. Firstly, it needed to be established what the

prevalence of IOTB in the study area was and whether HIV infection had any effect on this. To realise this a collaboration was entered into with the Division of Molecular Biology and Human Genetics and specifically the DST/NRF Centre for Excellence in Biomedical TB Research (CBTBR), which is one of the Centres of Excellence for research in South Africa, as it provided access to the QuantiFERON TB-Gold test (QFT) that was not yet available in our clinical setting. Secondly, it needed to be determined whether the QFT was indeed superior to a tuberculin skin test (TST) given the highly endemic environment for both TB and HIV infection. Lastly, it presented an opportunity to evaluate a newly proposed revised international classification system of IOTB and, in so doing, identify areas for possible future improvement in this system.

The pilot study showed that herpes viruses were common causes of uveitis in our environment and that viral causes of infectious uveitis also needed to be studied. However, based on publications from the Netherlands and Thailand,[2-10] it was realised that both rubella virus and HIV *per se* needed to be studied as well. The literature suggested that PCR alone would miss the diagnosis of viral uveitis in a significant proportion of cases and that Goldmann-Witmer coefficient calculation could improve diagnostic accuracy significantly but the challenge was that GWC testing was not available anywhere in South Africa. A decision was made to approach researchers at the University Medical Center in Utrecht, The Netherlands, where most of the publications had emanated from, and a collaboration agreement was entered into, thereby providing access to diagnostic techniques that had never been evaluated in the Southern African setting.

As the study progressed, collaborative research assisted us to gain access to diagnostic imaging modalities, previously thought to be out of our reach. Most importantly, positron emission tomography with computed tomography (PET/CT) became available to assist in an attempt to differentiate between pulmonary TB and sarcoidosis in uveitis patients. Those results were not included in this dissertation but will form a Master of Medicine dissertation for a registrar in Nuclear Medicine. An opportunity presented itself to re-evaluate the role of some older modalities, such as

chest radiography, in diagnosing the underlying causes of uveitis and a research paper presenting those findings will be submitted for publication in the near future by other members of the investigating team.

Another publication that had its origin in this study looked at the ocular and CSF penetration of anti-retroviral agents (ARV's) (Appendix B).[11] The initial reports on HIV-induced uveitis indicated that the condition was not responsive to corticosteroid treatment but responded dramatically to anti-retroviral agents. A literature search however revealed that very little was known about especially the ocular penetration of ARV's and it was decided to investigate this further. There is no doubt that further research needs to be conducted to better understand which ARV's achieve therapeutic levels in the eye. Given an increased awareness of the association between HIV infection and ocular inflammation we also described two new forms of immune recovery-based ocular inflammation during the course of this project. One paper described an immune recovery response to Cryptococcus neoformans (Appendix C) and the other the development of Mooren's corneal ulceration (Appendix D) after immune reconstitution.[12,13]

Apart from the aims and objectives defined at the outset we were therefore also able to address some additional questions and describe novel findings, thereby increasing the total impact of the study.

Critical appraisal

Chapter 3

This chapter presented the first ever report on the prevalence of intraocular tuberculosis in the Western Cape Province of South Africa using a revised classification system. Given the high prevalence of both TB and HIV in this setting it was not surprising to find that 1 in 3 patients with uveitis could be classified as having IOTB. The revised classification was not only found to be more useful than previous versions but certain shortcomings were identified that would need to be

addressed in future versions of the classification system. The addition of the category called possible IOTB was particularly useful as it allowed many patients, who would previously have been labelled as having idiopathic uveitis, to be diagnosed with IOTB. This is also the main reason why the reported prevalence is almost double that reported by Schaftenaar *et al* as all the cases of IOTB they diagnosed would be classified as probable IOTB.[14] It is likely that a significant proportion of the patients in their idiopathic group could have been diagnosed as having possible IOTB had they used the revised classification by Gupta et al.[15] As a result of this research our clinical index of suspicion for a diagnosis of IOTB has increased significantly and ophthalmologists are therefore much less likely than before to miss a diagnosis of IOTB.

Chapter 4

After utilising the revised IOTB classification in this study, Chapter 4 provides novel perspectives gained. Four main shortcomings were identified that would need to be addressed in order to further improve the accuracy of the classification. In short, given the protean manifestations of ocular TB, it was recommended that the number of clinical signs contained in the classification be increased in order for more manifestations to be compatible with a diagnosis of IOTB and that it should make provision for unusual manifestations of IOTB in immunocompromised patients. Furthermore, a CD4+ count of 100 x 10^6/L was identified to be an arbitrary cut-off value under which both QFT and TST results often become falsely negative. In those cases a positive QFT and/or TST result should not be mandatory in order to diagnose IOTB provided the rest of the criteria are met. Lastly, based on the extensive clinical experience gained in treating patients in the Western Cape, it was recommended that a positive 8 week trial of anti-TB treatment should still form part of the diagnostic criteria as we often rely on this to guide our decision making in clinical practice in the absence of a gold standard for the diagnosis of IOTB.

Chapter 5

Chapter 5 evaluated the utility of QFT and TST as diagnostic tests for IOTB in HIV+ and HIV- patients. For many years it was taught that there was no reason to use the TST in adults as they would all invariably be positive in a TB endemic area like the Western Cape Province of South Africa. We were therefore very excited when the first data regarding the use of QFT to diagnose IOTB was published and we hoped that it would improve our ability to accurately diagnose IOTB. However, once the data from this study had been analysed, it was concluded that QFT was not superior to TST in our highly endemic environment and that it therefore did not warrant the much higher cost involved in a limited resource setting. When a comparison was drawn between two countries with a high incidence of TB (South Africa and India) and 3 countries with a much lower incidence (Singapore, Korea and Spain) the conclusion was that the TST performs better in high incidence countries while QFT is more accurate in countries with a much lower incidence of TB. In practice, the number that has the most clinical significance is the negative predictive value of 92.1% of the TST as it means that a patient with a negative TST only has an 8% chance of still having IOTB. However, it has also been noted that in patients with a CD4+ count <100 x 10^6/L clinicians should be aware of the fact that the TST may be false negative.

Chapter 6

In Chapter 6 molecular methods were utilised to study the role of Epstein-Barr virus in uveitis as there was limited evidence in the literature to prove that the virus actually causes intraocular inflammation. How to interpret a positive result for EBV in a clinical setting was therefore questioned. Using both quantitative PCR and GWC no evidence of active intraocular replication or antibody production could be found. Furthermore, an alternative, more plausible cause of uveitis was identified in the majority of cases that had a positive multiplex PCR result for EBV. The recommendation therefore is that EBV should not be considered the sole cause of uveitis unless one can demonstrate either intraocular replication and/or intraocular antibody production.

Chapter 7

Chapter 7 reported a case of HIV-induced uveitis who, in contrast to the patients with positive EBV multiplex PCR results, had an intraocular HIV viral load more than 150 times higher than in the peripheral blood and therefore had evidence of intraocular viral replication. It also described a previously unreported finding of small fluffy nodules along the pupil margin which was present in photos of previous cases but not highlighted at the time. This case was published in the journal 'AIDS' in order to increase awareness of this highly treatable form of uveitis amongst all healthcare practitioners who work with people living with HIV infection.

Chapter 8

Chapter 8 describes the findings regarding the use of both PCR and GWC testing to diagnose infectious uveitis in HIV+ and HIV- patients and 3 main differences between these 2 groups were identified. Firstly, HIV+ patients were more likely to have positive PCR results than GWC results. The interpretation of this finding is that HIV+ patients, especially those with lower CD4+ counts, are more prone to having actual intraocular infections and the likelihood of finding a pathogen's DNA or RNA in ocular fluid is therefore higher. Secondly, HIV+ patients are more likely to have multiple infections in the same eye which again reflects their compromised immune system. Thirdly, HIV+ patients are more likely to have non-anterior uveitis than anterior uveitis and this fits in with the theory that non-anterior uveitis is more frequently infectious in origin while anterior uveitis is more frequently non-infectious. An interesting finding relating to herpetic uveitis was the fact that all cases of herpetic anterior uveitis were PCR-/GWC+ and all cases of herpetic non-anterior uveitis were PCR+/GWC-. Furthermore, GWC+ anterior uveitis occurred almost equally between HIV+ and HIV- individuals while PCR+ non-anterior uveitis occurred much more frequently in HIV+ individuals. Even though the numbers are small this would seem to suggest that in cases with suspected herpetic anterior uveitis a GWC would be more likely to provide a positive result while in suspected herpetic

non-anterior uveitis PCR should be more likely to provide a positive result – especially if the patient is HIV+.

Chapter 9

Chapter 9 contains a summary of the epidemiological findings of this study which provided an affirmative answer to the original research question. The objectives outlined at the beginning of the study were met and are discussed in this chapter. In patients with anterior and panuveitis it was demonstrated that the causes were different between HIV+ and HIV- cases. It was determined that 80% of HIV+ cases had infectious uveitis which illustrates the fact that clinicians should actively search for intraocular infections in all HIV+ cases as the majority of these respond well to antimicrobial therapy and poorly to corticosteroids alone. In contrast, only 5% of HIV+ cases had a non-infectious cause and 15% were idiopathic. In HIV- cases, 57.6% had an infectious cause while 24.2% had non-infectious causes such as sarcoidosis. It is noteworthy that no cases of sarcoidosis or rubella virus were diagnosed in HIV+ individuals as these occurred exclusively in HIV- study participants. Another finding with clinical implications is that VZV was the most commonly identified herpes virus in both the HIV+ and HIV- groups as this differs from what has been reported from elsewhere in the world where CMV infection is more prevalent and has specific therapeutic requirements.

Whilst it was not surprising to find that HIV+ patients had a high prevalence of infectious uveitis it was however remarkable to see how the patterns of infectious and non-infectious uveitis differed between HIV+ and HIV- patients. These differences were quantified for the very first time in our setting. In other parts of the world an underlying cause of anterior uveitis is not found in the majority of cases whereas in our study idiopathic uveitis was only third on the list of causes in HIV+ patients and second on the list in HIV- individuals. Moreover, in cases with panuveitis, idiopathic uveitis did not even reach the top 3 in HIV+ patients and was only the third commonest cause in HIV- cases. This suggests that in both patients with and without HIV infection there is a high likelihood

that an underlying cause will be found, if a thorough investigation is conducted. By identifying such an underlying cause clinicians should be able to treat the condition more effectively with ultimately a superior visual outcome for the patient.

Chapter 10

Chapter 10 explores the relationship between ocular and neurosyphilis by utilising traditional criteria as well as newer experimental methods. Neurosyphilis has been subdivided into confirmed and probable neurosyphilis for a number of years based on the results of certain CSF investigations – most specifically the CSF VDRL. More recently, it has been suggested that the diagnosis of probable neurosyphilis be based on slightly different criteria for HIV+ and HIV- individuals.[16] This has led to some interesting findings regarding the diagnosis of neurosyphilis in a few of our patients. According to the CDC criteria for neurosyphilis, 4 patients required treatment for neurosyphilis but according to Marra's algorithms on UpToDate a total of 6 patients should have been treated for neurosyphilis. The 2 additional patients were both HIV- and needed treatment based on raised CSF protein in one case and raised CSF WCC in the other. To make matters even more interesting, the immunoblot results suggested that a few more patients might have had neurosyphilis. In 2 cases, 1 HIV+ and 1 HIV-, a diagnosis of neurosyphilis could not be made if both sets of criteria were strictly applied. The HIV+ case had 18 lymphocytes/µL instead of 20/µL and the HIV+ case had 5/µL instead of >5/µL so they could both be labelled near misses. However, if one adds the positive CSF immunoblot results and utilise our proposed algorithm then both of these patients had confirmed neurosyphilis. It therefore appears as if immunoblotting of CSF to look for treponemal proteins may provide additional information to help confirm a diagnosis of neurosyphilis – perhaps more so in borderline cases.

To date, a diagnosis of ocular syphilis was based on positive blood serology and exclusion of other possible causes of uveitis. Identification of treponemal DNA or antibodies from the eye itself was therefore not required. Given the recent advances in diagnostic techniques we are of the opinion

that ocular syphilis should henceforth also be subdivided into confirmed and probable ocular syphilis based on the level of evidence obtained as outlined in our algorithm. Despite the disappointing results of our AH PCR, which should improve with further investigation, it was pleasing to demonstrate positive immunoblot results on AH in three cases which translates into a diagnosis of confirmed ocular syphilis. Immunoblotting therefore also appears to have a valuable role to play in distinguishing between confirmed and probable ocular syphilis and this role will become better defined as more research is conducted.

Conclusion

Addressing initial study objectives

This study has enabled us to demonstrate differences in the causes of intraocular inflammation between HIV+ and HIV- patients in the Western Cape Province of South Africa. Specifically in Chapter 9 we have presented the prevalence of all the causes of uveitis that were set out to achieve in the stated objectives. The secondary objectives have also been addressed by testing ocular and CSF samples of patients with positive blood serology for syphilis by PCR and immunoblotting and reporting on these in Chapter 10. Moreover, we have evaluated different types of investigations (radiological, serological, microbiological) to determine which tests are most useful in determining the etiology of infective uveitis. The results of the comparisons of different imaging techniques have not been presented in this dissertation as they will be published in the near future by some of our collaborators.

Future research directions

The majority of research in the near future will be focused on searching for, and also refining, new methods to accurately diagnose infectious causes of uveitis as these are by far the most prevalent in this setting. Biomarker analyses are already being performed on samples collected during the course of this study to determine whether characteristic patterns, or so-called bio-signatures, can be

identified to aid in the diagnosis of certain ocular infections. Attempts will be made to refine our syphilis PCR technique in order to improve its sensitivity. The syphilis immunoblot will also be studied further in order to better define both its role and value in clinical practice. Lastly, plans are underway to study the role of PCR with multiple targets such as MPB64, *protein b* and devR in addition to IS6110 to determine whether this approach could increase the sensitivity of TB PCR in accordance with what has already been published from elsewhere in the world.[17,18]

What this study has contributed to Ophthalmology

First and foremost, this study has quantified the prevalence of different causes of uveitis in the Western Cape Province which is something that was long overdue. We now have a much better understanding of what ophthalmologists are likely to encounter in a clinical setting from day to day and how to best go about confirming the diagnosis and prescribing the appropriate treatment. There are many areas that still require further investigation. Secondly, the majority of the data generated by this study has either already been published in international journals or has at least been submitted for peer review – thereby sharing our newly found knowledge with researchers and clinicians from around the world who have shown great interest in wanting to know about our experience at the tip of Africa. Thirdly, apart from publishing the data, we have also shared with, and in the process learnt from, colleagues in other parts of the world via personal visits, the internet, Skype conferences and International Workshops/Congresses. Data has been presented orally at the Ophthalmological Society of South Africa's annual congress in Port Elizabeth in 2017 and will be presented at the 14th Congress of the International Ocular Inflammation Society (IOIS) in Lausanne, Switzerland from 18 – 21 October 2017.

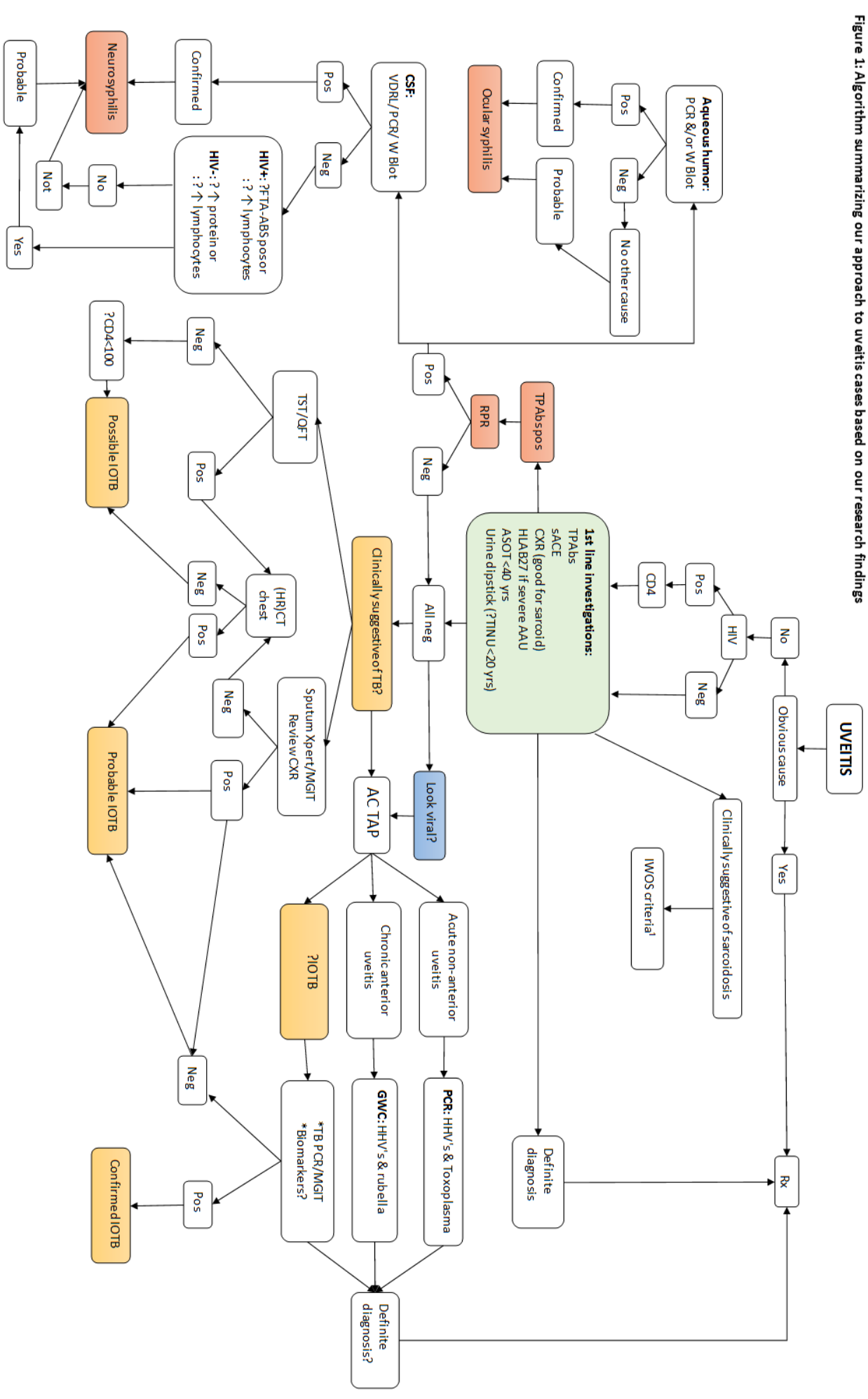
During the course of this study we have evaluated different investigations used in the diagnosis of ocular TB in our highly endemic setting and either have already or will in the near future disseminate this knowledge to other interested parties. We have described rare cases, mostly involving patients with HIV infection, and provided feedback to researchers elsewhere about classification systems that

they have been revising. We took particular interest in the role played by EBV in causing uveitis and have produced solid evidence to question whether EBV really does cause intraocular inflammation. We have provided more data to define the role of PCR and GWC as diagnostic techniques against the background of a high prevalence of HIV infection and we have introduced the idea of using immunoblotting as an additional diagnostic modality to study the relationship between ocular and neurosyphilis and, in so doing, have suggested that ocular syphilis should henceforth also be subdivided in a similar fashion to neurosyphilis.

Overarching conclusion

In South Africa, with its high prevalence of HIV infection, TB and other infectious diseases, most cases of intraocular inflammation are caused by underlying infections in both HIV+ and HIV- patients. It is therefore of utmost importance that clinicians dealing with these patients have a detailed understanding of what they are likely to encounter in order to accurately and cost-effectively diagnose these conditions and provide the best possible treatment. In this study we have established the patterns of uveitis in our immediate surroundings, we have evaluated existing diagnostic techniques and classifications and we have introduced novel techniques and proposed alterations to current classifications after applying these techniques. Based on these findings we have designed an algorithm that summarizes our current approach to uveitis cases (Figure 1; https://www.dropbox.com/s/qwjy5jmxaj0xf50/Algorithm.xlsx?dl=0). In the process we have gained a deeper understanding of how best to manage patients with potentially vision-threatening diseases. The good news is that, in contrast to other parts of the world where many cases of uveitis remain idiopathic, we have identified an underlying cause of inflammation in 83% of cases we studied and were therefore able to provide targeted rather than empiric treatment to those patients. We should, and we will, continue searching for answers to questions that currently have none until we reach the day when we can confidently say that uveitis is idiopathic no more.

Figure 1: Algorithm summarizing our approach to uveitis cases based on our research findings



Key: AAU = acute anterior uveitis, ASOT = anti-Streptolysin O titer, TINU = tubulo-interstitial nephritis and uveitis, AC = anterior chamber, HHV = human herpes virus, MGIT = mycobacterial growth inhibitor tube, W Blot = Western Blot

(HR)CT = (high resolution) chest computed tomography

* = areas of ongoing and future research

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