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MMED RESEARCH REPORT

A PROSPECTIVE, OBSERVATIONAL STUDY OF CRYPTOCOCCAL INFECTION AND CLINICAL AND OPHTHALMOLOGIC OUTCOMES

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A PROSPECTIVE, OBSERVATIONAL STUDY OF CRYPTOCOCCAL INFECTION AND CLINICAL AND OPHTHALMOLOGIC OUTCOMES

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Ophthalmology.

Johannesburg, 2006

DECLARATION

I, Natalia Lydia Maria Ehlers, hereby declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Ophthalmology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



27 day of OCTOBER 2006

The work reported in this dissertation was carried out in the Departments of Ophthalmology and Internal Medicine at the Johannesburg Hospital, South Rand Hospital and Selby Park Hospitals, Johannesburg, South Africa.

The project was approved by the Ethics Committee, University of the Witwatersrand.

DEDICATION

To my parents, Renate and Jack, and my brother, Richard

Thank you so much for all the years of support and encouragement.

PRESENTATIONS ARISING FROM THIS STUDY

1. Presentation at the Ophthalmological Society of South Africa (OSSA) Congress in Durban, February 2003.

   Title: Cryptococcal Infection and Ophthalmological and Clinical Outcomes

   Presenter: Natalia Ehlers

ABSTRACT

Cryptococcosis is a serious fungal infection. Neurological, pulmonary and cutaneous complications are more commonly found in the immunocompetent host. Long-term ocular sequelae have not been well investigated.

Objectives:

To determine the prevalence and nature of ocular involvement in patients with cryptococcal infection. To determine risk factors for vision loss and to correlate this with outcome.

Method:

This was a cross-sectional, observational study. From July 2002 to December 2004 sixty-four patients with a laboratory diagnosis of cryptococcal infection were entered into the study. Ocular findings were recorded on admission and at 1, 6 and 12-month follow-up visits.

Results:

All patients had cryptococcal meningitis with positive latex agglutination tests. Ophthalmic involvement was seen in 84% of patients. The commonest findings were papilloedema (60%), abducens nerve palsies (30%) and choroiditis (14%). Poor visual prognostic factors included optic neuritis (21%), choroiditis (21%) and optic atrophy (10%). Underlying infection with the *Human immunodeficiency virus (HIV)* was seen in 67% of patients. All patients who could be traced had a poor outcome. The average time of survival of patients was 7 months. Vision loss did not correlate with outcome.

Conclusion:

A range of ocular manifestations is seen in cryptococcal meningitis. Risk factors for loss of vision include optic neuritis, choroiditis and optic atrophy. Cryptococcal meningitis carries a high morbidity and mortality.

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PREFACE

I first became interested in cryptococcal infection of the eye when I treated a young man at St John Eye Hospital with optic neuritis related to cryptococcal meningitis. I subsequently saw several patients with optic neuritis related to HIV infection at both the Johannesburg Hospital and St John Eye Hospital. I then presented a talk on Optic Neuritis at one of our Friday Ophthalmology Academic meetings.

Shortly before my final examinations Professor Carmichael mentioned that the Department of Medicine was involved in a multi-centre study on cryptococcal meningitis. They had approached Ophthalmology to conduct ocular examinations on these patients. The study included centres in the United States, India, Taiwan and Australia. He encouraged my participation and suggested that I could do an MMed research report simultaneously. I was very excited at the idea and started the project soon thereafter.

In February 2003 I presented preliminary data as a case series at the annual Ophthalmological Society of South Africa (OSSA) Congress in Durban. Initially patients presented only to the Johannesburg Hospital. Later in the study they were referred on admission to either South Rand or Selby Park Hospitals for chronic management. They were then seen and followed up at one of the three hospitals.

Cryptococcosis is a debilitating disease carrying a high mortality. It was often a challenge trying to obtain patients' cooperation as they were so ill. But as the study unfolded and more pathology was seen it became interesting comparing findings in our setting with that seen in centres abroad. Follow up was often disappointing as

many patients were too weak to come in or had demised in the interim. In addition, on several occasions laboratory data had been lost in the system and I wondered if the study would materialise and come to fruition. I am most grateful to Professor Carmichael and Professor Feldman for their invaluable encouragement, support and advice through those trying times.

Although the study was of ocular involvement in cryptococcal infection, all the patients, eventually turned out to have cryptococcal meningitis.

It remains a challenging illness to treat. With early recognition and improved prophylactic programmes in place, this challenge stands a better chance of being met successfully.

1.0 INTRODUCTION

Cryptococcosis is a serious infection carrying a high mortality. It is caused by the ubiquitous yeast-like fungus *Cryptococcus neoformans*. This is also known as *Torula histolytica,* hence the name Torulosis for infection with this organism.[1]

1.1 Epidemiology

Cryptococcosis occurs more commonly in patients with cell-mediated immunodeficiency, but can affect immunocompetent hosts.[1-4] Defects in T-cell mediated immunity or underlying disease are found in 40-85% of patients.[2-7]

It is the first Acquired Immune Deficiency Syndrome (AIDS) defining opportunistic infection in 40% of cases.[8] It is the fourth most common opportunistic infection in AIDS patients. It occurs in patients with CD_4 counts of less than 100 cells/μL and especially in those with less than 50 cells/μL.[9-11] It has been described in 30% of patients with AIDS in Africa and Southeast Asia.[9] In industrialised countries it occurs less often in 2-10% of patients with AIDS.[8;9;12] Two thirds of these patients develop cryptococcal meningitis.[1;2;9;11]

It ranks as the commonest life-threatening fungal pathogen in AIDS.[9] Cryptococcal meningoencephalitis is the third most common neurological opportunistic infection after *Human immunodeficiency virus (HIV)* encephalopathy and toxoplasmosis.[9;13] However, since the introduction of Highly Active Antiretroviral Therapy (HAART), there has been a decline in the rates of all opportunistic infections in those with AIDS.[6] The atypical presentation of these cases needs to be studied more closely.

Predisposing factors to cryptococcal infection include: [1;4;6;7;14]

- infection with HIV
- lymphoreticular malignancies such as leukaemia and lymphoma (notably Hodgkin's disease)
- transplantation immunosuppression
- diabetes mellitus
- chronic renal failure
- cirrhosis
- corticosteroids either as an endogenous increase in production or as exogenous corticosteroid therapy for sarcoidosis and collagen vascular diseases[1;6;14-17]
- idiopathic lymphocytopenia with low CD_4 counts (known as the CD_4 Syndrome)[1;18]
- smoking (disrupts the respiratory epithelium)[5]
- outdoor occupations[5]

The incidence and disease spectrum of cryptococcosis in South Africa has not been well investigated to date.

1.2 Organism

The most common human pathogen of the group is *Cryptococcus neoformans*. *Cryptococcus albidus* and *C. laurentii* have been reported to cause disease.[1] The organism measures 3.5 to 8.0 microns in diameter and is of variable shape (oval to round).[1] It is unique in that it is a budding yeast with a polysaccharide refractile capsule composed of one of four capsular antigens A, B, C and D. Based on this there are two serovariations (serovars) which differ in epidemiologic, biochemical, genetic and virulence aspects.[1;18-20]

(1) *C. neoformans* var. *neoformans* – containing serotypes A, D or AD and infecting immunosuppressed patients
(2) *C. neoformans* var. *gattii* – containing antigens B or C and occurring mainly in immunocompetent patients

Some authors further classify serotype A to *C. neoformans* var. *grubii*.[18] It has also been suggested that var. *gattii* may represent a distinct species from var. *grubii* and var. *neoformans* and should not be considered an opportunistic pathogen.[18] It has been proposed that var. *gattii* be reclassified as *Cryptococcus gattii*.[18] The molecular basis of these unique predilections of cryptococcal infections is largely unknown.[18]

Cultures of *C. neoformans* var. *neoformans* are isolated throughout the world from soil contaminated with avian faeces. Classically it is associated with pigeon droppings, but can also be found in the excreta of other birds including bats, starlings and turkeys.[1]

It colonises the gastrointestinal tracts of these birds, but does not cause invasive disease. One explanation for the particularly high numbers found in pigeon droppings is that pigeon faeces contain a large concentration of creatinine, which is used as a source of nitrogen by the organism.[1]

- *Serotype A* is more common in Canada, United States, Argentina, New Zealand and Japan[19;20]
- *Serotype D* is more prevalent in Europe[19;20]

C. neoformans var. *gattii* species is virtually restricted to tropical and sub-tropical regions such as Hawaii, Brazil, southern California, central Africa, Cambodia, Thailand, Vietnam and Papua New Guinea.[19;20]

- *Serotype B and C* (88%) are more common in southern California, central Africa and China[19;20]
- Kwon-Chung and Bennett found *serotype B* to be 4.5 times more prevalent than *serotype C* [20]

C. neoformans var. *gattii* has been cultured mainly from clinical isolates and from air samples, leaf and bark debris around the Forest Red Gum (*Eucalyptus tereticornis*) and River Red Gum (*Eucalyptus camaldulensis*) in California and southern Australia.[1;3;21-23] These trees grow along the banks of large rivers in the tropics and subtropics.[1] Export of contaminated seeds may be responsible for the worldwide dissemination of this variety.[21] Mammals and birds associated with the gum trees may also passage cryptococci through their gut, and desiccated yeast cells from these excreta are then inhaled.[1;23]

The epidemiology of *Cryptococcus neoformans* serotypes in South Africa has not been well described.

1.3 Pathophysiology

The polysaccharide capsule is the main virulence factor of *C. neoformans*.[1] It interferes with immune cell function as shed capsular antigens inhibit phagocytosis of fungal cells and enhance the intracellular survival of fungal cells within macrophages.[18]

Spores or desiccated yeast cells from contaminated soil are aerosolised and inhaled.[18] These are known as basidiospores.[22] The primary site of infection is the lung. In HIV-infected persons it is not clear whether acute infection, reactivation of latent infection, or both cause cryptococcosis. These patients often have a reduction in total immunoglobulin levels.[24] Fungaemia occurs in 70% of cases and the pathogen may reach any organ via haematogenous dissemination.[9] The most common manifestation is meningitis or meningoencephalitis which occurs in 60-90% of patients.[1;9] Infection of the prostate is of interest as it may serve as a nidus for relapse in patients who have been successfully treated with antifungal therapy.[9]

The organism reproduces throughout the meninges and brain parenchyma with the basal ganglia and grey matter most heavily involved.[1] This predilection for the central nervous system (CNS) may be explained by the theory that the organism uses dopamine as a substrate for melanin production and that there is an absence of an inflammatory response to cryptococcus in the brain.[1] The typical lesion is a cyst-like collection of organisms without associated inflammation. Outside the CNS the organism is usually surrounded by inflammatory cells such as lymphocytes, plasma cells, giant cells and macrophages, but not well-formed granulomas.[1]

Hydrocephalus is found in 9-58% of patients with cryptococcal meningoencephalitis.[25] The primary deficit is a failure of cerebrospinal fluid (CSF) absorption as yeast cells are large and may occlude the channels and valves of the subarachnoid villi. Cryptococcal meningitis itself may also alter the rate of CSF production.[25] Vasculitis and cerebral infarction may be associated.[25] The absence of ventricular dilatation in the presence of elevated CSF pressure may be due to the coexistence of cerebral oedema and high outflow resistance.[25] Sixty percent (60%) of AIDS patients with cryptococcal meningitis have opening pressures > 250mm water. Thirty percent (30%) have pressures >350mm water.[26]

1.4 Clinical features of cryptococcal infection

The disease presentation is affected by the immune status of the patient. In immunocompromised individuals it is due to the *neoformans* variant of *Cryptococcus*, while the *gattii* variant has been attributed to causing disease in the immunocompetent host.[3] Immunocompetent patients with var. *gattii* infection have a higher incidence of focal neurological, pulmonary and cutaneous sequelae, but a lower mortality rate.[3;21;23] Loss of vision is present in up to 50% of these patients.[27] They are also more likely to undergo neurosurgical procedures such as ventricular shunting and optic nerve decompression.[3] Patients with var. *neoformans* infection are more likely to have widely disseminated disease.[3]

Since 1996 and the introduction of HAART, there have been modifications in the incidence and clinical presentation of symptomatic HIV infection and opportunistic infections such as cryptococcosis.[28] This may range from multiple relapses with poor immune recovery to enhanced local immune activation with the "immune restoration

syndrome".[28] In the latter case lymph node involvement and multiple abscesses may manifest in the absence of multi-organ dissemination.[28] Culture assays are frequently negative.[28]

Poor immune recovery may be part of the "dissociated virological and immunological" response to HAART. The viral load decreases, but there is a failure in recovering the immune defences as expressed by low CD_4 lymphocyte counts. This may be attributed to advanced age, severity of prior immunodeficiency or decreased residual thymic function.[28] "Immune restoration syndrome" is a rapid recovery of immune competence with enhanced local inflammatory responses.[28;29]

To date there are no data available on the serotypes and disease spectrum of cryptococcosis in South Africa. It is not known if ocular complications or loss of vision are problematic in our setting. Furthermore, it is unknown whether ocular infection may be a nidus for relapse in this population.

1.4.1 Reported ophthalmic symptoms in cryptococcal meningitis patients

These may include: [30;31]

- blurred vision
- retrobulbar pain
- photophobia
- diplopia

1.4.2 Reported ophthalmic signs in cryptococcal meningitis patients

Abnormal ocular findings have been reported in 50% of patients. These include: [30;31]

- papilloedema (the commonest finding in 30% of patients)[1;30-32]
- increased blind spot
- optic atrophy
- extraocular muscle paresis (20% of patients)[1;17;30]
- nystagmus
- anisocoria
- amblyopia
- nodules in the eyelid[33] and conjunctiva[34] as well as the limbus and iris[35] (cutaneous lesions may predate dissemination)[33]
- vitreoretinal masses
- choroiditis, chorioretinitis, neuroretinitis, retinal neovascularization[36-39]
- diffuse intraocular infection[40;41]

less frequently:

- internuclear and supranuclear ophthalmoplegia[30;31]
- Horner's syndrome[30;31]

The most frequent ocular signs are papilloedema (31%) and abducens palsy (9-14%). In immunosuppressed patients loss of vision (9%) and optic atrophy (2.5%) are reportedly less common than in immunocompetent patients (22% and 8% respectively).[30;32]

C. neoformans invasion of the intraocular structures is rare and is the result of haematogenous dissemination of the organisms to the choriocapillaris. In contrast,

optic neuropathy develops by extension from the central nervous system.[32;42;43] It is rare for ocular cryptococcosis to occur without systemic involvement.[35] The posterior segment is more likely to be involved than the anterior segment due to its proximity to the optic nerve and the abundance of short posterior ciliary arteries compared with the two long ciliary arteries supplying the anterior segment.[35]

1.4.3 Pathogenesis of vision loss

Long-term ocular and neurological complications after cryptococcal meningitis have not been well studied. Reports indicate that vision loss is the most devastating and catastrophic complication following cryptococcal meningitis and is often irreversible.[30;42;43] The pathogenesis of this loss of vision in cryptococcal meningitis is not well known. In the absence of other ocular lesions two distinct patterns have been described: rapid and slow.[42]

- *Rapid vision loss* is caused by direct invasion of the optic nerve, optic tracts or lateral geniculate nuclei by the organisms.[30;31;42;44] It is profound and develops within hours to days (1 to 3 days). This is usually shortly before or after the initiation of antifungal treatment.[42] Associated intraneuronal oedema may exacerbate this by causing compression and compromise of the vessels supplying the optic nerve.[42]
- *Slow vision loss* develops later during therapy (usually after day 3). It is progressive and may be caused by the effects of raised intracranial pressure (ICP).[42;43;45] Often there are associated transient obscurations of the visual field such as increased blind spots, constricted fields and central scotomas.[42] As damage to the nerve fibres develops, profound loss of visual acuity and optic atrophy result.[42]

Proposed mechanisms for *sudden bilateral vision loss* include direct infiltration and necrosis of the optic nerves or chiasm, adhesive arachnoiditis, cerebral vasculitis, intracranial hypertension resulting in reduced cerebral perfusion pressure and Amphotericin B neurotoxicity.[1;30;43;45-49] Loss of vision due to Amphotericin B may be caused by neurotoxicity or an idiosyncratic reaction to the drug.[30;46]

Occipital lobe hypoperfusion and cortical blindness is another proposed mechanism for loss of vision in cryptococcosis.[47;48;50]This may result in cerebral infarction. The pathogenesis includes: [50]

- strangulation of vessels transversing the exudates at the base of the brain with vasculitis, vasospasm and thrombosis
- spreading of meningeal inflammatory exudates leading to necrotizing panarteritis, secondary thrombosis and occlusion
- hydrocephalus with dilated ventricles stretching compromised vessels and developing an infarction

A decrease in occipital lobe blood flow in patients with cryptococcal meningitis and loss of vision has been demonstrated using ^{99m}Tc-HMPAO SPET (Tc-hexamethyl propylene amine oxime single photon emission tomography)[47;48] In these situations anatomical imaging studies like CT (computerized tomography) and MRI (magnetic resonance imaging) are not as sensitive in detecting minor fluctuations in cerebral blood flow and are usually normal or not conclusive in the presence of vision loss.[47;48]

1.4.4 Differential rates of vision loss

There is a differential rate of visual loss in immunosuppressed and immunocompetent patients. In AIDS patients with *C. neoformans* var. *neoformans* infection visual loss is uncommon, occurring in up to 9% of cases.[32] In contrast, loss of vision has been found in 52% of *C. neoformans* var. *gattii* survivors with normal immunity.[27,51] This discrepancy may be due to the following:

(1) Absence of an inflammatory response in immunosuppressed patients may result in a lower frequency of optic atrophy and hence visual loss in these patients.

(2) Diagnostic awareness in immunocompromised patients may lead to a shorter time interval between the onset of symptoms and the initiation of therapy for cryptococcal meningitis.

(3) *C. neoformans* var. *gattii* may be more resistant to conventional therapy than *C. neoformans* var. *neoformans*. This may predispose patients to more ocular and neurological sequelae with this serovar.[21]

To date there are no data on ophthalmic involvement in cryptococcosis in South Africa. The present study investigates the nature of cryptococcal-related ocular manifestations and loss of vision in this setting. It is postulated that an immune response is central to the risk of visual loss in cryptococcal meningitis.[51]

1.5 Diagnosis

The diagnosis of cryptococcal meningitis rests on clinical suspicion and laboratory testing.[1]

1.5.1 India Ink (Nigrosin) staining

A drop of cerebrospinal fluid (CSF) is stained with India ink. Microscopy shows the thick polysaccharide capsule as a halo around the organism against a black background. This is only positive in 50-60% of cases.[1] PAS and mucicarmine staining may also be used to delineate the capsule.[1]

1.5.2 Latex agglutination test

This is a fast, sensitive and reliable way of identifying *C. neoformans* capsular antigen in clinical specimens. Latex beads are coated with polyclonal rabbit antibodies specific for the capsular polysaccharide. Agglutination of the beads indicates a positive response.[1] Latex agglutination is positive in 99% of cases and can be confirmed by culture on Sabouraud agar or biopsy of suspicious sites such as lung or skin.

1.5.3 Culture

Cryptococcus neoformans produces mucoid, flat or raised colonies with smooth borders on most media.[1] They are generally cream-coloured, but may turn tan with age.[1] Media include 5% sheep blood agar and Sabouraud dextrose agar at 37°C.

Culture on Niger- birdseed agar produces colonies which are brown in colour due to melanin production.[18] Cycloheximide which is often added to fungal media to inhibit bacterial growth, will inhibit growth of the organism.[1] *Cryptococcus neoformans* is distinguished from non-pathogenic cryptococci by its ability to grow at 37^0C.[1] Variety identification of isolates can be determined by growth in canavanine-glycine-bromothymol blue agar.[1;21]

1.5.4 Caffeic acid disk test

Caffeic acid is degraded to melanin by phenoloxidase producing a brown-black colour. *C. neoformans* is the only yeast known to produce phenoloxidase.[1] The organism also produces large quantities of *urease* which is used for rapid presumptive identification. In addition, *C. neoformans* is also known to assimilate dextrose, maltose, sucrose, galactose, inositol, xylose, dulcitol, raffinose and cellobiose.[1] Another feature used in identification includes an absence of pseudomycelia on cornmeal agar.[1]

1.5.5 Serology

Titres can be performed, but variable results are often seen due to the organism's irregular stimulation of antibody production.[1;24] A decrease in titre is used to monitor treatment success. False positive results may be due to: [1]

- rheumatoid factor
- concomitant malignancy
- *Trichosporon beigelii*
- *Stomatococcus mucilaginosis*

1.5.6 Histology

The yeast is seen as a grey form surrounded by a clearing of tissue, a halo, representing the capsular material.[1]

1.5.7 Ocular tests

When tests of cerebrospinal fluid (CSF), serum, sputum and blood are negative and there is a high index of suspicion of ocular cryptococcosis the following may be undertaken to obtain tissue specimens for histological examination and culture: [35;39]

- anterior chamber paracentesis or vitreous tap
- vitrectomy
- eye wall biopsy

1.6 Treatment

Three groups of anti-fungal compounds are in clinical use:

(1) polyene antibiotics (Amphotericin B, Nystatin)

(2) azole derivatives (Fluconazole, Itraconazole, Ketoconazole, Miconazole)

(3) allylamines-thiocarbamates[53]

These interact with ergesterol, the major sterol in the fungal plasma membrane. Amphotericin B binds to ergesterol, weakening the cell wall and leading to collapse. The azole antifungals inhibit ergesterol synthesis. Trimethoprim, Sulphamethoxazole and Flucytosine (5-FC) inhibit fungal nucleic acid synthesis. Newer antifungal agents

such as the Nikkomycins and Echinocandins inhibit cell wall synthesis by blocking the enzyme 1.3 β-synthetase.[53]

Amphotericin B is given intravenously as it is poorly absorbed orally and is highly toxic. In 3-10% of patients there may be side effects such as nausea, vomiting, fever, chills, malaise, kidney toxicity, renal tubular acidosis, cardiac arrhythmias, anaemia, rigors, phlebitis and bacterial superinfection (especially *Staphylococcus aureus*).[1;54;55] Increasing fluid intake should be encouraged to minimise damage to the kidneys.

The National Institute of Allergy and Infectious Diseases Mycosis Study Group (NIAID MSG) has published guidelines for the management of *Cryptococcus neoformans* infection.[54] The choice of treatment depends on the anatomic site of disease and the host's immune status.[54]

1.6.1 Immunocompetent hosts

(i) Immunocompetent hosts with non-central nervous system (CNS) disease:

- pulmonary disease
- urinary tract disease
- cutaneous disease
- non-CNS isolated cryptococcaemia
- positive serum cryptoccocal antigen titre >1:8

Recommended treatment is either: [54]

(1) Fluconazole (200-400mg per os (p.o.)/day) for 3-6 months or

(2) Itraconazole (200-400mg p.o./day) for 6-12 months (if intolerant to Fluconazole) or

(3) Amphotericin B (0.5mg intravenous infusion (IVI)/kg/day) for 6-10 weeks (for severe disease)

Occult meningitis must be excluded in each case.[54]

(ii) Immunocompetent hosts with CNS disease

For **healthy** hosts with **CNS** disease treatment is either: [54]

(1) Amphotericin B (0.7mg IVI/kg/day) with Flucytosine (100mg p.o./kg/day) for 6-10 weeks or

(2) Amphotericin B (0.7mg IVI/kg/day) with Flucytosine (100mg p.o./kg/day) for two weeks followed by oral Fluconazole maintenance for a minimum of ten weeks. Fluconazole therapy may then be continued for 6-12 months depending on the clinical status of the patient.

1.6.2 Immunocompromised hosts

(i) *Human immunodeficiency virus (HIV)*-negative immunocompromised hosts should be treated with the above-mentioned protocol as for CNS disease regardless of the site of involvement.[54]

(ii) HIV-infected hosts: [54]

(a) HIV-infected hosts with non-CNS disease

- pulmonary disease
- urinary tract disease

Recommended treatment is either:

(1) Fluconazole (200-400mg p.o./day) or

(2) Itraconazole (200-400mg p.o./day) (if unable to tolerate Fluconazole)

This should be continued lifelong as maintenance therapy.

For **severe non-CNS disease**:

(3) Fluconazole (400mg p.o./day) with Flucytosine (100mg p.o./day) for ten weeks followed by Fluconazole maintenance therapy.

(b) HIV-infected hosts with cryptococcal meningitis

For patients with **HIV-infection and cryptococcal meningitis** the primary objectives are control of infection, prevention of relapse and sequelae, and decrease in early mortality.[9;54] Either of the following is recommended: [54;56]

(1) *Induction* with Amphotericin B (0.7mg IVI/kg/day) and Flucytosine (100mg p.o./kg/day) for 2 weeks followed by Fluconazole (400mg p.o./day) for ten weeks. This is the **treatment of choice.** After ten weeks Fluconazole may be reduced to 200mg p.o./day lifelong depending on the patient's clinical status.

(2) Amphotericin B (0.7mg IVI/kg/day) with Flucytosine (100mg p.o./kg/day) for 6-10 weeks followed by Fluconazole *maintenance* therapy.

Patients without AIDS may clear the infection after the initial therapy (induction), but patients with HIV infection require lifelong suppressive therapy (maintenance).[57]

The following should be noted: [54-57]

- The combination of Amphotericin B and Flucytosine is more effective with less relapses and more rapid sterilization of the CSF than Amphotericin B alone or Fluconazole monotherapy [54-58]
- The addition of Flucytosine does not increase toxicity, it lowers the risk of relapse and is associated with better initial and long-term responses[54;56]
- With the above treatment regimen 60-90% of CSF cultures are negative within two weeks of treatment and there is a 15-20% failure rate[54;56]

- Failure to achieve negative CSF cultures at week two are associated with five-fold greater chance of failure at week ten[59]
- Intrathecal or intraventricular Amphotericin B may be considered if intravenous therapy is ineffective, but is highly toxic[54]
- Induction with an azole alone is discouraged as it is less effective[54] and there is a higher two-week mortality, delayed CSF clearance of the organism and lower success rate (50%) with Fluconazole as induction agent alone[60]
- Ketoconazole is ineffective for either induction or maintenance therapy
- In renal dysfunction a lipid formulation of Amphotericin B (AmBisome 4mg/kg/day) may be used, but is expensive[54]
- An alternative to Amphotericin B is Flucytosine (100mg/kg/day) with Fluconazole (400-800mg/day) for six weeks, but this is not as effective as Amphotericin B and toxicity is high[54]
- Flucytosine should not be used as monotherapy for induction or maintenance as it leads to resistance[54]
- A response to treatment is defined as two negative cerebrospinal fluid cryptococcal cultures by ten weeks of treatment[54]
- The California Collaborative Treatment Group (CCTG) and AIDS Clinical Trials Group (ACTG) have shown Fluconazole to be the best agent to use for maintenance therapy[10;54;55;61]
- Fluconazole is better able to penetrate the vitreous body and CSF than Itraconazole which has a relapse rate of 24% and a risk of Jarisch-Herxheimer reactions[41;54]
- Fluconazole is also superior to Amphotericin B which has shown high rates of toxicity, relapse (17%) and bacterial infection
- Survival rates are the same with Fluconazole or Amphotericin B[55;62]

- The relapse rate with Fluconazole maintenance therapy is 3% as compared with 37% in patients without maintenance therapy[54;55]
- With treatment there is a 6% mortality rate and without treatment mortality rates are around 14-25%, thereby confirming that maintenance therapy lowers the relapse rate and improves survival[54]
- Relapse of cryptococcosis indicates failure to eradicate the organism from the meninges or an extrameningeal site[54]
- In 20% of relapses the prostate has been considered to be a nidus for infection[9]
- It is not known if ocular infection may be similarly responsible for a relapse

In addition to antifungal maintenance therapy, a response to HAART will lower relapse rates. If viral loads decrease or CD_4 T-cell counts increase within 12-18 months on HAART, antifungal maintenance therapy may be discontinued.[54] Cryptococcus is thus one of the opportunistic infections for which a response to HAART may confer protection.

1.6.3 Raised intracranial pressure

In all cases of cryptococcal meningitis the management of **raised intracranial pressure** is imperative.[54] It is both life-threatening and vision-threatening.[9] It is present in 50% of cases and is defined as a CSF opening pressure of greater than 200mm water in the lateral decubitus position. In 25-30% of patients this is greater than 350mm water.[26;54] It may be caused by:

- meningeal inflammation
- cryptococcomas
- hydrocephalus (communicating or obstructive)

Neurological manifestations include: [26;54]

- abnormal mentation or headache
- papilloedema
- loss of vision
- deafness
- pathologic reflexes
- raised CSF titres
- positive CSF India ink stain

Elevated intracranial pressure (ICP) at baseline affects the probability of CSF clearance at two weeks.[26] It is recommended that if the intracranial pressure is above 250mm water that measures be taken. These measures became mandatory if the ICP is over 350mm water or if the patient has severe nausea, headache or papilloedema.[26;54]

Treatment options include: [9;54]

(1) Serial lumbar punctures removing 25-30ml of CSF or a lumbar drain

(2) Ventriculoperitoneal shunt

(3) Administration of Acetazolamide (Diamox) 250mg four times daily which inhibits the production of CSF

(4) Optic nerve sheath decompression[63]

The target is to decrease the opening pressure by 50% of its initial value.[54]

Medical treatment with Acetazolamide or Mannitol is controversial as some studies have not shown their use to be effective.[54]

By decreasing extracellular fluid pressure and normalizing axoplasmic flow, optic nerve sheath fenestration is thought to relieve papilloedema and prevent progression of visual loss.[31] Persistent loss of vision despite optic nerve sheath fenestration may suggest that there is direct invasion of the optic nerve or tract by the organism.[31] In these cases loss of vision is most likely immune-mediated and systemic steroids may be of more benefit than CSF shunting procedures.

Mortality rates due to cryptococcal meningitis have decreased due to improved standards of care. These include:

(1) Using higher doses of Amphotericin B with Flucytosine for induction[56]

(2) Using Fluconazole rather than Amphotericin B for maintenance[54]

(3) Reducing intracranial pressure in patients presenting with dangerously high opening pressures in their CSF[26;54]

1.6.4 Corticosteroids

The use of **corticosteroids** is controversial. It is thought that they reduce the production of pro-inflammatory cytokines and subsequent excess fluid in the CSF space.[26] However, their use is not recommended in HIV-infected patients due to the intense fungal burden and large amount of replication.[54] In AIDS patients with raised intracranial pressure their use is associated with higher mortality rates than in patients who do not receive them.[16;26] However, they have been shown to be of benefit in immunocompetent patients with var. *gattii* meningitis and loss of vision.[27,52] This may be due to their anti-inflammatory or immunomodulatory effects preventing the development of optic atrophy post optic disc swelling.[27;52] Two mechanisms are described: [27]

(1) The reduction of arachnoid adhesions around the optic nerve leads to reperfusion of compromised neural tissue

(2) The reduction of oedema, fibrosis and scarring in the optic nerve by decreasing the inflammatory reaction to cryptococcal invasion in the optic nerve

Corticosteroid use is suggested if there is: [42]

- rapid, progressive loss of vision
- dull eye pain suggestive of optic neuritis
- loss of vision before or coincident with the initiation of antifungal therapy
- deterioration of visual acuity to hand movements or worse

1.6.5 Intra-ocular cryptococcosis

Additional strategies for the treatment of **intra-ocular cryptococcosis** may include:

- parenteral Amphotericin B alone or with Flucytosine[54]
- intracameral Amphotericin B with Flucytosine for anterior segment disease[35]
- Fluconazole with its high penetration and low toxicity may be considered[54]
- Miconazole which is effective for CNS cryptococcosis and unresponsive cases[16]
- intravitreal Amphotericin B 5 μg[16;39] for posterior segment disease
- transconjunctival cryopexy for accessible choroidal lesions[39]
- pars plana vitrectomy for organism identification and to facilitate intravitreal therapy[39]
- surgical excision or partial eye-wall resection for subretinal inflammatory masses[39]
- sustained release device by intravitreal implant [39]

There are no studies to date on the benefits or risks with corticosteroids for loss of vision in *Cryptococcus neoformans* infection in South Africa.

1.6.6 Additional treatment options

(1) **Interferon-γ (INF-γ)** at the site of infection increases the rate of clearance of organisms in cryptococcal meningitis.[66] Cytokine secretion peaks at day three after initiation of treatment. This suggests increased immune stimulation after commencement of antifungal therapy. It may result from the release of antigens from dying fungal cells or the immunostimulatory properties of Amphotericin B. This supports the rationale for adjunctive INF-γ in the treatment of refractory HIV-associated cryptococcosis.[66]

(2) **Antibody replacement** or targeted prophylaxis should be considered in HIV-infected individuals with lower immunoglobulin levels.[24]

1.6.7 Prophylaxis

Prophylaxis with Fluconazole remains controversial due to the dangers of encouraging antifungal drug resistance, especially with *Candida*, as well as concerns about the expense involved.[10] HIV-infected patients with CD_4 T-cell counts <200 cells/μL on prophylactic therapy showed a significant reduction in the frequency of serious and life-threatening cryptococcal infections from 10% to 2.5%, a 75% reduction in relative risk. In 78% of the invasive fungal infections the CD_4 T-cell count was less than 50 cells/μL.[10]

Despite the dramatic reduction in serious and invasive infections seen with Fluconazole, routine antifungal prophylaxis is not yet recommended for everyone with CD_4 T-cell counts below 200 cells/μL for the following reasons:

(1) Most serious and invasive infections occur in patients with CD_4 T-cell counts below 25 cells/μL and almost all in patients with counts below 50 cells/μL. Thus the efficacy of using Fluconazole at higher CD_4 levels remains unclear.[10;54]

(2) Widespread or inappropriate early use of Fluconazole selects for the development of Fluconazole resistant *Candida* species, many of which become resistant to all the azoles and can only be treated with weekly Amphotericin B.[10;54]

(3) Fluconazole prophylaxis is expensive.[10;54]

For these reasons many physicians take a cautious approach with Fluconazole prophylaxis.

It is proposed that further investigations are needed to determine the frequency of antifungal-resistant *Cryptococcus neoformans* and to correlate the *in vivo* response and the *in vitro* susceptibility data.

1.7 Outcome measures

1.7.1 Risk factors for mortality

C. neoformans is life-threatening and carries a high mortality rate in both immunocompromised and immunocompetent patients.[65] Mortality up to 38% in immunocompetent patients and 85% in the immunosuppressed may occur.[1;3;6;9;14;21;65-67] The 12-month survival rate ranges from 30-60%.[9]

A high mortality may result from the untreated effects of raised intracranial pressure. This leads to tentorial herniation and cardiorespiratory arrest.[65] An improvement may reflect current management practices of systemic therapy, surgery and the era of HAART.[54;65]

The median survival for patients with AIDS varies. In one study it was shown to be nine months for AIDS patients compared with two months for those with neoplastic disease.[68] In another study patients had a median survival of just over five months after diagnosis with cryptococcal meningitis.[8] However, most outcome analyses of cryptococcosis have been difficult to verify as they have combined all disease manifestations in patients with or without AIDS.[68]

Risk factors and markers of poor prognosis vary in the two groups. They can help identify patients who might benefit from more aggressive therapy. In *immunocompetent* patients they may include: [7;58;65]

- signs of severity on admission including altered mentation, seizures and systolic blood pressure >150mmHg[65]

- males (especially in the West, less in the tropics)[23;65]
- older age (>60 years)[7;65]
- infection with *C.neoformans* var. *gattii*[58]
- delayed treatment

Cerebrospinal fluid opening pressures are raised in both immunocompetent survivors and those who die and are thus not a good predictor of outcome.[65]

In *immunosuppressed* patients poor prognostic markers include: [8;9;15;55;67]

- altered mental status at presentation (confusion, lethargy or absence of headache which leads to delayed diagnosis and treatment)[9;61]
- raised serum or CSF cryptococcal antigen titre >1:32 [15] (Some authors state CSF cryptococcal antigen titre >1:1024[9;62] or >1:10 000)[67]
- positive CSF cryptococcal cultures after two weeks of treatment[60]
- cryptococci isolated from extraneural sites[8;15]
- lymphoreticular malignancy[4;15]
- corticosteroid therapy[15;26]
- relapse (before the HAART era this was associated with 100% mortality)[67]
- infectious choroiditis[38]
- hyponatraemia[8]
- lack of suppressive or maintenance therapy or a delay in initiating therapy[8]
- age less than 35 years[9;61]

Initial high CSF opening pressures, low CSF glucose levels and low CSF leukocyte counts have not been found to be *consistently* reliable prognostic factors in the

immunosuppressed.[67] Some studies correlate their presence with increased mortality while other studies do not support this finding.[9;15;26;61;67]

After the diagnosis of infectious choroiditis is made, the average survival time has been shown to be 25 days.[38] Proper therapy may prolong survival up to a year.[69] In one study ocular signs such as papilloedema or cotton wool spots due to cryptococcosis or concomitant HIV infection were ***not*** related to a significant difference in survival.[32] One of the objectives of the present study is to correlate loss of vision and ocular features in cryptococcosis to outcome.

AIDS is an independent risk factor for mortality with the following poor prognostic indicators: [59;70]

- increased viral load (>100 000 copies/ml)
- low CD_4 T-cell count (0-49 cells/μL)
- low haemoglobin (<10g/dl)

Survivors of cryptococcal meningitis have higher levels of CSF IL-6, IL-8, IFN-y and TNF-α.[64] Interferon-γ is independently associated with the rate of clearance of cryptococcal infection. The proportion of patients in HIV-associated cryptococcal meningitis with a negative CSF culture at two weeks is higher in those receiving adjunctive IFN-γ compared with those on chemotherapy alone.[64]

The outcome of infection with *C. neoformans* appears to be influenced by both the ***virulence*** of the organism and the ***immune status*** of the host.[18;24;58] These should both be considered when determining the prognostic factors in a patient.

1.7.2 Risk factors for relapse

In immunocompetent patients this ranges from 2-30%.[4;7] In immunosuppressed patients with cryptococcal meningitis relapse occurs in up to 40-50% of those who complete therapy and are culture negative.[67] In HIV-infected patients relapse is a true recrudescence due to subclinical foci of infection.[9]

No clear risk factors for relapse have been identified in *immunocompetent* patients. In the *immunosuppressed* the following risk factors have been identified: [7;15;54;56;57;60;67]

- high titres of CSF cryptococcal antigen after treatment(>1:8)
- incomplete induction or maintenance therapy

On completion of treatment with negative cultures, high CSF antigen titres may represent dead, nonviable antigen agglutinating.[65]

The following *do not consistently* affect the rates of relapse: [67]

- height of *serum* antigen titres
- CSF leukocyte counts
- CSF glucose levels
- sites of extraneural infection[61;67]
- absent cryptococcal antibody
- failure of serum or CSF titres to decrease during therapy

1.7.3 Risk factors for vision loss

Loss of vision varies from 1-9% in AIDS patients to 20-55% in the immunocompetent host.[23;30;32;42;51;65] Blindness has been described in 4-42% of cases.[23;51] An underlying disease is present in 75% of cases with rapid vision loss, but only 20% with slow vision loss.[51]

Risk factors include: [51]

- immunocompetence
- raised CSF antigen titres (>1:1024)
- abducens nerve palsies

Raised CSF opening pressure and CSF leucocyte counts have not been shown to be reliable indicators of vision loss.[51]

Rex *et al*,[42] described papilloedema and positive CSF India ink staining to be risk factors associated with increased visual loss. However, data collected in this review were retrospective, not homogeneous and incomplete and therefore these risk factors need to be more carefully reviewed in a prospective study.

There are no data for poor prognostic factors for mortality, relapse or vision loss related to cryptococcosis in South Africa. The present study attempts to assess predictors for vision loss. It further attempts to assess the relation of vision loss to outcome.

2.0 STUDY DESIGN

2.1 Introduction

This research was conducted as part of an international, multicentre, cross-sectional observational study of cryptococcal infection. The following centres enrolled patients: University of Pittsburgh Medical Centre USA, Bowman Gray School of Medicine USA, M.D. Anderson Medical Centre USA, Vanderbilt University Medical Centre USA, Microbiology Laboratory New Zealand, National Taiwan University Hospital Taiwan, All India Institute of Medical Sciences India. All patients enrolled in the study were required to have a complete ophthalmologic examination.

2.2 Objectives

(1) To determine the incidence of ocular involvement and blindness associated with cryptococcal infection.

(2) To describe the ocular manifestations associated with cryptococcosis. To assess the neurological sequelae, response to therapy and the outcome.

(3) To determine the risk factors for loss of vision, including cryptococcal fungaemia, positive CSF India Ink staining and positive CSF cryptococcal antigen.

(4) To correlate loss of vision with outcome.

2.3 Recruitment and follow-up procedures

(1) The period of recruitment and follow-up was twenty-eight months, from July 2002 to November 2004.

(2) Fluid samples to determine susceptibility of *C. neoformans* were taken from tests for routine clinical care. No blood, cerebrospinal fluid, urine, peritoneal fluid or bronchial fluid was taken only for the purpose of the study.

(3) The microbiology laboratory at the Johannesburg Hospital was surveyed twice-weekly for the positive culture of *C. neoformans* recovered from sterile sites.

(4) The patients recruited were either admitted to the Johannesburg Hospital or transferred to South Rand and Selby Park Hospitals. Patients entered into the study were informed about the research project and gave written consent if they were able.

(5) Ocular examination was done within two days of presentation at the bedside with a handheld and an indirect ophthalmoscope, as patients were too ill to be formally assessed in the clinic. Visual acuities were checked from the foot of the bed at a distance of 1.5m (5ft) with a Snellen chart reduced to a fourth of its original size.

(6) Neurological findings were recorded simultaneously. Detailed questionnaires for the multicentre study were completed. Follow-up was done at 1,3,6,12 months if there were positive ocular findings on admission.

(7) Underlying diseases, immunologic status and administration of antifungal therapy prior to the onset of cryptococcal infection were

recorded.

(8) The cryptococcal isolates from the initial cultures were saved at -70^0C. All isolates recovered after 7 days into therapy or from follow-up visits were also saved.

2.4 Inclusion criteria

(1) Positive cryptococcal antigen titre of greater than or equivalent to1:4 from serum or CSF.

(2) Patients with positive culture for *C. neoformans* from sterile sites.

(3) Histology showing yeast with capsule or positive India Ink or Meyer's mucicarmine stain compatible with *C. neoformans* in patients with negative culture.

2.5 Exclusion criterion

Patients refusing to participate in the study.

A flow sheet of the questionnaire for the multicentre cryptococcal study is presented in Appendix 7.1

2.6 Definitions

2.6.1 Definition of vision loss

Loss of vision on admission was defined as mild, moderate or severe.

Mild loss of vision was a visual acuity of 6/9 or 6/12.

Moderate loss of vision was a visual acuity of 6/18-6/36.

Severe loss of vision was a visual acuity of less than 6/36.

Blindness was having no light perception (NLP).

2.6.2 Clinical characteristics of the subtypes of papilloedema

Table 2.1: Clinical characteristics of the subtypes of papilloedema[71]

Early papilloedema	Established papilloedema	Chronic papilloedema
* mildly hyperaemic, elevated discs, indistinct margins * absent spontaneous venous pulsation	* optic discs very hyperaemic, elevated, indistinct margins * venous engorgement, peripapillary haemorrhages, cotton wool spots * retinal oedema and folds * hard exudates at fovea	* elevated, champagne cork-like discs * absent cotton wool spots * absent haemorrhages * opto-ciliary shunts at disc

2.7 Risks and benefits

There were no risks as a result of participation in the study. No lumbar punctures were done for research purposes. No procedures or tests were performed solely for the purposes of this study.

Any information obtained from this research was kept confidential. Records were stored in a locked filing cabinet. A case number rather than a name was used to indicate patient identity. Safety monitoring was implemented by the Principal Investigator to ensure that confidentiality of research data was maintained.

2.8 Ethics clearance number

Ethics clearance number: M02-02-09

Reference number: R14/49 Feldman

2.9 Costs and payments

Patients were charged in the usual manner for any procedures performed for routine medical care. There were no monetary incentives for the participants of this study.

3.0 RESULTS

3.1 Demographics

Sixty-four patients were enrolled into the study from July 2002 to November 2004 (see Table 3.1). Thirty patients were male and 34 were female. The ages ranged from 19-58 years with a mean age of 35 years (average age for males was 36 years and for females 34 years). Racial distribution showed all patients to be Black Africans. All patients entered into this study presented with cryptococcal meningitis and in all cases the diagnosis was made on CSF.

Table 3.1 Demographic features of patients with cryptococcal infection

Number of patients	64
Males (%)	30 (47)
Females (%)	34 (53)
Age range and mean (years)	19-58 (35)
Race	All Black Africans
Site of *C. neoformans* isolation	Cerebrospinal fluid in all patients

3.2 Clinical characteristics

3.2.1 Visual acuities

Vision ranged from 6/6 to no light perception (refer to Table 3.2). Normal vision was present in 23% of eyes (30 of 128 eyes). Loss of vision was seen in 77% of eyes (98 of 128 eyes). There was mild loss of vision in 45 eyes (36%), moderate loss of vision in18 eyes (14%) and severe loss of vision in 27 eyes (21%). Eight eyes were blind with no light perception (6%). This is illustrated in Figure 3.1.

Table 3.2 Visual acuity on admission

Visual acuity on admission	Total number of eyes (%)
Normal	30 (23%)
Mild reduction (6/9 or 6/12)	45 (36%)
Moderate reduction (6/18-6/36)	18 (14%)
Severe reduction (<6/36)	27 (21%)
No light perception (NLP)	8 (6%)
Total number of eyes	128 (100%)

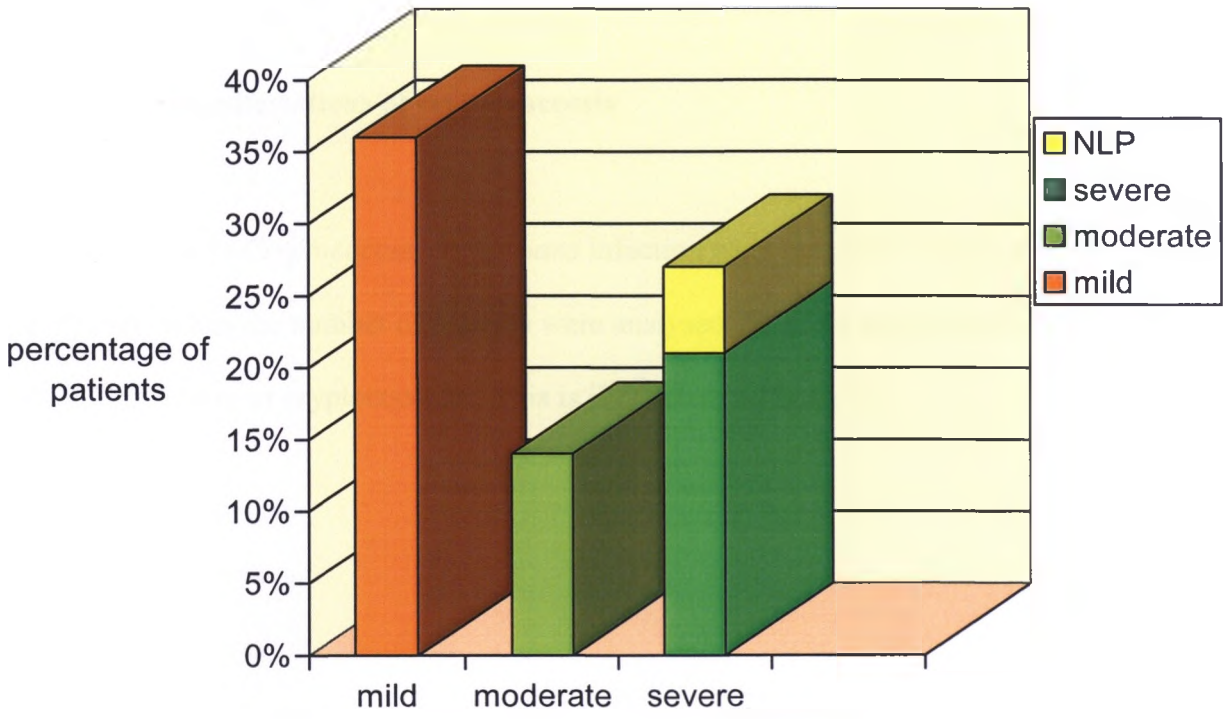


Figure 3.1: Vision loss in *C. neoformans* infection

Visual acuities were also expressed in terms of number of patients examined. In these cases the visual acuity of the eye with the most severe loss of vision was recorded. Of the 64 patients examined, 54 (84%) had loss of vision. In 25 patients this was mild (39%), in 8 patients this was moderate (12%) and in 21 patients (33%) there was severe loss of vision. Of the 21 patients with severe loss of vision 3 were blind (6% overall). Bilateral loss of vision occurred in 44 of 64 patients (68.8%). In 12 of these patients (18.8%) this visual acuity was less than 6/36.

In the 25 patients with mild loss of vision, 24 (96%) had photophobia and four (16%) had decreased mentation. The true vision may have been normal, but was recorded as low due to the effects of abnormal mentation and photophobia. For

this reason the **analysis** of data for vision loss will be done in terms of **moderate-severe loss of vision (less than 6/12)** in 29 patients (45%).

3.2.2 Ocular manifestations of cryptococcosis

Features related to *Cryptococcus neoformans* infection were present in 100 of 128 eyes (78%). When the number of patients were analysed, 54 of 64 patients (84%) had ocular features of cryptococcosis. This is illustrated in Figure 3.2.

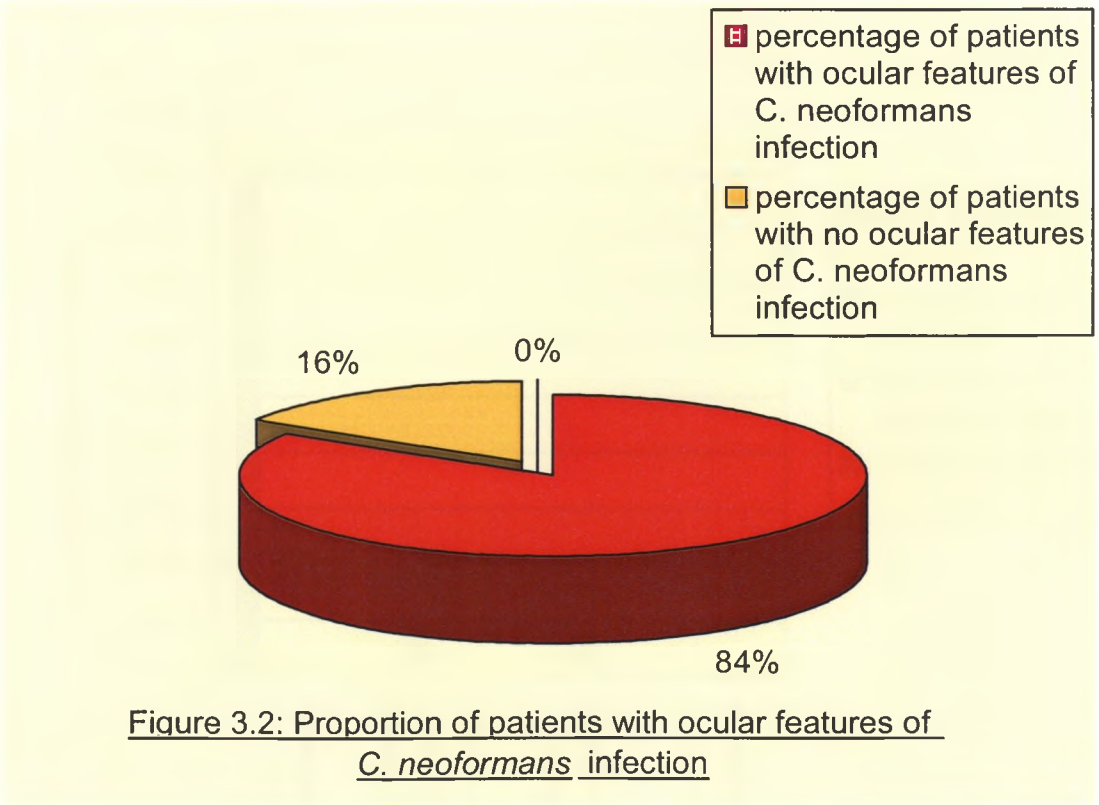


Figure 3.2: Proportion of patients with ocular features of *C. neoformans* infection

The commonest ocular finding was **papilloedema.** This was seen in thirty-eight patients (60%). Thirty-four of the 38 patients (89%) had early papilloedema, three patients (8%) had established papilloedema and one patient (3%) had chronic papilloedema. The clinical characteristics used to classify each subtype are listed in Table 2.1. The subtypes of optic disc oedema are shown in Figure 3.3.

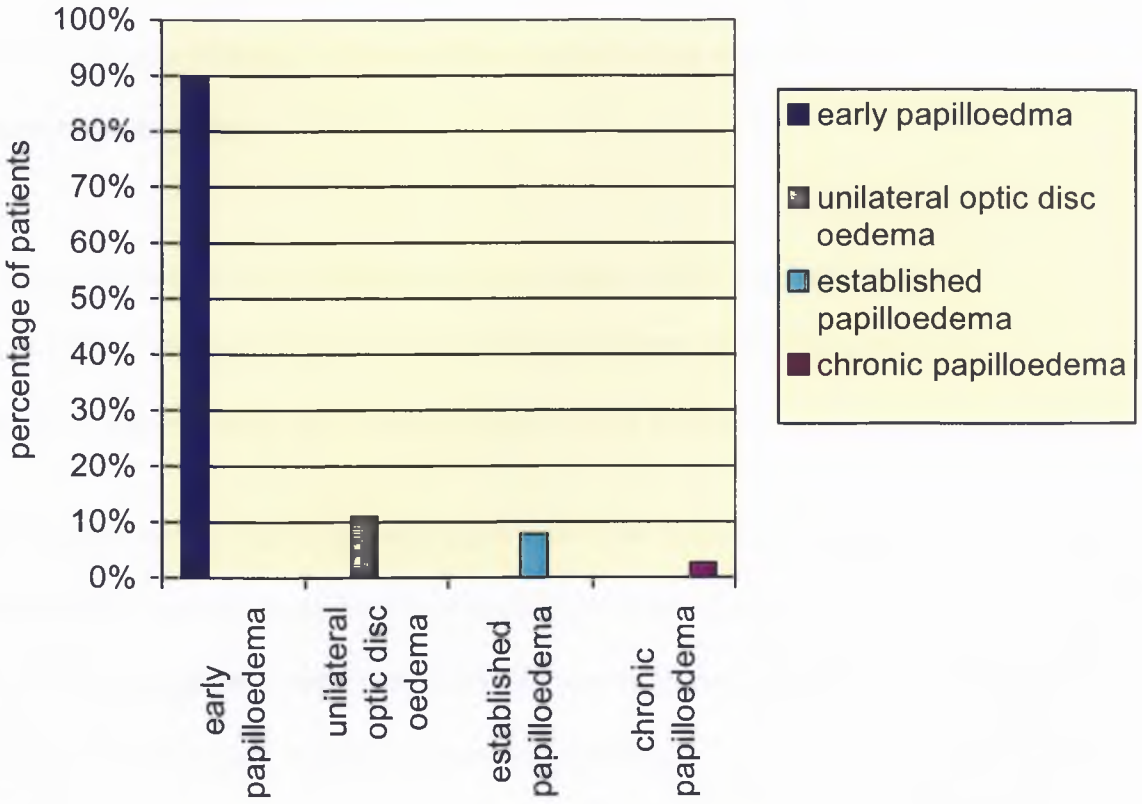


Figure 3.3: Subtypes of optic disc oedema

Cranial nerve palsies were the second commonest finding and were seen in thirty-two patients (50%).

Abducens nerve palsies were the commonest cranial nerve palsy, occurring in nineteen of the thirty-two patients (59%) and in 30% of the total 64 patients entered in the study. Sixteen patients had bilateral abducens nerve palsies and three had unilateral abducens nerve palsies.

Six of the thirty-two patients (19%) or 9% overall had oculomotor nerve palsies. Three of these were partial and three were total pupil-involving oculomotor nerve palsies.

Seven of the thirty-two patients (22%) or 11% overall had facial nerve palsies, two of which were bilateral. In three of these patients there was associated **exposure keratopathy.**

The third commonest ocular finding was **choroiditis,** which was found in nine patients (14%). This manifested as pale, creamy infiltrates of the choroid. In six patients this was unilateral and in three patients it was bilateral.

Optic neuritis with a relative afferent pupil defect was seen in six patients (9%). **Unilateral disc oedema** consistent with papillitis was noted in five patients (8%). **Secondary optic atrophy** was found in three patients (5%). In one of these patients the optic atrophy was noted bilaterally.

Other ocular findings not caused by cryptococcosis included cotton wool spots in four patients (6%). These were most likely due to ***Human immunodeficiency***

virus (HIV) **retinopathy**. This was seen in both eyes in one of these patients. ***Cytomegalovirus (CMV)*** **retinitis** was seen bilaterally in two patients (3%) and **progressive outer retinal necrosis (PORN)** was found in one patient (2%).

***Molluscum contagiosum*-like lesions** of the lids were seen in two patients (3%). **Papular dermatitis** of the face was noted in two patients (3%) and **ecchymoses** of the lid (possibly the start of Kaposi's sarcoma) were seen in one patient (2%). One patient had **episcleritis** (2%) and one patient had **squamous cell carcinoma** of the conjunctiva (2%). Two patients (3%) had features of **allergic conjunctivitis**.

Figure 3.4 shows the ocular manifestations in patients with cryptococcal meningitis.

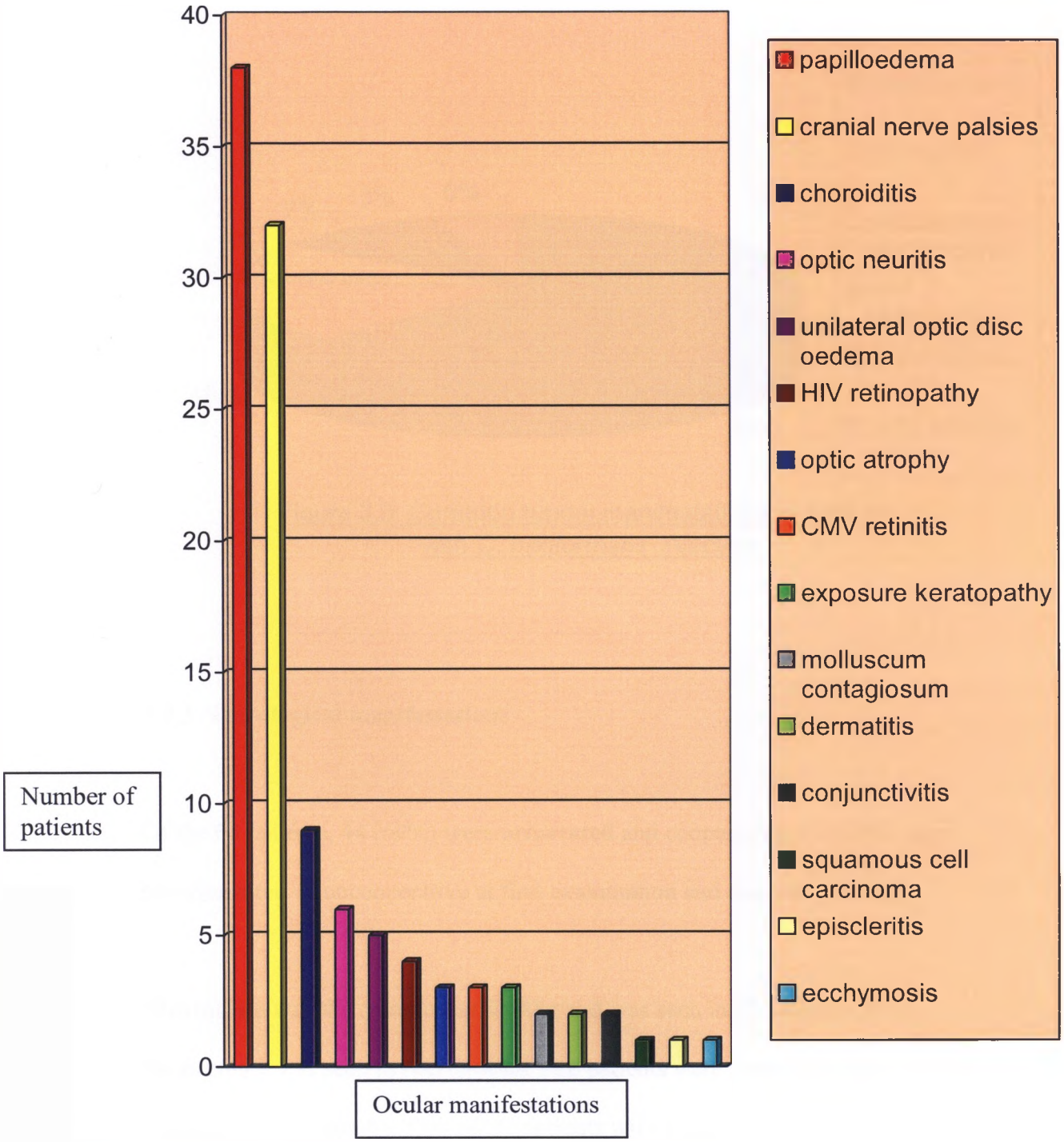


Figure 3.4: Ocular manifestations in patients with cryptococcal meningitis

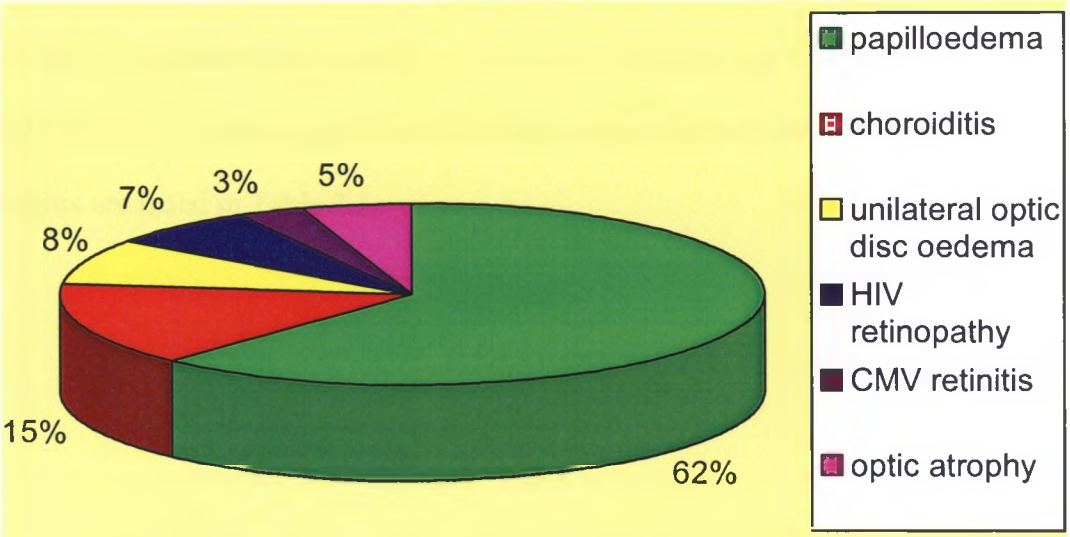


Figure 3.5: Common fundal manifestations in patients with *C. neoformans* infection

3.2.3 Neurological manifestations

Of the 64 patients, 44 (69%) were **orientated** and cooperative, 19 (30%) were **disorientated** or uncooperative at first examination and one was **psychotic.**

Meningism was the commonest finding and was seen in 62 patients (97%). **Hemiplegia** was seen in two patients, two patients were **paraplegic** and one had **peripheral neuropathy.** One of the patients with paraplegia and concomitant bilateral facial nerve palsies had progressive multifocal leucoencephalopathy and early hydrocephalus on CT of the brain. The second patient with paraplegia had the CT brain scan as seen in Appendix 7.1, showing bilateral frontal lobe infarcts, superior and inferior sagittal sinus thromboses and a left posterior fossa subdural haematoma.

Loss of vision was seen in 54 of the 64 patients (84%). **Blindness** was seen in 5 patients (8%). This was associated with diffuse bilateral *Cytomegalovirus* retinitis in one patient, bilateral choroiditis in another patient and optic neuritis in the last three patients. **Deafness** was noted in 12 patients (19%) and was bilateral in 7 patients (58%). The neurological manifestations associated with cryptococcal meningitis are listed in Table 3.3.

Table 3.3: Neurological manifestations associated with cryptococcal meningitis

Neurological manifestations	Number of patients
Meningism	62
Vision loss	54
Obtunded	19
Deafness	12
Blind	5
Hemiplegia	2
Paraplegia	2
Peripheral neuropathy	1
Psychosis	1

3.2.4 Concurrent diseases

At least 43 of 64 patients (67%) were infected with the ***Human Immunodeficiency Virus (HIV)***. This was confirmed on ELISA testing. HIV testing had not been performed on the remaining twenty-one patients.

Of the 43 patients who were HIV positive, **CD_4 T-cell counts** were done in 13 (30%). The average **CD_4 T-cell counts** ranged from 1 to 62 cells/μL (see Table 3.4) with a mean count of 22 cells/μL.

Table 3.4: CD_4 T-cell counts obtained in thirteen patients

Age of patient (years)	CD_4 count (cells/μL)
22	20
32	17
38	62
33	22
24	11
33	10
42	49
39	9
27	1
45	60
29	7
23	8
29	10

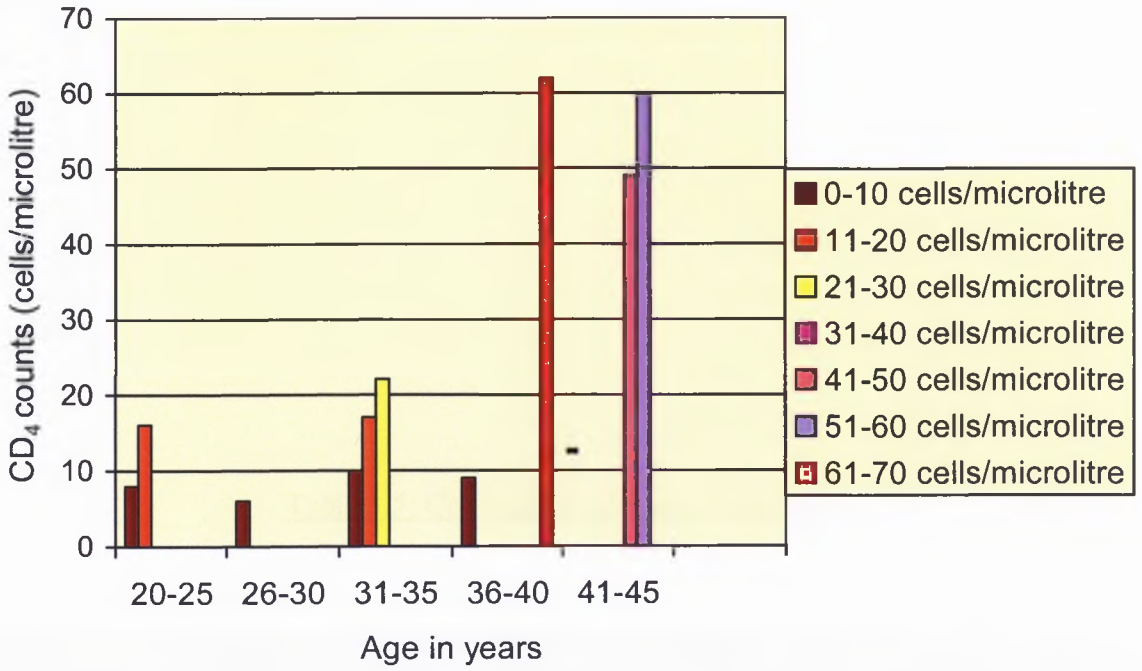


Figure 3.6: Average CD_4 T-cell counts (in cells/microlitre) for the different age-groups

None of the patients in this study had been diagnosed with an underlying lymphoreticular malignancy. None had undergone organ transplantation and none was receiving corticosteroids prior to admission.

Haemoglobin was tested in 57 of the 64 patients (89%). It ranged from 5 to 18g/dL with a mean of 11.43g/dL. Of the 57 tested patients, 30 were female and 27 were male. In 34 of the tested patients (60%) the haemoglobin level was <12g/dL and in 12 patients (21%) it was <10g/dL.

White cell counts were performed in 57 patients with a range of 1.7-28.9 x.10^9/L and a mean of 5.72 x 10^9/L.

Concurrent illnesses at admission are listed in Table 3.5.

Table 3.5: Concurrent diseases at admission

Concurrent disease	*Number of patients*	*Concurrent disease*	*Number of patients*
Lymphoreticular disease	0	*Organ transplantation*	0
Human immunodeficiency virus infection	43	*Corticosteroid use*	0
Pulmonary Tuberculosis	32	*Urinary tract infection*	2
Tuberculous meningitis	1	*Hepatomegaly*	2
Bronchopneumonia	8	*Malignant hypertension*	1
Dermatitis	8	*Septicaemia*	1
Herpes simplex labialis	1	*Gastroenteritis*	3
Anaemia of chronic disorders	2	*Dementia*	1
Typhoid fever	1	*Psychosis*	1
Renal dysfunction	2	*Otitis media*	1

3.3 Special investigations

All patients enrolled in the study had **cryptococcal meningitis** confirmed on CSF tests. None of the patients entered into this study had *Cryptococcus* isolated from any other site such as sputum, urine, blood, skin, or the eye.

Cerebrospinal fluid (CSF) tests showed the following:

- Positive **India ink** staining was found in 63 of the 64 patients (98%)
- **Latex agglutination** was positive in all 64 patients entered into the study
- Sixty-two of the sixty-four patients (97%) had positive **CSF cultures** for *C. neoformans* and the other two were negative
- **CSF protein** was measured in 55 patients with a range of 0.15-2.52 mmol/L and a mean of 0.93 mmol/L
- **CSF glucose** was performed in 56 patients with a range of 0.3-3.8 g/L and a mean of 2.21 g/L
- **CSF polymorphonucleocytes (PMN)** measured in 48 patients ranged from 0-170 cells/mm^3 with an average of 9 cells/mm^3
- **CSF lymphocytes** measured in 48 patients ranged from 0-930 cells/mm^3 with a mean of 69 cells/mm^3

CSF titres and **serotyping** of *C. neoformans* are not available at the Johannesburg Hospital and could not be done on any of the specimens collected.

Blood cultures were performed on 11 patients and 4 of the 64 patients in the study (6%) were positive for *C. neoformans.* One patient had *Staphylococcus aureus* isolated on blood culture. It should be noted that this patient had received intravenous Amphotericin B which predisposes to this superinfection.[61]

Computerised Assisted Tomography (CT) of the brain was performed in 13 of 64 patients (20%). In two patients (15%) it was normal and in 3 patients (23%) there was paranasal sinus opacification only. The remaining 8 patients (62%) had the features listed in Table 3.6. Some of these features are shown in the Appendix 7.2.

Table 3.6: Features noted on CT brain

Patient	**Features on CT of the brain**
Patient 7	Two calcified granulomata in the posterior fossa
Patient 12	Progressive multifocal leukoencephalopathy (PML), with leukoencephalomalacia, prominent CSF spaces, subependymal oedema, early hydrocephalus
Patient 28	Nonobstructive hydrocephalus
Patient 41	Bilateral frontal lobe infarcts, superior and inferior sagittal sinus thrombosis, posterior fossa subdural haematoma
Patient 42	Small deep lacunar infarcts at the level of the lateral ventricles
Patient 47	Left internal capsule infarct
Patient 52	Raised intracranial pressure, attenuated basal cisterns
Patient 53	Calcification of the basal ganglia

3.4 Response to therapy

All patients in the study were treated with Fluconazole at admission. Amphotericin B was given intravenously to 16 of the 64 patients (25%). Seven patients received simultaneous oral Bactrim (11%). One patient received oral Prednisone and one patient received Solucortef. Three patients receiving Amphotericin B reported nausea and vomiting and one developed a concomitant *Staphylococcus aureus* infection. One patient receiving Fluconazole reported nausea and vomiting.

Five of the 64 patients were known to have **relapsed** with a recurrent episode of cryptococcal meningitis (8%). The average time to relapse was 4.2 months after their first admission. (See Table 3.7). Three of these five patients demised at their second admission.

Table 3.7: Time to relapse in five patients

Patient	**Date of admission**	**Date of relapse**	**Time to relapse**
Patient 2	20/08/2002	06/10/2002	6 weeks
Patient 8	07/2002	05/2003	10 months
Patient 27	21/04/2003	27/06/2003	2 months
Patient 36	15/03/2002	10/06/2002	3 months
Patient 55	09/2003	04/01/2004	4 months

Only 2 of 54 patients (4%) with ocular features related to cryptococcosis attended their **one-month follow-up** visit and the defaulters were difficult to trace. Many had no telephone numbers or proper addresses on file. Some had given addresses or telephone numbers of friends who reported not knowing their whereabouts. This made follow-up quite ineffective and frustrating.

3.5 Outcome

Twenty-one of sixty-four patients were known to have **demised** (33%). The time of survival from onset of treatment ranged from one day to sixteen months. In six patients this averaged three days after admission. In the other 15 patients the mean time of survival was six months (see Table 3.8). The remaining 43 patients were untraceable (67%).

Table 3.8: Duration of survival from onset of treatment in 21 patients

Patient	**Date of onset of treatment**	**Date of demise**	**Duration of survival**
Patient 36	15/03/2002	13/06/2002	3 months
Patient 1	12/06/2002	10/10/2003	16 months
Patient 14	23/06/2002	24/06/2002	1 day
Patient 6	07/2002	03/2003	8 months
Patient 7	07/2002	01/2003	6 months
Patient 20	09/08/2002	10/2003	14 months
Patient 2	20/08/2002	07/10/2002	2 months
Patient 3	02/10/2002	09/2003	11 months
Patient 9	20/11/2002	02/2003	3 months
Patient 19	16/02/2003	06/2003	4 months
Patient 18	22/02/2003	23/02/2003	1 day
Patient 15	24/02/2003	09/2003	7 months
Patient 45	12/06/2003	13/06/2003	1 day
Patient 50	15/06/2003	12/2003	6 months
Patient 44	17/06/2003	10/2003	4 months
Patient 48	22/06/2003	28/06/2003	6 days
Patient 55	09/2003	04/01/2004	4 months
Patient 53	26/10/2003	28/10/2003	2 days
Patient 54	09/11/2003	11/11/2003	2 days
Patient 56	04/01/2004	02/2004	1 month

3.6 Risk factors for loss of vision

Table 3.9 lists the frequency of features that were seen in patients with loss of vision.

Table 3.9: The frequency of features noted in patients with and without loss of vision

Associated features	Loss of vision (29 patients)	Normal vision (35 patients)
Papilloedema	18 (62%)	20 (57%)
Optic atrophy	3 (10%)	0
Optic neuritis	6 (21%)	0
Choroiditis	6 (21%)	1 (3%)
Cytomegalovirus (CMV) retinitis	2 (7%)	0
Progressive outer retinal necrosis (PORN)	1 (3%)	0
Human immunodeficiency virus (HIV) retinopathy	1 (3%)	3 (9%)
Bilateral abducens nerve palsies	11 (38%)	4 (11%)
Oculomotor nerve palsy	6 (21%)	0
Facial nerve palsy	2 (7%)	0
HIV infection	17 (59%)	26 (74%)
White cell count<$4x10^9$ cells/L	8 (28%)	13 (37%)
Haemoglobin <12g/dL	15 (52%)	19 (54%)
Age <35 years	13 (45%)	24 (69%)
Abnormal mentation	12 (41%)	6 (17%)
Amphotericin B	7 (24%)	8 (23%)

In this study papilloedema (62%), abnormal mentation (41%) and bilateral abducens palsies (38%) were seen frequently in patients with vision loss at admission. These cannot be described as risk factors for vision loss as this was not assessed over a time-period as a cohort study. In addition, univariate analysis has not been done independently on each associated feature.

Of the 29 patients with vision loss, 15 (52%) had a haemoglobin level <12g/dL and six patients (21%) had a haemoglobin <10g/dL.

Intravenous Amphotericin B was given in 16 of the 64 patients (25%). Loss of vision was noted in 14 patients. Of these 14 patients, eight had early papilloedema, one had bilateral optic atrophy, one had optic neuritis, and two had choroiditis. The remaining two patients had mild vision loss and a normal ocular examination.

3.7 The correlation of loss of vision with outcome

The time of survival was similar for those with and without vision loss. In ten of the 29 patients with loss of vision the average time of survival from onset of treatment was seven months. In 11 of the 35 patients with normal vision the average survival time was six months. The outcome for the remaining patients could not be determined.

4.0: DISCUSSION

Sixty-four patients were entered into the study during a twenty-eight month period from July 2002 to November 2004. The patients had an average **age** of 35 years (range was 19-58 years), which is similar to reports in the literature that show a greater incidence of cryptococcosis in the third and fourth decades of life.[2;4;5] Rozenbaum and Goncalves found the median age of non-immunosuppressed patients, patients with AIDS, and those with other diseases or on immunosuppressive therapy to be 32, 35 and 38 years, respectively.[2]

The **gender** distribution (see Table 3.1) was almost equal with 34 females (53%) and 30 males (47%). A preponderance of females (73%) was seen in the study by Kiertiburanakul *et al.*[6] In addition, studies in the tropics demonstrate a female preponderance with a generally younger age group.[23] This is in contrast to most reports where cryptococcosis has more commonly been noted in males.[1-5] Some authors suggest that women are potentially protected from cryptococcal meningitis by endogenous oestrogens,[22] but the findings in this study are not in keeping with this theory. Another possible explanation for the gender distribution noted in the literature could be a difference in the host's immune response between men and women.[6]

All patients in this study were **black Africans**. Hajjeh *et al*, noted a preponderance of African-Americans in their study.[5] The results in this study may be a selection bias because of the patient base that the Johannesburg, South Rand and Selby Park hospitals serve.

Cryptococcal meningitis confirmed on CSF tests was the **presenting feature** in all of the patients recruited. This is in keeping with meningitis being the commonest presentation of cryptococcal infection, but is higher than the rate of 60-90% reported in the literature.[1;2;3;9;21] It may be a reflection of the host defense mechanisms in this patient population.[1] CSF is a good growth medium for *C. neoformans* and is devoid of soluble anticryptococcal factors that are present in serum. In addition, the inflammatory response to cryptococci is absent in the brain. This is due to an absence of local chemotactic and opsonic factors and complement activity. This may allow the progress of lesions in the brain whilst infectious foci outside the CNS are cleared.[1] Finally, high levels of dopamine within the brain serve as a substrate for melanin production favouring the proliferation of more virulent yeasts.[1]

Loss of vision (visual acuity <6/12) occurred in 29 of 64 patients (45%) as noted in Table 3.2. In the 25 patients with mild vision loss (6/9, 6/12), the vision may have been normal, but may have been recorded as low due to decreased mentation (4 patients) or photophobia (24 patients). They were not included for data analysis in the study.

The incidence of visual deterioration reported by Seaton *et al* in their study was 70%.[27] Their patient population was immunocompetent and mostly infected with *C.neoformans* var. *gattii*.[27] This is in keeping with other reports in the literature of the high incidence of visual loss in immunocompetent patients.[2;3;23;51] This may be because of an aggressive inflammatory response causing adhesive arachnoiditis and constriction of the optic nerves or due to the inflammatory reaction to cryptococci in the visual pathway.[27]

In this study 67% of patients had HIV infection and the average CD_4 T-cell count in 13 tested patients was 22 cells/μL. In the remaining patients there was a high index of suspicion of advanced immunosuppression, but tests had not been done. This does not easily explain the high incidence of loss of vision in this patient population. The literature reports rates of 1-9% for loss of vision in AIDS patients.[32;42;53]

Variety *gattii* is the serovar which usually causes vision loss in immunocompetent patients. Despite the high prevalence of HIV infection, its presence cannot be excluded in the present study as some of these patients may have originated from subtropical areas. In addition, the presence of hybrid strains of *C. neoformans* infection cannot be excluded.

Bilateral loss of vision was seen in 69% of patients with visual loss in this study. Nineteen percent (19%) of these patients had a visual acuity worse than 6/36 at admission. Seaton *et al*, reported a higher rate of 96% for bilateral visual loss in their study.[51]

Three (6%) of the patients in the present study were **blind** (bilaterally). This is similar to the 9.9% of patients in the study by Seaton *et al*, who were blind bilaterally *on admission.* [51] However, it is much less than the 50% and 31% noted by Seaton[51] and Lalloo[23] respectively, in immunocompetent patients after following up patients *for several months.* It is closer to the 1.1% and 2.5% recorded by Rex[42] and Kestelyn[32] respectively in their studies on AIDS patients in California and Tanzania. Two-thirds of the patients in this study had HIV infection.

This was a cross-sectional observational study. Most of the patients presented shortly after onset of their symptoms and were examined at admission only. Of the 54 patients with ocular features related to cryptococcosis, only two (4%) arrived for their one-month follow-up visit. None of the patients were seen at 3, 6 or 12 months after their first admission. Some of these cases may have deteriorated and might have subsequently developed optic atrophy. If this had been monitored, the incidence of blindness might have been higher. Thus bias has been introduced into the study due to loss of patients to follow-up.

In the study by Seaton *et al*,[27] patients were followed over several months. A peak visual loss was noted at day 21 with a range of less than 24 hours to 150 days.[27] Optic atrophy has been described as following optic disc swelling in 61% of these patients.[51]

The **prevalence of ocular pathology** in patients with cryptococcal infection was 84% (see Figure 3.2). **Papilloedema** was the commonest finding (refer to Figure 3.4) in 38 of the patients (60%), which is similar to the 64% noted by Rex,[42] but is higher than the 32% and 33% noted by Kestelyn[32] and Okun[30] respectively in their studies. In all three studies papilloedema was the commonest finding.

The visual sequelae vary with the stage of the papilloedema.[42] In the early stages vision may be normal or slightly reduced. With progressive damage to the nerve fibres loss of visual acuity and optic atrophy result.[42] The majority of the patients in this study presented with early papilloedema (34 of 38 patients). This may

reflect a short time interval between onset of symptoms and admission. Most of these patients (15 of 34) had visual acuities better than 6/18. Only one patient presented with chronic papilloedema and the vision in this patient was normal.

Cranial nerve palsies were the next commonest finding and were noted in 32 patients (50%). These most commonly affected the **abducens nerve** (30%), oculomotor nerve (9%) and facial nerve (11%). This is similar to the findings of Rozenbaum,[2] Lalloo[23] and Seaton[51] where abducens nerve palsies were the commonest cranial nerve palsy noted in 22%, 20.4% and 25.5% of cases respectively. The high rate of abducens nerve palsies and papilloedema found in this study may be a reflection of raised intracranial pressure present at admission.

Choroiditis was the third commonest finding and was seen in nine patients (14%). Morinelli *et al*,[38] found 8% of patients with AIDS to have infectious choroiditis. Of these, 39% had cryptococcal choroiditis (or 3% of the total number of patients with AIDS).[38] In this study choroiditis appeared as multiple, pale, spherical choroidal infiltrates which is in keeping with other reports.[37,38] Other presentations may include a subretinal mass with surrounding exudation[39] or associated retinal telangiectasia and neovascularization.[36] All the patients with cryptococcal choroiditis in this study had a vision of counting fingers or worse.

Choroiditis usually occurs with endogenous dissemination of infectious emboli to the choriocapillaris.[38;72] Cryptococcal choroiditis may be the first sign of systemic dissemination and is associated with a poor prognosis.[38] Arevalo[37] and Morinelli[38] noted an average survival time of 3 weeks and 25 days respectively. In the present study six of the seven patients with cryptococcal choroiditis were

lost to follow-up and might have demised. The remaining patient survived for three months post diagnosis.

Other ocular findings included **optic neuritis** in six patients (9%) and **unilateral disc oedema** in five patients (8%). **Optic atrophy** was noted in three patients (5%). This is similar to studies by Kestelyn[32] and Okun[30] who reported a prevalence of optic atrophy of 2.5% and 8.0% respectively. In this study optic atrophy was bilateral in one of the patients. All three cases had severe vision loss with only counting fingers or hand movements possible.

HIV retinopathy occurred bilaterally in four patients (6%) and **CMV retinitis** was seen bilaterally in two patients. This is similar to the 5% of cases with CMV retinitis seen by Kestelyn *et al.*[32] **PORN** was noted in one patient. These findings are in keeping with reports that multiple pathogens may be present in patients with cryptococcal meningitis, especially if there is accompanying immunosuppression.[32;40] While *C. neoformans* typically causes choroidal involvement, *Cytomegalovirus, Human immunodeficiency virus and Herpes zoster virus* primarily infect the retina.[40]

Optic neuropathy was the cause of blindness in all five patients who presented with no light perception. Three patients had optic neuritis, while one patient had bilateral CMV retinitis and one had bilateral choroiditis. Both of the last two patients had optic nerve involvement. This is in keeping with reports in the literature that optic neuropathy is the commonest cause for loss of vision in cryptococcosis,[27;32;51] while CMV retinitis is an important cause of blindness in patients with AIDS.[36] The role of HIV itself as an aetiologic agent in optic nerve

disease is unknown. The mechanisms described for sudden bilateral blindness may include fulminant necrosis of both optic nerves, vasculitis, adhesive arachnoiditis, and bilateral occipital lobe hypoperfusion.[1;30;73-75]

Reports in the literature indicate that 10-15% of patients with cryptococcosis have **cutaneous involvement**.[33] In this study skin lesions were seen in 5 patients (8%). The usual presentation is described as umbilicated papules with a central haemorrhagic crust similar to *Molluscum contagiosum,* on the neck, face and less frequently trunk. Two patients in the study had *Molluscum contagiosum*-like lesions of the lids, two patients had papular dermatitis of the face and one patient had ecchymoses of the lids. The latter may have been the start of Kaposi sarcoma and is in keeping with concomitant immunosuppression or HIV infection.

Eyelid nodules of cryptococcosis have been described to be a **sentinel sign** preceding dissemination.[33] Their presence alerts to the need for rapid initiation of therapy.[33] Unfortunately the one patient who had this was lost to follow-up and his outcome could not be assessed.

Adnexal lesions included a conjunctival mass resembling squamous cell carcinoma in one patient, episcleritis in another and allergic conjunctivitis in two patients. Conjunctival cryptococcosis may be very similar in appearance to squamous cell carcinoma. Its presence in ill patients may be overlooked unless histology is done.[34]

On examination of the mental status of patients at admission 44 were orientated (69%), 19 were disorientated (30%) and one patient was frankly psychotic.

Rozenbaum and Goncalves,[2] reported that **altered states of consciousness** were less frequent in patients with AIDS than in the non-immunosuppressed (24% and 50% respectively). The prevalence of 30% disorientated patients in this study is in keeping with this finding since the majority of the patients in the present study (67%) were infected with the human immunodeficiency virus.

Meningism was present in most patients (97%) as the patients were selected with CSF involvement (refer to Table 3.3). This is also in keeping with extrapulmonary dissemination of Cryptococcosis which is commoner in immunosuppressed patients and usually takes on the form of meningoencephalitis.[2] Other features included **focal neurologic deficits.** Rozenbaum and Goncalves[2] reported 21%, 18% and 11% neurologic deficits in non-immunosuppressed patients, AIDS patients and in those with other underlying diseases respectively. Shih *et al*,[4] reported hemiplegia in 5 of his 94 patients (5%). In the present study a similar 5 of 64 patients (8%) had neurologic deficits (hemiplegia, paraplegia and peripheral neuropathy).

Deafness was the other devastating complication. This was seen in 12 patients (19%), which was higher than the 9% found by Shih *et al* in their 94 patients.[4] The reason for this may be that the diagnosis of deafness was based on clinical findings rather than formal testing. Objective testing would have separated patients with conductive loss from those with sensorineural deafness. It may also have revealed those who had complete deafness rather than depressed hearing. This may have resulted in lower numbers. Hearing loss has been observed in the literature to occur more frequently in those with CSF opening pressures >250mm water.[26]

A study from the tropics shows the infection to occur in healthy hosts, whereas 69-85% of cases in temperate climates have a predisposing disease associated with immunosuppression, mostly due to lymphoma.[23] In these cases the disease also presents more rapidly with more frequent dissemination.[23]

In the present study 43 of the patients (67%) had underlying **HIV infection.** The remaining 33% of patients in this study had not been tested for HIV and it is likely that almost all were HIV positive. **CD_4 T-cell counts** performed in 13 of these 43 patients (30%) ranged from 1-62 cells/μL with a mean of 22 cells/μL (refer to Table 3.4).

Due to the high rate of HIV infection and budget constraints at the Johannesburg Hospital the Department of Medicine is unable to perform HIV tests on all suspicious cases. It has adopted a policy of only requesting HIV tests if it is absolutely essential, if the diagnosis is uncertain and if management decisions will alter significantly depending on the result. Pre-test counselling and patients' consent are required in all cases.

The average CD_4 T-cell count of 22 cells/μL in this study indicates profound immunosuppression. Higher counts (40-62 cells/μL) were seen in patients above the age of 38 years (see Figure 3.7). There was a high index of suspicion that the remaining untested 21 patients might have had advanced AIDS due to their clinical presentation. The majority showed features of wasting, oral candidiasis and bronchopneumonia. Many patients were too ill to sit up and examination at a slit lamp was not possible. However, despite this clinical suspicion no conclusions

could be made about the immune status of the remaining 21 patients in the absence of appropriate special investigations.

In 32 patients (50%) concomitant underlying **pulmonary tuberculosis** was confirmed on sputa and chest radiography. Pulmonary cryptococcosis may also have been present, but was not actively diagnosed. It is the second most frequently involved site in AIDS patients, but its presence is often underestimated.[2] Tuberculosis may cause focal or multifocal choroiditis similar to that of cryptococcal choroiditis and it would not be possible to differentiate the two in the absence of invasive tests.

The mean **haemoglobin level** in 57 tested patients ranged from 5 to 18g/dL with a mean of 11.43g/dL. In 12 of the 57 patients (21%) the haemoglobin level was <10g/dL. Of these 12 patients, six (50%) had loss of vision and seven were female. Jabs *et al*,[70] showed lower haemoglobin levels (<10g/dL) to be associated with increased mortality. The outcome in eight of these patients was unknown. The average survival time in the remaining four patients was six months.

None of the patients had a lymphoreticular malignancy. None had undergone organ transplantation and none had received corticosteroids prior to their admission.

The diagnosis of cryptococcal meningitis in the present study was confirmed on CSF tests. **India ink** was positive in 63 patients (98%), **latex agglutination** was positive in all 64 patients and **culture** was positive for *Cryptococcus* in 62

patients (97%). This is in keeping with reports from the literature that in cryptococcal meningoencephalitis CSF India ink preparations, latex tests and cultures are usually positive.[2] The latex agglutination test in particular is highly sensitive and specific in the diagnosis of infection with *C. neoformans*. In contrast *serum* cryptococcal antigen testing is far less reliable, especially in non-HIV-infected patients with extraneural cryptococcosis.[1;7] No comment can be made about **titres** of *C. neoformans* at presentation as these were not performed at the Johannesburg Hospital.

Findings such as abnormal mentation (30%), papilloedema (60%), loss of vision (84%) and deafness (19%) that were present in the study may have resulted from raised intracranial pressure.[26] However, **opening CSF pressures** were not documented on any of the patients. This practice is unusual as the literature reports a high occurrence (50%) of raised intracranial pressure in cryptococcal meningitis. Loss of vision and a decrease in CSF clearance of the organisms at two weeks is associated in these patients.[26]

Documenting raised intracranial or opening pressures is mandatory to determine if aggressive intervention such as optic nerve sheath fenestation is needed to minimise neurologic sequelae.[63] Current practice guidelines recommend decreasing pressures by 50% if they are above 350mm water.[54] Unfortunately no conclusions could be made correlating survival and loss of vision with possible intracranial hypertension in this study.

Serotyping of the responsible organism is not available at the Johannesburg Hospital. In addition, no data were available regarding the **origin** or **occupation**

of the patients entered into the study. This information would give an indication of the serovar causing infection in this study population. It would be significant in accurately determining prognostic factors for ocular outcome.

Focal neurologic and ocular complications are more frequently reported in infection with *C. neoformans* var. *gattii*.[3;21;27;53] There is no strong evidence for an ecological niche for variety *gattii* in South Africa. However, it cannot be excluded completely as it reportedly occurs more commonly in the rural areas in the tropics and subtropics.[77] Some of the patients in this study may have originated from these places. In addition, with the large amount of ocular involvement in this study, infection with var. *gattii* remains a possibility.

Avian droppings, especially those of pigeons are responsible for the dissemination and urbanization of *C. neoformans* var. *neoformans*.[2] It is postulated that variety *neoformans* may also be an infecting agent in this environment. This is due to the high proportion of patients in this study with HIV infection (67%) and the low CD_4 T-cell counts noted. However, infection with var. *neoformans* would not correlate with the high rate of vision loss seen in this study. Further investigations with serotyping of the organism are needed to determine which serovar is responsible for vision loss in the South African setting and if this is of prognostic significance.

Most patients with AIDS or underlying immunosuppression have disseminated cryptococcal disease.[9] **Fungaemia** occurs in up to 70% of cryptococcosis patients in the literature.[9] In this study patients did not report symptoms suggesting sites of infection outside the CNS. Tests to exclude **extrameningeal**

sites of infection were also not performed routinely by the physicians. This was due to budget constraints at the Johannesburg Hospital and that this investigation was not indicated in most of the patients. **Blood cultures** were taken in 11 of the 64 patients (17%) and were positive for *Cryptococcus* in four of these 11 patients (36%). Fungaemia could thus not be used as a prognostic determinant of outcome or vision loss in this study.

CT scanning of the brain was done in 13 of 64 patients (20%). In two patients this showed hydrocephalus and in one patient raised intracranial pressure. Three patients had infarcts which were seen in the internal capsule, frontal lobes and around the lateral ventricles (refer to Table 3.6). Although these infarcts were not in the occipital lobes there may have been an associated decrease in cerebral blood flow to these areas which was not visible on CT scan.[47;48] This could contribute to decreased vision. Access to ^{99m}Tc-HMPAO SPET may be useful to detect such patients at risk for loss of vision.[47;48]

Follow-up of patients was problematic. They often did not return on arranged dates due to lack of transport or funds or as they may have died. Contacting them was also difficult if they had no telephones or stayed in remote areas. This made follow-up incomplete. Only two of 54 patients (4%) with ocular features related to cryptococcosis arrived for their **one-month follow-up** visit.

The known **relapse rate** with a recurrent episode of cryptococcal meningitis was (8%). It may have been higher than this but the other patients could not be traced. Reports in the literature of relapse rates in immunosuppressed patients vary from 35-50%.[67] The average time to relapse was 4.2 months after the first admission

(refer to Table 3.7). Risk factors for relapse may have included high cryptococccal titres (>1/8) post treatment, recrudescence of subclinical foci of infection such as the prostate or eye and incomplete maintenance therapy due to non-compliance.[7;15;54;56;57;67] Cryptococcal titres are not done at the Johannesburg Hospital so these could not be monitored to identify patients with treatment failure.

Twenty-one of sixty-four patients were known to have **demised** (33%). This is very high if it considered that all the patients that could be traced had a poor outcome. It is, however, in keeping with mortality rates of 30-85% in immunosuppressed patients recorded in the literature.[9;14;66;67] In six patients this averaged three days after admission. In the other 15 patients the mean time of survival from onset of treatment was six months (refer to Table 3.8). This is in keeping with a 12-month survival of 30-60% in the literature with a median survival of nine months in AIDS patients.[9;68]

The low rate of survival may be due to a number of factors. There was a high index of suspicion that many of these patients may have been in an advanced state of **immune-compromise** and thus at risk for poor outcome.[59;70] A low CD_4 T-cell count where tested and haemoglobin levels less than 10g/dL further contributed to AIDS as an important risk factor for mortality.[59;70]

Secondly, incomplete suppressive or maintenance therapy is a risk factor for mortality.[54] Recommended guidelines[54] suggest that a combination of **Amphotericin B and Flucytosine** is the best induction therapy for such patients. This should be for two to six weeks followed by Fluconazole 400mg daily

maintenance therapy which could be reduced to 200mg daily after ten weeks depending on the clinical status of these patients.[54]

However, most of these patients in this study received Fluconazole monotherapy as an induction agent. This has been shown to be as effective as Amphotericin B monotherapy, but is inferior to the combination therapy of Amphotericin B and Flucytosine.[54] The guidelines from the NIAID MSG advise against the use of an azole as an induction agent. Fluconazole monotherapy has an increased rate of two-week mortality (seen in six patients) and reduced CSF clearance at two weeks.[60] Such patients have a five-fold increased risk of delayed CSF clearance at ten weeks and this is associated with poorer outcome.[59] It is not known whether repeated CSF cultures were done on these patients. However, with the poor follow-up attendance record of these patients this is unlikely. It has been recommended that repeat cultures are done at two weeks and again at ten weeks and that therapy is optimised according to the results.[59]

Thirdly, none of the patients in this study had **CSF opening pressures** recorded at the time of their admission. Raised intracranial pressure is associated with neurological sequelae and poorer outcome.[42;43;47] Extreme elevation of CSF pressure is associated with increased mortality at two weeks.[26]

The high prevalence of papilloedema (60%), abducens nerve palsies (30%) and deafness (19%) recorded in this study suggests that raised intracranial pressure may have been present in these patients at admission. None of these patients had interventions such as CSF taps, ventriculoperitoneal shunts or optic nerve sheath fenestrations. The high rate (50%) of raised intracranial pressure with

cryptococcal meningitis recorded in the literature would make such monitoring and procedures seem desirable as aggressive reduction of raised intracranial pressure is known to be associated with improved outcomes.[42;43]

Fourthly, **serotyping** of the organism is important in determining the virulence of the offending agent. Studies indicate that var. *gattii* may not be a true opportunistic agent as is var. *neoformans* and that it should be reclassified as *Cryptococcus gattii*.[18] It has been suggested that patients with infection with this serovar be treated with higher doses of therapy and for more prolonged periods to lessen complications.[3;21] Serotyping was not done in this study population. There was a high prevalence of vision loss (45%) in this study which is more in keeping with var. *gattii* infection.[27] However, as many patients appeared to be immune-compromised one cannot exclude the possibility of hybrid strains of antigen A or D with B or C in this setting.

Fifthly, **relapse** is associated with higher mortality rates.[9;67] This is usually due to recrudescence of extra-meningeal foci of infection.[9] The prostate has been described as one such site. With the high rate of ocular involvement in this study this cannot be excluded as another possible source for relapse in male patients. Further studies are needed to evaluate this.

Other factors predictive of mortality in the literature include **age less than 35 years** and **altered mental status** of the patient at presentation. The average age of patients in this study was 35 years and altered mental status was seen in 30% of patients at admission.

The study undertaken was a cross-sectional observational study. Features that were seen frequently in patients with loss of vision included papilloedema (62%), abnormal mentation (41%) and bilateral abducens palsies (38%) (refer to Table 3.9). The literature reports CSF antigen titres >1:1024, bilateral abducens palsies and immunocompetence to be the factors associated with loss of vision.[51]

To accurately determine if the findings in this study were risk factors for loss of vision each variable would need to be assessed independently as part of a cohort study where risk factors were measured at baseline. The high rate of HIV infection found in this study is a confounder and is not in keeping with reports from the literature that loss of vision is more frequent in immunocompetent patients in the tropics and subtropics.[27;51] In addition, the effect of reduced cerebral blood flow and the serovar responsible for infection would each need to be assessed independently.

Raised intracranial pressure has been shown by some authors to correlate with visual loss,[42;43;45] but not others.[51] CSF opening pressures were not recorded in the present study thus the contribution of intracranial pressure to loss of vision cannot be made.

Amphotericin B has been associated with acute profound loss of vision shortly after onset of therapy.[32;48] This is mostly with intrathecal use and may be due to direct neurotoxicity, an idiosyncratic reaction and adhesive arachnoiditis.[32;48;77] Loss of vision was noted in 14 of the 16 patients who received Amphotericin B in this study. In 12 of these patients there was an ocular finding which would have contributed to loss of vision. In the remaining two patients ocular examination was

normal and loss of vision mild (vision 6/12 or better). It is unlikely that Amphotericin B contributed to vision loss in this study. In most of the cases visual loss occurred before its administration, indicating that the disease process itself contributed to optic nerve damage.

Corticosteroids were employed in two patients most likely to dampen the febrile response to intravenous Amphotericin B in these patients. Both patients had bilateral no light perception at admission before the use of corticosteroids. One patient demised at admission, while the other was lost to follow-up. Seaton *et al,*[27] demonstrated that corticosteroids may have a role in preventing or halting loss of vision in var. *gattii* infection. This could be considered with the high rate of visual deterioration in this study, but only if the testing of immune status, serotyping and CSF opening pressures of these patients were available. The high prevalence of concomitant pulmonary tuberculosis (50%) noted in the present study would, however, make the use of steroids inadvisable. Their use may be indicated in tuberculous meningitis, but this was not a concomitant finding in any of the patients in this study. In addition, if high CSF pressures are present, the use of corticosteroids may be associated with significantly higher mortality.[26]

In 10 of the 29 patients with loss of vision the average time of survival from onset of treatment was seven months. Only 11 of the 35 patients with normal vision could be traced after discharge. The average survival time in these patients was six months. The remaining patients were untraceable. With such a small sample population and this being a cross-sectional study, it was not possible to correlate loss of vision over time with outcome.

5.0: CONCLUSIONS

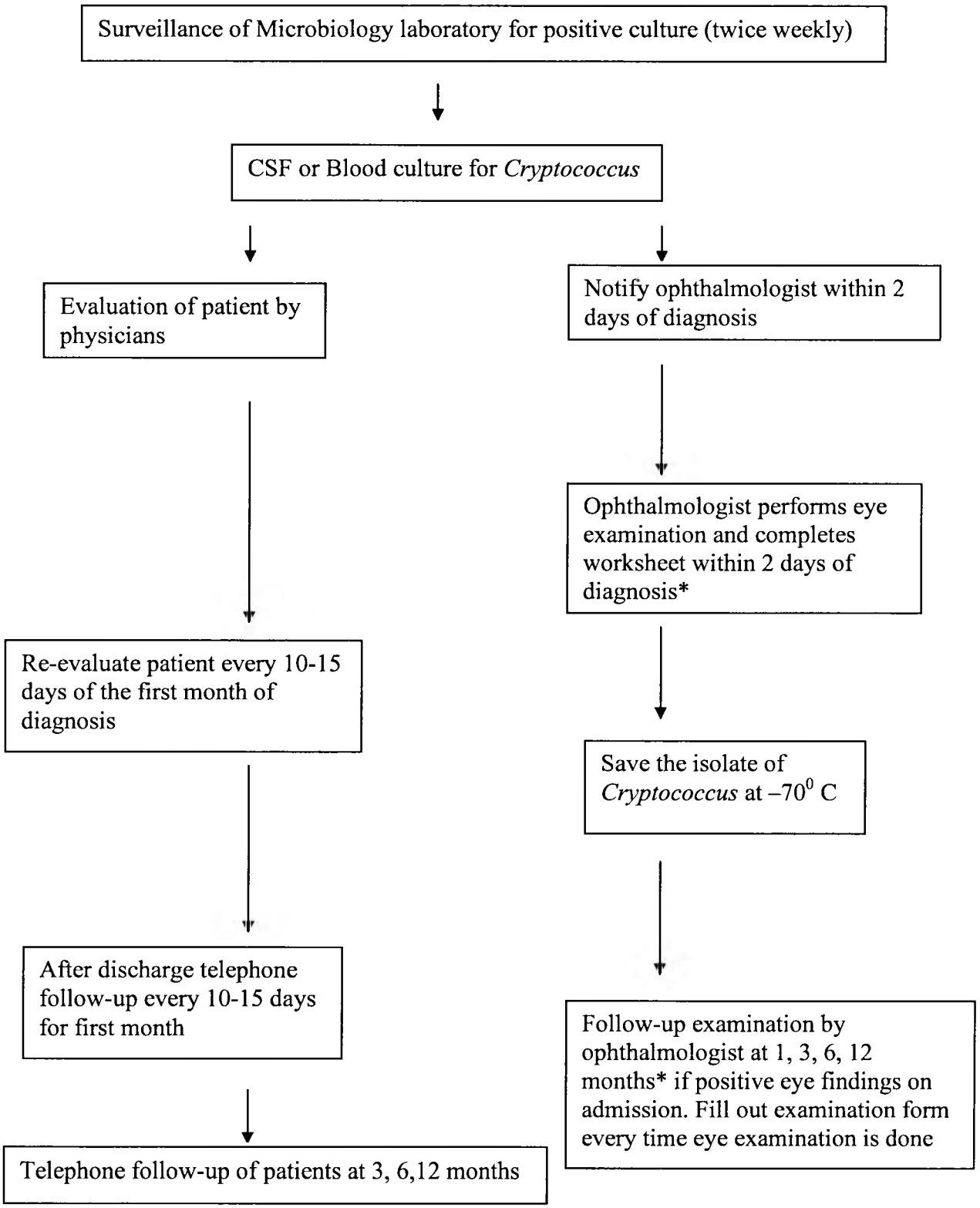
- The prevalence of ocular involvement in cryptococcal meningitis patients was 84%
- Loss of vision in cryptococcal meningitis occurred in 45% of patients
- Papilloedema was the commonest ocular finding (60%) followed by abducens nerve palsies (30%) and choroiditis (14%)
- HIV infection was the commonest underlying condition (67%) and active tuberculosis was present in 50%
- Meningism was the commonest neurological feature in 97% of cases as might have been expected
- Blindness and deafness were the commonest neurological sequelae in 6% and 19% of patients respectively
- Poor visual prognostic factors that were not found in patients with normal vision included optic neuritis (21%), choroiditis (21%) and optic atrophy (10%)
- All patients who could be traced had a poor outcome (33%)
- Loss of vision did not correlate with time of survival

6.0: CONSIDERATIONS FOR FUTURE RESEARCH

- Monitoring of CSF opening pressures in cryptococcal meningitis suspects on admission to identify patients at risk of sequelae from high pressures
- Serotyping of *C. neoformans* organisms on admission and to correlate with loss of vision
- Assessment of CSF cryptococcal titres on admission, to identify patients at risk of treatment failure and to correlate with loss of vision
- HIV testing or assessment of immune status on admission to correlate with loss of vision
- Monitoring cerebral blood flow to determine patients at risk for cortical blindness
- Offering patient incentives to return for follow-up

7.0: APPENDIX

APPENDIX 7.1: FLOW SHEET FOR PATIENTS WITH CRYPTOCOCCAL INFECTION



* after initial positive CSF or blood culture

APPENDIX 7.2: CT SCANS

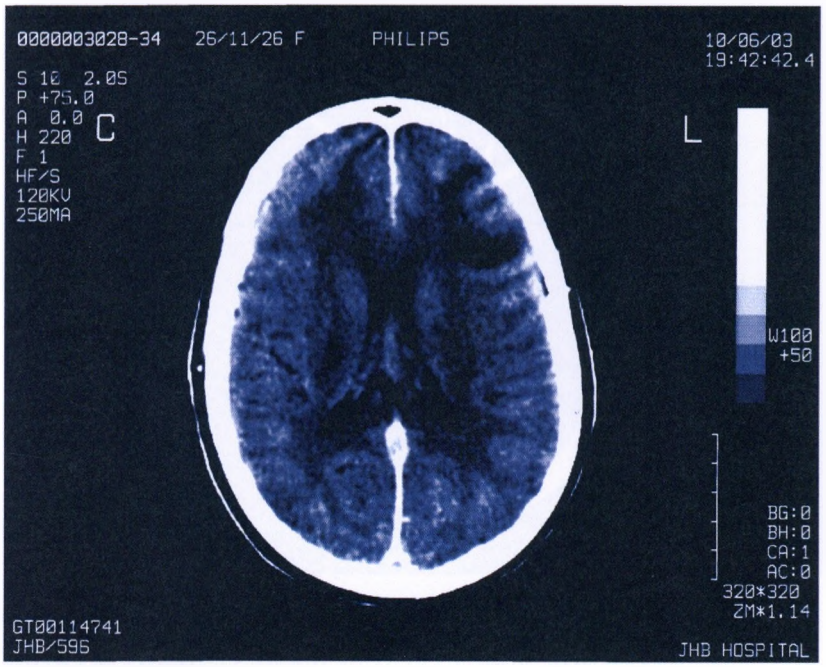


Figure 7.1 Axial CT brain showing left frontal lobe infarct and superior sagittal sinus thrombosis in cryptococcal meningitis

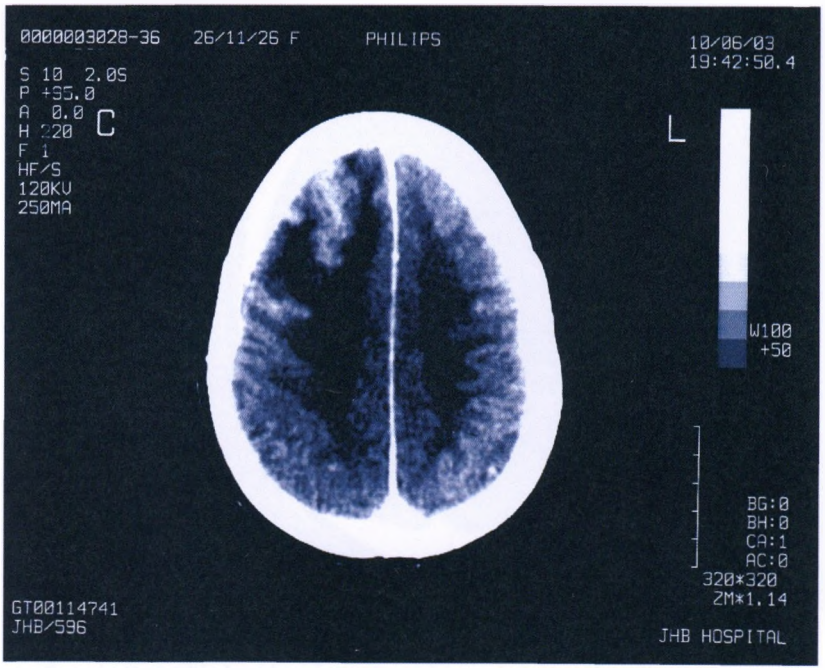


Figure 7.2 Axial CT brain showing right frontal lobe infarct and superior sagittal sinus thrombosis in cryptococcal meningitis

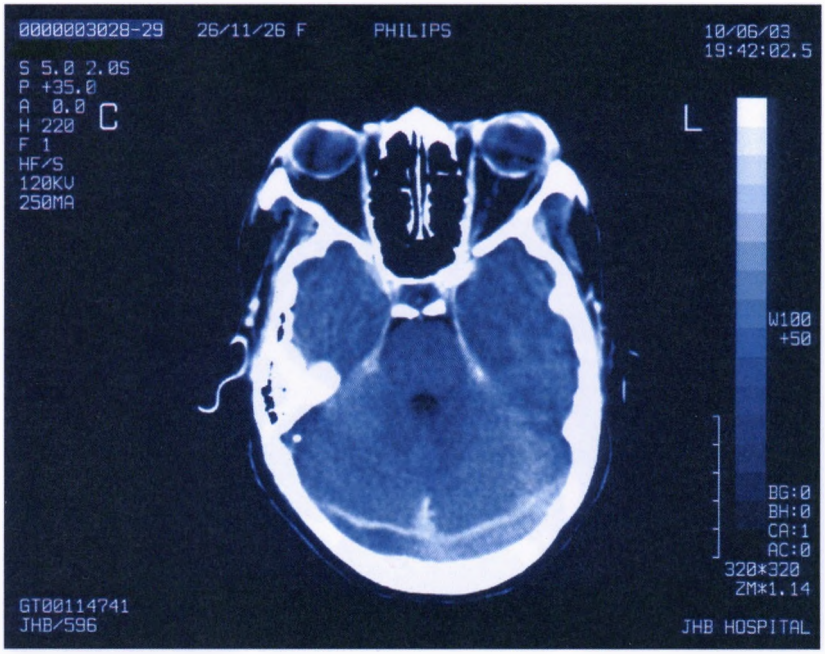


Figure 7.3 Axial CT brain showing a subdural haemorrhage in the left posterior fossa in cryptococcal meningitis

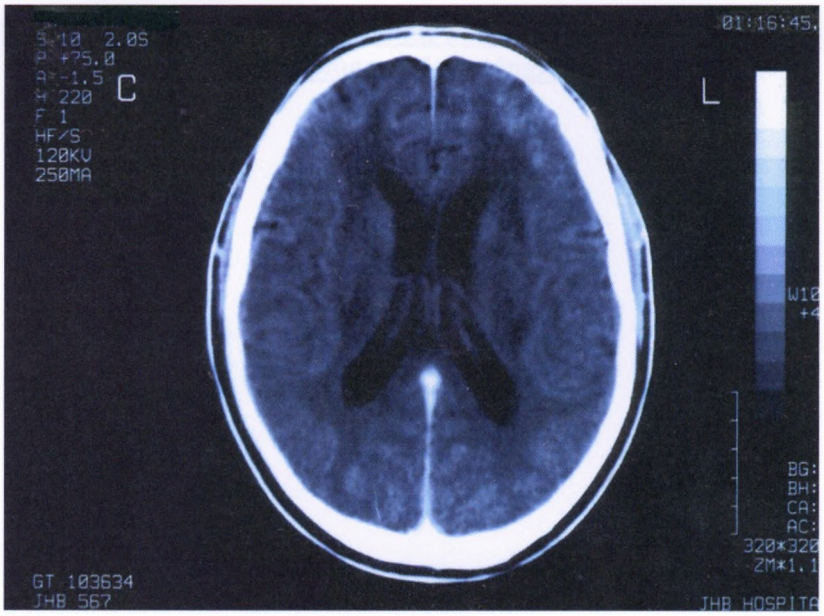


Figure 7.4 Axial CT brain showing non-obstructive hydrocephalus with cerebral atrophy in cryptococcal meningitis

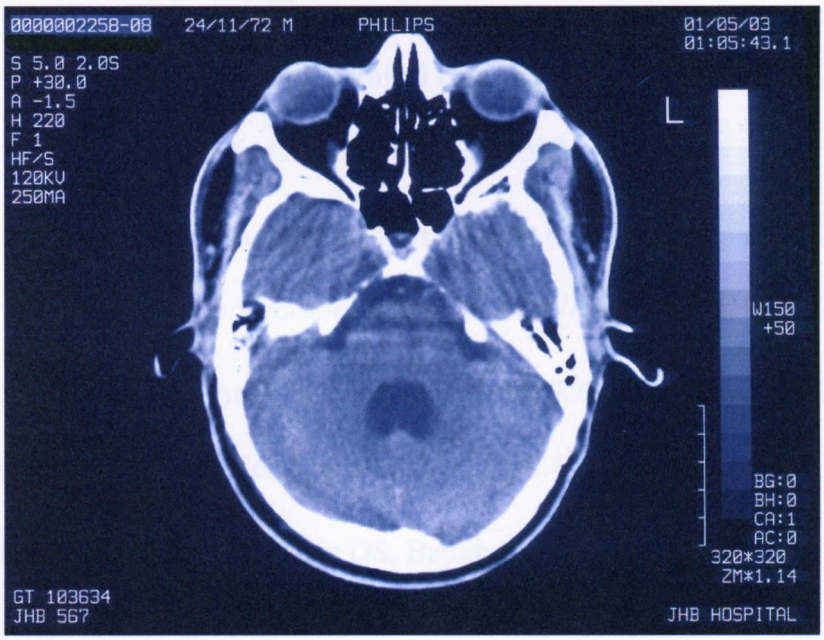


Figure 7.5 Axial CT brain showing non-obstructive hydrocephalus in cryptococcal meningitis

8.0: REFERENCES

1. Diamond RD. *Cryptococcus neoformans*. Principles and Practice of Infectious Diseases. Mandell G, Bennett J, Dolin R. Fourth Edition, Churchill Livingstone Inc. Pg 2331-2340, 1995.
2. Rozenbaum R, Goncalves AJ. Clinical epidemiological study of 171 cases of cryptococcosis. *Clin Infect Dis* 1994; **18**:369-380.
3. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clin Infect Dis* 1995; **21**:28-34.
4. Shih CC, Chen YC, Chang SC, Luh KT, Hsieh WC. Cryptococcal meningitis in non-HIV-infected patients. *Q J M* 2000; **93**:245-251.
5. Hajjeh RA, Conn LA, Stephens DS, Baughman W, Hamill R, Graviss E, *et al,* and the Cryptococcal Active Surveillance Group. Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus –infected persons. *J Infect Dis* 1999; **179**:449-454.
6. Kiertiburanakul S, Wirojtananugoon S, Pracharktam R, Sungkanuparph S. Cryptococcosis in human immunodeficiency virus- negative patients. *Int J Infect Dis* 2006; **10**:72-78.
7. Pappas PG, Perfect JR, Cloud GA, Larsen RA, Pankey GA, Lancaster DJ, *et al.* Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin Infect Dis* 2001; **33**:690-699.
8. Chuck SL, Sande MA. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. *N Engl J Med* 1989; **321**:794-799.
9. Powderly WG. Cryptococcal meningitis and AIDS. *Clin Infect Dis* 1993; **17**: 837-842.

10. Pinner RW, Hajjeh RA, Powderly WG. Prospects for preventing cryptococcosis in persons infected with human immunodeficiency virus. *Clin Infect Dis* 1995; **21**:S103-S107.

11. Darras-Joly C, Chevret S, Wolff M, Matheron S, Longuet P, Casalino E, *et al.* *Cryptococcus neoformans* infection in France: epidemiologic features of and early prognostic parameters for 76 patients who were infected with human immunodeficiency virus. *Clin Infect Dis* 1996; **23**:369-376.

12. Currie BP, Casadevall A. Estimation of the prevalence of cryptococcal infection among patients infected with the human immunodeficiency virus in New York City. *Clin Infect Dis* 1994; **19**:1029-1033.

13. Gabuzda DH, Hirsch MS. Neurologic manifestations of infection with human immunodeficiency virus. Clinical features and pathogenesis. *Ann Intern Med* 1987; **107**:383-391.

14. Perfect JR, Durack DT, Gallis HA. Cryptococcemia. *Medicine* 1983; **62**: 98-109.

15. Diamond RD, Bennett JE. Prognostic factors in cryptococcal meningitis. A study in 111 cases. *Ann Intern Med* 1974; **80**:176-181.

16. Schulman JA, Leveque C, Coats M, Lawrence L, Barber JC. Fatal disseminated cryptococcosis following intraocular involvement. *Br J Ophthalmol* 1988; **72**:171-175.

17. Ferreira RC, Phan G, Bateman JB. Favorable visual outcome in cryptococcal meningitis. *Am J Ophthalmol* 1997; **124**:558-560.

18. D'Souza CA, Hagen F, Boekhout T, Cox GM, Heitman J. Investigation of the basis of virulence in serotype A strains of *Cryptococcus neoformans* from apparently immunocompetent individuals. *Curr Genet* 2004; **46**:92-102.

19. Bennett JE, Kwon-Chung KJ, Howard DH. Epidemiologic differences among serotypes of *Cryptococcus neoformans*. *Am J Epidemiol* 1977; **105**:582-586.

20. Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. *Am J Epidem* 1984; **120**:123-130.

21. Fisher D, Burrow J, Lo D, Currie B. *Cryptococcus neoformans* in tropical northern Australia: predominantly variant *gattii* with good outcomes. *Aust N Z J Med* 1993; **23**:678-682.

22. Seaton RA, Hamilton AJ, Hay RJ, Warrell DA. Exposure to *Cryptococcus neoformans* var. *gattii* – a seroepidemiological study. *Trans R Soc Trop Med Hyg* 1996; **90**:508-512.

23. Lalloo D, Fisher D, Naraqi S, Laurenson I, Temu P, Sinha A, *et al.* Cryptococcal meningitis (*C. neoformans* var. *gattii*) leading to blindness in previously healthy melanesian adults in Papua New Guinea. *Q J M* 1994; **87**:343-349.

24. Fleuridor R, Lyles RH, Pirofski L. Quantitative and qualitative differences in the serum antibody profiles of human immunodeficiency virus- infected persons with and without *Cryptococcus neoformans* meningitis. *J Infect Dis* 1999; **180**:1526-1535.

25. Denning DW, Armstrong RW, Lewis BH, Stevens DA. Elevated cerebrospinal fluid pressures in patients with cryptococcal meningitis and acquired immunodeficiency syndrome. *Am J Med* 1991; **91**:267-272.

26. Graybill JR, Sobel J, Saag M, van der Horst C, Powderly W, Cloud G, *et al.* Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. *Clin Infect Dis* 2000; **30**:47-54.

27. Seaton RA, Verma N, Naraqi S, Wembri JP, Warrell DA. The effect of corticosteroids on visual loss in *Cryptococcus neoformans* var. *gattii* meningitis. *Trans R Soc Trop Med Hyg* 1997; **91**:50-52.

28. Manfredi R, Pieri F, Pileri SA, Chiodo F. The changing face of AIDS-related opportunism: cryptococcosis in the highly active antiretroviral (HAART) era. Case reports and literature review. *Mycopathologia* 1999; **148**:73-78.

29. Powderly WG. Prophylaxis for opportunistic infections in an era of effective antiretroviral therapy. *Clin Infect Dis* 2000; **31**:597-601.

30. Okun E, Butler WT. Ophthalmic complications of cryptococcal meningitis. *Arch Ophthalmol* 1964; **71**:52-57.

31. Ofner S, Baker RS. Visual loss in cryptococcal meningitis. *J Clin Neuro-Ophthalmol* 1987; **7**:45-48.

32. Kestelyn P, Taelman H, Bogaerts J, Kagame A, Aziz MA, Batungwanayo J, *et al.* Ophthalmic manifestations of infections with *Cryptococcus neoformans* in patients with the acquired immunodeficiency syndrome. *Am J Ophthalmol* 1993; **116**:721-727.

33. Coccia L, Calista D, Boschini A. Eyelid Nodule: a sentinel lesion of disseminated cryptococcosis in a patient with acquired immunodeficiency syndrome. *Arch Ophthalmol* 1999; **117**:271-272.

34. Waddell KM, Lucas SB, Downing RG. Conjunctival cryptococcosis in the acquired immunodefiency syndrome. *Arch Ophthalmol* 2000; **118**:1452-1453.

35. Charles NC, Boxrud CA, Small EA. Cryptococcosis of the anterior segment in acquired immune deficiency syndrome. *Ophthalmology* 1992; **99**:813-816.

36. Saran BR, Pomilla PV. Retinal vascular nonperfusion and retinal neovascularization as a consequence of cytomegalovirus retinitis and cryptococcal choroiditis. *Retina* 1996; **16**:510-512.

37. Arevalo JF, Fuenmayor-Rivera D, Giral AE, Murcia E. Indocyanine green videoangiography of multifocal *Cryptococcus neoformans* choroiditis in a patient with acquired immunodeficiency syndrome. *Retina* 2001; **21**:537-541.

38. Morinelli EN, Dugel PU, Riffenburgh R, Rao NA. Infectious multifocal choroiditis in patients with acquired immune deficiency syndrome. *Ophthalmology* 1993; **100**:1014-1021.

39. Biswas J, Gopal L, Sharma T, Parikh S, Madhavan HN, Badrinath SS. Recurrent cryptococcal choroiditis in a renal transplant patient. *Retina* 1998; **18**:273-275.

40. Crump JR, Elner SG, Elner VM, Kauffman CA. Cryptococcal endophthalmitis: case report and review. *Clin Infect Dis* 1992; **14**:1069-1073.

41. Denning DW, Armstrong RW, Fishman M, Stevens DA. Endophthalmitis in a patient with disseminated cryptococcosis and AIDS who was treated with Itraconazole. *Rev Infect Dis* 1991; **13**:1126-1130.

42. Rex JH, Larsen RA, Dismukes WE, Cloud GA, Bennett JE. Catastrophic visual loss due to *Cryptococcus neoformans* meningitis. *Medicine* 1993; 72: 207-224.

43. Claus JJ, Portegies P. Case report. Reversible blindness in AIDS- related cryptococcal meningitis. *Clin Neurol Neurosurg* 1998; **100**:51-52.

44. Kupfer C, McCrane E. A possible cause of decreased vision in cryptococcal meningitis. *Invest Ophthalmol* 1974; **13**:801-804.

45. Tan CT. Intracranial hypertension causing visual failure in cryptococcus meningitis. *J Neurol Neurosurg Psychiatry* 1988; **51**:944-946.

46. Li PK, Lai KN. Amphotericin B induced ocular toxicity in cryptococcal meningitis. *Br J Ophthalmol* 1989; **73**:397-398.

47. Torres OH, Negredo E, Ris J, Domingo P, Catafau AM, Contreras JL. ^{99M}Tc-HMPAO SPET: a method to study visual loss in cryptococcal meningitis. *Acta Neurol Scand* 2000; **102**:340-341.

48. Silverman IT, Galetta SL, Gray LG, Moster M, Atlas SW, Maurer AH, *et al.* SPECT in patients with cortical visual loss. *J Nucl Med* 1993; **34**:1447-1451.

49. Winward KE, Hamed LM, Glaser JS. The spectrum of optic nerve disease in human immunodeficiency virus infection. *Am J Ophthalmol* 1989; **107**: 373-380.

50. Lan SH, Chang WN, Lu CH, Lui CC, Chang HW. Cerebral infarction in chronic meningitis: a comparison of tuberculous meningitis and cryptococcal meningitis. *Q J M* 2001; **94**:247-253.

51. Seaton RA, Verma N, Naraqi S, Wembri JP, Warrell DA. Visual loss in immunocompetent patients with *Cryptococcus neoformans* var. *gattii* meningitis. *Trans R Soc Trop Med Hyg* 1997; **91**:44-49.

52. Kestelyn P, Taelman H. Visual loss and cryptococcal meningitis. *Trans R Soc Trop Med Hyg* 1997; **91**:727-728.

53. Antoniadou A, Kontoyiannis DP. Status of combination therapy for refractory mycoses. *Curr Opin Infect Dis* 2003; **16**:539-545.

54. Saag MS, Graybill RJ, Larsen RA, Pappas PG, Perfect JR, Powderly WG, *et al,* for the Mycoses Study Group Cryptococcal Subproject. Practice guidelines for the management of cryptococcal disease. *Clin Infect Dis* 2000; **30**:710-718.

55. Powderly WG, Saag MS, Cloud GA, Robinson P, Meyer RD, Jacobson JM, *et al*, The NIAID AIDS Clinical Trials Group, and the NIAID Mycoses Study Group. A controlled trial of Fluconazole or Amphotericin B to prevent relapse of cryptococcal meningitis in patients with the acquired immunodeficiency syndrome. *N Eng J Med* 1992; **326**:793-798.

56. Bennett JE, Dismukes WE, Duma RJ, Medoff G, Sande MA, Gallis H, *et al.* Mycosis Study Group. A comparison of Amphotericin B alone and combined with Flucytosine in the treatment of cryptococcal meningitis. *N Engl J Med* 1979; **301**:126-131.

57. Dismukes WE, Cloud G, Gallis HA, Kerkering TM, Medoff G, Craven PC, *et al.* Treatment of cryptococcal meningitis with combination Amphotericin B and Flucytosine for four as compared with six weeks. *N Engl J Med* 1987; **317**: 334-341.

58. Mitchell DH, Sorrell TC, Allworth AM, Heath CH, McGregor AR, Papanaoum K, *et al.* Cryptococcal disease of the CNS in immunocompetent Hosts: influence of cryptococcal variety on clinical manifestations and outcome. *Clin Infect Dis* 1995; **20**:611-616.

59. Robinson PA, Bauer M, Leal MA, Evans SG, Holtom PD, Diamond DM, *et al.* Early mycological treatment failure in AIDS-associated cryptococcal meningitis. *Clin Infect Dis* 1999; **28**: 82-92.

60. Saag MS, Powderly WG, Cloud GA, Robinson P, Grieco MH, Sharkey PK, *et al,* and the NIAID Mycoses Study Group and the AIDS Clinical Trials Group. Comparison of Amphotericin B with Fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. *N Engl J Med* 1992; **326**:83-89.

61. Powderly WG. Therapy for cryptococcal meningitis in patients with AIDS. *Clin Infect Dis* 1992; **14**: S54-S59.

62. Dromer F, Mathoulin S, Dupont B, Brugiere O, Letenneur L, Chardon H, *et al.* Comparison of the efficacy of Amphotericin B and Fluconazole in the treatment of cryptococcosis in human immunodeficiency virus-negative patients: retrospective analysis of 83 cases. *Clin Infect Dis* 1996; **22**:S154-S160.

63. Garrity JA, Herman DC, Imes R, Fries P, Hughes CF, Campbell RJ. Optic nerve sheath decompression for visual loss in patients with acquired immunodeficiency syndrome and cryptococcal meningitis with papilledema. *Am J Ophthalmol* 1993; **116**:472-478.

64. Siddiqui AA, Brouwer AE, Wuthiekanun V, Jaffar S, Shattock R, Irving D, *et al.* IFN-γ at the site of infection determines rate of clearance of infection in cryptococcal meningitis. *J Immunol* 2005; **174**:1746-1750.

65. Seaton RA, Naraqi S, Wembri JP, Warrell DA. Predictors of outcome in *Cryptococcus neoformans* var. *gattii* meningitis. *Q J M* 1996; **89**:423-428.

66. Laurenson IF, Trevett AJ, Lalloo DG, Nwokolo N, Naraqi S, Black J, *et al.* Meningitis caused by *Cryptococcus neoformans* var. *gattii* and var. *neoformans* in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1996; **90**: 57-60.

67. Zuger A, Louie E, Holzman RS, Simberkoff MS, Rahal JJ. Cryptococcal disease in patients with the acquired immunodeficiency syndrome. Diagnostic features and outcome of treatment. *Ann Intern Med* 1986; **104**:234-240.

68. White M, Cirrincione C, Blevins A, Armstrong D. Cryptococcal meningitis: outcome in patients with AIDS and patients with neoplastic disease. *J Infect Dis* 1992; **165**:960-963.

69. Studies of Ocular Complications of AIDS Research Group, in collaboration with the AIDS Clinical Trials Group. Mortality in patients with the acquired immunodeficiency syndrome treated with either foscarnet or ganciclovir for cytomegalovirus retinitis. *N Engl J Med* 1992; **326**:213-220.

70. Jabs DA, Holbrook JT, Van Natta ML, Clark R, Jacobson MA, Kempen JH, *et al,* for the Studies of Ocular Complications of AIDS Research Group. Risk

factors for mortality in patients with AIDS in the era of highly active antiretroviral therapy. *Ophthalmology* 2005; **112**:771-779.

71. Kanski JJ. Clinical Ophthalmology. Fourth Edition 1999; 599-601. Butterworth-Heinemann.
72. Avendano J, Tanishima T, Kuwabara T. Ocular cryptococcosis. *Am J Ophthalmol* 1978; **86**:110-113.
73. Cohen DB, Glasgow BJ. Bilateral optic nerve cryptococcosis in sudden blindness in patients with acquired immune deficiency syndrome. *Ophthalmology* 1993; **100**:1689-1694.
74. Torres OH, Negredo E, Ris J, Domingo P, Catafau AM. Visual loss due to cryptococcal meningitis in AIDS patients. *AIDS* 1999; **13**:530-533.
75. Lipson BK, Freeman WR, Beniz J, Goldbaum MH, Hesselink JR, Weinreb RN, *et al.* Optic neuropathy associated with cryptococcal arachnoiditis in AIDS patients. *Am J Ophthalmol* 1989; 107:523-527.
76. Johnston SR, Corbett EL, Foster O, Ash S, Cohen J. Raised intracranial pressure and visual complications in AIDS patients with cryptococcal meningitis. *J Infect* 1992; 24:185-189.
77. Rinaldi MG, Drutz DJ, Howell A, Sande MA, Wofsy CB, Hadley WK. Serotypes of *Cryptococcus neoformans* in patients with AIDS. *J Infect Dis* 1986; 153:642.

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27 October 2006

Corrected research report submitted in partial fulfilment of
Master of Medicine (Ophthalmology)

Student name: NLM Ehlers
Student number: 8700833/M

Title: A prospective, observational study of cryptocccal infection and clinical and ophthalmologic outcomes.

This is to confirm that I am satisfied that the above-mentioned student has made the necessary corrections to the research report in accordance with the examiner's recommendations.

Yours sincerely

Professor Trevor R Carmichael
Head: Ophthalmology
Department of Neurosciences

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Abstract and key bibliographic data *(i.e. from submission form)*

9.3 I acknowledge that I am not entitled to the return of the copies of the ~~dissertation or~~ research/~~project~~ report ~~or thesis~~ or other work I have submitted for the degree.

10. Did your research involve animal experimentation or the use of human subjects, human tissue or other material, or patient records?

☒ Yes

☐ No

If yes, please certify that clearance was obtained from the relevant, approved, University ethics committee: YES

Clearance number(~~s~~): M02-02-09

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