HUMAN IMMUNODEFICIENCY VIRUS AND CD_4 COUNT IN OCULAR SURFACE SQUAMOUS NEOPLASIA

by

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Declaration

I Dr Thenushka Jogi declare that

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

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Definition of key terms

Epidemiology

Incidence – The number of new cases that develop over a given period of time.

Prevalence – The percentage of a population affected by a certain disease at a given time.

Population

A **couple** – This refers to two people in an on-going sexual relationship; each of these people is referred to as a partner in the relationship. How individuals define their relationships will vary according to their cultural and social context.

Serodiscordant couples – Couples in which one partner is living with HIV and the other is HIV negative.

Statistical

Epidemic – An outbreak of disease within a community or region. It may be seasonal, such as outbreaks of influenza and Ebola.

Pandemic – An outbreak of a disease across different countries – or in global proportions. A new virus or new sub-strain of a virus, such as Spanish flu and HIV/AIDS, usually causes it.

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Overview

During my time working at various eye clinics in Kwazulu-Natal, I observed that more often than not, patients with disfiguring ocular surface squamous neoplasia (OSSN) were Human Immunodeficiency virus (HIV) positive and generally quite ill, often requiring radical ophthalmic surgery for the tumour and urgent referral to the local Centre for Disease control (CDC) and HIV/Acquired Immunodeficiency Syndrome (AIDS) clinics, whereas the patients with conjunctival intraepithelial neoplasia (CIN) were usually HIV negative or HIV positive but well looking.

This sparked the question of whether or not there may be a relationship between Cluster of Differentiation 4: a glycoprotein found on the surface of immune cells (CD_4) counts and OSSN in HIV positive patients and could these findings – should they be in the affirmative – be used as a CDC case defining criteria for AIDS and streamlet referral of these patients for Highly Active Antiretroviral Therapy (HAART).

Although HIV/AIDS is a global pandemic and has been linked to OSSN, an extensive literature search found no studies specifically looking at CD_4 counts in these patients. My study titled "Human Immunodeficiency Virus and CD_4 count in ocular surface squamous neoplasia" was thus born.

I elected to perform my study in two parts. Firstly a prospective descriptive study to determine the prevalence of HIV in OSSN in my study population from September 2012 to December 2014, and secondly a case control study to determine the odds ratio of CD_4 counts in HIV positive patients with OSSN (cases) and those without OSSN (controls)

I hypothesized that:

1. >50% of patients with OSSN have HIV and

2. At least 90% of HIV patients with OSSN have a CD_4 count <350 cells/µl.

It was unfortunate that the number of cases recruited in the given time fell short of the ideal number required as outlined in my protocol, however a minimum of 3 controls per case (as opposed to one) all matched for age and gender were selected from the national data base in order to improve the statistical significance and proceed with the study.

I had hoped that the results from this study could be used to include OSSN as a CDC case defining condition for AIDS but unfortunately, although illustrative of the possibility, larger studies will have to be conducted to prove this. I was however, able to achieve the listed aims of the study and prove both of the hypotheses. It is hoped that in doing so, the holistic management of these patients with OSSN will be improved with their referral for HIV and AIDS screening being streamlined and emphasized.

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Part One: Introduction

Ocular surface squamous cell neoplasia (OSSN) is the most frequently encountered conjunctival malignancy[1] and occurs more frequently in the tropics than in temperate regions[1]. Although the exact cause for OSSN is unknown, it has been found to be associated with increased ultraviolet exposure, Human papilloma virus (HPV) infection, Immunodeficiency syndrome, cigarette smoking, petroleum products, Caucasian decent and long standing inflammation. In fact it has been documented that patients with Acquired Immunodeficiency Syndrome (AIDS) have a 13 fold increased risk of developing conjunctival epithelial malignancies and present at a younger age[2].

With the association of ocular surface squamous neoplasia and human immunodeficiency virus (HIV), the high prevalence of HIV and the growing incidence of OSSN in HIV patients, it has become difficult to ignore the role HIV infection may play in the morbidity and mortality of patients with this tumour.

Although most studies involving OSSN and HIV have been conducted in Africa an extensive literature search has found few studies looking specifically at Cluster of Differentiation 4: a glycoprotein found on the surface of immune cells (CD_4) counts in these patients, with OSSN not included in the Expanded Case Definition for AIDS[3].

Background Epidemiology

Since the advent of the HIV/AIDS epidemic in Sub Saharan Africa, the epidemiology of the disease appears to have changed dramatically with OSSN more often being diagnosed in younger women[4]. In one retrospective study examining clinical modalities for OSSN in Gaborone, Botswana from 1998-2008, it was found that the estimated annual incidence of OSSN in Botswana had risen from 0.13 cases per 100 000 (1.3 per 1 000 000) in 1993 to a peak of 7.6 per 100 000 (76 per 1 000 000) in 2004[5]. Even though this was only an estimated incidence rate for OSSN, it is considered high for Sub Saharan Africa. Other countries in the region showed an incidence of 2.2 per 100 000 (22 per 1 000 000) persons in Tanzania and 2.1 per 100 000 (21 per 1 000 000) in Uganda[5]. These differences in OSSN prevalence may mirror differences in HIV prevalence, with Botswana having an estimated adult HIV prevalence of 23.0, Tanzania 5.1 and Uganda 7.2[5].

Historically, the average incidence of OSSN of conjunctiva and cornea was estimated to be 0.13/100 000 (1.3 per 1 000 000) in tribal groups in Uganda by Templeton in a study published in 1967, and 1.9/100 000 (19 per 1 000 000) population per year in the Brisbane metropolitan area of Australia by Lee in 1992[6]. Sun and co-workers reported an average incidence of 0.3 million per year in the U.S in 1997[6]. Most recently in a study published in 2012, an incidence of 37.3 per 10 000 000 (0.37 per 1 000 000) was reported for all eye cancers and 8.4 per 10 000 000 (0.84 per 1 000 000) for Squamous cell carcinoma (SCC)[6]. This was predominantly a tumour of

adults with the average reported age being 56 years but cases have ranged from 4-96 years. Younger age of presentation of OSSN is seen among patients suffering from Xeroderma Pigmentosum and HIV infection[5].

In Africa there is a strong association between HIV and OSSN with the mean age of presentation for invasive OSSN from 32-37 years and the proportion of female patients ranges from 55-70%[5]. Several studies have reported OSSN as the first clinical presentation of HIV in young patients and these tumours occurring in HIV patients are more aggressive and invasive, requiring enucleation or even exenteration[6], however minimal mention of CD_4 counts were found in these studies. A retrospective cohort study conducted in Botswana from 1998-2008 only 180 (38.5%) HIV positive patients had CD_4 counts available during the study period 85 (47.2%) had CD_4 counts <200 and 152 (84.4%) had CD_4 counts <400 cells/μL[5]. Of the 85 patients with CD_4 counts <200 cells/μL at any time during the study period, 17 (20%) were on high dose antiretroviral therapy (HAART) prior to OSSN surgery, 13 (15.3%) were initiated on HAART within one year after surgery, 9 (10.6%) were initiated on HAART later in the study period and 46 (54.1%) had no evidence of HAART initiation during the study period[5]. In another study of the review of literature in Sub Saharan Africa and published in Current Opinion 2010, it was found that the mean CD_4 count level was significantly lower among patients with OSSN as compared to patients with other ocular pathologies[7].

With documented increasing incidence of OSSN in Sub Saharan Africa and the association with HIV as well as increased morbidity of these patients, I believe that the accessibility and initiation of HAART in this region may need further addressing.

Treatment Guidelines

HIV: - Currently, the Antiretroviral Treatment Guidelines for 2014 published by the South African HIV Clinicians Society in the 2014 December issue of the SAJHIVMED, states that all patients with CD_4 counts <350 cells/μL should be advised and encouraged to start ART without delay[7]. If CD_4 counts are between 350-500 cells/μL ART is recommended if the patient is ready and motivated to start[7]. ART is deferred if CD_4 >500 cells/μL. ART is also recommended in WHO clinical stage 3 and 4 (see Appendix E and F), other severe HIV related disorders for example immune thrombocytopenia, thrombotic thrombocytopenic purpura, polymyositis and lymphocytic interstitial pneumonitis as well as non-HIV related disorders for example malignancies (excluding local malignancies), Hepatitis B co-infection, Hepatitis C co-infection. Having an HIV infected partner in a serodiscordant relationship is also important and ART is recommended in these patients regardless of CD_4 count or clinical diagnosis. (See Figure 3). It is important to note that malignancies are listed under non-HIV related disorders and localized malignancies are excluded in the criteria for commencement of ART in South Africa despite evidence presented in a number of papers from Sub Saharan Africa that OSSN, a locally invasive malignancy, demonstrates a high morbidity in patients with HIV and is related to HIV infection and lower CD_4 counts.

To combat a potential social disaster in high burden countries, calls were made in 2003 to start the formidable task of getting ARVs to people who needed them[8]. Research conducted by Uebel et al stated that by 2006 it was clear that universal access to antiretrovirals (ARVs) in

South Africa would take more than five years to achieve[8]. Currently 54% of all people living with HIV in the region of East and Southern Africa are accessing ARVs[8]. Although ARV roll-out is on the increase in South Africa it is still inadequate for the burden of the disease.

OSSN: - Treatment of OSSN includes surgical excision alone, with or without additional adjunctive therapy. Resulting surface defects may need reconstruction with amniotic membrane transplantation, limbal stem cell grafts or skin grafts. Adjunctive therapies include cryotherapy, chemotherapy, radiotherapy, immune therapy, and amniotic membrane transplants to kill residual malignant cells at the excision margins or any that may have been seeded during excision and there are also reports of these being used as primary therapy[15]. In a Cochrane review by Gichuhi, quoting Shields et al it was found that advanced disease may require enucleation or exenteration to save a life[9].

Recurrence rates may be higher in Africa due to late presentation, exposure to solar radiation, and lack of many of the adjunctive therapies[9]. It is not clear whether the efficacy of these interventions is different in people with HIV infection or what the effects of HAART are on OSSN[9]. No randomised control trials of interventions currently used against conjunctival squamous cell carcinoma in HIV-infected individuals were identified[9].

Problem Statement and Aims

Mittal et al reported in a study published by the Saudi Journal of Ophthalmology in 2013 that, in Africa, OSSN has been strongly associated with HIV[6]. HIV infection is now established as a risk factor for the development of squamous cell neoplasia of the conjunctiva based on studies from Rwanda, Malawi and Uganda[6] . A significant increase in the incidence of OSSN is also reported in patients with HIV/AIDS in the United States[6]. Thee are several studies that have reported OSSN as the first clinical presentation of HIV in young patients [6]. But an extensive literature search found no study specifically looking at CD_4 counts in these patients. OSSN occurring in these patients are more aggressive and invasive requiring enucleation or even exenteration [6] thus showing significant morbidity and mortality in these patients. With the above problems highlighted in the literature, the aims of this two part study were to determine what percentage of patients with OSSN have HIV infection in our setting and to determine the odds ratio of HIV seropositivity with OSSN (from the cases), and patients without ocular surface squamous neoplasia (from the controls) specifically for CD_4 counts <200 cells/μL, 200-350 cells/μL and >350 cells/μL.

Research Question and Purpose

This study was to determine the prevalence of HIV in patients with OSSN in our setting and whether HIV positive patients are more likely to have a low CD_4 count if they have OSSN, with the hypothesis, based on clinical experience, that more than 50% of patients with OSSN have HIV and at least 90% of HIV patients with OSSN have a CD_4 count <350 cells/μL hence proposing that OSSN could be used as an Expanded Case Definition for AIDS.

In 1981, the centres for Disease Control in the United States Department of Health and Human Services began surveillance for a newly recognized constellation of diseases, now termed Acquired Immunodeficiency Syndrome[10]. CDC developed a surveillance case definition for this syndrome in 1982 and received case reports directly from health care providers and state and local health departments[10] with the goals of AIDS surveillance to monitor trends in the number of AIDS cases and monitor the scope of severe morbidity due to infection with Human Immunodeficiency Virus[10]. For example: - From 1980-1981 the CDC received its first reports of 5 cases involving homosexual males with Pneumocystis Carinii Pneumonia (PCP) due to severe immunodeficiency and by 1983, 51% of cases reported PCP without Kaposis sarcoma[10]. PCP was hence included as an opportunistic illness associated with this syndrome and remains a condition in the CDC's expanded case definition for AIDS.

The CDC case definition for AIDS was expanded in 1993[3]. It now includes all HIV infected persons who have a CD_4 count of less that 200 cells/μL, or a recurrent pneumonia and invasive cervical cancer[3] and retains the 23 clinical conditions in the AIDS surveillance case definition published in 1987. The objectives of these changes are to simplify the classification of HIV infection, to reflect current standards of medical care for HIV infected persons, and to categorize more accurately, HIV related morbidity[3], thus more accurately reflect the number of persons with severe HIV related immunosuppression and those at higher risk for severe HIV related morbidity[3].

According to the Adult Antiretroviral Therapy Guidelines 2014, published by the South African HIV clinician's society, all patients with a CD_4 count <350 cells/μL should be advised and encouraged to start ART without delay. There is clinical evidence that this reduces mortality: a randomised trial in Haiti demonstrated reduced mortality and incident tuberculosis in patients starting ART at a CD_4 count threshold of <350 cells/μL compared with patients waiting to commence therapy at a threshold of <200 cells/μL[7].

Currently OSSN is not included in the CDC expanded case definition for AIDS [See supplementary file Figure 4] and neither is it included in the guidelines for commencement of ART [See supplementary file Figure 3]. Despite this study not being powered to prove inclusion of OSSN in the expanded case definition for AIDS, it may be warranted that further, larger studies be looked at in this regard.

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Part Two: Submission Ready Manuscript

HUMAN IMMUNODEFICIENCY VIRUS AND CD_4 COUNT IN OCULAR SURFACE SQUAMOUS NEOPLASIA

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Background
Few studies have been done specifically looking at CD_4 counts in patients with OSSN. Local clinical experience has suggested that there may in fact be a close relationship with OSSN and CD_4 counts as more patients are seemingly presenting to our eye clinics with lower CD_4 counts if they have this tumour as opposed to their OSSN naïve counterparts.

Objectives
The aims of this study were
1. To determine the prevalence of HIV in patients with OSSN.
2. To determine whether HIV positive patients are more likely to have a low CD_4 count if they have OSSN**,** with the objective of including OSSN as a CDC case defining condition.

Method
This study was conducted in two parts. First a prospective descriptive study to determine what percentage of patients with OSSN have HIV, and secondly a case control study to determine the odds ratio of HIV patients with OSSN and patients without OSSN for CD_4 counts in the ranges of <200, 200 – 350 and >350 cells/µL. Statistical planning advised 105 cases and 210 controls.

Results
Of the 13 cases collected, 100% were found to be HIV positive confirming the initial study hypothesis. 46% of cases were found to have a CD_4 count <200 cells/µL, 46% a CD_4 count between 200 – 350 cells/µL and 8% a CD_4 count >350 cells/µL. Analysis of 6 000 controls showed an inverse relationship with the CD_4 count – where a higher percentage of patients without OSSN had CD_4 counts above 350 cells/µL at 56% compared to the 8% of cases with OSSN and 44% of patients without OSSN had CD_4 counts <350 cells/µL compared to 92% of patients with tumour.

Conclusion
Although a small study sample, this study highlights the association OSSN has with HIV and specifically with lower CD_4 counts and the need for prompt antiretroviral treatment in these patients due to the high morbidity and mortality of these patients in the setting of HIV seropositivity. Larger studies are however recommended to ascertain if OSSN can indeed be used as a criterion in the CDC expanded case definition for AIDS.

Introduction

Despite a number of studies regarding Ocular Surface Squamous Neoplasia (OSSN) and Human Immunodeficiency Virus (HIV) coming from Central and Sub Saharan Africa, few studies have specifically looked at Cluster of Differentiation 4: a glycoprotein found on the surface of immune cells (CD_4) counts in these patients. This gap in literature, along with the clinical question of the apparent relationship of OSSN with HIV and CD_4 count is what prompted this study.

Background of Ocular Surface Squamous Neoplasia (OSSN)

Clinically: - Squamous cell carcinoma of the conjunctiva is a rare, slow-growing tumour of the eye, normally affecting elderly men around 70 years of age. In Africa, however, the disease is different. The incidence is rising rapidly, affecting young persons (around 35 years of age), and usually affecting women. It is more aggressive with a mean history of three months at presentation. This pattern is related to the coexistence of the Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) pandemic, high Human Papilloma Virus (HPV) exposure, and solar radiation in the region. Various interventions exist, but despite therapy, there is a high recurrence rate (up to 43%) and poor cosmetic results in late disease[1].

OSSN encompasses a spectrum of precancerous and cancerous lesions of the conjunctiva, from conjunctival intraepithelial neoplasia (CIN) to frankly invasive tumour with disfiguring orbital destruction and intracranial invasion although distant metastases are rare[2]. Patients usually present with redness, irritation, visual impairment, a mass of increasing size in the affected eye or sometimes may even be asymptomatic with the finding being incidental. Currently OSSN is the most common indication for exenteration performed in adults in Africa[3].

The tumour usually appears as a nodular or gelatinous plaque on the nasal corneal/conjunctival surface and maybe pigmented or not, often has a leukoplakic surface and prominent conjunctival feeder vessels. Most lesions are unilateral and can be mistaken for benign lesions for example, pterygium, pinguecula, or squamous papilloma. As mentioned progression of ocular surface squamous neoplasia is usually over weeks and months and is historically a slow growing tumour of elderly men living in areas of high ambient sunlight[4].

Other risk factors known to be associated with ocular surface squamous neoplasia are infection with HPV types 16 and 18, smoking and HIV infection, ultra violet radiation exposure and other causes of immunosuppression.

Epidemiologically: - An increase in the incidence of ocular surface squamous neoplasia since the HIV pandemic, has suggested that HIV infection increases the risk of OSSN[2].

Figure 1: Image of the anterior ocular surface showing nodular gelatinous OSSN with large feeder vessels and some pigmentation. (Reproduced from eyworld.org)

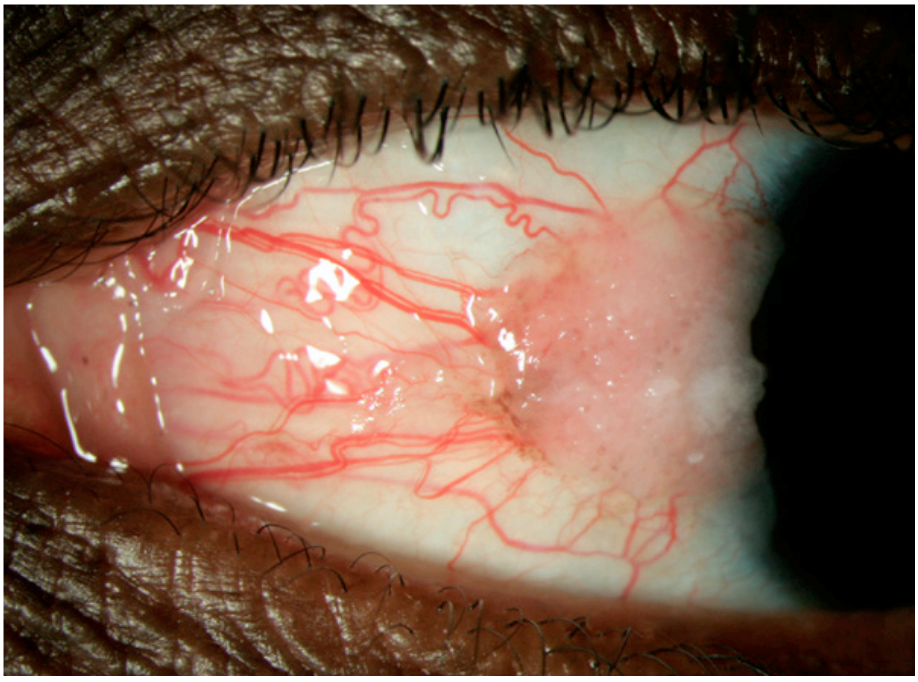
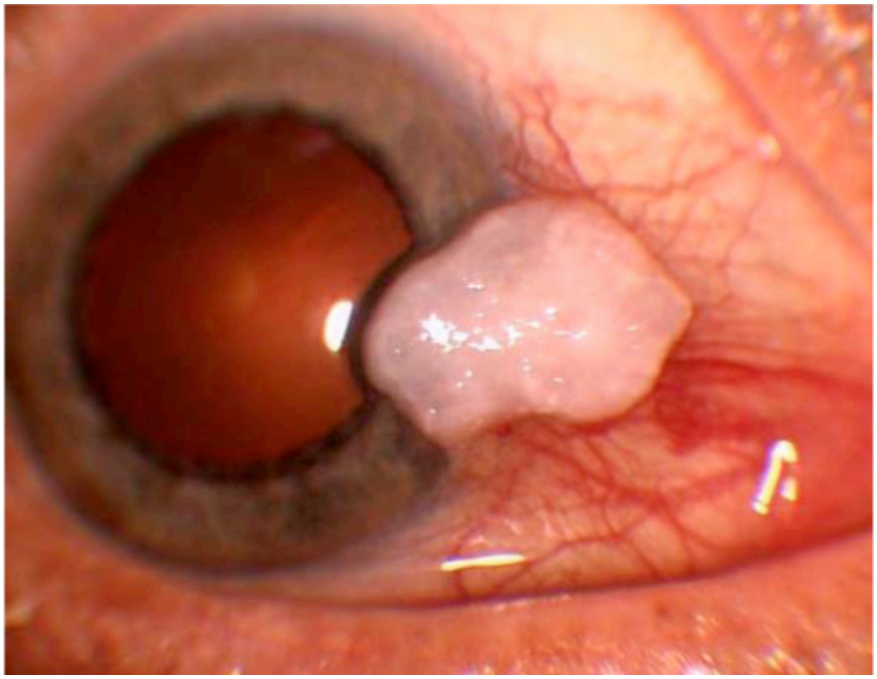


Figure 2: Image of the anterior ocular surface showing leukoplakic surface of OSSN (Reproduced from cdn.intechweb.org)



Background of Human Immunodeficiency Virus (HIV)

Clinically: - HIV Seroconversion presents as a flulike illness, consisting of fever, malaise and sometimes a generalized rash. Patients may also be asymptomatic. Generalized lymphadenopathy is common and may also be a presenting symptom. AIDS manifests with recurrent, severe and occasionally life threatening illnesses and/or opportunistic malignancies. The Human Immunodeficiency Virus itself does cause some sequelae, including AIDS-associated dementia/encephalopathy and HIV wasting syndrome with chronic diarrhoea and weight loss[5].

This disease is spread in a number of identifiable ways, for example through unprotected intercourse with an infected partner, sharing intravenous drug paraphernalia, receipt of infected blood products, mucosal contact with infected blood or needle stick injuries, and maternal HIV infection which may cause infection in the new-born, infant or child if precautions are not taken[5].

Epidemiologically: - Although HIV and AIDS has become a global pandemic and global statistics as of December 2015 show an average of 36.7 million people living with HIV globally, 19 million of these were living in Eastern and Southern Africa. (See Appendix F and G). In Eastern and Southern Africa, 10.3 million were accessing antiretroviral therapy, 54% (50-58) of all people living with HIV in the region (see Appendix F). HIV over the course of 35 years has infected 78 million people and killed 39 million people[6]. At present, a cure for this disease is still being sought[6]. The current Adult Antiretroviral Treatment Guidelines (2014) by the South African HIV clinicians Society are as follows[7]: -

Figure 3: ART Recommendations by the South African HIV clinicians society[7]

Clinical diagnosis (irrespective of CD4⁺ count)	
WHO clinical stage 3 and 4†	ART recommended
Other severe HIV-related disorders, e.g.:‡ • immune thrombocytopenia • thrombotic thrombocytopenic purpura • polymyositis • lymphocytic interstitial pneumonitis	ART recommended
Non HIV-related disorders:§ • malignancies (excluding localised malignancies) • hepatitis B co-infection¶ • hepatitis C co-infection	ART recommended
Any condition requiring long-term immunosuppressive therapy	ART recommended
CD4⁺ counts	
<350 cells/μL	ART recommended
350 - 500 cells/μL (two counts in this range)	ART recommended if patient is ready and motivated to start
>500 cells/μL	Defer ART
HIV-infected partner in serodiscordant relationship	
Regardless of CD4⁺ count or clinical diagnoses	Offer ART and discuss safe sex (discussion should ideally involve all partners)

ART = antiretroviral therapy; WHO = World Health Organization.

*Note that EITHER listed clinical diagnoses OR CD4⁺ strata would be an indication for ART.

Expanded surveillance case definition for Acquired immunodeficiency syndrome (AIDS)

The definition for AIDS includes all HIV-infected individuals with CD_4 counts of <200 cells/µL (or CD_4 percentage <14%) as well as those with specific HIV related conditions and symptoms[8]

Figure 4: Clinical categories for HIV/AIDS[8]

CD4 Cell Count Categories	Clinical Categories		
	A Asymptomatic, Acute HIV, or PGL	B* Symptomatic Conditions, not A or C	C# AIDS-Indicator Conditions
(1) ≥500 cells/ µL	A1	B1	C1
(2) 200-499 cells/µL	A2	B2	C2
(3) <200 cells/ µL	A3	B3	C3
Abbreviations: PGL = persistent generalized lymphadenopathy			

Category B: Symptomatic conditions for adolescents or adults must meet one of the following criteria:-

1. Must be attributed to HIV or indicate a defect in cell mediated immunity.
2. Must be considered to have a clinical course or management that is complicated by HIV infection.

Examples include, but are not limited to, the following: - Bacillary angiomatosis, oropharyngeal candidiasis (thrush), vulvovaginal candidiasis - persistent or resistant, pelvic inflammatory disease (PID), cervical dysplasia (moderate or severe)/cervical carcinoma in situ, hairy leukoplakia-oral, herpes zoster (shingles) - involving two or more episodes or at least one dermatome, idiopathic thrombocytopenic purpura, constitutional symptoms - fever (>38.5^{o}C) OR diarrhoea lasting >1 month, peripheral neuropathy[9]

Category C: AIDS-indicator conditions

Bacterial pneumonia, recurrent (two or more episodes in 12 months)
Candidiasis of the bronchi, trachea, or lungs
Candidiasis, oesophageal
Cervical carcinoma, invasive, confirmed by biopsy
Coccidiodomycosis, disseminated or extra pulmonary
Cryptococcosis, extra pulmonary
Cryptosporidiosis, chronic intestinal (>1 month in duration)
Cytomegalovirus disease (other than liver, spleen or nodes)
Encephalopathy, HIV related
Herpes simplex: chronic ulcers (>1 month in duration), or bronchitis, pneumonia, or esophagitis
Histoplasmosis, disseminated or extra pulmonary
Isosporiasis, chronic intestinal (>1 month in duration)
Kaposi sarcoma
Lymphoma, Burkett, immunoblastic, or primary central nervous system
Mycobacterium avium complex (MAC) or *Mycobacterium kansasii*, disseminated or extra pulmonary
Mycobacterium tuberculosis, pulmonary or extra pulmonary
Mycobacterium, other species or unidentified species, disseminated or extra pulmonary
Pneumocystis jiroveci (formerly *carinii)* pneumonia (PCP)
Progressive multifocal leukoencephalopathy (PML)
Salmonella septicaemia, recurrent (non-typhoid)
Toxoplasmosis of brain
Wasting syndrome caused by HIV (involuntary weight loss >10% of baseline body weight) associated with either chronic diarrhoea (two or more loose stools per day for >/= 1 month) or chronic weakness and documented fever for >/= 1 month

Methods

Study design

This research was conducted in two parts.
PART 1: A prospective descriptive study with cases collected from September 2012 to December 2014
PART 2: A case control study with cases recruited from part 1 and controls from the National Health Laboratory Services (NHLS).

Study Procedure

Inclusion criteria

For Part 1, only new, adult patients from the eye clinics at approved sites with biopsy proven OSSN (ocular surface squamous neoplasia) and who consented to participation were to be included. Only the HIV positive portion of this group would serve as cases for the second part of the study.

For Part 2, controls would be sourced from the national health laboratories database and matched for gender and age within 5 years of current age. Controls were to be tumour free and sourced from September 2012-2014. Patients from eye clinics and eye wards were excluded so as to negate accidental inclusion of patients with OSSN in the control group as the National Health Laboratories database did not have records of primary or ancilliary diagnoses for all patients. All patients that satisfied the criteria were entered into the study. All patients would be HIV positive and CD_4 counts would be looked at.

Data collection

After statistical review of the recent literature during the protocol phase of the project, it was elected that the study should aim for 105 cases from part 1 and 210 controls in part 2, to be statistically significant based on the global incidence of OSSN. During part 2 of the study, patient results were sourced with the aid of convenient sampling, from the NHLS and matched for gender and age. Due to the enormity of the database, 6 000 controls were easily acquired for analysis.

Study Population

Only OSSN treatment naïve, adult patients from approved sites, namely St Aidan's, Addington, Edendale and Greys Hospitals in KwaZulu-Natal were used.

Ethical considerations

Data Management

To maintain confidentiality during Part 1 of the study, a study questionnaire with a random study number was allocated to each patient at the time of data collection or admission for the recording of the required data. Only the attending doctor and myself as the principal investigator had access to the file and patient number that matched the study number. Anonymity was maintained during Part 2 of the study by using age within five years and gender. Patients from the eye clinic or eye ward were excluded and no names or other identifiers were used.

Consent

Patient consents for participation in the research and procedure of surgical tumour excision as well as the drawing of bloods was obtained prior to inclusion in the study. With the help of Ms Mary Gordon from the University of Kwazulu-Natal Humanities Department, consent forms were translated into isiZulu for those patients whose mother-tongue was isiZulu. Consent for Part 2 was obtained from Dr B. Malope-Kgokong, the manager for the academic research corporate office to access the NHLS database for acquisition of the required controls. (See Appendix E)

Biomedical Research Ethics Committee (BREC) approval

Ethical approval was obtained from The Biomedical Research Ethics Committee of the University of KwaZulu-Natal. The ethics approval number allocated to the study was BE057/13. (See Appendix C)

Data Analysis

Data was recorded in Microsoft Excel and entered into Statistical Packages for the Social Sciences (SPSS) version 24. A p-value <0.05 was considered statistically significant. Descriptive statistical analysis of the data (means, standard deviations, ranges, frequencies and percentages) was initially conducted prior to conducting interferential statistics. A logistical regression was run to determine the odds ratio of lower CD_4 counts in HIV patients with OSSN and patients without OSSN.

Results

Overview of Results

One of the aims of the study was to determine the prevalence of HIV in OSSN in our target population from September 2012 to December 2014 and although a smaller than required sample size was recruited due to poor data collection at the various approved hopsital sites with patients having to be excluded due to missing data, the following was found: - Of the 13 cases from Part 1 of the study, 8 were female and 5 were male. 100% were HIV positive. See Table 1 below

Table 1: An overview of results showing cases and controls

	Male	Female	With OSSN	Without OSSN
Number	5	8	**13**	***6 030***
Tumour	100%	100%	**100%**	***0%***
HIV+	100%	100%	**100%**	***100%***
HIV-	0%	0%	**0%**	***0%***

Analysis of CD_4 counts

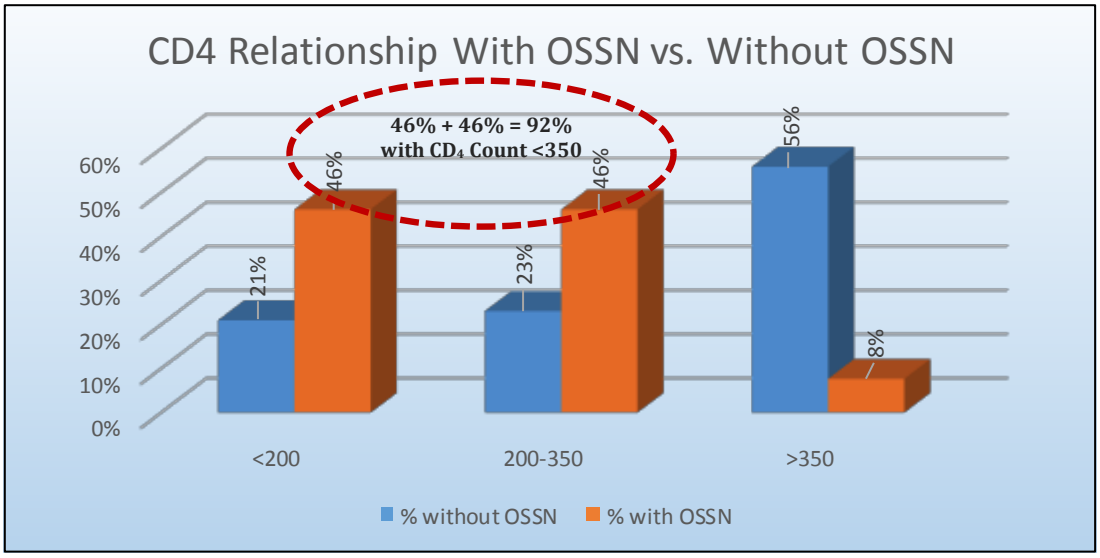
CD_4 count categorization for HAART

It was found that more patients (56%) had CD_4 counts >350 cells/µL if they did not have OSSN with just 8% of patients with OSSN (cases) having a CD_4 count >350 cells/µL. 46% of patients with OSSN had a CD_4 count of between 200 – 350 cells/µL and 23% were without OSSN in this CD_4 count range, with 46% of patients with OSSN also having a CD_4 count of <200 cells/µL but just 21% of patients without OSSN in this range. See Table 2 below

Table 2: Analysis of CD_4 count for HAART implementation in cases and controls

Variable CD4 Count	<200	200-350	>350	Total
Count	1 292	1 421	3 443	6 156
% without OSSN	21%	23%	56%	100%
Count	6	6	1	13
% with OSSN	46%	46%	8%	100%
Count	1 298	1 427	3 444	6 169
Total Sample	21,0%	23,1%	55,8%	100%

Figure 5: Graph of CD_4 counts relationship with OSSN versus without OSSN for the HAART sub-categories



A consolidated analysis of the CD_4 count results showed that 92% of patients with OSSN had a CD_4 count <350 cells/µL and only 44% of those without OSSN had CD_4 counts <350 cells/µL. This supports the second aim of the study in determining whether patients who are HIV positive are more likely to have a lower CD_4 count if they have OSSN. It was also found that 92% of patients had a CD4 count <350 cells/µL which is in support of the second hypoyhesis although it is to be noted that the sample size was smaller than required. See Figure 5 above.

Logistic Regression

The odds analysis of the data using cases against controls in the required CD4 count ranges found that patients with OSSN were 15.9 times more likely to have CD_4 counts less than 200 cells/µL than CD_4 counts greater 350 cells/µL, and 14.5 times more likely to have CD_4 counts in the range of 200-350 cells/µL than CD_4 counts greater 350 cells/µL. This was found to be statistically significant with a greater than 95% confidence index. See Table 3 below.

Table 3: Logistic regression to determine odds ratio as outlined in the protocol

VARIABLE CD COUNT	ODDS RATIO	P VALUE	95% CI
<200	15.989	0.010	1.923-132,938
200-350	14.583	0.013	1.749-120,862
>350	REF(1)		

CD_4 Count categorization for CDC expanded surveillance case definition for AIDS

I have included the CD_4 count categorization in HIV/AIDS. As previously mentioned AIDS is defined as a CD_4 count <200 or the presence of certain category B or C AIDS defining conditions. It is interesting to note that 46% of patients with OSSN had a CD_4 count <200 cells/µL compared to just 21% of those without tumour. See Table 4 below

Table 4: Analysis of CD_4 counts in the CDC expanded case definition for AIDS CD_4 categories for cases and controls

CD4 Count	With OSSN	Without OSSN
CD4: <200	**46%**	***21%***
CD4: 200-499	**54%**	***45%***
CD4: >/= 500	**0%**	***34%***

Discussion

The Centre for Disease Control (CDC) Expanded Surveillance case definition for HIV/AIDS, since the advent of the disease in the United States in the early 1980s, has been revised several times to respond to diagnostic advances. Both the laboratory and the clinical criteria have evolved to include HIV "negative" individuals who may be in the window period and additional AIDS defining conditions for example: - invasive cervical cancer, recurrent pneumonia and pulmonary *Mycobacterium Tuberculosis* infection. The definition of AIDS includes all HIV infected individuals with CD_4 counts <200 cells/μL (or CD_4 percentage <14%) as well as those with certain HIV-related conditions and symptoms[8].

Symptomatic conditions occur in the HIV infected adolescent or adult and should either be attributed to
1. The HIV infection or indicate a defect in cell mediated immunity, or
2. Have a clinical course or management that is complicated by HIV infection

In this study it was found that 46% of patients had a CD_4 count <200 cells/μL. This aligns with other studies conducted in the region. For example, in a study conducted in sub Saharan Africa, the median CD_4 count within one year of OSSN surgery was 192 cells/μL (IQR 122-288) and more than half of the OSSN patients had CD_4 counts <200 cells/μL, consistent with a diagnosis of AIDS[10]. A recent study from Tanzania reported that 85% of OSSN patients had CD_4 counts <200 cells/μL and the median CD_4 count of patients at the time of presentation was 71 cells/μL[10]. Similarly in Uganda, the median CD_4 count among HIV-infected OSSN patients was 111 cells/μL (IQR 62-221) and 65% of HIV-infected OSSN patients died of AIDS related complications at a median of 20 months after OSSN diagnosis[10]. Although these studies allude to the fact that there is a linear relationship between CD_4 count and OSSN, these could be incidental findings. The association with HIV suggests that immunosuppression plays a role in OSSN; however, a linear association between CD_4 lymphocyte count and OSSN has not been confirmed[11].

Although this study did not look at the grades of OSSN as well as the course and outcome for patients with CD_4 counts in the different categories, it was a general observation that those patients with more invasive OSSN and requiring more radical ophthalmic surgery for the removal of tumour were indeed more ill in terms of their HIV progression. It has been shown in a review article published in Current Opinion Oncology 2010, September that data from Uganda, in which the recurrence rate after a median follow up of 32 months (range 0-81) was 3.2%[4]. 64% of these patients were HIV seropositive. It was also stated in the same paper that OSSN appears to be more aggressive in the setting of HIV disease. In another study conducted in Malawi it was found that OSSN was indeed the first presenting symptom for HIV in the majority of the study patients[9]. It does however remain a fact that an extensive literature search, to my knowledge, has found no study specifically looking at CD_4 counts in OSSN with morbidity and mortality in these patients and it is hence a recommendation that this warrants more investigation.

It is a limitation of my study that OSSN is in fact a rare tumour as far as ocular tumours go but with a 100% of my study cases being HIV positive, and 92% having a CD_4 count <350 cells/μL,

there have been other papers with evidence to support the association of HIV with OSSN thus supporting the need for a holistic approach to the management of these patients. In fact, in one study that may be regarded as supportive to mine, conducted in Harare, Zimbabwe, it was clearly stated in its recommendations that OSSN is an HIV/AIDS defining tumour in regions experiencing an HIV/AIDS epidemic[12]. Patients with OSSN fit in the WHO stage 3 and 4 of HIV/AIDS disease and can be started on antiretroviral therapy without a CD_4 count if the test is not readily available[12].

Although the CDC expanded surveillance case definition for AIDS is specific in its criteria and currently does not include OSSN as either a category B-symptomatic or category C-clinical condition[7], evidence presented from papers originating mostly in Sub Saharan Africa, suggest that OSSN be further investigated for inclusion in the CDC Expanded Surveillance Case definition for AIDS.

Squamaous cell carcinoma of the conjunctiva has been shown to be a marker of HIV seropositivity or AIDS[13] and it is hoped that more comprehensive studies be conducted to thoroughly investigate the relationship of OSSN and CD_4 counts in HIV positive patients with a view to reduce morbidity and mortality in this disease.

Conclusion

This study, despite small sample size, found that more than 50% of patients with OSSN will be HIV positive and more than 90% of HIV positive patients with OSSN having CD_4 counts <350 cells/μL. Significant knowledge gaps have been highlighted with regards to OSSN and CD_4 counts in HIV positive patients with no published studies looking at these vaiables. The current literature reitterates the asociation of OSSN with HIV with some studies proving OSSN as the initial presenting feature for HIV/AIDS. Escalation of the morbidity of OSSN on the background of HIV, highlights the importance of this tumour in ophthalmology.

References

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APPENDICES

Appendix A - The Protocol

APPLICATION FOR CONDUCT OF SELF INITIATED RESEARCH STUDY

RESEARCH STUDY FOR HIGHER DEGREES ✓ **MMED** ✓

SECTION 1: ADMINISTRATIVE DETAILS

NAME:	Dr Thenushka Jogi
NAME: Co-investigator	N/A
Professional status (if student, year of study)	Medical Doctor – Registrar year 3 in 2013
UKZN Department	Ophthalmology
Hospital / Institution where employed	Grey's Hospital
Full Postal address	14 Calendula Crescent, Buffelsdale, Tongaat, 4399
Contact telephone and fax numbers	+27 83 3059893
Email Address	thenushkajogi@yahoo.com
Full time/part time employment	Full Time
Current HPCSA Number (or equivalent statutory health council registration no. as appropriate) – if	MP0588636

registration is pending, submit proof of application.	

1.1 TITLE OF PROJECT in full:
Human immunodeficiency virus and CD_4 count in ocular surface squamous neoplasia

1.2 WHERE WILL THE RESEARCH BE CARRIED OUT? *(Interaction with participants)*

(Please furnish the name of hospital/institution and department.

Departments of Ophthalmology at Addington + St Aidan's + Grey's + Edendale Hospitals

1.3 PURPOSE OF RESEARCH:

To determine whether **ocular surface squamous neoplasia (OSSN)** could be included in the WHO Expanded Surveillance Case Definition for AIDS

Postgraduate Degree: ***Masters***

1.4 STUDENT: YES STUDENT NO: 982176913

1.5 PRINCIPAL INVESTIGATOR/CO-INVESTIGATOR/S (state exact role/s in the study):

Name/Dept	Role	Signature
Thenushka Jogi	Principal Investigator	

1.6 FUNDING

Has funding been secured? **No**

Can this project proceed without funding? **Yes**

Give a brief explanation: **All procedures done will form part of normal clinical work**

Please note that any contractual undertaking that involves the Faculty of Health Sciences must be processed through KwaZulu-Natal University Health (Pty) Ltd – contact Mr. T Govender, Tel: 033-260 4476 - e-mail govenderreg@ukzn.ac.za

Contracts from other Faculties should be routed through Mrs D Latchmanan, Research Office – Tel: 033-260 2333 – latchmanand1@ukzn.ac.za

SECTION 2: DISCLOSURES

1. Has this study been, or is it likely to be, submitted to any other ethics review committee? ***No***
2. Have you been previously/are you presently being investigated in regard to alleged misconduct relating to research-related activities? ***No***
3. Are any of your intended research participants in other research studies and/or trials? ***No***
4. Are you presently involved in other research and/or clinical trial activities? ***No***
5. ***No*** tissues are to be stored
6. ***No*** tissues are to be exported
7. Conflict of Interest:

   Investigators should have no undisclosed conflict of interest with their study collaborators, sponsors or participants. Conflicts can arise, for example, when a commercial or other sponsor may not wish research results detrimental to their corporate image / interest to be disclosed, especially when the investigator is being remunerated by the sponsor for the research in question; when research subjects are being rewarded for their participation in the research; or when an investigator has a vested interest in, or is an employee / shareholder / director in the sponsor's corporate entity. Investigators should note that the duty to disclose a conflict of interest to the ethics review committee begins during application for ethical approval and continues until the research in question is complete and the research results are submitted to the sponsor / published (if applicable).

   If the investigator(s) has / have / foresees any such conflict of interest, please provide details here: ***None foreseen***

SECTION 3: THE PROTOCOL

Type of study: The research will be conducted in 2 parts

PART 1 : PROSPECTIVE DESCRIPTIVE STUDY

PART 2 : CASE CONTROL STUDY

3.1 THE PROJECT:

1. Aims:

   1) To determine the prevalence of HIV in patients with **ocular surface squamous neoplasia (OSSN)**.

2) To determine whether HIV positive patients are more likely to have a low CD_4 count if they have OSSN.

2. Hypothesis to be tested:

   1) More than 50% of patients with ocular surface squamous neoplasia (OSSN) have HIV.

   2) At least 90% of HIV patients with OSSN have a CD_4 count less than 350/µl.

3. Summary of the proposed research

This study will be conducted in two parts:

PART 1: A prospective descriptive study to determine what percentage of patients with OSSN have HIV.

PART 2: Case control study to determine the odds ratio of HIV patients with OSSN (from cases above) and patients without OSSN (controls) for:

CD_4 count less than 200/µl vs.

CD_4 count 200/µl to 350/µl vs.

CD_4 count more than 350/µl:

	CD_4 < 200/µL	**CD_4 200 - 350**	**CD_4 > 350/µL**
HIV positive *with* OSSN	46%	46%	8%
HIV positive *without* OSSN	21%	23%	56%

4. Background and Literature:

The world wide incidence of Conjunctival Squamous Cell Carcinoma as of November 2010 was found to be 0.03 – 3.5 per 100 000 people/yr. [1]

Although the exact cause for OSSN is unknown, it has been found to be associated with increased UV exposure, HPV, Immunodeficiency Syndrome, cigarette smoking, petroleum products, Caucasian decent and long-standing inflammation. In fact it has been documented that patients with AIDS have a 13 fold increased risk of developing conjunctival epithelial malignancies and present at a younger age. [1]

Most studies involving OSSN and HIV have been conducted in Africa. In 1 study of case-control design it was found that in 50% of the study population, conjunctival malignancy was the first presenting sign for AIDS[2]. In the same study it was found that seropositivity for HIV was significantly ($p < 0.01$) higher, 92.3% in patients with OSSN/CIS than in the control group with benign conjunctival lesions [2]

According to the UN Global Report for 2010, an estimated 33 300 000 adults + children were living with HIV in 2009. Of these 22 500 000 are estimated to be in Sub Saharan Africa. [1]

However, an extensive literature search has found no study looking at CD_4 counts in these patients. OSSN is also not included in the Expanded Surveillance Case Definition for AIDS [3].

5. Keywords:

conjunctiva; squamous cell; CD_4; HIV; AIDS; HPV; Expanded Surveillance Case Definition

6. Key References:

1. Kvitsinadze, L., D. Tvildiani, and G. Pkhakadze, *HIV/AIDS prevalence in the Southern Caucasus.* Georgian Med News, 2010(189): p. 26-36.

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3.2 PLAN OF INVESTIGATION:

(a) Design and/or experimental procedures:

Consulted: Dr C.H Kruse (supervisor)

Only new patients from the eye clinics with biopsy proven squamous neoplasia will be included as cases. The patients are to be counseled and consented on the study, to have an excision or incision biopsy of the lesion, as well as an HIV test, CD_4 count and questionnaire.

The surgery, treatment and follow up will be standard. The tissues taken will undergo histological testing.

In **Part 1** of the study the percentage of HIV positive patients of the OSSN cases will be determined. Only the HIV positive portion will serve as the cases for the second part of the study.

In **Part 2** of the study the cases (HIV + OSSN) will be compared to the controls (HIV without OSSN) with regards to CD_4 counts. Controls will be sourced from Virology and Haematology labs, IALCH, with selection from anonymous data using the national database – Johannesburg. Convenient sampling matched for age and gender will be used.

Confounders: The following factors will be assessed as possible confounders for the case-control study:

- Gender: Controls will be matched for gender
- Age: Matched within 5 years
- ARV use: Immunological staging of HIV/AIDS disease often reverses with successful Antiretroviral Therapy. This confounder is hence negated by categorizing patients according to their CD_4 counts(immunological status)
- Smoking: Although smoking is associated with OSSN, there is as yet no documented literature to prove that it is causative.
- Current or previous HPV infection: Although HPV 6,11,16 and 18 have been associated with OSSN at a prevalence of 33.8%, data suggests that HPV is not necessary for initiating the disease, although maybe a cofactor in a susceptible host, hence this should have little impact on the study results [4]
- Exposure to petroleum products: The expected number of patients that have been significantly exposed to petroleum products is expected to be very low and should also have minimal impact on the study results.

(b) Statistical Planning:

Has this project been discussed with a professional statistician? *Yes, Mr B Tlou*

Using the formula above where $n = \left(\frac{r+1}{r}\right)(\bar{p})(1-\bar{p})\frac{(Z_{\beta}+Z_{\alpha/2})^2}{(p_1-p_2)^2}$

n = is the sample size

r = ratio of controls to cases

z-beta is the desired power

z-alpha is the desired level of statistical significance

p cap is a measure of variability

(p1 – p2) is the difference in proportions

- For 80% power, Z_{β} =.84
- For 0.05 significance level, Z_{α} =1.96
- r=2(ratio of cases and controls)
- The proportion exposed in the control group is 50%

Then n = 315 ie 105 cases and 210 controls

(c) Participants:

Clinical data: Please indicate the source and age of the participants to be used:

Source: Eye Outpatients (all adults): Patients with biopsy proven OSSN

Will you have control groups? Yes. Matched for Age (within 5 yrs) and Gender

Inclusion:

- Patients with biopsy proven ocular surface squamous neoplasia (OSSN)
- Consent to surgical excision or incision biopsy
- Controls matched for age and gender

Exclusion:

- Previous treatment for OSSN (surgical or medical)
- Inability or refusal to give consent for the study.

(d) The Environment:

1. Is this a multi-national study? **No**

2. **List all sites in South Africa in which the project will be carried out.**

Grey's Hospital
Edendale Hospital
St Aidan's Hospital
Addington Hospital
Inkosi Albert Luthuli Hospital

3. Can the project have any negative consequences on the physical environment (incl. the laboratory), participants, researchers or members of the public? **No**

4. How many hours/week can be devoted to this project?

± 2 hours/week

3.3 ETHICAL ASPECTS:

(a) Responsibility

Ensuring that no patient names will be used in the study will optimize patient confidentiality. Only the doctor caring for the patient as well as the author shall have access to personal patient information.

Standard pre- and post-test counseling will be carried out prior to HIV testing.

(b) Incentives / Reimbursement

1. List any undue incentives, explicit and implicit, that have been offered to study participants, either to recruit or to remain within the study. **None**

2. List reimbursement / compensation for participation in the study (e.g. travel costs, out of pocket expenses, etc.). **None**

(c) Potential risk or discomfort:

Compared with participants with similar conditions indicate, for each study group, the potential additional

Risks – Patients in the study, and those who do not want to be in the study, will be medically and surgically treated identically.

Confidentiality: At the completion of the study hard copies of identifying study data will be destroyed and electronic data will be permanently removed or encrypted using 128 bit encryption.

Discomfort – Blood drawn.

(d) Health Service Utilisation:

Compared with participants with similar conditions indicate, for each study group, the likely additional:

Duration of hospital stay (days): 0

Outpatient attendances (number): 0

Laboratory services used: 3 (HIV, CD_4, FBC + differential)

Extent of nursing involvement: Translation, Drawing bloods

Have the nursing team who will be involved in the study been informed of the study and the nursing involvement that will be required? **Yes**

(e) Management:

In the case of participants drawn from patient populations, indicate, in respect of each sub-group, how management differs from that usually offered to patients with similar conditions. **Identical**

(f) Community Consultation:

In the case of community based studies, explain what consultation is planned within the community in the: **N/A**

(g) State the expected benefits arising from this study under the following headings:

1. Clinical care: Knowledge to compare this specific condition to the stage of the HIV infection. This can streamline care for the patients and expedite referral to the correct clinic for treatment.

2. Public health: Update of the Expanded Surveillance Case Definition for AIDS. Most international HIV programs are based on this classification.

3. Financial: Health planning for HIV and AIDS treatment in Southern Africa

SECTION 4: STUDY QUESTIONNAIRE – Dr T Jogi

"HIV and CD_4 count in ocular surface squamous neoplasia"

Study Number: _____

Age (Years): _____

Race: Black ☐ Indian ☐ White ☐ Coloured ☐

Current Occupation: _____

Gender: MALE ☐ FEMALE ☐

Smoker (Ever): YES ☐ NO ☐

Pack Years: _____

Drug History: _____

ARVs Commenced: YES ☐ NO ☐

☐ ☐

HPV History: YES NO

Surgery Booked For: _____/_____/20_____

Biopsy Findings:_____

_____

Bloods Drawn: HIV ☐ CD_4 ☐

FBC and DIFF ☐

RVD status and CD_4 count pos ☐ neg ☐ _____cells/µl

Doctor's Initials: _____ Date: _____

SECTION 4 cont :

To maintain confidentiality, each patient will have a random "study number" allocated to them at the time of specimen collection/admission.
This number will be printed on stickers and attached to:

1. The out patient file
2. The questionnaire
3. The histology request form
4. The CD_4 count request form and
5. The Pathology request form

Stickers will be printed and allocated to all hospitals participating in the study.

SECTION 5: DECLARATION

CONFLICT OF INTEREST:

Investigators should have no undisclosed conflict of interest with their study collaborators, sponsors or participants. Conflicts can arise, for example, when a commercial or other sponsor may not wish research results detrimental to their corporate image / interest to be disclosed, especially when the investigator is being remunerated by the sponsor for the research in question; when research subjects are being rewarded for their participation in the research; or when an investigator has a vested interest in, or is an employee / shareholder / director in the sponsor's corporate entity. Investigators should note that the duty to disclose a conflict of interest to the ethics review committee begins with application for ethical approval and continues until the research in question is complete and the research results are submitted to the sponsor / published (if applicable).

If the investigator(s) has / have / foresees any such conflict of interest, please provide details.

Is there any conflict of interest – financial or otherwise - to your involvement in this study?

Oversight of study: Will this study be overseen by a professional Clinical Research Organisation or study sponsor? Please give details:

I understand and accept that I will be required to submit half-yearly progress reports for pharmaceutical studies and annual reports for other studies. Where applicable, all reports from the Data Safety Monitoring Boards (or similar committees) will be provided to the Biomedical Research Ethics Committee within 7 days.

I agree to provide monitoring data if and when required.

I expect the project to be completed by *(Date)*: 31 December 2014

I agree to abide by the guidance contained in the SA Department of Health (2004) *Ethics in Health Research: Principles, structures and processes* and the (2006) *South African Good Clinical Practice Guidelines* and the UKZN Terms of Reference and Standard Operating Procedures of the UKZN Biomedical Research Ethics Committee. All are available at http://research.ukzn.ac.za/ResearchEthics11415.aspx

I understand and accept that all information pertaining to this application is a true reflection of the project proposed and I take full responsibility should there be any transgression.

SIGNATURE OF PRINCIPAL INVESTIGATOR:.....
DATE: .....

APPENDIX 1

(This Document will be translated into IsiZulu once it has been ethically approved)

INFORMATION BOOKLET - CONJUNCTIVAL/CORNEAL MASS

"HIV and CD_4 count in ocular surface squamous neoplasia"

Thank you for agreeing to participate in this study and help the doctors learn about this disease by answering a few short questions and having a few blood tests done. You may choose to leave the study at any time if you wish and you will still receive the same therapy as those patients inside the study at all times.

As part of the study you will be having your bloods taken for a Full Blood Count, HIV test and a CD_4 count. All results will be confidential. These results will help us determine your immune status, which will in turn aid us in deciding the optimal treatment for you i.e. Whether or not to start ARVs.

A surgical procedure called a biopsy will be performed on your eye to remove all, or a piece of the mass. This surgery is done under local anaesthetic so you will be awake during the procedure. This tissue will be sent to the lab to be analysed and appropriate treatment will then be started. These results will again be confidential.

Ocular surface squamous neoplasia (OSSN) refers to abnormal, pre-cancerous cells as well as cancer of the surface of the eye.

Treatment involves attempted complete removal of the mass. If we do manage to remove the entire mass you will need to use eye drops for the next 6 weeks. These masses sometimes recur so your consistent follow up is important.

Please direct any questions or queries to Dr T. Jogi

St Aidan's Hospital

031 314 2200

Contact details of BREC Administrator or Chair – for reporting of complaints/ problems:

Biomedical Research Ethics

UKZN

Private Bag X54001

Durban 4000

Telephone: 031 260 4769 / 260 1074

Fax: 031 260 4609

Administrator: Ms D Ramnarain Email: BREC@ukzn.ac.za

APPENDIX 2

This document will be translated into IsiZulu once it has been ethically approved

INFORMED CONSENT: FOR PROCEDURE AND PARTICIPATION IN RESEARCH STUDY

"HIV and CD_4 count in ocular surface squamous neoplasia"

I, _____ (PATIENT NAME) hereby give my consent for RIGHT / LEFT conjunctival and/or corneal mass biopsy as well as voluntary testing and counselling for HIV and CD_4 count.

I understand that the results of the above tests will be used for research purposes whilst maintaining confidentiality, that appropriate treatment will be started once the results of testing are available and that my participation is voluntary.

The risks of surgery with regards to local anaesthesia and eye surgery have been explained to me.

You may contact the **Biomedical Research Ethics Office** on **031-260 4769 or 260 1074** or Email BREC@ukzn.ac.za if you have questions about your rights as a research participant.

NAME OF DOCTOR OBTAINING CONSENT: _____

SIGNATURE: _____ DATE:____/_____/ 20_____

I have provided the patient with the information booklet available in Zulu or English to explain the nature, risks and possible consequences of the medical procedures to be performed on the undersigned patient.

SIGNATURE OF PATIENT: _____ DATE: ____/_____/20_____

OR THUMBPRINT:

Witnesses and translator where applicable

1. WITNESS NAME: _____
   SIGNATURE: _____ DATE: _____/_____/20_____

2. TRANSLATOR NAME: _____ SIGNATURE: _____ DATE: _____/_____/20_____

APPENDIX 3

CURRICULUM VITAE

of

THENUSHKA JOGI

PERSONAL DETAILS:

Title: Dr

Gender: Female

DOB: 01 May 1980

Known As: Jogi

ID No: 8005010121086

Nationality: South African

Drivers license: Code B (1998)

Marital Status: Single

Race: Asian

Religion: Hinduism

Postal Add.: 14 Calendula Crescent, Buffelsdale, Tongaat, 4399

Residential Add.: 14 Calendula Crescent, Buffelsdale, Tongaat, 4399

Home Tel. No.: 032 9444452

Cell No.: 083 305 9893

E mail add.: jogi.thenushka@gmail.com

ACADEMIC RECORD:

TERTIARY EDUCATION-

-MBChB (Natal) (1998-2003)

POST GRADUATE EDUCATION-

-FC Ophthalmology Part 1 (March 2010)

Publication list over the past 3 years: 0

Details of all other research studies presently being conducted: 0

MEMBERSHIPS CURRENTLY HELD:

1.HPCSA – MP 0588636 ie. Health Professions Council of South Africa

2.MPS – 01/36636 ie. Medical Protection Society

3.SAMA – 71142 South African Medical Association

4.DISPENSING LICENCE – H.S.A 19761.Certificate no.: 05168

5.Please see GCP Certificate attached

EMPLOYMENT HISTORY:

Internship at Johannesburg General Hospital 2004

Community Service at Dundee Provincial Hospital 2005

Senior medical officer accident and emergency 2006 – 2008

Senior medical officer Ophthalmology 2009 - 2010

Registrar in Ophthalmology 2011 – 2014

CURRICULUM VITAE – Supervisor

Full name: Dr Carl-Heinz Kruse

Date of birth: 1975-06-29

Male/Female: Male

Telephone (Home): 031 811 8130

Telephone (Business): 033 897 3345

Cell: 084 011 0767

Fax No: 033 897-3111

E-mail Address: ruraleye@gmail.com

Current HPCSA No: MP 0532851

Present position: Head of Clinical Unit

Institution: Grey's Hospital

Department/Section: Ophthalmology

Nationality and Permanent residency: RSA

Previous positions held (last 10 years):

Principal Specialist: Ngwelezana Hospital (2008 – 2010)

Registrar: St Aidan's Hospital (2004 – 2007)

MO: Ermelo Hospital, Edendale Hospital (2002 – 2004)

Qualifications: MBChB: University of Pretoria 2000

MMed(Ophth): UKZN 2008

FCOphth: CMSA 2007

Area of study: Ophthalmology

Number of Postgraduate theses supervised (Masters): 0

Publication list over the past 3 years: 0

Details of all other research studies presently being conducted: 0

Feb 2009

Appendix B – Guidelines for Authorship

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- ***Manuscripts must be written in UK English.***
- ***The manuscript must be in Microsoft Word or RTF document format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).***
- ***Please make your article concise, even if it is below the word limit.***
- ***Qualifications, full affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.***
- ***Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.***
- ***Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).***
- ***Litres is denoted with an uppercase L e.g. 'mL' for millilitres).***
- ***Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.***
- ***Please be sure to insert proper symbols e.g. μ not u for micro, a not a for alpha, b not B for beta, etc.***
- ***Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.***
- ***Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'***
- ***Round brackets (parentheses) should be used, as opposed to***

square brackets, which are reserved for denoting concentrations or insertions in direct quotes.

- ***If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the only exception. Please DO NOT use fill, format lines and so on.***

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text.

Structured abstract

- ***This should be 250-400 words, with the following recommended headings:***
  - ***Background:** why the study is being done and how it relates to other published work.*
  - ***Objectives:** what the study intends to find out*
  - ***Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.*
  - ***Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.*
  - ***Conclusion:** must be supported by the data, include recommendations for further study/actions.*
- ***Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.***
- ***Do not include any references in the abstracts.***

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- ***Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed***
- ***Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.***
- ***Setting (within Methods): level of care, e.g. primary, secondary,***

number of participating centres.

- ***Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.***
- ***Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.***
- ***Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.***

Results

- ***Start with description of the population and sample. Include key characteristics of comparison groups.***
- ***Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.***
- ***Do not replicate data in tables and in text.***
- ***If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:***
- ***E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).***
- ***Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.***

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- ***Statement of principal findings***
- ***Strengths and weaknesses of the study***
- ***Contribution to the body of knowledge***
- ***Strengths and weaknesses in relation to other studies***

- ***The meaning of the study – e.g. what this study means to clinicians and policymakers***
- ***Unanswered questions and recommendations for future research***

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations/photos/scans

- ***If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.***
- ***Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.***
- ***Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).***
- ***All images must be of high enough resolution/quality for print.***
- ***All illustrations (graphs, diagrams, charts, etc.) must be in PDF form. Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.***
- ***Scans/photos showing a specific feature e.g. Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (HandE stain). –include an arrow to show the tumour.***
- ***Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.***

Tables

- ***Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.***
- ***Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.***
- ***Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.***
- ***Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.***
- ***Ensure each table has a concise title and column headings, and include units where necessary.***
- ***Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ \|\| then ** †† ‡‡ etc.***

***Do not:** Use [Enter] within a row to make 'new rows':*

Rather:

Each row of data must have its own proper row:

***Do not:** use separate columns for n and %:*

Rather:

Combine into one column, n (%):

***Do not:** have overlapping categories, e.g.:*

Rather:

Use <> symbols or numbers that don't overlap:

References

***NB:** Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must **not** be used.* **Authors must verify references from original sources.**

- ***Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]***
- ***All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).***
- ***Approved abbreviations of journal titles must be used; see the*** List of Journals in Index Medicus***.***
- ***Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.***
- ***Volume and issue numbers should be given.***
- ***First and last page, in full, should be given e.g.: 1215-1217 not 1215-17.***
- ***Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by*** CrossRef***:***
  - ***On the Crossref homepage, paste the article title into the 'Metadata search' box.***
  - ***Look for the correct, matching article in the list of results.***
  - ***Click Actions > Cite***
  - ***Copy the DOI between { }, which will always start with 10.***
  - ***Provide as follows: DOI:10.7196/07294.937.98x***

Some examples:

- ***Journal references:** Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. DOI:10.1000/hgjr.182*
- ***Book references:** Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.*
- ***Chapter/section in a book:** Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA,*

Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.

- ***Internet references:*** ***World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).***
- ***Legal references***
- ***Government Gazettes:***

National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

- ***Provincial Gazettes:***

Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- ***Acts:***

South Africa. National Health Act No. 61 of 2003.

- ***Regulations to an Act:***

South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- ***Bills:***

South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- ***Green/white papers:***

South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- ***Case law:***

Rex v Jopp and Another 1949 (4) SA 11 (N)

Rex v Jopp and Another: Name of the parties concerned

1949: Date of decision (or when the case was heard)

(4): Volume number

SA: SA Law Reports

11: Page or section number

(N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

NOTE: no . after the v

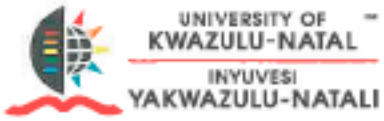
- ***Other references (e.g. reports) should follow the same format: Author(s). Title. Publisher place: Publisher name, year; pages.***
- ***Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.***
- ***Unpublished observations and personal communications in the text must <u>not</u> appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.***

Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. ***Named authors consent to publication and meet the requirements of authorship as set out by the journal.***
2. ***The submission has not been previously published, nor is it before another journal for consideration.***
3. ***The text complies with the stylistic and bibliographic requirements in Author Guidelines.***
4. ***The manuscript is in Microsoft Word or RTF document format. The text is single-spaced, in 12-point Times New Roman font, and contains no unnecessary formatting.***
5. ***Illustrations/figures are high resolution/quality (not compressed) and in an acceptable format (preferably TIFF or PNG). These must be submitted individually as 'supplementary files' (not solely embedded in the manuscript).***
6. ***For illustrations/figures or tables that have been published elsewhere, the author has obtained written consent to republication from the copyright holder.***
7. ***Where possible, references are accompanied by a digital object identifier (DOI) and PubMed ID (PMID)/PubMed Central ID (PMCID).***
8. ***An abstract has been included where applicable.***
9. ***The research was approved by a Research Ethics Committee (if applicable)***
10. ***Any conflict of interest (or competing interests) is indicated by the author(s).***

Appendix C – Bioethical Approvals



10 September 2013

Dr. T Jogi
14 Calendula Crescent
Buffelsdale
Tongaat
4399

PROTOCOL: Human Immunodeficiency Virus & CD4 count in ocular surface squamous neoplasia.
REF: BE057/13.

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 22 February 2013.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 26 August 2013 to queries raised on 20 August 2013 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 10 September 2013.

This approval is valid for one year from **10 September 2013.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **08 October 2013.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

Professor D Wassenaar (Chair)
Biomedical Research Ethics Committee
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban, 4000, South Africa
Telephone: +27 (0)31 260 2384 **Facsimile:** +27 (0)31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

10 March 2016

Dr. T Jogi
14 Calendula Crescent
Buffelsdale
Tongaat
4399

PROTOCOL: Human Immunodeficiency Virus & CD4 count in ocular surface squamous neoplasia. REF: BE057/13.

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	10 September 2015
Expiration of Ethical Approval:	09 September 2016

I wish to advise you that your application for Recertification dated 29 December 2015 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be ratified at the next full meeting to be held on 12 April 2016.

Yours sincerely

Mrs A Marimuthu
Senior Administrative Officer
Biomedical Research Ethics Committee

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website:http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

27 July 2016

Dr. T Jogi
14 Calendula Crescent
Buffelsdale
Tongaat
4399

PROTOCOL: Human Immunodeficiency Virus & CD4 count in ocular surface squamous neoplasia. REF: BE057/13.

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	10 September 2016
Expiration of Ethical Approval:	09 September 2017

I wish to advise you that your application for Recertification dated received 18 July 2016 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be ratified at the next full meeting to be held on 16 August 2016.

Yours sincerely

Mrs A Marimuthu
Senior Administrative Officer
Biomedical Research Ethics Committee

Appendix D – Site Approvals

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the Medical Superintendent/s / Hospital Manager for signature.

For King Edward VIII Hospital (KEH) and Inkosi Albert Luthuli Central Hospital (IALCH) studies please submit together with the following:

i) Two copies of the final, approved protocol
ii) Letter giving provisional ethical approval
iii) Details of other research presently being performed by yourself (individually or as a collaborator)
iv) Details of any financial or human resource implications to King Edward VIII Hospital
v) If a clinical trial, please produce proof of payment or intention thereof to KEH

Once the document has been signed it should be returned to this office so that full ethical approval can be granted.

To: Hospital Manager

PROTOCOL : Human Immunodeficiency Virus & CD4 Count in Ocular surface squamous neoplasia. REF: BE 057/13

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 1 address:

Greys Hospital
Private Bag X9001
Pietermaritzburg
3200

Investigator/s:

Principal: DR. T. JOGI
Co-investigator: N/A
Co-investigator: N/A

Hospital Manager: Dr K.B. Bolenge

Signature Hospital Manager:

Date: 08/07/2013

NB: Hospital Manager/s to send a copy of this document to Natalia.

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the Medical Superintendent/s / Hospital Manager for signature.

For King Edward VIII Hospital (KEH) and Inkosi Albert Luthuli Central Hospital (IALCH) studies please submit together with the following:

i) Two copies of the final, approved protocol
ii) Letter giving provisional ethical approval
iii) Details of other research presently being performed by yourself (individually or as a collaborator)
iv) Details of any financial or human resource implications to King Edward VIII Hospital
v) If a clinical trial, please produce proof of payment or intention thereof to KEH

Once the document has been signed it should be returned to this office so that full ethical approval can be granted.

To: Hospital Manager

PROTOCOL : Human Immunodeficiency Virus & CD4 Count in Ocular surface squamous neoplasia. REF: BE 057/13

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 2 address:

Edendale Hospital
P/Bag X509
Plessislaer, 3216
Pietermaritzburg

Investigator/s:

Principal: DR. T. JOOL
Co-Investigator: N/A
Co-Investigator: N/A

Hospital Manager: _____

Signature Hospital Manager: _____

Date: _____

NB: Hospital Manager/s to send a copy of this document to Natalia.

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the Medical Superintendent/s / Hospital Manager for signature.

For King Edward VIII Hospital (KEH) and Inkosi Albert Luthuli Central Hospital (IALCH) studies please submit together with the following:

i) Two copies of the final, approved protocol
ii) Letter giving provisional ethical approval
iii) Details of other research presently being performed by yourself (individually or as a collaborator)
iv) Details of any financial or human resource implications to King Edward VIII Hospital
v) If a clinical trial, please produce proof of payment or intention thereof to KEH

Once the document has been signed it should be returned to this office so that full ethical approval can be granted.

To: Hospital Manager

PROTOCOL : Human Immunodeficiency Virus & CD4 Count in Ocular surface squamous neoplasia. REF: BE 057/13

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 3 address:

St. Aidan's Hospital
33 Centenary Road
Durban
4000

Investigator/s:

Principal: DR. T. JOGI.
Co-investigator: N/A
Co-investigator: N/A

Hospital Manager: Dilra Ley

Signature Hospital Manager:

Date: 13/6/13

NB: Hospital Manager/s to send a copy of this document to Natalia.

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the Medical Superintendent/s / Hospital Manager for signature.

For King Edward VIII Hospital (KEH) and Inkosi Albert Luthuli Central Hospital (IALCH) studies please submit together with the following:

i) Two copies of the final, approved protocol
ii) Letter giving provisional ethical approval
iii) Details of other research presently being performed by yourself (individually or as a collaborator)
iv) Details of any financial or human resource implications to King Edward VIII Hospital
v) If a clinical trial, please produce proof of payment or intention thereof to KEH

Once the document has been signed it should be returned to this office so that full ethical approval can be granted.

To: Hospital Manager

PROTOCOL : Human Immunodeficiency Virus & CD4 Count in Ocular surface squamous neoplasia. REF: BE 057/13

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 4 address:

Addington Hospital
P.O. Box 977
DURBAN
4000

Investigator/s:

Principal: DR. T. JOGI
Co-investigator: N/A
Co-investigator: N/A

Hospital Manager: GBZ Khathi/LS

Signature Hospital Manager:

Date: 27/6/2013

NB: Hospital Manager/s to send a copy of this document to Natalia.

Appendix E – NHLS Approval

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
Fax: +27 (0)11 386 6296
Email: babatyi.kgokong@nhls.ac.za
Web: www.nhls.ac.za

18 August 2016

Applicant: Dr Thenushka Jogi
Institution: University of KwaZulu Natal
Department: Opthalmology
Email: jogi.thenushka@gmail.com
Cell: 083 305 9893

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **Human Immunodeficiency Virus & CD4 count in Ocular Surface Squamous Neoplasia** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you to conduct the proposed study as outlined in the submitted application.

Please note that the approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Ethics approval is obtained from a recognised SA Health Research Ethics Committee.
- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Department) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research. Any data related queries may be directed to Sue Candy, manager NHLS Corporate Data Warehouse, Tel: (011) 386 6036. Email: sue.candy@nhls.ac.za.

Yours sincerely,

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix F – Global HIV data[12]

Global HIV data

	2000	2005	2010	2011	2012	2013	2014	2015
People living with HIV	**28.9 million** [26.5 million–31.7 million]	**31.8 million** [29.4 million–34.5 million]	**33.3 million** [30.8 million–36.1 million]	**33.9 million** [31.4 million–36.7 million]	**34.5 million** [31.9 million–37.4 million]	**35.2 million** [32.6 million–38.1 million]	**35.9 million** [33.3 million–38.9 million]	**36.7 million** [34.0 million–39.8 million]
New HIV Infections (total)	**3.2 million** [2.9 million–3.5 million]	**2.5 million** [2.3 million–2.8 million]	**2.2 million** [2.0 million–2.5 million]	**2.2 million** [1.9 million–2.5 million]	**2.2 million** [1.9 million–2.4 million]	**2.1 million** [1.9 million–2.4 million]	**2.1 million** [1.9 million–2.4 million]	**2.1 million** [1.8 million–2.4 million]
New HIV infections (aged 15+)	**2.7 million** [2.5 million–3.0 million]	**2.1 million** [1.9 million–2.3 million]	**1.9 million** [1.7 million–2.1 million]	**1.9 million** [1.7 million–2.2 million]	**1.9 million** [1.7 million–2.2 million]	**1.9 million** [1.7 million–2.2 million]	**1.9 million** [1.7 million–2.2 million]	**1.9 million** [1.7 million–2.2 million]
New infections (aged 0–14)	**490 000** [430 000–560 000]	**450 000** [390 000–510 000]	**290 000** [250 000–350 000]	**270 000** [220 000–330 000]	**230 000** [190 000–290 000]	**200 000** [160 000–250 000]	**160 000** [130 000–220 000]	**150 000** [110 000–190 000]
AIDS-related deaths	**1.5 million** [1.3 million–1.8 million]	**2.0 million** [1.7 million–2.3 million]	**1.5 million** [1.3 million–1.7 million]	**1.4 million** [1.2 million–1.7 million]	**1.4 million** [1.2 million–1.6 million]	**1.3 million** [1.1 million–1.5 million]	**1.2 million** [990 000–1.4 million]	**1.1 million** [940 000–1.3 million]
People accessing treatment	**770 000**	**2.2 million**	**7.5 million**	**9.1 million**	**11 million**	**13 million**	**15 million**	**17 million**
Resources available for HIV (low- and middle-income countries)	**4.8 billion**	**9.4 billion**	**15.9 billion**	**18.3 billion**	**19.5 billion**	**19.6 billion**	**19.2 billion**	**19 billion**

Appendix G – Regional HIV data[12]

Regional data—2015

Region	People living with HIV (total)	New HIV infections			AIDS-related deaths (total)	Total number accessing antiretroviral therapy
		Total	Aged 15+	Aged 0–14		
Eastern and southern Africa	**19.0 million** [17.7 million–20.5 million]	**960 000** [830 000–1.1 million]	910 000 [790 000–1.1 million]	56 000 [40 000–76 000]	**470 000** [390 000–560 000]	**10 million**
Latin America and the Caribbean	**2.0 million** [1.7 million–2.3 million]	**100 000** [86 000–120 000]	100 000 [84 000–120 000]	2100 [1600–2900]	**50 000** [41 000–59 000]	**1.1 million**
Western and central Africa	**6.5 million** [5.3 million–7.8 million]	**410 000** [310 000–530 000]	350 000 [270 000–450 000]	66 000 [47 000–87 000]	**330 000** [250 000–430 000]	**1.8 million**
Asia and the Pacific	**5.1 million** [4.4 million–5.9 million]	**300 000** [240 000–380 000]	280 000 [220 000–350 000]	19 000 [16 000–21 000]	**180 000** [150 000–220 000]	**2.1 million**
Eastern Europe and central Asia	**1.5 million** [1.4 million–1.7 million]	**190 000** [170 000–200 000]	190 000 [170 000–200 000]	—*	**47 000** [39 000–55 000]	**320 000**
Middle East and North Africa	**230 000** [160 000–330 000]	**21 000** [12 000–37 000]	19 000 [11 000–34 000]	2100 [1400–3200]	**12 000** [8700–16 000]	**38 000**
Western and central Europe and North America	**2.4 million** [2.2 million–2.7 million]	**91 000** [89 000–97 000]	91 000 [88 000–96 000]	—*	**22 000** [20 000–24 000]	**1.4 million**

Appendix H – WHO stage 3 conditions[7]

WHO stage 3 conditions

- Unexplained severe weight loss (>10% of presumed or measured body weight)
- Unexplained chronic diarrhoea persisting for longer than 1 month
- Unexplained persistent fever (intermittent or constant for longer than 1 month)
- Persistent oral candidiasis
- Oral hairy leukoplakia
- Pulmonary TB (current)
- Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia, severe pelvic inflammatory disease)
- Acute necrotising ulcerative stomatitis, gingivitis or periodontitis
- Unexplained anaemia (<8 g/dL), neutropenia ($<0.5 \times 10^9$/L) and/or chronic thrombocytopenia ($<50 \times 10^9$/L).

Appendix I – WHO stage 4 conditions[7]

WHO stage 4 conditions

- HIV wasting syndrome
- *Pneumocystis* pneumonia
- Recurrent severe bacterial pneumonia
- Chronic herpes simplex virus infection (orolabial, genital or anorectal of more than 1 month's duration, or visceral at any site)
- Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
- Extrapulmonary TB
- Kaposi's sarcoma
- Cytomegalovirus infection (retinitis or infection of other organs)
- CNS toxoplasmosis
- HIV encephalopathy
- Extrapulmonary cryptococcosis including meningitis
- Disseminated non-tuberculous mycobacteria infection
- Progressive multifocal leukoencephalopathy
- Chronic cryptosporidiosis
- Chronic isosporiasis
- Disseminated mycosis (extrapulmonary histoplasmosis, coccidio-mycosis)
- Recurrent septicaemia (including non-typhoidal salmonella)
- Lymphoma (cerebral or B-cell non-Hodgkin's)
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy
- Symptomatic HIV-associated cardiomyopathy

Appendix J – Raw Data

Page 1 of 6 056 controls – Full list available on request

AGE_TESTED_YEARS	GENDER	PROVINCE_NAME	FACILITY_NAME	WARD_NAME	HIV_STATUS	CD4_COUNT	OSSN	ARVs
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	304	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	628	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	239	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	WARD NOT STATED	POSITIVE	979	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	7F PAEDIATRIC WARD	POSITIVE	756	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	OCC HEALTH & SAFETY (STAFF CLINIC)	POSITIVE	1006	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	48	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	520	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	453	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	217	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	445	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	7F PAEDIATRIC WARD	POSITIVE	947	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	173	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	394	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	308	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	402	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	817	N	U
24	F	KWAZULU-NATAL	GREY'S HOSPITAL	H2 MEDICAL WARD	POSITIVE	707	N	U
24	F	KWAZULU-NATAL	GREY'S HOSPITAL	CDC CLINIC	POSITIVE	282	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	359	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CRISIS CENTRE	POSITIVE	572	N	U
24	F	KWAZULU-NATAL	GREY'S HOSPITAL	M4 OBSTETRICS AND GYNAECOLOGY WARD	POSITIVE	134	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	370	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	284	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	245	N	U
24	F	KWAZULU-NATAL	GREY'S HOSPITAL	RENAL UNIT D2	POSITIVE	1506	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	480	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	595	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	WARD NOT STATED	POSITIVE	682	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	5	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	5B1 FEMALE MEDICAL WARD	POSITIVE	616	N	U
24	F	KWAZULU-NATAL	GREY'S HOSPITAL	MEDICAL ADMISSIONS WARD	POSITIVE	900	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	430	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	WARD NOT STATED	POSITIVE	482	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	WARD NOT STATED	POSITIVE	443	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	437	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	979	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	MEDICAL OPD	POSITIVE	115	N	U
24	F	KWAZULU-NATAL	GREY'S HOSPITAL	E1 PAEDIATRIC WARD	POSITIVE	647	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	523	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	500	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	OCC HEALTH & SAFETY (STAFF CLINIC)	POSITIVE	194	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	TEEN CLINIC	POSITIVE	469	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	540	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	332	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	5B1 FEMALE MEDICAL WARD	POSITIVE	2	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	530	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	463	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	631	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	178	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	7F PAEDIATRIC WARD	POSITIVE	525	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	707	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	295	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	161	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	830	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	686	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	5B1 FEMALE MEDICAL WARD	POSITIVE	2	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	444	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CRISIS CENTRE	POSITIVE	427	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	684	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	462	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	598	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	B WARD	POSITIVE	80	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	311	N	U
24	F	KWAZULU-NATAL	GREY'S HOSPITAL	E1 PAEDIATRIC WARD	POSITIVE	460	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	542	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	CDC CLINIC	POSITIVE	22	N	U
24	F	KWAZULU-NATAL	EDENDALE HOSPITAL	WARD NOT STATED	POSITIVE	782	N	U
24	F	KWAZULU-NATAL	GREY'S HOSPITAL	INTENSIVE CARE UNIT	POSITIVE	543	N	U

Page 1 – Showing 13 Cases

AGE_TESTED_YEARS	GENDER	PROVINCE_NAME	FACILITY_NAME	WARD_NAME	HIV_STATUS	CD4_COUNT	OSSN	ARVs
43	M	KWAZULU-NATAL	SAH	EYE CLINIC	POSITIVE	124	YES	YES
39	M	KWAZULU-NATAL	SAH	EYE CLINIC	POSITIVE	251	YES	YES
34	F	KWAZULU-NATAL	ADH	EYE CLINIC	POSITIVE	45	YES	YES
44	F	KWAZULU-NATAL	GREYS	EYE CLINIC	POSITIVE	243	YES	YES
31	M	KWAZULU-NATAL	GREYS	EYE CLINIC	POSITIVE	303	YES	NO
31	F	KWAZULU-NATAL	GREYS	EYE CLINIC	POSITIVE	277	YES	NO
39	M	KWAZULU-NATAL	GREYS	EYE CLINIC	POSITIVE	241	YES	YES
49	F	KWAZULU-NATAL	GREYS	EYE CLINIC	POSITIVE	444	YES	YES
44	F	KWAZULU-NATAL	GREYS	EYE CLINIC	POSITIVE	195	YES	YES
32	F	KWAZULU-NATAL	GREYS	EYE CLINIC	POSITIVE	43	YES	YES
28	F	KWAZULU-NATAL	EDH	EYE CLINIC	POSITIVE	181	YES	U
41	F	KWAZULU-NATAL	EDH	EYE CLINIC	POSITIVE	101	YES	NO
40	M	KWAZULU-NATAL	EDH	EYE CLINIC	POSITIVE	307	YES	YES

Appendix K – Abbreviations and acronyms

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
CD_4	Cluster of Differentiation 4: a glycoprotein found on the surface of immune cells
CIN	Conjunctival intraepithelial neoplasia
CDC	Centre for Disease Control
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
NHLS	National Health Laboratory Service
TB	Tuberculosis
UKZN	University of KwaZulu-Natal
WHO	World Health Organization
SPSS	Statistical Packages for the Social Sciences

SUPPLEMENTARY FILE – IMAGES

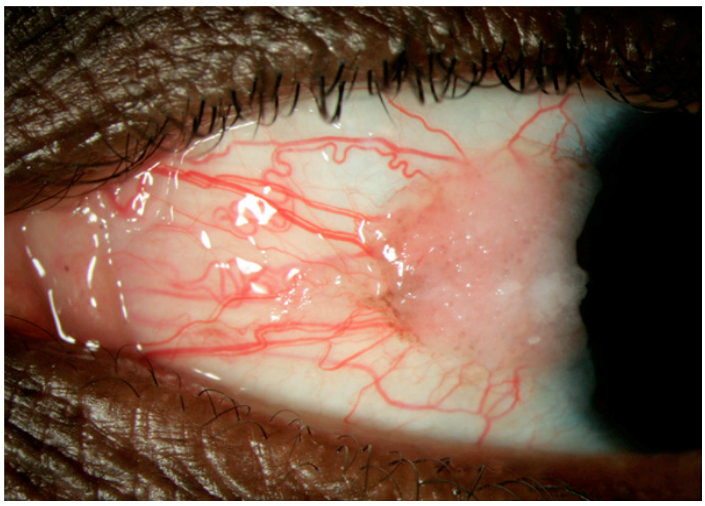


Figure 1: Image of the anterior ocular surface showing nodular gelatinous OSSN with large feeder vessels and some pigmentation. (Reproduced from eyworld.org)

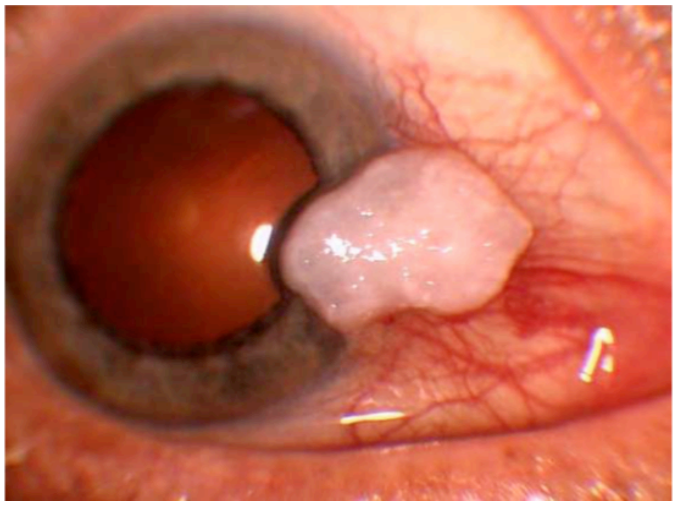


Figure 2: Image of the anterior ocular surface showing leukoplakic surface of OSSN (Reproduced from cdn.intechweb.org)

Clinical diagnosis (irrespective of CD4⁺ count)	
WHO clinical stage 3 and 4†	ART recommended
Other severe HIV-related disorders, e.g.:‡ • immune thrombocytopenia • thrombotic thrombocytopenic purpura • polymyositis • lymphocytic interstitial pneumonitis	ART recommended
Non HIV-related disorders:§ • malignancies (excluding localised malignancies) • hepatitis B co-infection¶ • hepatitis C co-infection	ART recommended
Any condition requiring long-term immunosuppressive therapy	ART recommended
CD4⁺ counts	
<350 cells/μL	ART recommended
350 - 500 cells/μL (two counts in this range)	ART recommended if patient is ready and motivated to start
>500 cells/μL	Defer ART
HIV-infected partner in serodiscordant relationship	
Regardless of CD4⁺ count or clinical diagnoses	Offer ART and discuss safe sex (discussion should ideally involve all partners)

ART = antiretroviral therapy; WHO = World Health Organization.

*Note that EITHER listed clinical diagnoses OR CD4⁺ strata would be an indication for ART.

Figure 3: ART Recommendations by the South African HIV clinicians society[6]

CD4 Cell Count Categories	Clinical Categories		
	A Asymptomatic, Acute HIV, or PGL	B* Symptomatic Conditions, not A or C	C# AIDS-Indicator Conditions
(1) ≥500 cells/ µL	A1	B1	C1
(2) 200-499 cells/µL	A2	B2	C2
(3) <200 cells/ µL	A3	B3	C3
Abbreviations: PGL = persistent generalized lymphadenopathy			

Figure 4: Clinical categories for HIV/AIDS[7]