A STUDY OF THE JOHN CUNNINGHAM VIRUS (JCV) SEROPREVALENCE AMONG ZAMBIAN ADULTS PRESENTING WITH "MENINGOENCEPHALITIS" TO THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

By

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DECLARATION

I, Atiyah Patel, declare that the dissertation represents my own work and has not previously been submitted for a degree, diploma or other qualification at this or any other university.

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CERTIFICATE OF APPROVAL

The dissertation of Atiyah Patel has been approved as partial fulfilment of the requirements for the award of Masters in Internal Medicine by University of Zambia.

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ABSTRACT

The John Cunningham virus (JCV) is an opportunistic virus, which leads to the development of progressive multifocal leukoencephalopathy (PML). Infection with the JCV occurs in childhood and the virus remains quiescent in the body, activating during immunosuppression. Exposure to the virus can be detected by testing for JC virus specific antibodies in an ELISA test. One of the major unanswered questions of JC virus epidemiology is whether it is less frequent in Africa than in the West. Our aim was to determine the JCV seroprevalence and factors associated with its positivity among Zambian adults presenting to the University Teaching Hospital (UTH) with suspected meningoencephalitis and to assess the JCV ELISA test as a possible tool for PML risk stratification.

This was a cross sectional nested study in the TB meningitis in Zambia (TMZ) study which looked at improving ways of TB diagnosis in patients with meningoencephalitis. It included adults 18 years and older who presented with suspected meningoencephalitis and had undergone a lumbar puncture as part of their evaluation. Confirmed PML cases were also recruited based on clinical features, confirmed by JCV DNA PCR of CSF. Data was analysed using Epi Info 7. Descriptive statistics were used to determine patient characteristics and JCV seroprevalence and compared using chi-square tests. Multiple logistic regression was used to determine the significance of factors associated with JCV positivity as well as for stratifying PML risk by comparing features of the HIV positive JCV positive group with confirmed PML cases.

Final analysis for JCV seroprevalence was done in 96 patients and noted to be 46% (95% CI, 35.62 – 56.31). The JCV seroprevalence in the HIV positive group was 40.82% and in the HIV negative group was 51.06 % but there was no statistical difference (*p*-value 0.31). None of the other factors studied had any impact on the JCV seroprevalence. There was a bimodal distribution of age associated with JCV seropositivity; with one peak occurring in the 18 to 20 years age group and the second peak occurring in the 55 to 60 years age group. 14 (3.2%) confirmed PML cases, based on clinical features and JCV DNA CSF positive, were all JCV seropositive and HIV positive with advanced immunosuppression (CD4<200/mm^3). Memory impairment was associated with a 6 fold increased likelihood of having PML in advanced HIV disease with JCV which further, increased to over 20 fold after adjusting for age , gender and TBM diagnosis. After adjusting for other variables TBM was associated with an 87% less likelihood of having PML (p-value 0.03). Female gender was associated with increased risk of having PML (p-value 0.02) and a younger age was protective for PML (p-value 0.03).

The prevalence of anti-JCV antibodies in patients with suspected CNS infection (meningoencephalitis) was 46%. Anti- JCV antibody prevalence did not differ significantly by age, gender, HIV status or CD4 count. Memory impairment in JCV seropositive, advanced HIV disease patients with meningoencephalitis was the most important variable associated with having PML. After adjusting for other variables, male gender, a younger age and diagnosis of TBM were protective of having PML.

***Key words**: John Cunningham Virus (JCV), Progressive Multifocal Leukoencephalopathy (PML)*

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ABBREVIATIONS

AAN	American Academy of Neurology
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
CNS	Central Nervous System
CSF	Cerebrospinal fluid
DKFZ	German Cancer Research Centre
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
HIV	Human Immunodeficiency Virus
IQR	Interquartile Range
JCV	John Cunningham Virus
JCVAb	John Cunningham Virus antibodies
PCR	Polymerase chain reaction
PICT	Provider initiated Counseling and testing
PML	Progressive Multifocal Leukoencephalopathy
TBM	Tuberculosis Meningitis
TH2	T helper 2
TMZ	Tuberculosis Meningitis in Zambia Study
UTH	University Teaching Hospital

CHAPTER 1

INTRODUCTION

1.1 Background

The John Cunningham virus (JCV) is an opportunistic virus which leads to the development of progressive multifocal leukoencephalopathy (PML) (Padgett *et al.*, 1971). PML is a lytic infection of mainly oligodendrocytes of the central nervous system in immunosuppressed patients (Brew *et al.*, 2010). Rarely, it is also known to occur in immunocompetent patients (Naess *et al.*, 2010, Guerini *et al.*, 2008, Tan *et al.*, 2011).

The JCV is a ubiquitous human pathogen and exposure is unavoidable. After asymptomatic primary infection with the JCV, which occurs in childhood, the virus remains quiescent in the kidneys, bone marrow, and lymphoid tissue and becomes activated when the host becomes immunosuppressed (Monaco *et al.*, 1998,Tan and Koralnik, 2010, Randhawa, Shapiro and Vats, 2005). Once infected, exposure to the virus can be detected by testing for JC virus specific antibodies. One of the major unanswered questions of JC virus epidemiology is whether it is less frequent in Africa than in the West. While multiple studies have shown that about 55% of adult are JCV seropositive in Europe and the US (Egli *et al.*, 2009) nothing is known about JCV seroepidemiology in Africa.

As JCV infection is a prerequisite for the development of PML, antibody tests for the JCV are now successfully being carried out to determine the subsequent risks of developing PML in different populations (Lee *et al.*, 2013).

With the advent of the HIV pandemic, and the increasing use of immunosuppressive agents the incidence of PML has risen sharply, but very few cases from Africa have been reported, even though this continent carries a very high burden of HIV infection (Tan and Koralnik, 2010). Two cases of PML have been diagnosed by autopsy among HIV+ patients in Uganda and Gambia each (Chima *et al.*, 1999, Berger *et al.*, 2013).

PML prevalence in an autopsy series from Ivory Coast, conducted on 271 HIV positive patients who died from any cause within the study period, was 1.5% (Sylvester C Chima *et al.,* 1999). In the study of CNS opportunistic infections in HIV+ Zambian adults (COINZ), JCV DNA was detected in 20/331 (6%) of CSF samples of HIV+ Zambians presenting with signs and symptoms consistent with CNS infection (Siddiqi *et al.*, 2014).

Explanations for the paucity of reports of PML from Africa include non-recognition of the condition, diagnostic challenges, death due to other infections which occur at higher CD4 counts such as TB, as well as suggestions of decreased seroprevalence and neurovirulence of African JC virus types (Shankar *et al.*, 2003, Rgen *et al.*, 2000).

In this study, the seroprevalence of JCV was determined amongst the Zambian population who present with features of a central nervous system (CNS) infection (meningoencephalitis) and factors affecting the JCV seroprevalence will be studied. We will also asses the possible role of the JCV antibody test in stratifying PML risk in affected populations.

1.2 Statement of the Problem

Despite several studies in the West on JCV seroepidemiology, nothing is known about JCV seroepidemiology in Africa where the burden of HIV disease is high. In addition, there is now an increasing use of immunosuppressive agents such as monoclonal antibodies for various chronic auto-immune conditions such as Crohn's disease and Multiple Sclerosis thus, increasing the number of people potentially at risk of developing PML.

1.3 Study Rationale

PML is a fatal disease with a 9% survival rate at 1 year without any intervention and early identification is key (Berger *et al.*, 1998).This study will thus, give us an insight on the seroprevalence of JCV amongst Zambian adults presenting to UTH and identify factors affecting seropositivity. It will also help us establish who will be at risk of developing PML.

1.4 Research Question

What is the seroprevalence of JCV in Zambian adults who present to UTH with a suspected CNS infection and what factors would affect the JCV seropositivity.

1.5 Aims

1.5.1 General

1. To determine the seroprevalence of JC Virus (JCV) amongst Zambian adults with meningoencephalitis presenting to the University Teaching Hospital (UTH).

1.5.2 Specific

1. To identify any difference in the seroprevalence of JCV amongst HIV positive and HIV negative Zambian adults.

2. To identify any other factors that may affect JCV antibody positivity.

3. To determine the validity of the JC Virus Elisa test as a tool for stratifying PML risk in affected populations.

CHAPTER 2

LITERATURE REVIEW

2.1 The JC Virus

Natural History

The JC virus (JCV) was first isolated from the brain of a patient with Hodgkin's disease in 1971 and was identified as a cause of a demyelinating encephalopathy subsequently named progressive multifocal encephalopathy (PML) (Padgett *et al.*, 1971). JCV, a human polyoma virus, is a double stranded DNA virus. Inhalation and ingestion of contaminated water have been suggested as major modes of transmission of the virus (Bofill-Mas *et al.*, 2001; Monaco *et al.*, 1998). The primary infection is presumably asymptomatic and most likely occurs early in childhood. Most adults develop antibodies to the virus and the virus remains latent in the kidney, bone marrow and lymphoid tissue (Monaco *et al.*, 1996; Tan *et al.*, 2009; Randhawa, Shapiro and Vats, 2005).

Periods of viral replication without any clinical symptoms occur and can be detected when it is shed in the urine with prevalence of shedding dependent on age and gender. Usually, severe deficiency of T-cell immunity (cellular immunity) is necessary for reactivation of JCV (Gheuens *et al.*, 2010). When reactivation occurs viral replication ensues, causing dissemination to the brain and results in a lytic infection of the oligodendrocytes (Gheuens *et al.*, 2010). The reactivation process for JCV has yet to be conclusively described. Immune suppression is thought to create conditions suitable for changes in the JCV regulatory region due to unchecked replication and escape from the immune system. It results in the rearrangement of the virus from its latent "archetype" form to the more pathogenic "prototype" form which is haematogenously disseminated to the brain, infecting the oligodendrocytes causing cell lysis and demyelination (White and Khalili, 2011).

PML has also been documented to occur in patients who are immunocompetent, although it is rare (Naess *et al.*, 2010, Gourineni *et al.*, 2014, Tan *et al.*, 2011). The exact proportion of JCV infected immunocompetent individuals who go on to develop PML is not known. Individual case reports have been documented. Naess et al reports of a previously healthy 35-year-old male with a CD4+ count of 994 cells/μL who was diagnosed with PML by brain biopsy (Naess *et al.*, 2010). Goureneni et al report a case of a 62-year-old immunocompetent woman with no past medical history diagnosed with PML (Gourineni *et al.*, 2014).

JC Virus Serology

Because infection by JCV is a prerequisite for PML development, serologic detection of anti-JCV antibodies prove to be a sensitive means to determine current or past infection with JCV. These tests have been employed successfully to determine the seroprevalence of JCV and subsequent risk for developing PML in multiple sclerosis patients who are on *natalizumab* (Lee *et al.*, 2013). The JCV antibody level generally remain stable throughout the life of an individual. However, just before the onset of PML there is a mild to moderate increase in anti-JCV antibody levels as was noted in a cohort of multiple sclerosis patients who were followed up closely for development of PML (Warnke *et al.*, 2013). However, these antibodies offer no protection against the development of PML. Monitoring of anti-JCV antibody levels could potentially be used as a tool for prediction or earlier diagnosis of PML in patients with severe immunosuppression (cd4<200) or in patients on immunomodulatory therapy.

Various studies have been done to determine the seroprevalence of JCV and the rates varied from 39% to 91% depending on assay methodology and population studied. A European study conducted in Switzerland amongst 400 healthy blood donors indicated that anti-JCV seropositivity was 58% in 20-29-year age group and increased to 68% in the 50-59-year age group (Egli *et al.*, 2009). A large multinational study amongst Multiple Sclerosis patients selected from Europe, Canada and Australia found the

prevalence to be 57.1 % (Bozic *et al.*, 2014). However, nothing is known about JCV seroepidemiology in Africa.

Age, gender and geographical location are the most important factors associated with JCV seropositivity. Increasing age and the female gender were associated with increased JCV seroprevalence in large multinational studies carried out amongst multiple sclerosis patients (Bozic *et al.*, 2014, Olsson *et al.*, 2013). However, a subsequent smaller study in Portugal in multiple sclerosis patients found a similar association with age but not with gender (da Silva and Santos, 2014).

As a means of detecting JCV seropositivity, the FDA has approved the second generation ELISA test called the Stratify JCV Antibody ELISA test (Lee *et al.*, 2013). The validated second-generation JCV antibody ELISA offers improved assay design as a kit and enhanced performance characteristics that advance routine clinical use of the assay as a PML risk stratification tool. This is the test that we will employ in our study to detect JCV antibodies.

The sensitivity of JCV DNA detection in blood vs. JCV antibody testing using Stratify was tested in a group of Multiple sclerosis patients. In the study 72% of patients in the higher risk category for PML had not been identified by PCR but had positive JCVAb status (Kinsella *et al.*, 2012).

2.2 Progressive Multifocal Leucoencephalopathy (PML)

PML is a rare demyelinating disease of the white matter in the brain, caused by a lytic infection of the oligodendrocytes by the JCV. It occurs mostly in patients with immunosuppression but is also known to occur in immunocompetent patients as described above.

Risk Factors

HIV has been identified as the major risk factor for the development of PML and according to studies constitutes 80% of patients with PML. Other risk factors identified

by Gheuens et al include hematologic malignancies (13%), organ transplant recipients (5%), and autoimmune diseases treated with immunomodulators (3%) (Gheuens *et al.*, 2010).

Prior to the widespread use of HAART, PML prevalence was 1 to 5 percent in United States and Europe (Levy, Bredesen and Rosenblum, 1985, Holman *et al.*, 1991). Since the widespread use of HAART, the incidence of PML in HIV has decreased (Hansen *et al.*, 2009, Sacktor, 2002). In a population based study from Denmark involving a nationwide cohort of patients aged 16 and older with HIV infection, the incidence of PML declined over three observational periods as follows: (Hansen *et al.*, 2009)

- 1995 to 1996 (pre-HAART): 3.3 cases/1000 patient-years at risk (PYR)
- 1997 to 1999 (early HAART): 1.8 cases/1000 PYR
- 2000 to 2006 (late HAART): 1.3 cases/1000 PYR

In Denmark the seroprevalence of JCV was carried out amongst Multiple Sclerosis patients and was noted to be 52.6% (Olsson *et al.*, 2013).

PML Epidemilogy

One of the major unanswered questions of PML epidemiology is whether it is less frequent in Africa than in the West. It is estimated that approximately 5% of JCV seropositive HIV patients will develop PML (Saribas *et al.*, 2010). A study carried out amongst Multiple Sclerosis patient showed that if a patient tested positive for the antibodies then their risk of PML increased to 1 in 500. For those that tested negative their risk was close to zero (My-ms.org, 2019).

Africa, and specifically sub-Saharan Africa, has a very high burden of HIV infection. However, there are very few reports of PML. Only a few PML cases have been diagnosed by autopsy among HIV positive patients in Uganda and Gambia (S C Chima *et al.*, 1999). PML prevalence from an autopsy series from Ivory Coast by Lucas et al among 271 HIV positive patients showed that PML occurred in only 4 cases (1.5%) (Sylvester C Chima *et al.*, 1999). From Malawi, two cases of PML diagnosed by CT of the brain were found in an investigational diagnosis of stroke in the HIV population

(Kumwenda *et al.*, 2005). Another South African report described an HIV positive young woman with a CD4 count of 7 cells/mm^3 and a high viral load who had presented with seizures. CT of the brain showed lesions typical of PML and diagnosis was later confirmed on post mortem (Modi, 2008).

The seroprevalence of HIV in Zambia is 12% (ZAMPHIA, 2019). In the study of CNS opportunistic infections in HIV positive Zambian adults (COINZ), JCV DNA was detected in 20/331 (6%) of CSF samples of HIV positive Zambians presenting with signs and symptoms consistent with CNS infection (Siddiqi *et al.*, 2014).

Diagnosis and Treatment

PML diagnosis itself is a challenging task for clinicians. The consensus statement from the Neuroinfectious Disease Section of the American Academy of Neurology (AAN) released in April 2013 specifies PML diagnostic criteria providing two diagnostic algorithms (Berger *et al.*, 2013):

- one clinical; based on clinical manifestations, imaging tests and laboratory results; and
- one histopathological; based on histopathological findings.

Unequivocal diagnosis of PML through the clinical algorithm requires presence of characteristic symptoms together with imaging findings and JCV DNA identified in the CSF by PCR. Histopathological algorithm requires demonstration of the histopathological triad (demyelination, bizarre astrocytes and enlarged oligodendroglial nuclei) and JCV detection either by immunohistochemistry or electron microscopy, or/and by tissue PCR for JCV.

Characteristic clinical findings include:

- Cognitive impairments
- Motor dysfunctions
- Visual deficits

Imaging evidence from MRI or CT include:

- Subcortical white matter lesions
- Non-contrast enhancing

Laboratory detection of JCV DNA or protein in CSF

- PCR analysis of CSF
- In situ DNA hybridization in brain tissue
- In situ immunocytochemistry in brain tissue

There is no specific treatment for PML, which has a high mortality rate. Therefore, the main approach is restoring the host adaptive immune response, a strategy that appears to prolong survival. Implementation of this strategy differs according to the clinical setting:

- Initiating or optimizing effective antiretroviral therapy for patients with HIV infection.
- Withdrawing immunosuppressive drugs (when possible) for patients without HIV infection.

CHAPTER 3

METHODOLOGY

This study was nested in the TB meningitis in Zambia (TMZ) study, which was carried out at UTH over a period of 3 years (2015 – 2018). The TMZ study looked at improving ways of diagnosing TB meningitis in the Zambian population who presented with signs and symptoms consistent with a CNS infection. It compared CSF Xpert MTB / RIF and LAM lateral flow dipstick to standard TB cultures. It consisted of 550 patients, ages 18 and older, divided into HIV positive and HIV negative groups who had presented with features of meningoencephalitis and had received a lumbar puncture as part of their evaluation.

3.1 Study Design

This was a cross sectional study.

3.2 Study Population

Study subjects were selected from the parent TMZ study as stated above. They were all 18 years and older, black Zambians who had presented with features of meningoencephalitis and had received a lumbar puncture. For our study, the first 50 HIV negative patients from the TMZ study, who met our study criteria were consecutively selected. The HIV positive group was then matched based on age and gender.

3.3 Study Size

A total of 100 patients were selected divided into 50 HIV positive and 50 HIV negative patients from the two arms of the TMZ study.

Sample size was calculated using the power calculation with an estimation of approximately 30% JCV seropositivity in non- PML patients in Zambia vs 55% in historical controls in the West as determined by Gorelik et al (Gorelik *et al.*, 2010a).

Sample size of 100 provided us a 97.7% power to detect a 25% difference in JCV seroprevalence between the two populations.

14 confirmed PML cases based on clinical, laboratory and radiological criteria were also selected. There was an intention to select 20 confirmed PML patients; however, from the TMZ study only 14 patients were confirmed as PML. The sample size of 20 was selected from the previous COINZ study carried out at UTH where 6% of patients were found to have PML. Thus, of the 363 HIV positive study participants from the TMZ study, we were expected to recruit 20 patients with PML.

3.4 Study Site

Inpatient and outpatient population of patients who presented to department of medicine at University Teaching Hospital during the timeframe of the study.

3.5 Inclusion Criteria

GROUP A– 100 (50 HIV+, 50 HIV -) PATIENTS WERE SELECTED FROM THE MEDICAL WARD.

1. African adults ages 18 and above.
2. Willingness to do the HIV test.
3. Consent for lumbar puncture, bloods and urine.

GROUP B

1. 20 confirmed PML cases.

3.6 Exclusion Criteria

1. Unwilling to give consent.
2. Zambians of non- African origin

3.7 Case Definition of PML

Diagnosis of PML was established using the AAN guidelines stated above (Berger *et al.*, 2013). These included: 1) a compatible clinical picture; 2) typical radiological findings and 3) PCR detection of JCV in the CSF.

Histopathological diagnosis was not used in our study.

Clinical criteria included:

- A sub-acute clinical presentation with focal neurologic deficits, such as weakness, speech difficulties, unsteady gait hemiparesis, dysarthria, memory impairment and behavior changes.
- Ophthalmic symptoms, such as homonymous hemianopia which progresses to cortical blindness.

Radiological criteria included:

- CT images, which were, hypo dense lesions in the white matter without enhancement and mass effect in the occipitoparietal regions.
- In the more sensitive T2-wighted MRI, they would be hyper intense, without gadolinium enhancement.

Each case meeting the clinical and radiological criteria had PML confirmed by PCR detection of JCV in the CSF.

3.8 Procedure:

Ethics approval was sought from the University Of Zambia School Of Medicine Biomedical Research Ethics Committee and the Director of UTH (IRB00001131 of IORG0000774). Patients were then selected from the TMZ study.

All patients recruited in the TMZ study had undergone an HIV test as part of Provider Initiated HIV counseling and Testing (PICT) carried out at UTH and were divided into

two arms; HIV positive and HIV negative. Using the exclusion and inclusion criteria above, patients were selected from the two arms of the TMZ study.

Based on our sample size calculation for the JCV study we selected 50 patients each from the two arms. In addition, a further 14 confirmed PML cases were recruited from the parent study. In addition to clinical presentation, confirmation of PML diagnosis was made by a positive JCV DNA in the CSF. Imaging was only possible in two of the fourteen patients as the MRI and CT machine were not functional during most of the course of the study.

Sampling of the participants was done by selecting the first consecutive 50 HIV negative patients recruited in the TMZ study which met the inclusion criteria. The HIV positive group was then matched based on **gender** and **age**. The PML patients were selected based on convenience sampling as PML was noted to be relatively rare. There was an intention to recruit 20 confirmed PML patients in this study; however, only 14 patients were diagnosed as PML from the original TMZ study.

At the time of enrollment of the TMZ study, a detailed history and a full neurological examination was performed on all participants. Patients who had already received a lumbar puncture as part of their routine evaluation were asked if excess CSF be used as part of this study. The CSF from all HIV positive and HIV negative underwent qualitative testing to detect the presence of JCV by using DNA PCR (Qiagen molecular kit). Those with a positive JCV DNA in CSF were diagnosed as PML together with a compatible clinical picture. CNS imaging was only possible in two patients. A blood sample was also collected from the consenting patients and a CD4 count done on all the participants. The remaining blood was stored and sera aliquoted and kept frozen at -20 C. Frozen sera was sent to the German Cancer Research Centre (DKFZ) in Heidelberg, Germany and JCV serology was performed using commercial JCV ELISA (Quest Diagnostics). The samples were analysed at a dilution of 1:10000 and an antibody titre to the JCV capsid (VP1) >70 indicated JCV seropositivity. From the 114 samples sent to Germany, four were excluded from the final analysis, as they did not meet quality control (samples leaked out in the shipping process). These included three samples from the HIV negative group and one sample from the HIV positive group.

Except for the JCV ELISA assay all the other tests were done here in Zambia at the molecular biology laboratory already established at UTH. The cost of sending and analyzing 114 samples in Germany was 4,680 USD.

A summary of the study methodology is shown in the figure below:

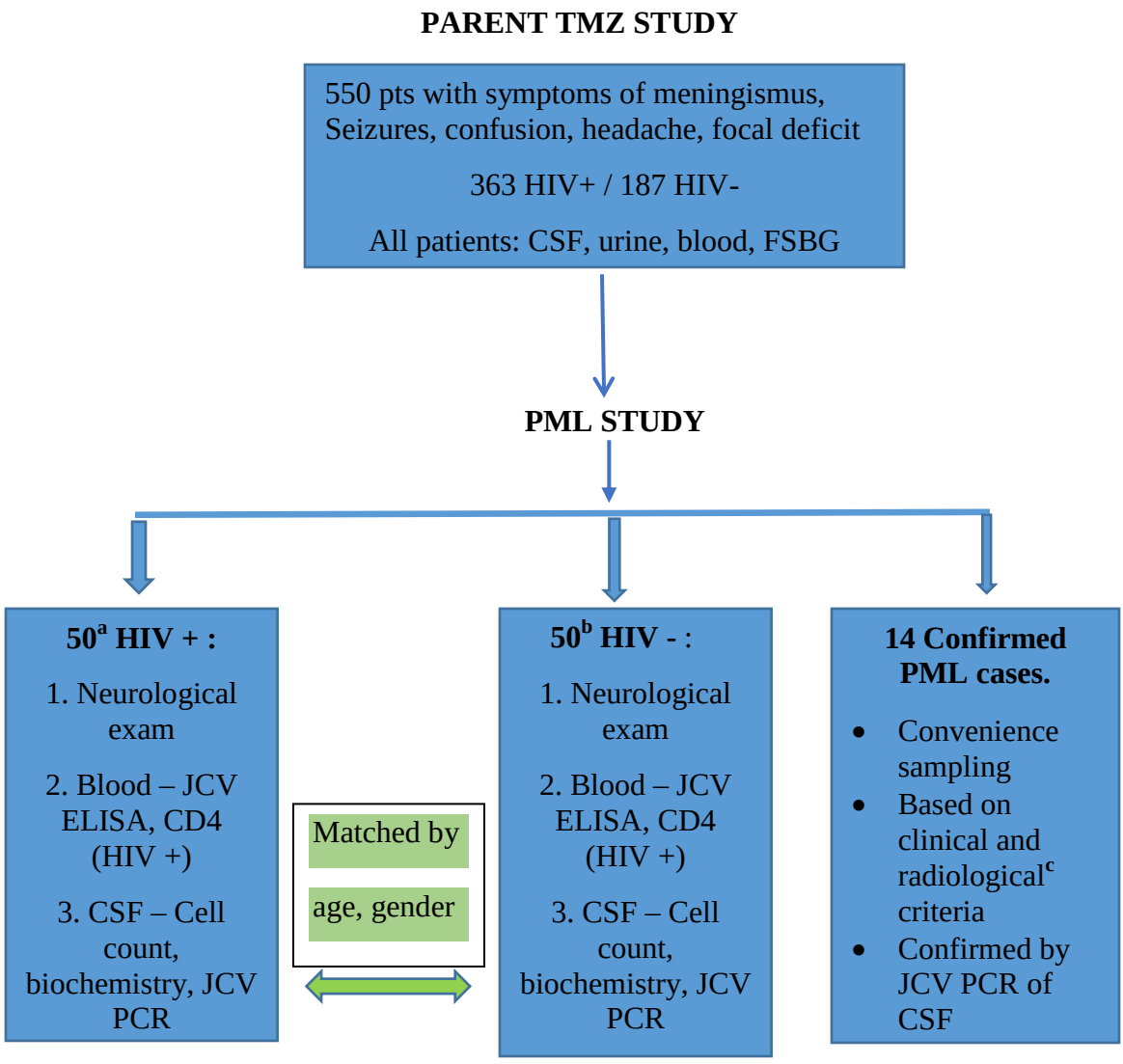

Figure 1: Summary of study Procedure

a: 49 samples analysed, 1 excluded (did not meet quality control)

b: 47 samples analysed, 3 excluded (did not meet quality control)

c: CNS imaging only possible in 2 patients due to non-functioning CT and MRI during study period.

3.9 Data Analysis

3.9.1 Outcome variables

Dependent Variables:

- JC Virus positive serostatus

Independent Variables:

- age
- sex
- HIV status
- Immune status CD4
- Memory Impairment
- GCS
- Vision loss
- Headache
- Fever
- Irrelevant speech
- Seizures
- TB Meningitis

3.9.2 Statistical Analysis

Results were analysed using Epi Info7. Patients with anti-JCV test results were included in the study. Descriptive statistics were used for overall prevalence and prevalence by demographics and clinical characteristics. Our study sample was compared to the parent TMZ study using Chi square tests. Factors associated with JCV seroprevalence were analysed using multiple logistics regression using odds ratio as a measure of association.

The multiple logistic regression model was also used for PML risk stratification was by comparing the advanced HIV JCV seropositive patients with the PML patients in the initial model and then comparing only the borderline insignificant variables in the final model (p-value ≤ 0.06).

CHAPTER 4

RESULTS

4.1 Patient Characteristics

In our study, 100 patients were selected from the parent TMZ study. The selection was matched for age and gender. *Table 1* shows the characteristics of our study sample in comparison to the parent TMZ study.

Table 1: Characteristics of the patients in the JCV study in comparison to TMZ study.

VARIABLE	JCV STUDY N (%)	TMZ STUDY[β] N (%)	P Value
Gender	M = 50 (50.0) F = 50 (50.0)	M = 294 (50.9) F = 284 (49.1)	0.231
Age[a]	39 ± 14.5	37 ± 10.4	0.173
HIV Positive*	**50 (50)**	**509 (88.3)**	**<0.001**
Cd4 (cells/mm^3) [Median IQR]	350 [IQR 123-707]	387 [IQR 48 – 434]	0.690
Headache	72 (72.0)	387 (67.0)	0.712
Seizures	18 (18.0)	104 (18.0)	0.915
Fever	49 (49.0)	328 (56.8)	0.058
Irrelevant Speech	45 (45.0)	294 (50.8)	0.577
Focal Neurological Deficit	25 (25.0)	155 (26.8)	0.339
GCS (Mean ±SD)	12.64 ± 2.68	12.42 ± 3.15	0.809
Memory Impairment*	**42 (42.0)**	**181 (31.3)**	**0.011**
TB Meningitis	26 (26)	151 (26.1)	0.876

* Statistically significant

β Parent Study (TB Meningitis Study)

Mean ±SD

Median [25%IQR-75%IQR]

In the TMZ study, 88% of the patients were HIV positive. The average age of our study population (JCV study) was 39 ± 14.5 years. The average CD4 count was 350 cells /mm^3 (IQR 123 -707). 26 (26%) had a confirmed diagnosis of TBM. Memory impairment was noted to be borderline significant different between the two study populations (p- value 0.011) with slightly more patients recruited into JCV study from the main TMZ study.

3.5 JCV Seroprevalence

The JCV seroprevalence in our study population was found to be 46% (95% CI, 35.62 – 56.31). This was done in 96 patients as four patients were excluded because their samples went missing (possibly leaked) in the shipping process.

Factors that would possibly affect the JCV antibody positivity were analysed as shown in *table 2*.

The JCV seroprevalence in the HIV positive group was noted to be 40.82% and in the HIV negative group was 51.06 % but there was no statistical difference (*p*-value 0.31). Patients with meningoencephalitis who were HIV positive were 34% less likely to be JCV positive but chance could not be ruled out (crude OR, 0.66; 95% CI, 0.29 – 1.48).

None of the variables studied had any significant effect on determining JCV seropositivity individually as well as after adjusting for the other variables studied. This included gender as well as age.

We found a bimodal distribution of age associated with JCV seropositivity in our study population of patients with suspected meningoencephalitis; with one peak occurring in the 18 to 20 years age group and the second peak occurring in the 55 to 60 years age group as shown in *figure 2*.

Table 2: Factors associated with JCV antibody positivity

VARIABLE	CRUDE OR (95% CI)	*P* value	ADJUSTED OR (95% CI)	*P* value
Gender	1.10 (0.49 – 2.44)	0.82	1.06 (0.45 – 2.48)	0.89
Age (years)	1.00 (0.97 – 1.03)	0.90	1.00 (0.97 – 1.03)	0.96
Age Category (years)				
0 = <30	1.00		1.00	
1 = 30 – 54	0.82 (0.32 - 2.05)	0.66	0.84 (0.33 - 2.16)	0.72
2 = ≥ 55	1.25 (0.41 - 3.75)	0.70	1.14 (0.35 - 3.72)	0.83
HIV Status	0.66 (0.29 - 1.48)	0.31	0.68 (0.26 - 1.74)	0.42
TBM confirmed	0.82 (0.33 - 2.04)	0.31	0.99 (0.34 - 2.91)	0.98
Vision loss	1.20 (0.28 - 5.10)	0.80	1.02 (0.19 - 5.49)	0.98
Seizures	0.93 (0.33 - 2.62)	0.90	1.10 (0.35 - 3.45)	0.88
Headache	1.33 (0.54 - 3.28)	0.53	1.17 (0.42 - 3.25)	0.77
Fever	0.70 (0.31 - 1.58)	0.39	0.85 (0.35 - 2.08)	0.72
Irrelevant Speech	1.14 (0.50 -2.55)	0.76	1.41 (0.57 - 3.47)	0.45
Focal Neurological Deficit	1.96 (0.77 - 5.00)	0.16	1.68 (0.56 - 5.04)	0.35
Memory Impairment	0.62 (0.27 - 1.40)	0.25	0.61 (0.23 - 1.62)	0.32
GCS	1.07 (0.92 – 1.25)	0.37	1.07 (0.92 – 1.25)	0.39
GCS Category				
0 = 13 – 15	1.00		1.00	
1 = 9 – 12	0.87 (0.36 - 2.11)	0.76	0.87 (0.36 - 2.11)	0.69
2 = < 9	0.43 (0.08 - 2.38)	0.33	0.43 (0.08 - 2.38)	0.22
Cd4 (cells/mm^3)	1.00 (1.00 – 1.00)	0.67	1.00 (1.00 – 1.00)	0.70
Cd4 category (cells/mm^3)				
0 = >500	1.00		1.00	
1 = 350 – 499	1.19 (0.28 - 5.10)	0.82	1.15 (0.26 - 5.02)	0.86
2 = 200 -349	0.71 (0.21 - 2.44)	0.59	0.71 (0.20 - 2.52)	0.59
3 = <200	0.59 (0.23 - 1.50)	0.26	0.62 (0.24 - 1.59)	0.32
INPATIENT OUTCOME	0.55 (0.21 - 1.45)	0.22	0.95 (0.27 - 3.31)	0.94
1 YEAR OUTCOME	0.49 (0.21 – 1.12)	0.09	0.61 (0.23 - 1.64)	0.33

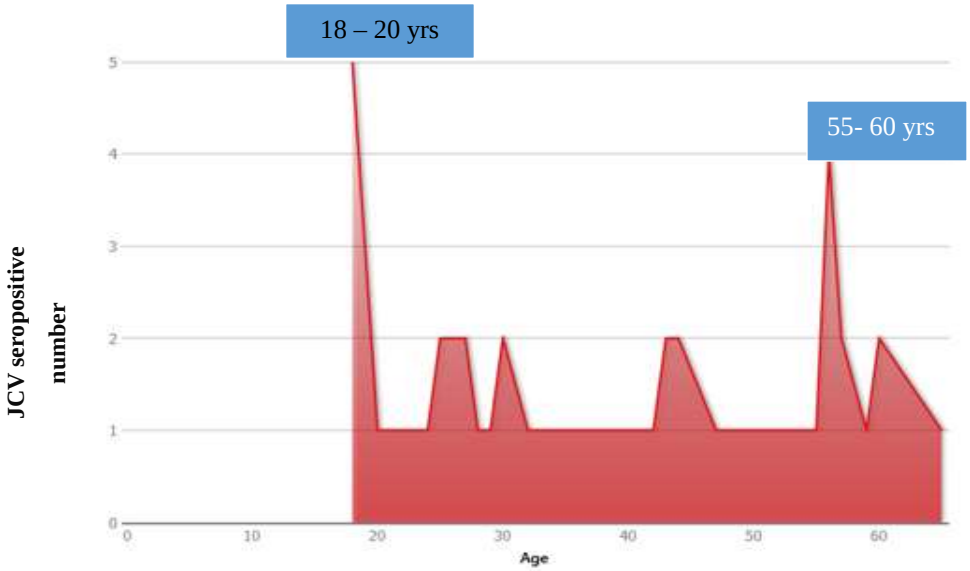

Figure 2: Relationship of age with JCV seropositive status

3.6 PML

Fourteen confirmed PML patients were recruited in the study from the parent TMZ study. Brain imaging was only possible in two of the patients and showed findings typical of PML. These are shown in *figure 2 and 3* respectively. Because of the limitation of availability of brain imaging during the course of the study (see methods section) we used a case definition of CSF PCR positive for JC virus as PML cases based on another study in the institution (Siddiqi *et al.*, 2014).

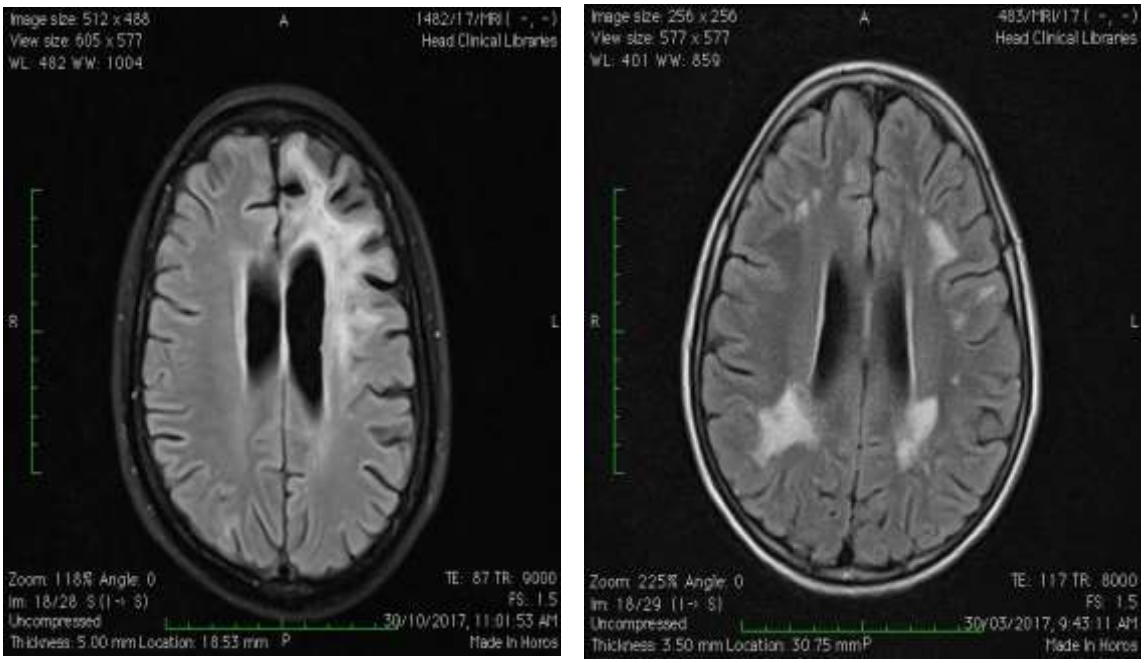

*Figure 3 and 4**:** MRI findings in 2 PML patients:* The axial images in fluid attenuated inversion recovery (FLAIR) show PML lesions in the left frontal lobe (left image) and in the right and left parietal lobes and the left frontal lobe of another (right image).

3.7 PML risk stratification

All the PML patients were JCV serology positive as well as HIV positive with severe immunosuppression (Cd4 < 200cells/mm^3). We therefore limited our comparison of the control group to HIV positive and JCV seropositive patients to the case group of confirmed PML patients in order to come up with a risk stratification score. We initially included all the variables in the model (see *table 3A*). From this model we then removed all the absolutely insignificant variables and kept the borderline insignificant variables namely: gender, memory impairment, GCS and confirmed TB meningitis (see *table 3B*).

Table 3A: PML Risk Stratification (Initial Model)

VARIABLE	CRUDE OR (95% CI)	*P* value	ADJUSTED OR (95% CI)	*P* value
Gender	1.80 (0.56 - 5.75)	0.32	10.46 (0.91 – 120.62)	0.06
Age (years)	0.98 (0.93 - 1.02)	0.26	0.88 (0.78 – 1.00)	0.06
Age Category (years)				
0 = <30	1.00		1.00	
1 = 30 – 54	1.79 (0.52 – 6.21)	0.36	1.19 (0.30 – 4.479)	0.81
2 = ≥ 55	0.01 (0.00 – 1.02)	0.96	0	0.96
TBM confirmed	0.41 (0.10 -1.65)	0.21	0.05 (<0.01 – 0.79)	0.03
Seizures	1.24 (0.29 -5.38)	0.77	0.52 (0.04 – 6.11)	0.60
Headache	0.43 (0.13 – 1.44)	0.17	0.49 (0.08 – 3.19)	0.46
Fever	0.59 (0.18 – 1.95)	0.39	1.07 (0.17 – 6.63)	0.94
Irrelevant Speech	1.95 (0.57 – 6.64)	0.28	3.05 (0.24 – 38.72)	0.39
Focal Neurological Deficit	0.27 (0.03 – 2.32)	0.23	1.05 (0.04 – 26.31)	0.98
Memory Impairment	5.98 (1.48-24.21)	0.01	14.24 (0.90 – 224.79)	0.06
GCS	0.81(0.65 – 1.01)	0.06	0.72 (0.47 – 1.11)	0.14
GCS Category				
0 = 13 – 15	1.00		1.00	
1 = 9 – 12	3.17 (0.87 – 11.58)	0.08	2.77(0.73 – 10.59)	0.14
2 = < 9	1.76 (0.16 – 19.48)	0.64	4.39 (0.24 – 79.82)	0.32
[β]Cd4 (cells/ml)	0.99 (0.99- 1.00)	0.04		
[β]Cd4 category (cells/ml)				
0 = >500	1.00		1.00	
1 = 350 – 499	3.00 (0.25 – 35.33)	0.38		
2 = 200 -349	0.75 (0.08 – 7.21)	0.80		
3 = <200	1.83 (0.28 – 12.19)	0.53		
INPATIENT OUTCOME	1.42 (0.37 – 5.41)	0.61	4.35 (0.24 – 79.36)	0.32
1YEAR OUTCOME	0.82 (0.36 – 1.87)	0.63	0.21(0.02 -2.27)	0.21

* Only HIV positive patients analysed as no HIV negative patients had confirmed PML

* Vision loss not analysed as had none observed for PML confirmed.

[β] Cd4 was excluded as PML patients were all severely immunosuppressed.

Table 3B: PML risk stratification (Final Model) - HIV + with severe immunosuppression

VARIABLE	CRUDE OR (95% CI)	*P* value	ADJUSTED OR (95% CI)	*P* value
*Gender	1.80 (0.56 - 5.75)	0.32	7.12 (1.28 – 39.70)	0.02
*Age (years)	0.98 (0.93 - 1.02)	0.26	0.92 (0.85 – 0.99)	0.02
**Memory Impairment	5.98 (1.48-24.21)	0.01	20.85 (3.07 – 141.68)	<0.01
*TBM confirmed	0.41 (0.10 -1.65)	0.21	0.13 (0.02 – 0.82)	0.03

Memory impairment was noted to be the most important variable associated with PML development in our study population of patients with suspected meningoencephalitis. There was a 6 fold increased risk of having PML with memory impairment which increased to over 20 fold increased risk after adjusting for age, gender and confirmed TB meningitis.

In our study population (i.e. meningoencephalitis patients with advanced HIV disease presenting to UTH) we found female gender to have an 80% association with PML but chance could not be ruled out. However, this association went up to a 7 fold increased risk and became statistically significant after adjusting for age, memory impairment and underlying TB meningitis (p-value 0.02).

A confirmed diagnosis of TBM was associated with a 60% reduction in the likelihood of having PML though this was statistically insignificant (p-value 0.21). However, the associated protection went up to 87% and became statistically significant after adjusting for age, gender and memory impairment (p-value 0.03).

A younger age group was associated with a 2% protection for PML though chance could not be ruled out (p-value 0.26). The protection increased to 8%, which became statistically significant after adjusting for gender, memory impairment and a confirmed diagnosis of TBM (p-value 0.02).

CHAPTER 5

DISCUSSION AND LIMITATIONS

5.1 Discussion

Worldwide the seroprevalence of JCV varies according to the geographical region. In our study population of patients with meningoencephalitis, the overall seroprevalence rate was found to be 46%, which was slightly lower than that of the West. There is no documentation of JCV seroprevalence in Africa. A large multicenter study conducted in nine countries in Europe showed an overall prevalence of 57.6% (Olsson *et al.*, 2013). Gorelik et al. observed higher prevalence of anti-JCV antibody in Europe and North America compared to Australia and New Zealand (Gorelik *et al.*, 2010). This difference could be attributed to the differences in the study populations. The European and North American studies were carried out in predominantly Caucasian patients with multiple sclerosis. Environmental differences could also be attributed to this difference. Our population consisted of patients from a predominant urban setting with suspected CNS infection.

None of the factors we studied were associated with JCV seroprevalence such as age and gender. Interestingly JCV seroprevalence was noted to have a bimodal peak with age in our study. Highest seroprevalence was noted in the 18-20 years age group followed by the 50-55 year age group. This differs from the trends in previous studies where increasing age is the main factor associated with JCV seropositivity. A European study indicated that anti-JCV seropositivity was 58% in 20–29-year age group and increased to 68% in 50–59-year age group (Egli *et al.*, 2009). This was subsequently confirmed by two other studies (Olsson *et al.*, 2013, Bozic *et al.*, 2014). This differing trend could be due to the difference in our study population which consisted of African patients with meningoencephalitis. We also had a smaller sample size compared to the European studies.

No significant difference in gender and JCV seroprevalence was noted in our study. This is in contrast to two European studies where male gender was noted to be a significant association to JCV seropositivity (Bozic *et al.*, 2014, Olsson *et al.*, 2013). In Olsson's study the seroprevalence of females to males was 55.8% versus 61.9%; $p < 0.0001$. This again could be due to the differences in population studied, as our patients were all Africans with suspected meningoencephalitis and from a mostly urban setting.

Overall, we found there was no apparent difference in anti-JCV antibody prevalence between HIV positive and HIV negative patients and the level of immunosuppression (cd4 count) which is consistent with literature on how JCV is acquired (Bofill-Mas *et al.*, 2001).

14 (3.2%) patients were diagnosed with PML from the TMZ study. This interestingly differs from the previous COINZ study carried out at the same institution in a similar population which showed an incidence of 6% (Siddiqi *et al.*, 2014). This could possibly be due to improved PICT and early treatment with ART for HIV positive patients. However, it could also be explained by fact that we excluded confirmed cryptoccocosis patients which were part of the previous study population. A similar trend was observed in a Danish study of HIV patients where the incidence rate of PML was 3.3,1.8 and 1.3 cases per 1000 person-years at risk in 1995-1996, 1997-1999, and 2000-2006 respectively (Hansen *et al.*, 2009). This improvement was due to the evolution of the anti-retroviral treatment in this population. Another possibility of the lower incidence, is that our study mostly consisted of patients with meningoencephalitis. PML patients, who may have mimicked 'strokes', may have been missed, as they might not have undergone CSF studies.

As JCV infection is a prerequisite for PML, as expected, all the PML patients were seropositive for JCV. They were all HIV positive with severe immunosuppression ($CD4<200$ cells/mm^3).

PML is a fatal disease and without any intervention (pre ART) has a survival rate of 9% at one year (Berger *et al.*, 1998). Early ART treatment in cases of HIV or withdrawal of immunosuppressive drugs in other cases of PML, has been shown to improve survival

rates: up to 30% at 1 year (Study *et al.*, 2009). Various PML risk assessment scores have been used in order to predict the likelihood of patients having PML. However, these studies have been done in multiple sclerosis patients receiving *natalizumab* treatment and looked at factors such as JCV antibody status (anti-JCV antibody index), previous immunosuppressant use and treatment duration (Ho *et al.*, 2017). We looked at our study population (advanced HIV positive , JCV seropositive) and tried to see if they were any factors associated with the likelihood of having PML in patients with severe immunosuppression (CD4 <200mm^3) either alone or after adjusting for other variables. We noted that memory impairment was the most important factor associated with a 6 fold increased risk of having PML independently which increased to over 20 fold increased risk after adjusting for age, gender and confirmed TB meningitis.

After adjusting for age, gender, memory impairment and confirmed TBM diagnosis, it was noted that the female gender was associated with a 7 fold increased risk of PML and a younger age group was associated with an 8% protection for PML. A confirmed diagnosis of TBM was associated with an 87% protection against the likelihood of having PML while adjusting for age, gender and memory impairment. However independently TBM did not appear to protect against development of PML. This shows that possibly patients with a definitive alternative diagnosis for their presentation like TBM are less likely to have an additional infection like PML than those with no alternative diagnosis.

5.2 Limitations

Our study had several limitations:

- This was a nested study in the TMZ study. As such, the study population and their characteristics (patients with symptoms of meningoencephalitis) limited our study. In addition, any findings are suggestive but not conclusive for assoociations as the strength of the study was limited by the design.

- The cost of carrying out the ELISA test for JCV antibodies is expensive and needed a specialized laboratory (DKFZ in Heidelberg, Germany). This limited our sample size.
- We were unable to do CNS imaging on all of the PML patients to add more weight to the diagnosis as both the CT and MRI machines were non-functional during the course of the study (24 months). Attempts were later made to contact the surviving PML patients to carry out the imaging but we were successful in only two of the patients.
- We were unable to carry out histopathological diagnosis of PML by virtue of brain biopsies as this procedure is not done in UTH.
- HIV testing on patients was based on antibody testing and not by PCR. Thus, it might have been possible to erroneously label some patients as HIV negative who might have been in the HIV window period.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The prevalence of anti-JCV antibodies in our study population of patients with suspected CNS infection (meningoencephalitis) was 46% (UTH, Lusaka, Zambia). This was slightly lower then figures obtained from the West. Anti- JCV antibody prevalence did not differ significantly by age, gender, HIV status or CD4 count. Memory impairment in JCV seropositive, advanced HIV positive patients with meningoencephalitis was the most important variable associated with the likelihood of having PML. After adjusting for other variables, male gender, a younger age and diagnosis of TBM were associated with a less likelihood of having PML. The latter association (TBM) needs to be explored further in future studies.

6.2 Recommendations

After completing our study, we had the following recommendations:

- We need to extend the JCV serology testing (if cost of test comes down) to other groups of people who may be at risk of getting PML e.g. patients on immunomodulatory therapy, which includes post organ transplant patients, cancer patients, auto-immune disease patients, Crohns disease and Multiple Sclerosis patients. Those who test positive will need to be followed up closely in order to make better informed benefit–risk evaluation and treatment decisions.
- There is a need to create a data bank of all the JCV positive immunosuppressed patients in our study so that they can be followed up closely to monitor for the development of PML.

- To extend the JCV seroprevalence testing to reported healthy adults in Zambian population to see if similar results are replicated.
- We need to develop and validate a tool for assessing early memory impairment in our setting for patients with HIV and low CD4 counts (< 200 cells/mm^3) to allow for early detection of PML.
- Further studies are needed to confirm and explore the mechanisms behind the possible protective association of TBM and PML that our study showed.

REFERENCES

- Avert. (2019). *HIV and AIDS in Zambia*. [online] Available at: https://www.avert.org/professionals/hiv-around-world/sub-saharan-africa/zambia [Accessed 27 Apr. 2019].
- Berger, J. R. *et al.* (2013) 'PML diagnostic criteria: consensus statement from the AAN Neuroinfectious Disease Section', *Neurology*. Lippincott Williams & Wilkins, 80(15), pp. 1430–1438. doi: 10.1212/WNL.0b013e31828c2fa1.
- Bofill-Mas, S. *et al.* (2001) 'Potential transmission of human polyomaviruses through the gastrointestinal tract after exposure to virions or viral DNA', *Journal of virology*. American Society for Microbiology, 75(21), pp. 10290–10299. doi: 10.1128/JVI.75.21.10290-10299.2001.
- Bozic, C. *et al.* (2014) 'Anti-JC virus (JCV) antibody prevalence in the JCV Epidemiology in MS (JEMS) trial', *European Journal of Neurology*. John Wiley & Sons, Ltd (10.1111), 21(2), pp. 299–304. doi: 10.1111/ene.12304.
- Brew, B. J. *et al.* (2010) 'Progressive multifocal leukoencephalopathy and other forms of JC virus disease', *Nature Reviews Neurology*. Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved., 6, p. 667. Available at: http://dx.doi.org/10.1038/nrneurol.2010.164.
- Chima, S. C. *et al.* (1999) 'Progressive multifocal leukoencephalopathy and JC virus genotypes in West African patients with acquired immunodeficiency syndrome: a pathologic and DNA sequence analysis of 4 cases', *Arch Pathol Lab Med*, 123(5), pp. 395–403. doi: 10.1043/0003-9985(1999)123<0395.
- Chima, S. C. *et al.* (1999) 'Progressive Multifocal Leukoencephalopathy and JC Virus Genotypes in West African Patients With Acquired Immunodeficiency Syndrome', *Archives of Pathology & Laboratory Medicine*. College of American Pathologists, 123(5), pp. 395–403. doi: 10.1043/0003-9985(1999)123<0395:PMLAJV>2.0.CO;2.
- Egli, A. *et al.* (2009) 'Prevalence of Polyomavirus BK and JC Infection and Replication in 400 Healthy Blood Donors', *The Journal of Infectious Diseases*,

199(6), pp. 837–846. doi: 10.1086/597126.

- Feruglio, S. L. *et al.* (2015) ‘Early dynamics of T helper cell cytokines and T regulatory cells in response to treatment of active Mycobacterium tuberculosis infection.’, *Clinical and experimental immunology*. Wiley-Blackwell, 179(3), pp. 454–65. doi: 10.1111/cei.12468.
- Gheuens, S. *et al.* (2010) ‘Progressive multifocal leukoencephalopathy in individuals with minimal or occult immunosuppression’, *Journal of neurology, neurosurgery, and psychiatry*. 2009/10/14, 81(3), pp. 247–254. doi: 10.1136/jnnp.2009.187666.
- Gorelik, L. *et al.* (2010) ‘Anti-JC virus antibodies: implications for PML risk stratification.’, *Annals of neurology*. United States, 68(3), pp. 295–303. doi: 10.1002/ana.22128.
- Gourineni, V. C. *et al.* (2014) ‘Progressive multifocal leukoencephalopathy in a 62-year-old immunocompetent woman’, *Case reports in neurological medicine*. 2014/02/23. Hindawi Publishing Corporation, 2014, p. 549271. doi: 10.1155/2014/549271.
- Guerini, F. R. *et al.* (2008) ‘Analysis of CCR5, CCR2, SDF1 and RANTES gene polymorphisms in subjects with HIV-related PML and not determined leukoencephalopathy’, *Biomedicine and Pharmacotherapy*, 62(1), pp. 26–30. doi: 10.1016/j.biopha.2007.04.005.
- Hansen, A.-B. E. *et al.* (2009) ‘Incidence, Clinical Presentation, and Outcome of Progressive Multifocal Leukoencephalopathy in HIV-Infected Patients during the Highly Active Antiretroviral Therapy Era: A Nationwide Cohort Study’, *The Journal of Infectious Diseases*, 199(1), pp. 77–83. doi: 10.1086/595299.
- Ho, P.-R. *et al.* (2017) ‘Risk of natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: a retrospective analysis of data from four clinical studies’, *The Lancet Neurology*, 16(11), pp. 925–933. doi: https://doi.org/10.1016/S1474-4422(17)30282-X.
- Holman, R. C. *et al.* (1991) ‘Epidemiology of progressive multifocal leukoencephalopathy in the United States: analysis of national mortality and

AIDS surveillance data.', *Neurology*, 41(11), pp. 1733–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1944901 (Accessed: 13 April 2019).

- Kinsella, K. *et al.* (2012) '0854 The sensitivity of JC virus DNA detection vs JC virus antibody testing in a natalizumab-treated group of relapsing MS patients', *Journal of Neurology, Neurosurgery & Psychiatry*, 83(3), p. e1 LP-e1. doi: 10.1136/jnnp-2011-301993.35.
- Kumwenda, J. J. *et al.* (2005) 'Differential diagnosis of stroke in a setting of high HIV prevalence in Blantyre, Malawi.', *Malawi medical journal : the journal of Medical Association of Malawi*. Medical Association of Malawi, 17(4), pp. 107–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27528995 (Accessed: 13 April 2019).
- Lee, P. *et al.* (2013) 'A second-generation ELISA (STRATIFY JCV™ DxSelect™) for detection of JC virus antibodies in human serum and plasma to support progressive multifocal leukoencephalopathy risk stratification', *Journal of Clinical Virology*, 57(2), pp. 141–146. doi: https://doi.org/10.1016/j.jcv.2013.02.002.
- Levy, R. M., Bredesen, D. E. and Rosenblum, M. L. (1985) 'Neurological manifestations of the acquired immunodeficiency syndrome (AIDS): Experience at UCSF and review of the literature', *Journal of Neurosurgery*. Journal of Neurosurgery Publishing Group, 62(4), pp. 475–495. doi: 10.3171/jns.1985.62.4.0475.
- Modi, M. (2008) 'Progressive multifocal leucoencephalopathy - a case report ', *SAMJ: South African Medical Journal* . scieloza , pp. 263–264.
- Monaco, M. C. *et al.* (1996) 'JC virus infection of hematopoietic progenitor cells, primary B lymphocytes, and tonsillar stromal cells: implications for viral latency.', *Journal of Virology*, 70(10), p. 7004 LP-7012. Available at: http://jvi.asm.org/content/70/10/7004.abstract.
- Monaco, M. C. *et al.* (1998) 'Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection', *Journal of virology*. American Society for Microbiology, 72(12), pp. 9918–9923. Available at:

https://www.ncbi.nlm.nih.gov/pubmed/9811728.

- My-ms.org. (2019). *PML*. [online] Available at: http://www.my-ms.org/med_pml.htm [Accessed 27 Jun. 2018].
- Naess, H. *et al.* (2010) 'Progressive multifocal leucoencephalopathy in an immunocompetent patient with favourable outcome. A case report', *BMC neurology*. BioMed Central, 10, p. 32. doi: 10.1186/1471-2377-10-32.
- Olsson, T. *et al.* (2013) 'Anti-JC virus antibody prevalence in a multinational multiple sclerosis cohort', *Multiple Sclerosis Journal*. SAGE Publications Ltd STM, 19(11), pp. 1533–1538. doi: 10.1177/1352458513477925.
- Padgett, B. *et al.* (1971) 'CULTIVATION OF PAPOVA-LIKE VIRUS FROM HUMAN BRAIN WITH PROGRESSIVE MULTIFOCAL LEUCOENCEPHALOPATHY', *The Lancet*. Elsevier, 297(7712), pp. 1257–1260. doi: 10.1016/S0140-6736(71)91777-6.
- Randhawa, P., Shapiro, R. and Vats, A. (2005) 'Quantitation of DNA of Polyomaviruses BK and JC in Human Kidneys', *The Journal of Infectious Diseases*, 192(3), pp. 504–509. doi: 10.1086/431522.
- Rgen, H. È. *et al.* (2000) 'In¯uence of JC virus coding region genotype on risk of leukoencephalopathy', pp. 101–108.
- Sacktor, N. (2002) 'The Epidemiology of Human Immunodeficiency Virus-Associated Neurological Disease in the Era of Highly Active Antiretroviral Therapy', *Journal of Neurovirology*, 8(2), pp. 115–121. doi: 10.1080/13550280290101094.
- Saribas, A. S. *et al.* (2010) 'JC virus-induced Progressive Multifocal Leukoencephalopathy', *Future virology*, 5(3), pp. 313–323. doi: 10.2217/fvl.10.12.
- Shankar, S. K. *et al.* (2003) 'Low prevalence of progressive multifocal leukoencephalopathy in India and Africa: Is there a biological explanation?', *Journal of NeuroVirology*, 9(1), pp. 59–67. doi: 10.1080/13550280390195397.
- Siddiqi, O. K. *et al.* (2014) 'Molecular diagnosis of central nervous system opportunistic infections in HIV-infected Zambian adults', *Clinical infectious*

diseases : an official publication of the Infectious Diseases Society of America. 2014/03/25. Oxford University Press, 58(12), pp. 1771–1777. doi: 10.1093/cid/ciu191.

- da Silva, A. M. and Santos, M. E. (2014) 'JCV epidemiology in MS (JEMS)—Epidemiology of anti-JCV antibody prevalence in multiple sclerosis patients—Portuguese data', *Journal of the Neurological Sciences*. Elsevier, 337(1), pp. 119–122. doi: 10.1016/j.jns.2013.11.031.
- Study, for the S. H. I. V. C. *et al.* (2009) 'Incidence and Outcome of Progressive Multifocal Leukoencephalopathy over 20 Years of the Swiss HIV Cohort Study', *Clinical Infectious Diseases*, 48(10), pp. 1459–1466. doi: 10.1086/598335.
- Tan, C. S. *et al.* (2009) 'Detection of JC virus DNA and proteins in the bone marrow of HIV-positive and HIV-negative patients: implications for viral latency and neurotropic transformation', *The Journal of infectious diseases*, 199(6), pp. 881–888. doi: 10.1086/597117.
- Tan, C. S. and Koralnik, I. J. (2010) 'Progressive multifocal leukoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis', *The Lancet. Neurology*, 9(4), pp. 425–437. doi: 10.1016/S1474-4422(10)70040-5.
- Tan, I. L. *et al.* (2011) 'Progressive multifocal leukoencephalopathy in a patient without immunodeficiency', *Neurology*. Lippincott Williams & Wilkins, 77(3), pp. 297–299. doi: 10.1212/WNL.0b013e318225ab3f.
- Warnke, C. *et al.* (2013) 'Changes to anti-JCV antibody levels in a Swedish national MS cohort', *Journal of neurology, neurosurgery, and psychiatry*. 2013/03/05. BMJ Publishing Group, 84(11), pp. 1199–1205. doi: 10.1136/jnnp-2012-304332.

APPENDICES

Appendix 1

CSF Testing for TB and JCV in Zambia

(Lumbar Puncture Already Completed)

Information Sheet

• You have already had a test to remove water from your back because your doctors want to test whether you have an infection of your brain.

• Dr. Omar Siddiqi and other doctors are performing a study on the water surrounding your brain to check for other infections that are not normally tested for by the hospital. These include tests for tuberculosis (TB) and another infection called JC virus.

• If you agree to participate in this study we will test for TB and JC virus using water that is leftover from the regular testing.

• We would also like to take a sample of your blood and urine to see if you have signs of either of these infections in your body.

• If you have TB we would like to test your blood for markers to see how strong your body attacks the infection.

• You may also qualify for an MRI scan of the brain paid for by the study.

• There is a small risk of kidney problems from the dye used in the MRI so we will make sure you are not a risk by checking your kidney function from your blood.

• Some of the testing will be performed in the future and may not help you but the results of this study may improve the diagnosis of infections of the brain in the future.

• We may also review your medical record in the future for details related to your current presentation.

• Your personal information will not be shared with anyone outside of the research study. The information we collect from you will be stored on two password protected computers and in a locked file cabinet in the research study offices.

• To protect your privacy all of the samples we collect will be specially coded so that your name cannot be identified from the sample.

• You do not have to participate in this study..

• Your participation is voluntary and you will still receive medical care if you do not want to be a part of the study.

• Due to you your presentation, you may be offered HIV testing if you have not had this already. This is up to your treating doctors and is not a requirement for this study.

If you have any questions about this interview, you can contact any of the following people:

Dr. Omar Siddiqi
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University Teaching Hospital
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Tel: 0975015893
Email: osiddiqi@bidmc.harvard.edu

Dr. Masharip Atadzanov
Professor of Neurology
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Email: masharip@yahoo.com

For any further questions regarding the protection of human participants in the study, you may also contact:

Dr. K Babu Krishnamurthy
CCI
Beth Isreal Deaconess Medical Center
330 Brookline Avenue
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UNZA Biomedical Research Ethics Committee
Ridgeway Campus
P.O. BOX 50110
Lusaka, Zambia
Tel: (0) 211 256067

"I voluntarily agree to participate in this study, to allow additional testing for infections on my leftover spinal fluid as well as providing a sample of blood and urine. I also agree to an MRI scan if I am eligible and the study doctors feel that I am a safe candidate. I also agree to have my clinic records periodically reviewed by this research program"

----------	----------
Participant (print name)	Interview witness (print name)
----------	----------
Participant signature or thumb print	Interview Witness (signature)
----------	----------
Date signed	Date signed

Phone numbers:

.....

Please keep a copy of this form for your records

CSF Testing for TB and JCV in Zambia

(Lumbar Puncture Requested)

Information Sheet

• You have had a picture taken of your brain that, along with your symptoms, is concerning for an infection by JC virus.

• Dr. Omar Siddiqi and other doctors are performing a study to see if JC virus is different in Zambia compared to other parts of the world.

• You are eligible for this study because you have signs and symptoms concerning for JC virus infection.

• If you agree to participate in this study we would like to perform a lumbar puncture, if you have not had one already, to test if JC virus is in the water surrounding your brain.

• We would also like to take a sample of your blood and urine to see if you have been exposed to JC virus before.

• A lumbar puncture is a standard test for someone presenting with your symptoms and you should have this done even if you do not consent to enter this study.

• A lumbar puncture involves inserting a needle into your lower back in order to collect the water that surrounds your brain. We will collect a small amount of water from your back that is normally replaced in 2-3 hours.

• The testing will be performed in the future and may provide no extra benefit to you but the results of this study may improve the diagnosis of infections of the brain for other patients in the future.

• We may also review your medical record in the future for details related to your current presentation.

• Your personal information will not be shared with anyone outside of the research study. The information we collect from you will be stored on two password protected computers and in a locked file cabinet in the research study offices.

• To protect your privacy all of the samples we collect will be specially coded so that your name cannot be identified from the sample.

• You do not have to participate in this study..

• Your participation is voluntary and you will still receive medical care if you do not want to be a part of the study.

• Due to you your presentation, you may be offered HIV testing if you have not had this already.

If you have any questions about this interview, you can contact any of the following people:

Dr. Omar Siddiqi
Department of Medicine
University Teaching Hospital
P.O. Box 50110
Tel: 0975015893
Email: osiddiqi@bidmc.harvard.edu

Dr. Masharip Atadzanov
Professor of Neurology
University Teaching Hospital
P.O. Box 50110
Tel: (0) 211-250606
Email: masharip@yahoo.com

For any further questions regarding the protection of human participants in the study, you may also contact:

Dr. K Babu Krishnamurthy
Committee
CCI
Beth Isreal Deaconess Medical Center
330 Brookline Avenue
Boston, MA 02215
Tel: 1-617-667-4088
Fax: 1-617-667-2515
Email: bkrishna@bidmc.harvard.edu

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"I voluntarily agree to participate in this study. I agree to provide spinal fluid from a lumbar puncture as well as providing a sample of blood and urine to check for the presence of or past exposure to JC virus. I also agree to have my clinic records periodically reviewed by this research program"

Participant (print name)

Interview witness (print name)

Participant signature or thumb print

Interview Witness (signature)

Date signed

Date signed

Phone numbers:

.....

Please keep a copy of this form for your records

Appendix 2

Data Collection Tools

Name: _____(fname) text (sname) text Participant# _____

Date of Admission: _____date (DD,MM,YR)

Date of Enrollment: _____date (DD,MM,YR)

Demographics

1. Age _____ (years)

2. Gender ☐ Male ☐ Female

3. HIV diagnosis duration _____ days

4. Presenting symptoms

   ☐ headache 1

   neck ache 2

   ☐ seizure 3

   ☐ weakness 4

   ☐ loss of speech 5

   irrelevant speech/confusion/impaired consciousness/altered mental status 6

   ☐ loss of consciousness 7

   vision loss 8

   abnormal movements 9

   back pain 10

   memory problems 11

   ☐ Other _____ oth

5. Associated symptoms

- ☐ cough 1
- ☐ fever 2
- ☐ vomiting 3
- ☐ diarrhea 4
- ☐ Other _____ oth

6. Past medical history

- ☐ TB 1
- ☐ malaria associated with coma 2
- ☐ prior seizure/epilepsy 3
- ☐ meningitis 4
- ☐ stroke 5
- ☐ Other _____ oth

7. Medications

- ☐ None 0
- ☐ Septrin 1
- ☐ Carbamazepine 2
- ☐ Valproic Acid 3
- ☐ Phenobarbital 4
- ☐ Fluconazole 5
- ☐ ATT 6
- ☐ Other _____ oth

8. Anti-retroviral medications

- ☐ Stavudine d4T
- ☐ Lamivudine 3TC
- ☐ Efavirenz EFV

- ☐ Nevirapine NVP
- ☐ Tenofavir TDF
- ☐ Lopinavir/Ritonavir LPV/r
- ☐ Zidovudine ZDV
- ☐ Ritonavir RTV
- ☐ Abacavir ABC
- ☐ Truvada FTC/TDF
- ☐ Combivir 3TC/ZDV
- ☐ Other _____ oth
- ☐ None

9. HAART duration _____ days.

10. $CD4^+$ count _____ cells/µL

11. Clinical outcome

- ☐ Deceased 1
- ☐ Discharged 2
- ☐ Unknown 3
- ☐ Other _____ oth

Neurological Examination

Vitals Signs: Tmax/Tc ____/____ BP_____/_____ P____ RR____

1. Glasgow Coma Scale

Scale Definition - Eye opening

4 Opens eyes spontaneously

3 Opens eyes to voice

2 Opens eyes to pain

1 Does not open eyes

Scale Definition - Motor response

6 Follows commands

5 Makes localized movement in response to painful stimulation

4 Makes nonpurposeful movement in response to noxious stimulation

3 Flexes upper extremities/extends lower extremities in response to pain

2 Extends all extremities in response to pain

1 Makes no response to noxious stimuli

Scale Definition - Verbal response

5 Oriented to person, place, and time

4 Converses, confused

3 Replies with inappropriate words

2 Makes incomprehensible sounds Extends all extremities in response to pain

1 Makes no response

Total ____ /15

2a. Nuchal rigidity.

0 Absent

1 Present

2b. Kernig's sign.

0 Absent

1 Present

2a. Brudzinski's sign. Flexion of the neck resulting in flexion at the hips and knees.

0 Absent

1 Present

3a. Level of Consciousness. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation

Scale Definition

0	Alert; keenly responsive
1	Not alert, but arousable by minor stimulation to obey, answer, or respond
2	Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped)
3	Responds only with reflex motor or autonomic effects, or totally unresponsive, flaccid, areflexic

3b. Questions. Ask the patient, "what month is it?" and "how old are you?"

Scale	Definition
0	Answers both questions correctly
1	Answers one question correctly
2	Answers neither question correctly
4	Unable to assess

3c. Commands. Ask the patient, "open and close your eyes" and "make a fist with your hand"

Scale	Definition
0	Performs both tasks correctly
1	Performs one task correctly
2	Performs neither task correctly
4	Unable to assess

4a. Pupil size.

Right eye _____ mm

Left eye _____mm

4b.&4c. Pupil reaction.

Scale	Definition
0	no reaction
1	slow, sluggish
2	Normal
3	Fast

Right eye	0	1	2	3
Left eye	0	1	2	3

5. Best Gaze. Ask the patient, "Just using your eyes, follow my finger."

Scale Definition

0 Normal

1 Partial gaze palsy. This score is given when gaze is abnormal in one or both eyes, but where forced deviation or total gaze paresis are not present

2 Forced deviation or total gaze paresis not overcome by the oculocephalic maneuver

4 Unable to assess

6. Visual. Check four quadrants.

Scale Definition

0 No visual loss

1 Partial hemianopia

2 Complete hemianopia

3 Bilateral hemianopia (blind, including cortical blindness)

4 Unable to assess

7. Facial Palsy. Ask the patient, "smile, and show your teeth" and "close your eyes tight."

Scale Definition

0 Normal symmetrical movement

1 Minor paralysis (flattened nasolabial fold, asymmetry on smiling)

2 Partial paralysis (total or near total paralysis of lower face)

3 Complete paralysis (absence of facial movement in the upper and lower face)

4 Unable to assess

5. & 6. Motor Arm and Leg. Place limb in appropriate position. Ask patient to hold limb in air for 10 (arm) or 5 seconds (leg), and count out loud for the patient.

Scale Definition – ARM

0 No drift; limb holds 90 (or 45) degrees for full 10 seconds.

1 Drift; limb holds 90 (or 45) degrees, but drifts down before the full 10 seconds; does not hit bed or other support.

2 Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.

3 No effort against gravity; limb falls

4 No movement

9 Amputation, joint fusion, cannot assess; explain:_____

Left Arm	0	1	2	3	4	9
Right Arm	0	1	2	3	4	9

Scale Definition - LEG

0 No drift; leg holds 30 degrees for full 5 seconds.

1 Drift; leg falls by the end of the 5-second period, but doesn not hit bed.

2 Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity.

3 No effort against gravity; leg falls to bed immediately.

4 No movement.

9 Amputation, joint fusion, cannot assess; explain:_____

Left Leg	0	1	2	3	4	9
Right Leg	0	1	2	3	4	9

10. Limb Ataxia. Ask the patient, "Using your finger, touch my finger, and then your nose" Ask the patient to repeat this task a few times. Then ask the patient, "Using your heel, run your foot all the way down your leg, starting at your knee, and then back up again."

Scale Definition

0 Absent

1 Present in one limb

2 Present in two limbs. If present, is ataxia in:

3 Unable to assess

9 Amputation or joint fusion; explain:_____

Left Arm	Yes	No
Right Arm	Yes	No
Left Leg	Yes	No
Right Leg	Yes	No

11. Reflexes. Check bicep and patellar (knee) reflexes using reflex hammer.

Scale Definition

0 Areflexic

1 Hyporeflexic

2 normal

3 hyperreflexic

Left Arm	0	1	2	3
Right Arm	0	1	2	3
Left Leg	0	1	2	3
Right Leg	0	1	2	3

13. Romberg Test. Ask patient to stand with feet together.

0 Normal

1 Sways or feels unsteady

2 Falls or would fall if no caught

3 Unable to stand with feet together

4 Unable to assess

14. Gait

0 Normal

1 Walks slowly or with difficulty, but does not require assistance

2 Severe disturbance of gait, requiring assistance

3 Cannot walk at all, even with assistance

4 Unable to assess

CSF :

Glucose_____ Protein _____ WBC_____ RBC _____

Gram stain_____ Diff _____

India Ink _____ CrAg _____ Culture _____

Urine LAM_____(Pos/Neg)

CSF LAM_____(Pos/Neg)

TB Culture _____(Pos/Neg)

GeneXpert MTB/RIF _____ (Pos/Neg)

CSF JC virus _____ (Pos/Neg)

Blood JC virus serology _____ (Pos/Neg)

LTA4H genotype _____ (CC/TT/CT)

Was a NeuroImage obtained (Y/N)?

If yes, detail what study and when it was obtained: CT MRI Date_____

Appendix 3

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

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Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370 Fax:
+ 260-1-250753
E-mail: unzareúòunza.zm

Ridgeway Campus
P.O. Box 501 10
Lusaka. Zambia

Assurance No. FWA00000338

11000001131 of IORG0000774

5th November, 2013.

Your Ref: 014-06-13.

Dr. Omar Siddiqi,

University Teaching Hospital,

Department of Internal
Medicine, P/Bag RW I x,
Lusaka.

Dear Dr. Siddiqi,

RE: RE-SUBMITTED RESEARCH PROPOSAL: "CEREBRAL SPINAL FLUID DIAGNOSTICS IN HIV-ASSOCIATED NEUROLOGICAL DISEASES AT U.T.H" (REF. NO. 014-06-13)

The above mentioned research proposal was re-submitted to the Biomedical Research Ethics Committee with recommended changes on 7th October, 2013. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- Ensure that a final copy of the results is submitted to this Committee.

Yours sincerely,

Dr. J.C Munthali
CHAIRPERSON

Date of approval: 5 th November 2013. Date of expiry: 4 th November, 2014.

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

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Assurance No. FWA00000338

IRB00001131 of IORG0000774

2nd November, 2018.

Your Ref: 014-06-13.

Dr. Omar Siddiqi,

University of Zambia,

School of Medicine,

Department of Internal Medicine,

U.T.H,

Lusaka.

Dear Dr. Siddiqi,

RE: **RENEWAL FOR THE STUDY: "CEREBROSPINAL FLUID DIAGNOSIS IN HIV-ASSOCIATED NEUROLOGICAL DISEASES AT UTH" (REF. No. 014-06-13)**

We acknowledge receipt of your progress report and request for study renewal.

Renewal has been granted for the period 5th November 2018 to 4th November, 2019.

Yours sincerely,

Dr. S.H Nzala

VICE-CHAIRPERSON