Original Article

A Study of the John Cunningham Virus (JCV) Seroprevalence among Zambian Adults presenting with "Meningoencephalitis" to the University Teaching Hospital, Lusaka, Zambia

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ABSTRACT

Background: The John Cunningham virus (JCV) is an opportunistic virus which leads to the development of progressive multifocal leukoencephalopathy (PML), which is a lytic infection of oligodendrocytes of the central nervous system in immunosuppressed patients. 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. 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.

Methodology: 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. 50 HIV positive and 50 HIV negative patients were selected from the parent study and underwent testing for JCV serology. PML cases were also recruited from the parent study based on clinical features, confirmed by JCV DNA PCR of CSF.

Results: 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 such as age, gender, clinical presentation, CD4 count and co-morbid TB meningitis (TBM) diagnosis. 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 seropositive patients 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).

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

Conclusion: 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.

INTRODUCTION

The John Cunningham virus (JCV) is an opportunistic virus, which causes progressive multifocal leukoencephalopathy (PML).[1] PML is a lytic infection of mainly oligodendrocytes of the central nervous system in immunosuppressed patients.[2] The JCV is a ubiquitous human pathogen and exposure is unavoidable. After asymptomatic primary infection with the JCV in childhood, the virus remains quiescent in the kidneys, bone marrow, and lymphoid tissue and becomes activated when the host becomes immunosuppressed. [3,4,5]

Once infected, exposure to the virus can be detected by testing for JC virus specific antibodies and antibody tests for the JCV are now successfully being carried out to determine the subsequent risks of developing PML in at risk populations.[6]

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 nothing is known about JCV seroepidemiology in Africa.[7]

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. Two cases of PML have been diagnosed by autopsy among HIV+ patients in Uganda and Gambia each. [8, 9] 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%. [9] 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. [10]

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. [11, 12]

In this study, we sought to determine the seroprevalence of JCV amongst the Zambian population who present with features of a central nervous system (CNS) infection (meningoencephalitis) and factors associated with JCV seropositivity. We also assessed the possible role of the JCV antibody test in stratifying PML risk in affected populations, as PML is a fatal disease with a 9% survival rate at 1 year without any intervention.[2] Therefore, early identification is key.

METHODOLOGY

This was a cross sectional study 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 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.

Participants

100 patients were selected, divided into 50 HIV positive and 50 HIV negative patients from the 2 arms of the TMZ study, for determining JCV seroprevalence.

14 confirmed PML cases based on clinical, laboratory and radiological criteria (American Academy of Neurology criteria) were also selected from the TMZ study for the purpose of assessing

PML risk in affected populations. [13] Participants were selected from the inpatient and outpatient medical wards at UTH adult hospital.

Procedure

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.

At the time of enrollment of the TMZ study, a detailed history and a full neurological examination was performed on all participants by the attending neurologist. 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 and radiological picture. 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.

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 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).

Ethical approval

Ethics approval was obtained from the University Of Zambia School Of Medicine Biomedical Research Ethics Committee and the Director of UTH (IRB00001131 of IORG0000774). Written informed consent was obtained from each of the participants or the next of kin.

RESULTS

Patient Characteristics

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. 26 (26%) had a confirmed diagnosis of TBM.

TABLE 1: Characteristics of the Patients

VARIABLE	JCV STUDY N (%)
Gender	M = 50 (50.0) F = 50 (50.0)
Age[a]	39 ± 14.5
HIV Positive*	50 (50)
Cd4 (cells/mm^3) [Median IQR]	350 [IQR 123-707]
Headache	72 (72.0)
Seizures	18 (18.0)
Fever	49 (49.0)
Irrelevant Speech	45 (45.0)
Focal Neurological Deficit	25 (25.0)
GCS (Mean ±SD)	12.64 ± 2.68
Memory Impairment*	42 (42.0)
TB Meningitis	26 (26)

Mean ±SD
Median [25%IQR-75%IQR]

JCV seroprevalence

The JCV seroprevalence in our study population was found to be 46% (95% CI, 35.62 – 56.31) 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***.

TABLE 2: Factors associated with JCV antibody positivity

VARIABLE	CRUDE OR (95% CI)	*P* value	ADJUSTED OR (95% C	*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

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).

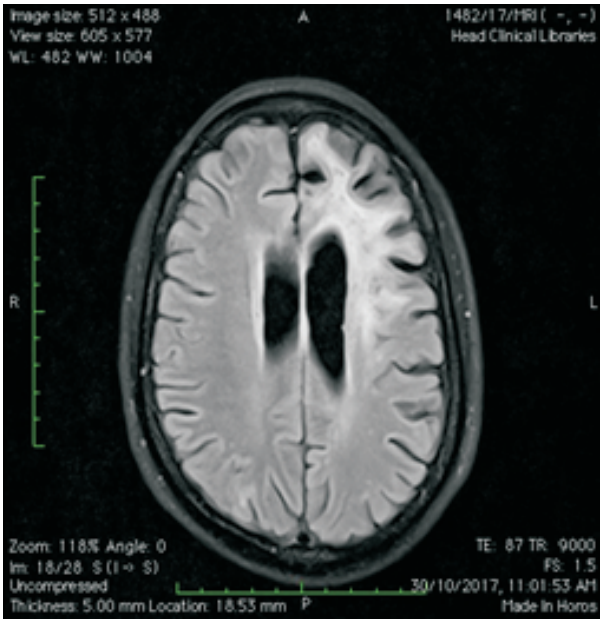
None of the variables studied had any significant effect on determining JCV seropositivity individually as well as after adjusting for the other variables studied. This included gender as well as age.

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***.

Figure 2*: Relationship of age with JCV seropositive status*
Count = JCV seropositive (number)

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 we used a case definition of CSF PCR positive for JC virus as PML cases based on another study in the institution. [10]



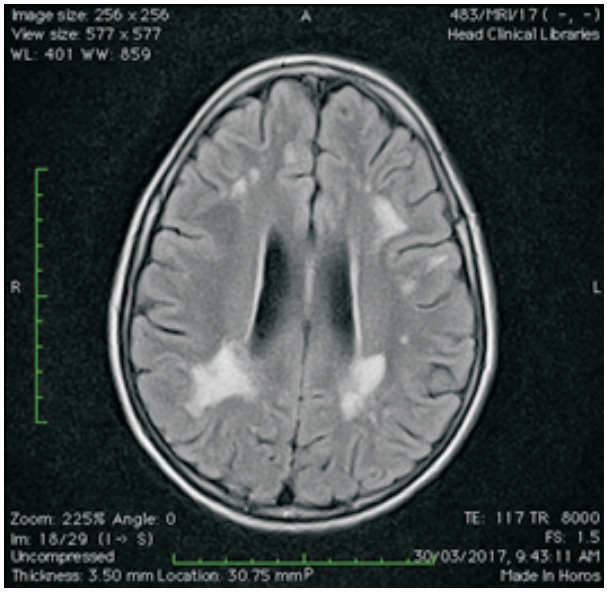


FIGURE 3 AND 4: MRI findings in 2 PML patients. The axial images in fluid attenuated inversion recovery (FLAIR) show PML lesions in the left frontal lobe (left image) and in the right and left parietal lobes and the left frontal lobe of another (right image).

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***).

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).

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
$^{\beta}$Cd4 (cells/ml)	0.99 (0.99- 1.00)	0.04		
$^{\beta}$Cd4 category (cells/ml)				
0 = >500				
1 = 350 – 499	1.00		1.00	
2 = 200 -349	3.00 (0.25 – 35.33)	0.38		
3 = <200	0.75 (0.08 – 7.21)	0.80		
	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.
$^{\beta}$ 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).

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%. [14] Gorelik et al. observed higher prevalence of anti-JCV antibody in Europe and North America compared to Australia and New Zealand.[15] 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 and from a mixed urban and rural setting. 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. [7] This was subsequently confirmed by two other studies. [14,16] This differing trend could be due to the differences 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 found in our study. This is in contrast to two European studies where male gender was noted to be a significant association to JCV seropositivity. [14,16] 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. [17]

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%. [10] This could possibly be due to improved PICT and early treatment with ART for HIV positive patients. 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. [18] 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. [2] 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. [19] 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. [20] 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.

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.

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