THE ASSOCIATION OF HYPERCOAGULABILITY STATE MARKERS IN ADULT HIV POSITIVE PATIENTS WITH ISCHAEMIC STROKE AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

STANLEY ZIMBA (MBChB)

A dissertation submitted to the **University of Zambia**

in partial fulfilment of the requirement of the degree in

Masters of Medicine (Internal Medicine)

University of Zambia

2016

DISSERTATION

THE ASSOCIATION OF HYPERCOAGULABILITY STATE MARKERS IN ADULT HIV POSITIVE PATIENTS WITH ISCHAEMIC STROKE AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

RESEARCHER: Dr Stanley Zimba

SUPERVISER: Prof Masharip Atadzhanov

CO-SUPERVISER: Dr Patrice Mukomena Ntanda

DECLARATION

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or at any other University.

Signed _____

Student: **Dr Stanley Zimba**

Signed _____

Supervisor: **Professor Masharip Atadzhanov**

Signed_____

Co-supervisor: **Dr Patrice Mukomena**

Signed _____

Head of department: **Dr Nason Lambwe**
Head of Department of Medicine
University Teaching Hospital
Lusaka, Zambia.

COPYRIGHT

Dr. Stanley Zimba
2016

DEDICATION

I dedicate this work to my family, for the patience you exhibited during the time I was away from you to collect data and do the write up; my wife Exildah Nkomba and my children Lillian and Stanley Zimba, God will surely reward you one day.

ACKNOWLEDGEMENTS

I am grateful to my teachers who have taught me Internal medicine and research with all their heart and to the best of their ability.

I thank the head of department of medicine Dr Nason Lambwe for ensuring that work is correctly done on time and UTH management to have given me the opportunity to practice medicine at one of the biggest hospital in sub-Saharan Africa.

I thank the patients and their relatives for availing themselves despite difficult moment during illness.

I wish to thank my supervisor Prof Masharip Atadzhanov and my co-supervisor Dr Patrice Mukomena who taught me research from the beginning.

I thank Dr Shabir Lahki for his great support during my training and in this stroke research.

I received very good advice and support from Dr Ben Andrews who brought new understanding of research methods to me.

I would also like to thank the Assistant Dean Postgraduate Dr Nzala, Vanderbilt University and SACORE grants through Prof Masharip Atadzhanov for having sponsored my study.

To all of you my senior colleagues and my fellow postgraduate students for your love and respect you showed me, may God Himself reward you as He alone knows one's heart.

To the government and the people of this peaceful nation, for ensuring a conducive environment during my study period, sincerely thanks.

Table of Contents

LIST OF FIGURES, A GRAPH AND TABLES

ABBREVIATIONS

AF	-	Atrial Fibrillation
AIDC	-	Adult Infectious Disease Centre
AMEU	-	Adult Medical Emergency Unit
ART	-	Anti-Retroviral Therapy
BMI	-	Body Mass Index
BP	-	Blood Pressure
CNS	-	Central Nervous System
CT scan	-	Computerised Tomography Scan
DBP	-	Diastolic Blood Pressure
DCM	-	Dilated Cardiomyopathy
ECG	-	Electrocardiography
GCS	-	Glasgow Coma Scale
GOS	-	Glasgow Outcome Scale
cART	-	Combination Anti-retroviral Therapy
Hb	-	Haemoglobin
HHD	-	Hypertensive Heart Disease
HIV	-	Human Immune Deficiency Virus
HIV+ve	-	Human Immune Deficiency Virus positive
HIV-ve	-	Human Immune Deficiency Virus negative
HLA	-	Human Leukocyte Antigen
ICH	-	Intracerebral Haemorrhage
IRIS	-	Immune Reconstitution Inflammatory Syndrome
IS	-	Ischemic Stroke
MRI	-	Magnetic Resonance Imaging
NIHSS	-	National Institute of Health Stroke Scale
NNRTI	-	Non-nucleoside Reverse Transcriptase Inhibitors
NRTI	-	Nucleoside Reverse Transcriptase Inhibitors
OR	-	Odd Ratio
PC	-	Protein C
PI	-	Protease Inhibitor
PS	-	Protein S
PML	-	Primary Multifocal Leukoencephalopathy
RBS	-	Random Blood Sugar
RHD	-	Rheumatic Heart Disease
RPR	-	Rapid Plasma Reagin
RVT	-	Retroviral Test
SAH	-	Subarachnoid Haemorrhage
SBP	-	Systolic Blood Pressure
SOL	-	Space Occupying Lesions
TIA	-	Transient Ischemic Attack

TOAST	-	Trial of Org 10172 in Acute Stroke Treatment
USA	-	United States of America
UTH	-	University Teaching Hospital
WHO	-	World Health Organisation
WBC	-	White Blood Cell

ABSTRACT

INTRODUCTION:

There has been a substantial and significant increase in patients hospitalized for ischaemic stroke with co-existing HIV infection. Little is known about the mechanism of stroke in these HIV+ve patients as no studies had been done in our region. Elsewhere, attributed mechanisms included vasculitis and hypercoagulability state with protein S deficiency being a prominent feature. Little is also known of the effect of antiretroviral drugs on patients with hypercoagulability state. Hence there is a need for this study to help understand the role of hypercoagulability state in HIV+ve ischaemic stroke patients and consequently help improve their management.

METHODOLOGY

A matched case control study was conducted in which a total of 52 HIV+ve patients with ischaemic stroke were prospectively compared with control groups for occurrence of protein S, protein C deficiencies, hyperhomocysteinaemia as well as other markers like hypercholesterolaemia and obesity. The control groups comprised an equal number of consecutive matched HIV-ve and HIV+ve patients with and without ischaemic stroke respectively.

RESULTS

Ischaemic stroke of undetermined aetiology occurred more frequently in HIV+ve compared to HIV-ve patients ($p<0.001$). In addition, protein S deficiency and Hyperhomocysteinaemia were more prominent in HIV+ve than HIV-ve ischaemic stroke patients ($P=0.011$). There was no difference in the presence of hyperhomocysteinaemia or protein S deficiency in HIV+ve patients with or without ischaemic stroke. Protein C deficiency was not noted to be significantly different between the cases and the two control arms.

CONCLUSION

There was a strong association between hypercoagulability state and ischaemic stroke in adult HIV+ve patients with traditional markers like smoking, sedentary lifestyle and obesity noted. Protein S deficiency and hyperhomocysteinaemia are strongly associated with HIV infection, and their presence in HIV+ve ischaemic stroke warrants them to be considered as important serum markers in the prevention of ischaemic strokes in the Zambian HIV+ve population.

CHAPTER 1 INTRODUCTION

Stroke is a sudden focal neurological deficit of vascular origin lasting more than 24 hours or leading to death.[1] It occurs due to inadequate blood supply to a part of the brain following vascular thrombosis or embolism (ischaemic stroke or cerebral infarction), or spontaneous haemorrhage into or over the brain substance following vascular compromise [haemorrhagic stroke (ICH)].[2]

Globally, stroke is the second leading cause of death and the most important cause of acquired adult disability.[3,4] Countries of low and middle income have the largest burden of stroke with more than 85% of stroke mortality worldwide.[5,6] The overall stroke incidence rate in low to middle income countries surpasses the level of stroke incidence seen in high-income countries by 20%.[7,8] In the world, approximately fifteen million people annually suffer a stroke with devastating effects.[9] One third of these die and another one third remain permanently disabled. The burden of non-communicable diseases like stroke and other vascular diseases is rising in sub-Saharan Africa, adding to the infectious and poverty related disease burden and further straining the limited resources channelled to the health sector.[10-12]

The commonest form of stroke is ischaemic stroke which accounts for about 80 to 85% of strokes in Whites and about 60 to 70% in Blacks and Asians.[13-15] In Nigeria, a retrospective hospital based study found 67.3% of stroke patients had cerebral infarction.[16,17] A study done at University Teaching Hospital (UTH) in Zambia showed 65% of patients had ischaemic and 35% haemorrhagic strokes.[18] As much as there are more ischaemic strokes in our population, there is a relatively larger proportion of ICH compared to the western population among whites.

Ischaemic stroke is classified according to the Trial of Org 10172 in acute stroke treatment (TOAST) classification.[19,20] There are five diagnostic subtypes of ischaemic stroke: large artery atherosclerosis, cardio-embolism, small vessel occlusion (lacuna), determined aetiology (vasculitis, hypercoagulability) and undetermined cause. These can also occur as multiple possible aetiologies. However, the major risk factors for stroke include excessive alcohol intake, previous stroke, family history of stroke, HIV infection, hypercholesterolaemia, tobacco smoking/sniffing, obesity, sedentary lifestyle, Diabetes mellitus, with hypertension being the most common risk factor for both ischaemic and haemorrhagic strokes.[11,18,21]

In the United States, there has been a substantial and significant increase in patients hospitalized for stroke with coexisting HIV infection.[22] This was echoed by studies conducted in the Department of Internal Medicine at UTH/University of Zambia (UNZA) that found 25.4% of all stroke patients were Human Immune Deficiency Virus positive (HIV+ve), and 83.3% of young patients with stroke were attributed to HIV infection.[18,23] This has significant public health and socioeconomic consequences as the people most affected are those in the productive age-group.[24]

Despite this high burden, the mechanism of stroke in these HIV+ve patients remains largely unknown, and no study has been done at UTH elucidating the possible mechanisms. Elsewhere, stroke in patients with HIV infection has been attributed to vascular abnormalities, coagulation disorders [particularly Protein S (PS) deficiency] and cardio-embolic disease in several clinical and pathological studies.[25] Nonetheless, stroke mechanisms are variable in HIV+ve patients with a relatively high incidence attributed to vasculitis and hypercoagulability. A study done in the USA on mechanisms of ischaemic

stroke in HIV+ve patients found that 45% had PS deficiency.[25] PS is produced by the liver and endothelial cells, and acts as a cofactor to activated Protein C in the inactivation of factor Va and factor VIIIa. The mechanism by which PS deficiency occurs is not fully understood, but is thought to result from the presence of autoimmune antibodies or because of inflammatory markers such as interleukin 1, cytokines and tumour necrosis factor alpha stimulated by HIV.[26] Other anticoagulation factors that may have a role in hypercoagulability include Protein C (PC), Homocysteine, Antithrombin III, factor V Leiden, lupus antibodies, anticardiolipin antibodies and prothrombin G20210A mutation.[27] Homocysteine levels are elevated in vitamin B_{12} and folic acid deficiency which is a common finding in HIV+ve patients who present with micronutrient deficiencies.

The management of thrombo-embolism in HIV+ve patients with PS deficiency remains a challenge. One study showed a high thrombotic recurrence rate and haemorrhagic complications using oral anticoagulants, and acetylsalicylic acid was successfully used for secondary prophylaxis.[26] In addition, there is scarce data on the relationship between stage of HIV disease and the level of serum PS although one study showed that PS levels were significantly lower at CD4+ T cell count below 100cell/ul.[28] Moreover, little is known of the effect of anti-retroviral therapy (ART) on a patient with PS deficiency. This is important because protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) increase the risk of ischaemic stroke and can have a compounding effect in a patient with PS deficiency.[29] One study suggested that screening for PS deficiency of asymptomatic HIV+ve patients should be indicated, and those with documented PS deficiency would benefit from aspirin primary prophylaxis so as to reduce the risk of ischaemic stroke in HIV infection and ensure that appropriate ART is instituted.[26]

Therefore, the purpose of this study was to determine the association of hypercoagulability state with ischaemic stroke in Zambian adult HIV+ve patients. The results of this study would help understand the role of hypercoagulability state in Zambian adult HIV+ve stroke patients and in their management.

CHAPTER 2 LITERATURE REVIEW

One in five strokes are fatal, and it is the leading cause of adult disability worldwide.[10] Black people are twice as likely to have stroke compared with whites.[13] With the highest burden of poverty and HIV infection, sub-Saharan African countries cannot afford to ignore the association between stroke and HIV.[30] It is imperative, therefore, to study the risk factors for ischaemic stroke in HIV and particularly the association of hypercoagulability state with ischaemic stroke in HIV infection.

According to Dobbs et al[10], HIV infection increases the risk of ischaemic stroke. The authors found that an increased risk was most apparent in the young HIV+ve patients who presented with stroke and with no evident cardiovascular risk factors other than being HIV+ve. Other risk factors included opportunistic infections, meningitides, vasculitides, altered coagulation, cardio-embolic events and cryptic. In another study done by Sen et al[29], HIV infection was strongly associated with ischaemic stroke in the young. Moreover, using data obtained from the nationwide inpatient sample in the USA, authors found an increase in the number of stroke hospitalizations in the HIV+ve population. They further suggested that the risk factor profile for stroke in HIV+ve and non-HIV differed, with HIV-associated stroke less likely to have risk factors like hypertension, diabetes, hyperlipidaemia and smoking. Ortiz et al[25], found that incidence of vasculitis and hypercoagulability is relatively high in HIV+ve patients. The authors reviewed records of consecutive HIV+ve patients with acute stroke admitted to a large metropolitan hospital between 1996 and 2004. Ninety-four percent of 82 patients had ischaemic stroke and the mean age for stroke was 42 years. The study deemed hypercoagulability state to only be responsible for 9% of their sample size although not all their patients had been tested for hypercoagulability state markers. This was a very serious

over-sight in this study as 45% of the 22 patients tested had PS deficiency and 29% of the 31 tested had anticardiolipin antibodies. This study also had contrasting information when it concluded that exposure to ART did not significantly influence the mechanism of stroke.

ART drugs like PIs and NRTIs are implicated in accelerating atherosclerosis and hyperlipidaemia, thus increasing the risk of ischaemic stroke.[31-33] They have a direct effect on lipid metabolism, endothelial and adipocyte cell function, activation of pro-inflammatory cytokines and mitochondrial dysfunction.[32] In this regard, it is important to study the impact of ART in HIV+ve patients to ascertain its role in increasing the risk of ischaemic stroke.

Various studies have been done to try to understand hypercoagulability state in HIV infection. A study by Alain et al[34] found that 31% of 71 HIV+ve patients in a prospective study over a period of one month had PS deficiency. Another study by Sorice et al screened 35 HIV+ve patients for PS deficiency and found that PS levels were significantly lower with lower CD4+ T cell counts especially below 100cell/ul.[28] The two studies had small sample sizes and make it very difficult to derive any definitive conclusions. Nonetheless, they provide a platform to predict that HIV induces a hypercoagulability state by causing PS deficiency. The mechanism by which it causes this PS deficiency is not fully understood and whether HIV has an influence on other hypercoagulability state markers like PC, Homocysteine, anticardiolipin antibodies and antithrombin III needs to be studied.

Although no major studies with large sample size are available to support this association of PS deficiency and HIV infection, a retrospective review on hypercoagulability due to PS in HIV+ve patients by Smego et al[26] recommended that HIV+ve patients should be screened for acquired PS deficiency, which contributes to hypercoagulability and risk of clinical thrombo-

embolic events. They further recommended that asymptomatic patients with reduced plasma free PS levels might benefit from aspirin primary prophylaxis. Before these measures are to be considered, it is essential that a large study is done on hypercoagulability in HIV infection with interest also in ischaemic stroke due to the burden of this problem in our setting.

In spite of shouldering the largest burden of stroke and HIV+ve patients in the world, Africa has seen few researches done on these topics, especially hypercoagulability state in HIV+ve patients with ischaemic stroke. Tipping et al[35] published a prospective study from 2000 to 2006, based on the review of stroke register of the Groote Schuur Hospital/ University of Cape Town stroke unit. According to the study, of 96% of HIV+ve patients with ischaemic stroke 19% had coagulopathy. The study did not clearly highlight the nature or cause of the coagulopathies.

Mochan et al[36] published a study on PS deficiency in HIV associated ischaemic stroke. It was a case control study with only 33 patients with HIV infection and ischaemic stroke whom they compared with 33 HIV+ve patients without stroke and 33 ischaemic stroke Human Immune Deficiency Virus negative (HIV-ve) patients. They measured hypercoagulability state markers in all these patients and found PS deficiency in HIV+ve patients with ischaemic stroke as well as in HIV+ve patients without stroke. This study was small and it did not address the role of ART in the patients with HIV infection. This is very important especially for patients on PIs and some NRTIs which have been attributed as a risk factor for stroke.

In Zambia, there are no data on the association of hypercoagulability state with ischaemic stroke in HIV+ve patients. The only studies available include Mukomena et al[18] on outcomes of stroke at UTH who found that HIV infection was independently associated with ischaemic stroke, and Lambwe et al[23] found that HIV infection was strongly associated with strokes in

the young adults (15 to 45years). However, none of these studies really ventured into the risk factors of stroke in these HIV+ve patients and thus necessitating the importance of this study.

CHAPTER 3

3.1 STATEMENT OF THE PROBLEM

The fact that stroke is the most important cause of acquired adult disability cannot be over emphasized, however, it is also the second leading cause of death worldwide.[2] The burden of stroke globally is on the rise with countries such as the USA reporting more than 780,000 new or recurrent strokes every year.[5,7] In sub-Saharan Africa, which has a high burden of infectious and poverty related diseases, stroke and other non-communicable diseases are an emerging public health problem.[8,24] Anecdotal reports at UTH strongly suggest an increase in the number of patients admitted with stroke with daily admissions at an all-time high.

The commonest form of cerebrovascular accident is ischaemic stroke and HIV infection appears to increase the risk of suffering this form of stroke. According to the United Nations 2012 report on HIV, sub-Saharan African is the region with the highest HIV+ve prevalence.[37] Therefore, it is not surprising that there has been an apparent increase in HIV+ve patients who present with ischaemic stroke with no evidence of cardiovascular or any other risk factors.[10] Hypercoagulability is a likely important factor in the mechanism of stroke in these patients although it is yet to be established in our population. This hypercoagulability state is likely to occur through PS deficiency induced in the presence of HIV as well as the influence of HIV on the other markers like PC, Homocysteine, cholesterol, obesity, sedentary lifestyle and smoking. This study is important to establish if there is an association between hypercoagulability with ischaemic stroke in HIV+ve patients as this will have a serious bearing on management and prevention measures.

3.2 STUDY JUSTIFICATION

A large part of the global burden of disease is attributed to stroke. The risk of stroke, especially ischaemic stroke is increased by the presence of HIV infection.[10] This increased risk has been demonstrated in the young HIV infected population where other risk factors for ischaemic stroke are hardly ever evident.[23] The association between HIV infection and ischaemic stroke was first suggested from autopsy and case series from the United States.[17,38] Population studies to determine the risk of this association soon followed. The majority of subsequent studies have demonstrated a number of risk factors, but hypercoagulability state has emerged as a very important risk factor even surpassing vasculitis in some studies.[32]

In Zambia, little is known on the mechanism of ischaemic stroke in adult HIV+ve patients in spite of the high prevalence rate of HIV infection. Health workers at the hospital are faced with more cases of stroke admissions than before with a high case fatality rate (40%) as shown by Mukomena.[18] The aetiology of ischaemic stroke in HIV infection affects prognosis, outcome, and management.[14,39] Hence the need to study the association of hypercoagulability state with ischaemic stroke in HIV+ve in order to put in place prevention and treatment guidelines. This may help reduce morbidity and mortality from ischaemic stroke in the HIV+ve population. Moreover, the results of this study could also be used by UTH managers and policy makers for planning and guiding future research.

3.3 HYPOTHESIS

Hypercoagulability state is one of the most important factors for ischaemic stroke in adult Zambian HIV+ve patients with a hypothetical proportion in these patients 2.5 folds more than the general population.

3.4 OBJECTIVES

3.4.1 General objective

To explore the association of hypercoagulability state in adult Zambian HIV+ve patients with ischaemic stroke.

3.4.2 Specific objectives

1. To describe the demographic and clinical characteristics of subtypes of ischaemic stroke in HIV+ve patients.
2. To identify main risk factors associated with ischaemic stroke in adult Zambian HIV+ve patients.
3. To assess for association of hypercoagulability state markers (PC function, PS levels, cholesterol, smoking, sedentary lifestyle, Homocysteine levels) in adult Zambian HIV+ve patients with ischaemic stroke.
4. To compare hypercoagulability state markers between HIV+ve ischaemic stroke patients with HIV-ve ischaemic stroke patients as well as HIV+ve without stroke.

CHAPTER 4 RESEARCH METHODOLOGY

4.1 Type of study

Matched case control study at UTH over an 8 months period from July 2014 to February 2015.

4.2 Setting and Target population

UTH, in Lusaka, is a third level hospital and the highest referral centre in Zambia with fifty six wards and over 1,500 bed spaces. According to the 2010 population census, the country had a total population of 13,046,508 with Lusaka's population at 2,198,999.[40] UTH functioned as a national referral hospital as well as a provincial and district hospital catering for five districts namely Kafue, Chongwe, Luangwa, Mumbwa and Lusaka districts with an estimated three million people.

In this study, the target population was adult patients seen at UTH either as in-patients or out-patients via Adult Medical Emergency Unit (AMEU), Clinic five, Adult Infectious Diseases Centre (AIDC), and physiotherapy department.

4.3 Inclusion criteria

4.3.1 Cases

a) 18 years and above
b) Diagnosis of ischaemic stroke confirmed by clinical assessment and brain imaging [Computer Tomography (CT) scan or Magnetic Resonance Imaging (MRI)], and stroke duration more than 48hours and up to one month
c) Informed consent given
d) HIV+ve

4.3.2 Control 1 (HIV-ve ischaemic stroke)

a) 18 years and above
b) Diagnosis of ischaemic stroke confirmed by clinical assessment and brain imaging [Computer Tomography (CT) scan or Magnetic Resonance Imaging (MRI)], and stroke duration more than 48hours, but up to one month
c) Informed consent given

4.3.3 Control 2 (HIV+ve no stroke)

a) 18 years and above
b) No clinical and/or brain imaging evidence of stroke or any other neurological disorder
c) Informed consent given

4.4 Exclusion criteria

a) Taking anticoagulant drugs, contraceptive drugs and hormonal replacement therapy
b) Intracerebral haemorrhage (ICH)
c) Focal neurological deficit of non-vascular origin [head injury, space occupying lesions (SOL), CNS infection, neurodegenerative disorders]
d) Refusal to do HIV test
e) Liver disease
f) Pregnancy

4.5 Study process

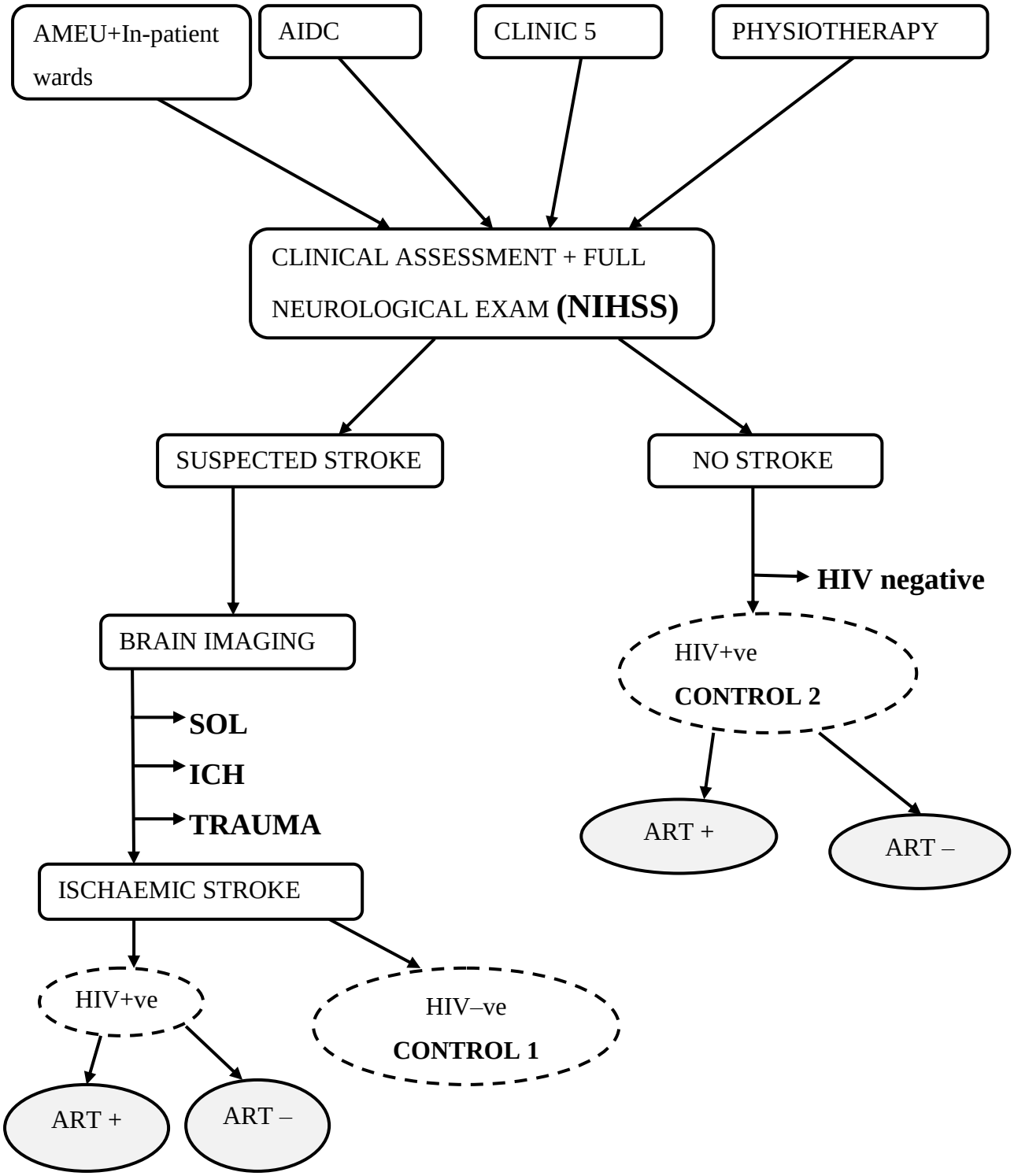

Figure 1: Study process for recruitment of patients

The study was publicized in hospital meetings and with study brochures being provided at the various departmental entry points for patients. Every morning, the unit on call in AMEU was educated about the study as well as doctors and nurses in Clinic five and AIDC so that stroke and HIV+ve patients were evaluated and enrolled in the study. Some patients were also recruited via the physiotherapy department. The participants or their relatives were informed about the nature of the study and consent was sought. They were also provided with an information sheet on the study.

The study included ischaemic stroke confirmed by clinical assessment and brain imaging. In the clinical assessment, patient's demographics as well as risk factors were documented. These included HIV infection, hypertension, diabetes mellitus, smoking/sniffing tobacco, alcohol intake, obesity, sedentary lifestyle, previous stroke and family history of stroke.

The HIV+ve patients were further classified into those who were not and those who were on ART, with and without ischaemic stroke. The markers of hypercoagulability state as well as serum glucose and Rapid Plasma Reagin (RPR) were measured after the acute phase of stroke. The markers measured included serum protein S levels, protein C function, Cholesterol and Homocysteine levels.

Two controls were recruited for each case and they were frequency matched for sex and age at +/- 5 years interval. Control 1 was HIV-ve with ischaemic stroke to assess the relationship of hypercoagulability state markers to HIV infection. Control 2 was HIV+ve no stroke (but free from neurological disorder and no previous history of coagulation disorder) to assess the relationship of hypercoagulability state markers with stroke.

4.6 Case definitions

Hypertension was defined in the study as current use of antihypertensive medication, history of being diagnosed as hypertensive by a doctor prior to stroke, documented blood pressure of greater than or equal to 140mmHg systolic or 90 mmHg diastolic before the stroke or persisting more than a week after the acute event (World Health Organization) or evidence of left ventricular hypertrophy on ECG or Echo.[41, 42]

Diabetes mellitus was diagnosed if patients are taking anti-diabetics drugs prior to stroke, if a doctor had diagnosed type I or type II diabetes before stroke or if the patient has a documented non fasting blood glucose of greater than 11.1 mmol/L or fasting blood glucose of greater than or equal to 7.0 mmol/L after the acute phase of stroke to exclude acute transient elevation of glucose as a stress response after stroke.

Hypercholesterolemia was defined as serum cholesterol > 5.2 mmol/L.[42]

Hyperhomocysteinaemia was defined as serum homocysteine levels > 14.4 umol/l, **Protein S deficiency** < 60% and **Protein C deficiency** < 70% as recommended by the International Federation of Clinical Chemistry (IFCC).[43]

We were unable to do **anticardiolipin antibodies**, but instead we did RPR which would give a false positive in the presence of these antibodies.

Laboratory investigations such as Full Blood Count (FBC) measured using FBC SYSMEX 2000 and 4000, CD4+ T cell count measured using FACS Calibur, Liver Function Tests (LFTs), cholesterol, urea and creatinine measured using Beckman Coulter AU480 were done at UTH as part of all patient routine work up. The HIV antibody tests were done on all recruited patients using a combination of Determine and Bioline test kits, with the Unigold kit as a tie-breaker. For PS, PC deficiencies and hyperhomocysteinaemia, venous blood sample was collected and immediately transported on ice to a private specialized laboratory (Lancet-Nkanza Laboratory) for separation of plasma and cellular components before the samples were flown while maintaining cold chain to Lancet Laboratory in Johannesburg, South Africa, for analysis using Hemosil^{TM} immunoassays.

Other risk factors included: **Cardiovascular diseases** (Dilated Cardiomyopathy (DCM), Rheumatic Heart Diseases (RHD), Atrial Fibrillation (AF), Hypertensive Heart Disease (HHD), peripheral vascular diseases defined as intermittent calf claudication or absent leg/pedal pulses, coronary artery disease).

Cigarette smoking was classified as current, former smoker for more than one year or never smoked, and **alcohol consumption** as never, ex-drinker for more than one year or current

alcohol use. History **of transient ischemic attack, previous stroke and a family history of stroke** were also obtained. HIV status and Sickle cell disease were considered as risk factors. The examination included a detailed neurological assessment with measurement of blood pressure, and examination for any evidence of vascular disease including hypertensive end-organ damage affecting the heart or fundal vessels and any potential sources of emboli. Assessment for signs of focal neurological deficit (hemiparesis, hemisensory loss, cranial nerves palsy, aphasia, level of consciousness, alexia, agraphia, and apraxia) was done together with the patient's National Institutes of Health Stroke Scale (NIHSS) at the time of examination. Patients were reviewed with all data collection sheets, scans and other investigations, and assigned a final diagnosis plus stroke type. The patients were classified using the TOAST classifications. [33, 34]

Stroke was defined according to the WHO criteria as rapidly developing signs of focal disturbance of cerebral function leading to death or lasting longer than 24hours, with no apparent causes other than vascular. The study included patients who had an ischaemic stroke within 1 month at recruitment irrespective of their reason for admission.

4.7 Ethical considerations

Ethical approval was obtained from ERES Converge IRB (ref. No. 2014 – Dec – 009) and permission was obtained from UTH management.

4.8 Data collection

Data was collected using a questionnaire. The questionnaire extracted information regarding patient social demographic factors, risk factors, general and neurological examinations, results, NIHSS score and Oxford handicap scale.

4.9 Sampling

The sample size was calculated using StatsToDo by first making the assumption that 19% of all HIV+ve ischaemic strokes were due to coagulopathies as reported by Tipping et al.[35] Then, the occurrence of coagulopathies in the general population was estimated at 4% taking into account Remkova's postulation that hereditary deficiencies of Antithrombin III, PC or

PS can be found in fewer than 5% of unselected patients.[44] The confidence interval was set at 95% and the power to detect a difference at 80%. There were three arms with the case being HIV+ve ischaemic stroke and two controls – one control being HIV-ve ischaemic stroke and the other being HIV+ve no stroke. A total of 51 subjects were required in each of the three arms.

4.10 Study variables

The independent variables were **hypercoagulability state** measured by serum levels of coagulation markers (PS, PC, cholesterol and Homocysteine), CD4+ T cell count, cART regimen, age and sex. The outcome variable was **ischaemic stroke in HIV+ve population.**

4.11 Analytical plan

All the data was entered and analyzed on SPSS statistics 2012 version 21. Analysis of characteristics, risks factors and serum level of hypercoagulability markers of ischaemic stroke and HIV infection was done. Parametric continuous variables were expressed as means with ranges. Some of the continuous variables such as serum creatinine, homocysteine, PC and PS were dichotomised before analysis based on values obtained from previous studies. Paired T-test was used for statistical significance.

Categorical variables were expressed as percentages or proportions. McNemar's Chi square test was used to measure the effect of each categorical variable (e.g. sex, hypertension, Diabetes mellitus and myocardial infarction) on the outcome (HIV+ve ischaemic stroke).

Conditional logistic regression step-down models were used to measure for the association of studied variables and HIV+ve ischaemic stroke. The variables for logistic regression were selected using biological plausibility on the basis of association with hypercoagulability state and also significant crude odds' ratios. A p-value of less than 0.05 was taken as the level of statistical significance.

CHAPTER 5 RESULTS

Between July 2014 and February 2015, 174 patients were approached as potential candidates for inclusion into the study. Of these, 18 patients were excluded from the study with 10 patients either HIV-ve with no stroke or had no brain imaging. Of the remainder, 1 patient refused consent and 7 patients had neurological findings other than ischaemic stroke. 156 patients were recruited of which 52 patients were HIV+ve and had ischaemic stroke. These patients were frequency matched for age (+/- 5 years) and sex with 52 HIV-ve ischaemic stroke patients and 52 HIV+ve no stroke patients as controls respectively.

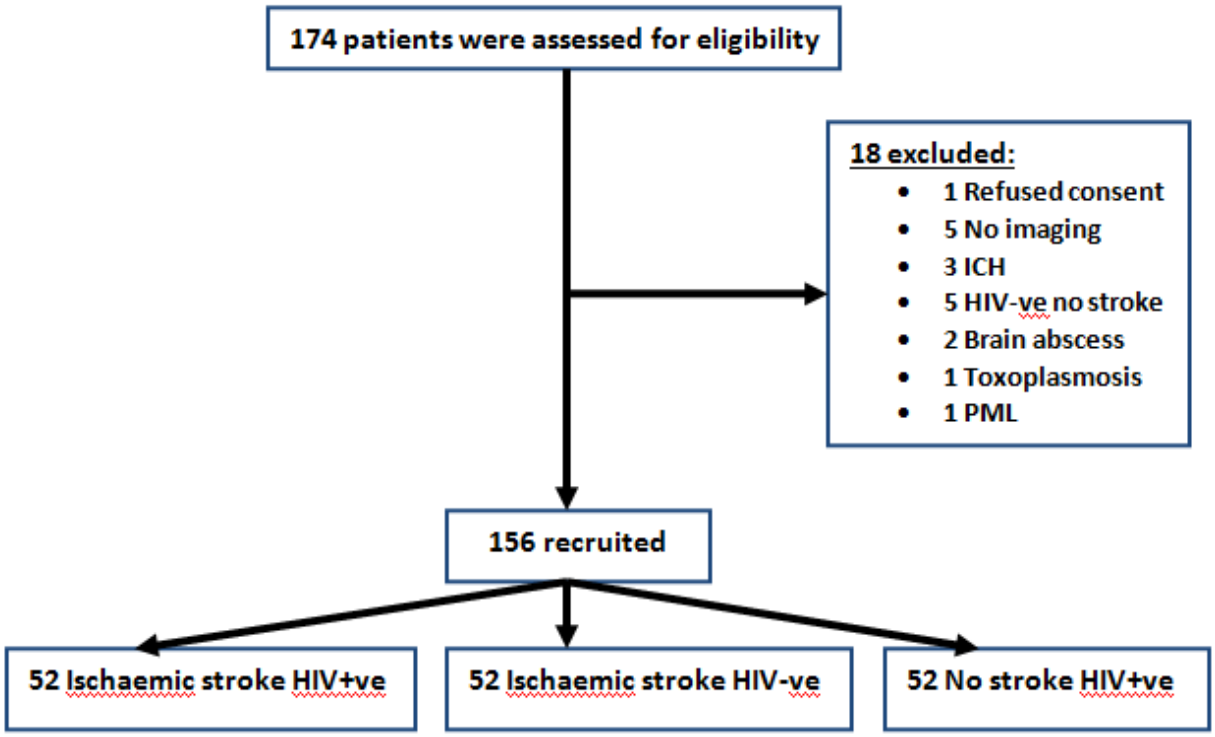

Figure 2: Recruitment process

The demographic and clinical findings of ischaemic stroke patients by HIV status are shown in **table 1a** and described briefly here. The difference between the mean age for the HIV+ve ischaemic stroke and the HIV-ve ischaemic stroke patients was statistically significant, but their ranges were comparable. This was because we had frequency matched the cases with controls by age (+/- 5 years), but as expected the HIV-ve ischaemic stroke patients tended to be older. The majority of the patients in both arms were females with no statistical difference between the two arms. Eight patients in the ischaemic stroke HIV-ve arm had previous ischaemic stroke compared to none in the other arm. Of the traditional risk factors, only hypertension was statistically significant as it was commoner in the HIV-ve ischaemic stroke patients. RPR was non-reactive in all the patients in both arms.

Table 1a: Comparison of baseline demographic and clinical characteristics between HIV+ve and HIV-ve ischaemic stroke patients

Characteristics	**Ischaemic stroke HIV+ve N=52**	**Ischaemic stroke HIV-ve N=52**	**P - value**
Age, years, mean (range)	52 (20 – 76)	61 (29 – 78)	< 0.01*
Sex, female, n (%)	30 (58)	34 (65)	0.42
Hypertension, n (%)	26 (50)	39 (75)	0.01*
Myocardial infarction, n (%)	0 (0)	1 (2)	0.99
Cardiac arrhythmias, n (%)	1 (2)	2 (4)	0.99
Previous ischaemic stroke/TIA, n(%)	0 (0)	8 (15)	< 0.01*
Alcohol, n (%)	10 (19)	6 (12)	0.28
Family history of stroke, n (%)	13 (25)	13 (25)	0.99
NIHSS score at enroll, n (range)	8 (2 – 15)	11 (4 – 19)	0.25
Oxford handicap at enroll, mean(%)	6 (12)	2 (4)	0.27
SBP, mmHg (range)	145 (110 – 180)	152 (117 – 187)	0.27
DBP, mmHg (range)	87 (66 – 108)	87 (69 – 106)	0.93
Hb, g/dl (range)	12.6 (10.5–14.6)	12.8 (10.7–15.0)	0.60
Serum creatinine(>120umol/l)n(%)	6 (17)	7 (23)	0.50

**statistically significant*

The subtypes of ischaemic stroke using TOAST classification were compared between the HIV+ve and HIV-ve patients using a bar chart (**figure 3**). Thirty-three percent of the patients in the HIV+ve ischaemic stroke arm compared to 8% in the HIV-ve arm had stroke of undetermined aetiology either due to negative evaluation or due to the presence of two or more causes. On Chi square test analysis, the difference between the two was statistically significant ($p<0.01$). Seventeen percent of the patients in the HIV+ve ischaemic stroke arm had stroke of undetermined aetiology due to incomplete evaluation compared to 21% patients in the other arm and this difference was not statistically significant ($p=0.31$). In addition, the HIV-ve arm had more of its patients with large artery atherosclerosis compared to the HIV+ve arm ($p=0.05$) as illustrated below.

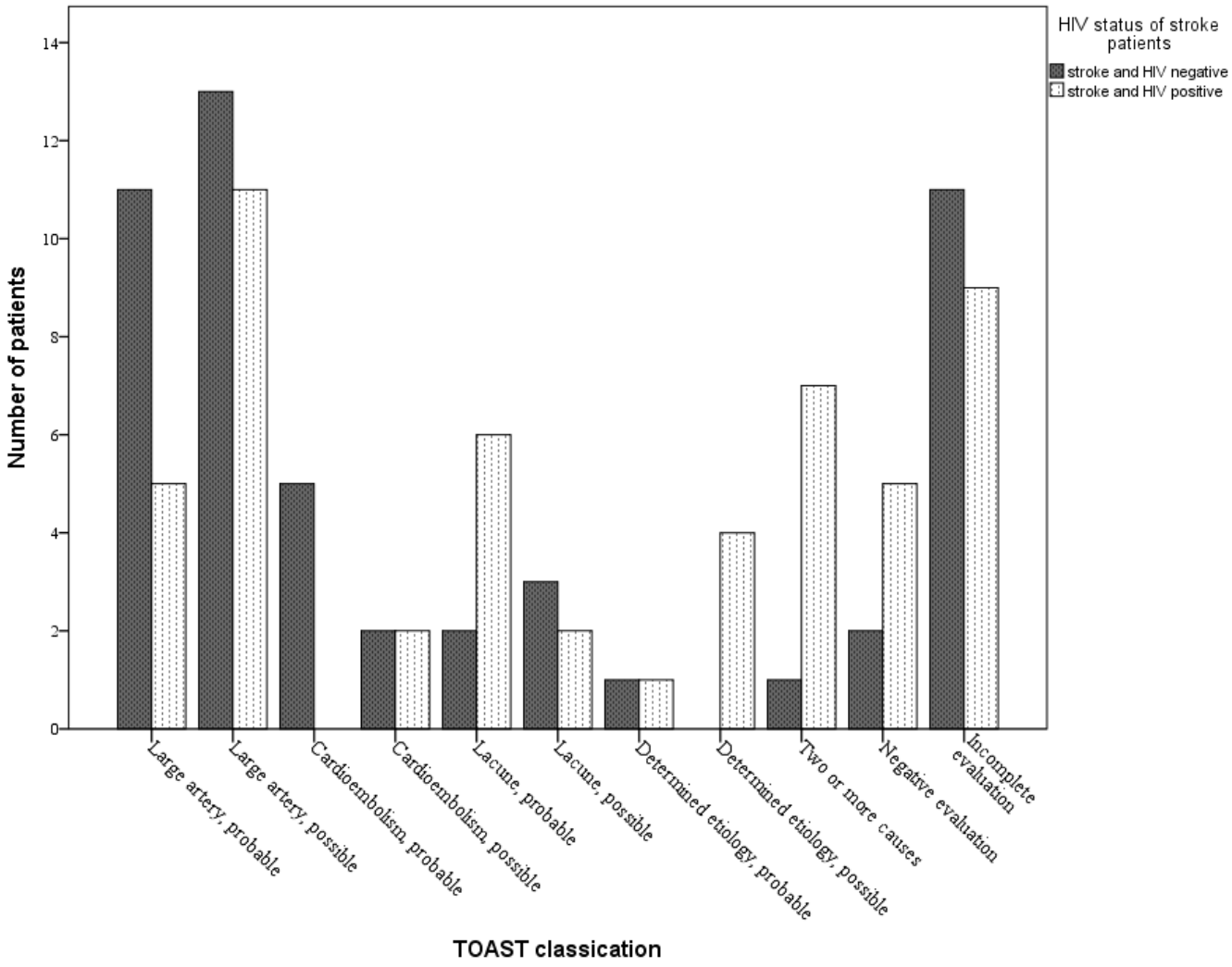

Figure 3: TOAST classification comparing ischaemic stroke by HIV status

Table 1b shows the demographic and clinical characteristics of the ischaemic stroke HIV+ve patients now compared to no stroke HIV+ve patients. The difference in mean ages between the two arms was statistically significant, but their ranges were comparable. As with the other control group, the difference was because the patients were frequency matched by age (+/-5years). There was no statistical difference in the gender of recruited patients between the two arms. The majority of the patients in the HIV+ve ischaemic stroke arm where on ART compared to the no stroke arms. The mean SBP and DBP together with hypertension were also statistically different between the two arms. RPR was negative in all the patients in both arms.

Table 1b: Comparison of baseline demographic and clinical characteristics between ischaemic stroke and no stroke HIV+ve patients

Characteristics	**Ischaemic stroke HIV+ve N=52**	**No stroke HIV+ve N=52**	**P - value**
Age, years, mean(range)	52 (20 – 76)	46 (20 – 71)	0.01*
Sex, female, n(%)	30 (58)	24 (46)	0.24
Hypertension, n(%)	26 (50)	9 (17)	< 0.01*
Cardiac arrhythmias, n(%)	1 (2)	0 (0)	0.99
Alcohol, n(%)	10 (19)	4 (8)	0.09
Family history of stroke, n(%)	13 (25)	10 (19)	0.48
ART use, n(%)	36 (69)	46 (88)	0.03*
NRTI use, n(%)	34 (65)	49 (94)	0.99
NNRTI use, n(%)	33 (63)	46 (88)	0.64
PI use, n(%)	1 (2)	3 (6)	0.64
ART duration(> 3months), n(%)	47 (90)	51 (98)	0.21
SBP, mmHg (range)	145 (110 – 180)	126 (107 – 144)	< 0.01*
DBP, mmHg (range)	87 (66 – 108)	77 (66 – 89)	< 0.01*
Hb, g/dl (range)	12.6 (10.5 – 14.6)	12.2 (9.7 – 14.7)	0.47
CD4+ T cell count, cells/ul (range)	431 (111 – 751)	422 (164 – 680)	0.89
Serum creatinine(>120umol/l),n(%)	6 (12)	4 (8)	0.32

**statistically significant*

Table 2 illustrates the comparison of hypercoagulability state markers between the cases (ischaemic stroke HIV+ve) and the two controls (ischaemic stroke HIV-ve and no stroke HIV+ve). Hyperhomocysteinaemia and PS deficiency were significantly present in the cases compared to the two controls. In addition, smoking, obesity and sedentary lifestyle were statistically found in the ischaemic stroke HIV-ve control compared to the cases and the no stroke HIV+ve control.

Table 2: Comparison of hypercoagulability state markers between ischaemic strokes HIV+ve and HIV-ve patients as well as between ischaemic stroke HIV+ve and no stroke HIV+ve patients

Variables	**Ischaemic stroke HIV+ve N=52**	**Ischaemic stroke HIV-ve N=52**	**No stroke HIV+ve N=52**	**Stroke HIV+ve vs. stroke HIV-ve P-value**	**Stroke HIV+ve vs. No stroke HIV+ve P-value**
Hyperhomocysteinaemia>14.4umol/l	33 (63)	20 (38)	27 (52)	0.01*	0.23
Protein S deficiency<60%	22 (42)	10 (19)	18 (35)	0.01*	0.42
Protein C deficiency<70%	5 (10)	5 (10)	0 (0)	0.99	0.06
Hypercholesterolaemia>5.2mmol/l	4 (8)	7 (13)	4 (8)	0.99	0.99
Smoking, n(%)	2 (4)	11 (21)	2 (4)	0.01*	0.99
Diabetes mellitus, n(%)	8 (15)	6 (12)	3 (6)	0.57	0.11
Obesity, n(%)	2 (4)	11 (21)	0 (0)	0.01*	0.50
Sedentary lifestyle, n(%)	46 (89)	50 (96)	18 (35)	0.27	0.01*

**statistically significant*

Tables 3a and **3b** show significant variables after step down binary logistic regression. The first table shows the variables for HIV+ve ischaemic stroke compared to HIV-ve ischaemic stroke patients and the second table shows HIV+ve ischaemic stroke compared to HIV+ve no stroke patients. This was done to assess for the association of hypercoagulability state markers and traditional risk factors with ischaemic stroke in HIV+ve patients. As expected, hypertension, sedentary lifestyle, smoking and obesity were associated with ischaemic stroke. Alcohol intake was noted to be protective against ischaemic stroke although we did not accurately quantify the amounts taken by our patients. . Protein S deficiency was associated with HIV infection, but not ischaemic stroke whereas Protein C deficiency and hyperhomocysteinaemia were neither associated with Ischaemic stroke nor HIV infection on logistic regression analysis. ART use was, however, noted to be associated with ischaemic stroke.

Table 3a: Binary logistic regression analysis of HIV+ve ischaemic stroke compared to HIV-ve ischaemic stroke patients

Variables	Crude OR	Adjusted OR	P – value (Adj. OR)
Age	0.94 (0.91 – 0.98)	0.93 (0.89 – 0.97)	< 0.01
Protein S deficiency	0.33 (0.13 – 0.78)	0.32 (0.10 – 0.96)	0.04
Smoking	6.71 (1.41 – 31.99)	20.7 (2.60– 164.40)	< 0.01
Obesity	6.71 (1.41 – 31.99)	4.13 (0.77 – 22.07)	0.10

Table 3b: Binary logistic regression analysis of HIV+ve ischaemic stroke compared to HIV+ve no stroke patients

Variables	Crude OR	Adjusted OR	P – value (Adj. OR)
Hypertension	4.78 (1.94 – 11.76)	4.51 (1.51 – 13.47)	0.01
Alcohol	0.35 (0.10 – 1.20)	0.15 (0.03 – 0.69)	0.02
ART use	5.43 (0.61 – 48.16)	8.77 (0.91 – 84.65)	0.06
Sedentary lifestyle	4.06 (1.64 – 11.31)	4.92 (1.42 – 16.99)	0.01

CHAPTER 6 DISCUSSION

Despite frequency matching the age between the HIV+ve and HIV-ve ischaemic stroke patients (+/- 5years), the difference between the two categories in terms of age was statistically significant. This observation may be explained by an earlier study at UTH by Lambwe who found that 83.3% of stroke in the young was attributed to HIV infection.[23] In Malawi, Heikinheimo et al found that HIV infection was a risk factor for ischaemic stroke for young people with no common risk factors.[45] This seems to be a universal occurrence as it has also been demonstrated in the USA by Sico et al in a study on HIV status and the risk of ischaemic stroke among men.[46] He found that HIV infection was associated with an increased ischaemic stroke risk among young HIV-infected compared with demographically and behaviourally similar uninfected male veterans.

We analyzed the subtypes of ischaemic stroke by HIV status on the basis of aetiology using TOAST classification. We found that 33% of the HIV+ve ischaemic stroke patients presented with stroke of undetermined aetiology due to negative evaluation compared to 8% in the HIV-ve group. The HIV-ve group had large artery atherosclerosis as the most important aetiological mechanism accounting for 46% of its patients whereas it only accounted for 30% in the HIV+ve group. This can be explained by Ortiz et al[25] who found that the incidence of vasculitis and hypercoagulability state is relatively high in HIV+ve patients and a likely mechanism for ischaemic stroke occurrence in these patients.

We compared variables that promote a hypercoagulability state between the HIV+ve and the HIV-ve ischaemic stroke patients, and an additional HIV+ve with no stroke patients. PC deficiency did not show any significant difference between these groups although hyperhomocysteinaemia and PS deficiency were significantly more in the HIV+ve ischaemic

stroke group compared to the HIV-ve ischaemic stroke group. Homocysteine levels are elevated in vitamin B_{12} and folic acid deficiency which is a common finding in HIV+ve patients who present with micronutrient deficiencies. Hence the reason why there was no noted difference in the presence of hyperhomocysteinaemia between the HIV+ve ischaemic stroke and HIV+ve with no stroke groups. This argument would have been strengthened if the body mass index (BMI) of all the patients we recruited had been done and compared as well as actual documentation serum B_{12} levels. Nonetheless, hyperhomocysteinaemia has been strongly linked to progression of generalized small-vessel disease as was evidenced by Kloppenborg et al who found that a role existed for homocysteine in the development of a generalized small-vessel disease in the brain.[49] Jeon et al, in looking at homocysteine, small-vessel disease and atherosclerosis also concluded that hyperhomocysteinaemia was associated with small-vessel disease of the brain and large-vessel disease of cerebral arteries.[50]

On the contrary, the study by Coria-Ramirez et al[51] added a new dimension to this discussion. They studied the effect of combination anti-retroviral therapy (cART) on homocysteine plasma concentrations in HIV-1 infected patients. They found that fasting and post-oral methionine load plasma homocysteine levels increased after 6 months of anti-retroviral treatment. Furthermore, they concluded that nutritional abnormalities were not responsible for hyperhomocysteinaemia, but suggested the enzymatic disturbances in the metabolic pathways of homocysteine that occurred after initiation of cART. The evidence from this study is worth taking into account considering 48% of the HIV+ve ischaemic stroke patients had been on ART for more than 3 months at the time of enrolment into our study. On logistic regression analysis, however, we found that hyperhomocysteinaemia was not significantly associated with HIV infection or ischaemic stroke. This was an unexpected finding which

needs further verification with a higher powered study, a larger sample size and also focusing on the role of genetic polymorphism particularly those related to homocysteine.

With regards to PS deficiency, we were able to demonstrate that PS deficiency is associated with HIV infection as was elicited in many studies including the Mochan et al study in South Africa, but we did not find a positive association with HIV+ve ischaemic stroke.[34-36] It was actually noted to be "protective" of HIV+ve ischaemic stroke – a finding which needs to be further studied. PS is produced by the liver and endothelial cells, and acts as a cofactor to activated PC in the inactivation of factor Va and factor VIIIa. The mechanism by which PS deficiency occurs is not fully understood, but is thought to result from the presence of autoimmune antibodies or because of inflammatory markers such as interleukin 1, cytokines and tumour necrosis factor alpha stimulated by HIV.[12]

The association of hypercoagulability state with ischaemic stroke in HIV infection is definitely a complex mechanism with many factors at play. Other anticoagulation factors that may have a role in this hypercoagulability state and need to be evaluated include Anithrombin III, factor V Leiden, lupus antibodies, anticardiolipin antibodies and prothrombin G20210A mutation.[13] In addition, genetic factors as well as polymorphisms are an important area of interest and serve as a limitation to our study. Moreover, there is evidence from twin and family-based studies which seems to suggest a substantial heritability for ischaemic stroke with different associations by ischaemic stroke subtypes.[26-29] This is exemplified by a large observational study in the United States of America which found that coagulation Factor XIII B-subunit contributed to risk of ischaemic stroke of cardioembolic subtype.[30] In this study, the HIV status of the participants was unknown and it is not entirely correct for these findings to be generalised to HIV+ve ischaemic stroke patients.

In summary, we assessed the association of hypercoagulability state markers and traditional risk factors with ischaemic stroke in HIV+ve patients. As expected, hypertension, sedentary lifestyle, smoking and obesity were significantly associated with ischaemic strokes irrespective of the HIV status. Alcohol intake was noted to be protective against ischaemic stroke although we did not accurately quantify the amounts taken by our patients. This is not an altogether surprising finding because alcohol in moderation is actually protective of ischaemic stroke as was demonstrated by Sacco et al.[52] There is need to research this finding in our population further, especially focusing on the relationship between alcohol abuse, dyslipidaemias and ischaemic stroke. Hyperhomocysteinaemia and PS deficiency are associated with HIV infection, but not ischaemic stroke.

6.1 STUDY LIMITATIONS

The funds were inadequate to allow for detailed investigation of the patients such that lipid profile, anticardiolipin antibodies, antithrombin III and HIV viral loads could not be done. Some findings were based on information from the routine care available at the hospital during the study. Failure to do brain imaging rendered some patients not to be included in the study due to technical difficulties. The BMIs for most patients could not be calculated because of lack of a proper weighing machine for bedridden patients. In addition, the assessment of the quantity of alcohol intake in the recruited patients was not adequate to properly derive a conclusion on its association with ischaemic stroke.

CHAPTER 7 CONCLUSION

The role of hypercoagulability state in ischaemic stroke in HIV infection cannot be under-estimated. Although the possible mechanisms by which it occurs may be complex and not fully understood, it is clear that there is a strong interaction between hypercoagulability state, ischaemic stroke and HIV infection. This is an area that requires extensive research and one that we cannot afford to ignore as it is an important bridge to all cardiovascular diseases**.** Protein S deficiency and hyperhomocysteinaemia are strongly associated with HIV infection although Protein S deficiency was surprisingly found to be "protective" of ischaemic stroke in these patients. The presence of these two markers in HIV+ve ischaemic stroke warrants them to be considered as important serum markers in the prevention of ischaemic strokes in the Zambian HIV+ve population

7.1 RECOMMENDATIONS

1. Further detailed exploration on these findings with emphasis on HIV acquisition and type and duration of ART use plus Human Leukocyte Antigen (HLA) studies and more comprehensive hypercoagulability markers especially with the unexpected finding of the "protective" nature of PS deficiency.
2. Accurate Blood Pressure measurement regularly and aggressive treatment of hypertension in all HIV positive patients.
3. Encourage lifestyle modification during ART clinic reviews.
4. Future studies to be undertaken to focus on the relationship between low CD4+ T cell count (especially below 100cells/ul) and HIV+ve ischaemic stroke.

REFERENCES

1. Strong K, Mathers C and Bonita R. **Preventing stroke: saving lives around the world**. *Lancet Neurol.* 2007;**6**:182–187. doi: 10.1016/S1474-4422(07)70031-5.

2. Hugh SM, Usman K and Jonathan B. **Differences in Stroke subtypes between Black and white patients with stroke**. *Stroke*. 2007;2:28-31.

3. Mackay J and Mensah G**. Atlas of Heart Disease and Stroke: World Health Organization**. *Stroke*. 2004;6:18-25.

4. Adamson J, Beswick A and Ebrahim S. **Stroke and Disability Journal of Stroke and Cerebrovascular Diseases**. International review of stroke. London: Academics; 2004 vol 13, No. 4 p.171 – 177.

5. Williams GR. **Incidence and characteristics of total stroke in the United States**. *BMC Neurol.* 2001; 1:2.

6. Kleindorfer D, Panagos P and Pancioli A. **Incidence and short-term prognosis of transient ischemic attack in a population based study**. *Stroke*. 2005; 36(4): p.720-3.

7. Graeme JH and Kennedy R. **Lees Stroke management in practice**. 3rd ed. London: Oxford University Press; 2001.

8. Warlow CP, Dennis MS and van Gijn J. **Stroke: A practical guide to management**. 2nd ed. Oxford: Blackwell Scientific publications; 2000.

9. Bonita R. **Stroke prevention: a global perspective**. In: Norris JW, Hachinski VC, editors. Stroke Prevention. New York: Oxford University Press; 2001: 259–274.

10. Connor MD, Walker R, Modi G, Warlow CP. **Burden of stroke in black Populations in sub-Saharan Africa**. *Lancet Neurol*. 2007; 6:269 –278.

11. Bonita R and Truelsen T. **Stroke in sub-Saharan Africa: a neglected chronic disease**. *Lancet Neurology*. 2003; 2:592.

12. Dobbs MR and Berger JR. **Stroke in HIV infection and AIDS**. *Expert Rev. Cardiovasc. Ther*. 2009;**7**:1263 – 1271.

13. Bamford J, Sandercock P and Dennis M. **A prospective study of acute cerebrovascular disease in the community: The Oxfordshire Community Stroke Project 1981-86.2.Incidence, case fatality rates and overall outcome at one year of cerebral infarction, primary intracerebral and subarachnoid hemorrhage**. *J Neurol Neurosurg Psychiatry*. 1990; 53: 16-22.

14. Connor M, Rheeder P, and Bryer A. **The South African stroke risk in general practice study**. *S Afr Med J*. 2005;95:334 –339.

15. Rosman KD. **The epidemiology of stroke in an urban black population**. *Stroke*. 1986; 17:667– 669.

16. Onwuchewa A, Bellgam H, Asekomeh G. **Stroke at the university of port Harcourt teaching hospital, rivers state, Nigeria**. *Trop Doct*. 2009 Jul; 39(3):150-2.

17. Caplan LR, Gorelick PB and Hier DB. **Race, sex and occlusive cerebrovascular disease: a review**. *Stroke*. 1986; 17:648–655.

18. Atadzhanov M, Mukomena PN, Lakhi S, et al. **Stroke Characteristics and Outcomes of Adults Patients Admitted to the University Teaching Hospital, Lusaka, Zambia**. *Open access*. 2012,**5**;3 – 8.

19. Adams HP, Bendixen BH and Kappelle LJ. **Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment**. *Stroke.* 1993; 24:35– 41.

20. Goldstein LB, Jones MR and Matchar DB. **Improving the reliability of stroke subgroup classification using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria editorial comment: classifying themechanisms of ischemic stroke**. *Stroke*. 2001; 32:1091–1097.

21. Worrall BB, Chen DT and Meschia JF. **Ethical and methodological issues in pedigree stroke research**. *Stroke*. 2001;**32**:1242–1249.

22. Ovbiagele B and Nath A. **Increasing incidence of ischaemic stroke in patients with HIV infection**. *Neurology*. 2011. DOI 10. 1212/ WNL.0b013e31820a0cfc.

23. Lambwe N. **Stroke in the young Adults at the University Teaching Hospital, Lusaka, Zambia**. *http//www.unzasom.zm* (2005). Accessed 20 Jan 2013.

24. Kengne AP and Anderson CS. **The neglected burden of stroke in sub-Saharan Africa**. *Int J Stroke*. 2006;1:180 –190.

25. Ortiz G, Koch S and Romano JG. **Mechanisms of ischaemic stroke in HIV-infected patients**. *Neurology*. 2007;**68**(16):1257 – 61.

26. Smego RA and Orlovic D. **Hypercoagulability due to protein S deficiency in HIV-seropositive patients**. *IJCRIMPH*. 2009.

27. Dichgans M and Markus HS. **Genetic association studies in stroke: methodological issues and proposed standard criteria**. *Stroke*. 2005;36:2027–2031. doi: 10.1161/01.STR. 0000177498. 21594

28. Sorice M, Griggi T and Arcieri P. **Protein S and HIV infection. The role of anticardiolipin and anti-protein S antibodies**. *Thromb Res*. 1994;**73**(3-4):165 – 75.

29. Sen S., Rabinstein AA and Elkind MSK. **Recent developments regarding Human Immunodeficiency Virus infection and stroke**. New York: Oxford University Press. 2012.

30. Longo M, Lelo T and Mbwuilu P. **Rates and predictors of stroke associated case fatality in black central African Patients**. *Cardiovasc J Afr*. 2008; 19: 72-6.

31. Rizza SA and Badley AD. **HIV protease inhibitors impact on apoptosis**. *Neurology*. 2008;3:75–79.

32. Barbaro G and Lacobellis G. **Metabolic syndrome associated with HIV and highly active antiretroviral therapy**. *J Neurol Psy*. 2009; 9:37 – 42.

33. Worm WS, Kamara DA, and Reiss P. **Evaluation of HIV protease inhibitors use and the risk of sudden death or nonhaemorrhagic stroke**. Copenhagen: Blackwell science. 2012.

34. Alain L, Alessi MC, and Poizot-Martin I. **Protein S deficiency and HIV infection**. *N Engl J Med*. 1991;**324**:1220.

35. Tipping B, de Villiers L, and Wainwright H. **Stroke in patients with human immunodeficiency virus infection**. *J Neurol Neurosurg Psychiatry*. 2007;**78**(12): 1320 – 1324.

36. Mochan A, Modi M and Modi G. **Protein S deficiency in HIV associated ischaemic stroke: an epiphenomenon of HIV infection**. *J Neurol Neurosurg Psychiatry*. 2005;**76**:1455 – 1456.

37. UNAIDS' World AIDS Day Report. 2012. *http//www.unaids.org*. Accessed 15 Feb 2013.

38. Connor MD, Lammie GA, Bell JE, et al. ***Cerebral Infarction in adult AIDS patients: Observations from the Edinburgh HIV Autopsy Cohort***. *Stroke*. 2000; 31: 2117 – 26.

39. Connor M and Bryer A. **Chronic diseases of lifestyle in South Africa since 1995 – 2005**. *S Afr Med J*. 2006;14:195 – 202.

40. Zambia Central Statistical Office. 2010 census. *http//www.cso.org.* Accessed 10 Jan 2013.

41. Matuja W, Janabi M, Kazema R, et al. **Strokes subtypes in black Tanzanians: Aretrospective Study of Computerized tomography scan diagnoses at Muhimbili National Hospital, Dar es salaam**. *Trop Doc*. 2004, 34:144-146.

42. Johannes M, Marianne AB, Emmanuel JB, et al. **Stroke Presentation and Outcome in Developing Countries: A Prospective Study in The Gambia Stroke**. *Top Curr Chem*. 2005; 36: 1388-1393.

43. Solberg HE. **Approved Recommendation on the Theory of Reference Values. Part 5. Statistical Treatment of Collected Reference Values.** *Determination of Reference Limits* 1987; **25** suppl 1: 645-656.

44. Remkova A. **Diagnostic approach to hypercoagulable states**. *Bratisl Lek Listy*. 2006;**107** (8): 292 – 295.

45. Heikinheimo T, Chimbayo D and Kumwenda JJ. **Stroke outcomes in Malawi, a country with high prevalence of HIV: a prospective follow-up study**. *Open access*. 2012;**7**:3.

46. Sico JJ and Justice CA. **HIV status and the risk of ischaemic stroke among men**. *Neurology*. 2015;84:1933–1940.

47. Sacco RL. **Preventing stroke among blacks: the challenges continue**. *JAMA*. 2003;289:3005–3007.

48. Mateen FJ, Post WS and Sacktor N. **Long-term predictive value of the Framingham Risk Score for Stroke in HIV-positive vs HIV-negative men**. *Neurology*. 2014;81:2094–2102.

49. Kloppenborg PR, Geerlings MI, Visseren FL, et al. **Homocysteine and progression of generalized small-vessel disease**. *Neurology*. 2014;82:777–783.

50. Jeon SB**,** Kang WD, Kim JS and Kwon SU. **Homocysteine, small-vessel disease, and atherosclerosis**. *Neurology*. 2014;83:695–701.

51. Coria-Ramirez E, Cisneros LN, Trevino-Perez S, et al**. Effect of highly active antiretroviral therapy on homocysteine plasma concentrations in HIV-1 infected patients**. *J Acquir Immune Defic Syndr*. 2010;**54**:477 – 481.

52. Sacco RL, Vermeulen M, et al. **The protective effect of moderate alcohol consumption on ischaemic stroke**. *JAMA*. 1999;12:5.

APPENDICES

A. Information Sheets

Information sheet for the association of hypercoagulability state with ischaemic stroke in adult Zambian HIV+ve patients

You are invited to take part in a study looking at the association of hypercoagulability state with ischaemic stroke in adult HIV+ve patients admitted to UTH. This study is being done as part of requirement for a master of internal medicine. Information about this study is supplied in this document. One of the study team will be on hand to explain the contents and answer all your questions. Please make sure that you understand everything in this document. If you decide to participate you will be asked to give consent before you take part.

Participation in this study is completely voluntary. You are under no obligation to take part. You are free to withdraw from this study at any time. This will have no consequences for your medical care. No financial reward will be given to any persons taking part in this study.

Title of study

THE ASSOCIATION OF HYPERCOAGULABILITY STATE WITH ISCHAEMIC STROKE IN ADULT HIV+ve PATIENTS AT UTH

- **Who is doing the study?**

Dr Stanley Zimba is the principal investigator under the supervision of Dr Patrice Mukomena and Professor Masharip Atadhzanov. The principle investigator is responsible for the day to day running of the study. We can be contacted via Department of medicine, University Teaching Hospital, **Lusaka, Zambia**

Tel: +260977437082, +260979832265, 0977775662

EMAIL: stanpaulzimba@yahoo.co.uk, patricemuko@yahoo.fr, masharip.atadzhanov@gmail.com

The study has been approved by ERES Converge IRB and the School of Medicine post graduate forum. They can be contacted on the following number: +260 955155633/4 or at this address: ERES Converge IRB, 33 Joseph Mwilwa Road, Rhodes Park, Lusaka, Zambia; email: eresconverge@yahoo.co.uk

- **What is the purpose of this study?**

Stroke is a major health problem in the world and increasingly in Zambia. Cerebrovascular accident may occur as a result of haemorrhage or infarction in the brain. It is an emergency and early diagnosis is important to save life. Patients presenting with stroke can have some risk factors such as diabetes, hypertension, high cholesterol, smoking and others may also have members of the family affected earlier on.

The aim of this project is to look at the association of hypercoagulability state with ischaemic stroke in HIV. This an important risk factor that needs to be explored so that treatment can be tailored towards the cause in the same way hypertension, diabetes mellitus, smoking and other risk factors are targeted in treatment.

- **What is stroke?**

Stroke is a sudden appearance of weakness of a part of the body due to narrowing, clogging or rupture of a blood vessel in or near the brain lasting more than 24 hours.

- **Procedure of the study**

1. If you agree to take part in this project you will be asked to sign or print a consent form. You will be given a copy of this information sheet and the consent form to keep.
2. You (patient or relatives if aphasic or unconscious) will then be interviewed. The interview will start with questions about your age, sex and place where you live. You will then be asked questions about your illness including the symptoms and length of time you have been unwell. You will be asked questions about your past medical history. You will be asked a few questions related to your general health and the health of yours parents and siblings. The interview will take about 30 minutes or less.
3. Your medical notes will be reviewed and your progress while you are an inpatient at UTH will be followed.
4. Blood samples to test for hypercoagulability will be collected and sent to the lab for analysis.
   The information you give in the interview and in the notes will be analyzed with the other results from the study.

- **Are there any risks for people taking part in this study?**

  Some of the questions in the interview related to health are personal and may cause distress. If the interview is distressing you we will not continue.

  Taking part in the interview will not interrupt your clinical care.

  While we will be reviewing your notes and investigations we will not be directly involved in your clinical care.

- **Benefits**

  The main benefit from this study will be a greater understanding of the association of hypercoagulability state with ischaemic stroke in HIV+ve patients admitted to UTH. We hope this will lead to adequate planning and care for these patients at UTH in the future.

- **Confidentiality**

All information that you give in the interview and we obtain from your records will be kept confidential. Your identity will not be disclosed in any report or publication that results from this study. The data we collect will be kept securely and it will only be accessible to medical staff taking

part in the research. The research ethics ERES Converge IRB and the department of medicine/UTH may review the data for verification purposes.

If you have any questions about this study please ask them now. If you have any later questions or concerns please contact, Dr Stanley Zimba, Dr Patrice Mukomena Ntanda or Professor Masharip Atadzhanov at the above address. Please keep this information sheet in a safe place, thank you.

Surrogate information sheet for the association of hypercoagulability state with ischaemic stroke in adult Zambian HIV+ve patients

Your patient is invited to take part in a study looking at the association of hypercoagulability state with ischaemic stroke in adult HIV+ve patients admitted to UTH. This study is being done as part of requirement for a master of internal medicine. Information about this study is supplied in this document. One of the study team will be on hand to explain the contents and answer all your questions. Please make sure that you understand everything in this document. If you decide to participate you will be asked to give consent before your patient takes part.

Participation in this study is completely voluntary. Your patient is under no obligation to take part. They are free to withdraw from this study at any time. This will have no consequences for their medical care. No financial reward will be given to any persons taking part in this study.

Title of study

THE ASSOCIATION OF HYPERCOAGULABILITY STATE WITH ISCHAEMIC STROKE IN ADULT HIV+ve PATIENTS AT UTH

- **Who is doing the study?**

Dr Stanley Zimba is the principal investigator under the supervision of Dr Patrice Mukomena and Professor Masharip Atadhzanov. The principle investigator is responsible for the day to day running of the study. We can be contacted via Department of medicine, University Teaching Hospital, **Lusaka, Zambia**

Tel: +260977437082, +260979832265, 0977775662

EMAIL: stanpaulzimba@yahoo.co.uk, patricemuko@yahoo.fr,
masharip.atadzhanov@gmail.com

The study has been approved by ERES Converge IRB and the School of Medicine post graduate forum. They can be contacted on the following number: +260 955155633/4 or at this address: ERES Converge IRB, 33 Joseph Mwilwa Road, Rhodes Park, Lusaka, Zambia; email: eresconverge@yahoo.co.uk

- **What is the purpose of this study?**

Stroke is a major health problem in the world and increasingly in Zambia. Cerebrovascular accident may occur as a result of haemorrhage or infarction in the brain. It is an emergency and early diagnosis is important to save life. Patients presenting with stroke can have some risk factors such as diabetes, hypertension, high cholesterol, smoking and others may also have members of the family affected earlier on.

The aim of this project is to look at the association of hypercoagulability state with ischaemic stroke in HIV. This an important risk factor that needs to be explored so that treatment can be tailored towards the cause in the same way hypertension, diabetes mellitus, smoking and other risk factors are targeted in treatment.

- **What is stroke?**

Stroke is a sudden appearance of weakness of a part of the body due to narrowing, clogging or rupture of a blood vessel in or near the brain lasting more than 24 hours.

- **Procedure of the study**

5. If you agree to take part in this project you will be asked to sign or print a consent form. You will be given a copy of this information sheet and the consent form to keep.
6. You (patient or relatives if aphasic or unconscious) will then be interviewed. The interview will start with questions about age, sex and place where the patient lives. You will then be asked questions about the patient's illness including the symptoms and length of time they have been unwell. You will be asked questions about their past medical history. You will be asked a few questions related to their general health and the health of their parents and siblings. The interview will take about 30 minutes or less.
7. Their medical notes will be reviewed and their progress while they are an inpatient at UTH will be followed.
8. Blood samples to test for hypercoagulability will be collected and sent to the lab for analysis. The information given in the interview and in the notes will be analyzed with the other results of the study.

- **Are there any risks for people taking part in this study?**

Some of the questions in the interview related to health are personal and may cause distress. If the interview is distressing you we will not continue.

Taking part in the interview will not interrupt clinical care.

While we will be reviewing the notes and investigations we will not be directly involved in the patient's clinical care.

- **Benefits**

The main benefit from this study will be a greater understanding of the association of hypercoagulability state with ischaemic stroke in HIV+ve patients admitted to UTH. We hope this will lead to adequate planning and care for these patients at UTH in the future.

- **Confidentiality**

All information that you give in the interview and we obtain from the patient's records will be kept confidential. Their identity will not be disclosed in any report or publication that results from this study. The data we collect will be kept securely and it will only be accessible to medical staff taking part in the research. The research ethics ERES Converge IRB and the department of medicine/UTH may review the data for verification purposes.

If you have any questions about this study please ask them now. If you have any later questions or concerns please contact, Dr Stanley Zimba, Dr Patrice Mukomena Ntanda or Professor Masharip Atadzhanov at the above address. Please keep this information sheet in a safe place, thank you.

KAMASULIDWE KA PUNZIRO LA CHIYANJANO PAKATI PA MATENDA YA "STROKE" [KUZIZILA KWA ZIBALO ZINA ZA MUTUPI] NDI KAYUMEDWE MAGADZI MU ANTHU ALI NDI KA LOMBO KA HIV ALI MU CHIPATALA CHA UNIVERSITY TEACHING HOSPITAL UTH .

Mwa itanidwa kutengako mbali mu punziro lo fufudza chiyanjano pakati pa matenda ya "stroke" [kuzizila kwa zibalo zina za mutupi] ndi kayumedwe magadzi mu anthu ali ndi ka lombo ka HIV ali mu chipatala cha University Teaching Hospital UTH. Punzilori ndi chi gawo cha mapunziro yo kuya you chedwa "Master of Internal Medicine". Kamasulidwe ka zochita mu punziro kali mu pepala ili. Umodzi wa oyandeletsa punzilori aza nkalapo ku masulila and kuyanka mafunso yonse. Ti kupenpani kuti mu nkale omasuka ku zunsa zones za mene mufuna pa punzilori. Ngati mwa vomela ku tengako mbali mu punzilo ili muzafunika kupasa chivomekezo mukalibe kutengako mbali.

Kutengako mbali mu punzilo ili chili kwani kopanda chikoka. Muli omasuka ku leka ntawi iliyonse kopanda chifukwa ndi kuza nkala kulibe chilango. Kulibe ndalama zaneme zika pasidwa kuli onse atengako mbali mupunzilo ili.

Ndina La Punziro

CHIYANJANO PAKATI PA MATENDA YA "STROKE" [KUZIZILA KWA ZIBALO ZINA ZA MUTUPI] NDI KAYUMEDWE MAGADZI MU ANTHU ALI NDI KA LOMBO KA HIV ALI MU CHIPATALA CHA UNIVERSITY TEACHING HOSPITAL UTH

- **Ndani oyendelesta punzilori**

A Dotolo Stanley Zimba ndiye oyendelesta ndipo ali ndi o ayanga nira a Dololo Patrice Mukomena ndi a Professor Marsharip Atazdhzanov. Oyendeletsa punziro munga apedze ku UTH chigawo cha Internal Medicine.

Ma numba ya ma Lamya yao ndi

+260977437082, +260979832265, 0977775662

EMAIL: stanpaulzimba@yahoo.co.uk, patricemuko@yahoo.fr,
masharip.atadzhanov@gmail.com

Iri punziro la vomekezedwa ndi a ERES Converge IRB ndi chigawo choyandanira mapunziro yakuya pa university of Zambia, School of Medicine ndi a ERES Converge IRB. Na lamya yawo ndi kwamene ba pezeka ndi +260 955155633/4 ERES Converge IRB, 33 Joseph Mwilwa Road, Rhodes Park, Lusaka, Zambia; email: eresconverge@yahoo.co.uk

- **Lingaliro La punziro**

“Stroke” ndi matenda yakulu ndi po yaku chuluka mu nzko la Zambia. Ngozi zo kuza mizipe za bombo imachitika paneme magadzi a taika kapena kuleka kuyenda chifukwa dzina zoletsa magaziwo kuyenda bwino. Izi ndi zofunikira kudziwa kotero kuti umtu a pulumuke. Anthu ali na “stroke” ama nkala ndi ndzina zinu zinga lengetse kuchuluka monga matenda ya Sugar [diabetes], BP you chuluka, mafuta, kukoka fodya kapena mubanja kuli ena anthu ana dwalapo matenda awa.

Muthu wa punzilori ndi ku fufudza chiyanjano kapati ka mwamene magadzi ya yumila muli anthu ali ndi “stroke” yamene ichokela ku chifukwa cha magadzi kusafika mukwanira ku bombo mu anthu alindi kalombo ka HIV. Ici ndi chifukwa chacikuru chofunikira kufufudza kotero kuti mankwala yo chilitsa ndi moyanganora anthuwa munga yanga niwapo ndi ku chilitsa bwino.

- **“Matenda ya “storke” ndi chain?**

Muntu ngati ali ndi matenda ya “stroke” chitantaudza kuti ku yamba kunvela kuzizila(kusowa mpanvhu) mwa dzidizdiz chifukwa magadzi mu midzipe si yayenda bwino chifukwa cha kuchepa, kapena ku valika, ndi kung’ambika.

- **Zdochitika mu pundilori**

9. Ngati mwa vomela kutengako mbali mu punzilori muza pempedwa ku lemba signature kapena ku fwatika ka pepala la chi bvumekezdo. Mudza tenga chigawo cha pepela iri ndi zoonze zo mastula punzilori
10. Inu (odwala kapena oimilira odwala) muza funsidwa mafunso pali zintu ngati kwamene munkala, zaka, mwamene matenda yanayambira ndi panene kusanvela bwino kuna yamba. Zingo ndi ku dwala kuli konse muna kala nako masiku yakumbuyo, ndi anthu amu banja lanu. Kufuza uku kuzatenga nthawi inga fike ku 30minute chabe
11. Mapela yanu ya chipata yaza yanga niwa ntawi ndi nthawi ndi mwa mene kulili pamene mukali mu chipatala.

Ti dza tengako ma gadzi ku pima kayumidwe, ndi zina chache ku labu.

- **Kodo Ku li zoyofwa ku kengako mbali mu pumzilori?**

Mafunso yamene ino simuli omasuka ku yanka muli omasuka kusa yanka

Kutengako mbali mu punzilori sikuza lengetsa bvuto lililonse mu ku yanga niwa ndi ku chilisidwa kwanu

Ngankali tiza yanga ma pepala yanu oyendeletsa punzilori sabaza tengako mbali mu ku chilisidwa kwanu

- **Zopedzamo**

Chikulu cho pezamo ndi ku dziwa chiyanjano pakati pa matenda ya “stroke” [kuzizila kwa zibalo zina za mutupi] ndi kayumedwe magadzi mu anthu ali ndi ka lombo ka HIV ali mu chipatala cha University Teaching Hospital UTH ndi ku dziwa mu chilisira matenda aya

- **Chisinsi**

Zonse zamene tiza tenga ndi kulemba ziza sungidwa mwa chisinsi. Madzina yanu siyaza ziwika ndi kupedzeka pali ponse mu lipoti ndi malemba yaza chokera mupunzilori. Oyendeletsa pumzilori nyi oka aza ziwa mwa chisinsi zonsezi.
Chigawo cha Medicine ku UTH ndi aku ERES ConvergeIRB adza yangana ku zones tiza tenga ngati tazindikila chivumekedzo.

Ngati muli namafunso funsani manje, ngati musogoro munga kambe naba Dr Stanley Zimba, Dr Patrice Mukomena Ntanda or Professor Masharip Atadzhanov. Sungani pepala ili bwino. Zikomo

B. Informed consent

Informed consent form for the association of hypercoagulability state with ischaemic stroke in HIV+ve study

1. I have been invited to take part in a research project being conducted at the University Teaching Hospital by Dr Stanley Zimba, Dr Patrice Mukomena Ntanda and Professor Atadhzanov department of medicine, UTH, Lusaka, Zambia; Tel: +260977437082 Email: **stanpaulzimba@yahoo.co.uk**, **patricemuko@yahoo.fr**, **masharip.atadzhanov@gmail.com**
2. The study is being supervised by professor Atadzhanov and Dr Patrice Mukomena Ntanda, Department of medicine, School of Medicine, UNZA. Tel 00260977775662.
3. I have been told the purposes of this research and understand the processes involved. I understand the potential distress that may occur.
4. I have been given a list of names and addresses of people and institutions I may contact in relation to this research.

   I have read the information in the **association of hypercoagulability with ischaemic stroke in HIV+ve information sheet** or have had it read or explained to me.
5. I have had the opportunity to ask questions and have had these answered satisfactorily.
6. I understand that I have the right to refuse to participate in this study or withdraw from the study at any time. I understand that refusing to take part or withdrawing from the study will in no way compromise my clinical care.
7. I agree to take part in the study

Participant's information:

Signature (or fingerprint): _____

Name: _____ (please print) **Date: _____**

The person who conducts the informed consent discussion must also sign and date this form.

Signature: _____

Name: _____ (please print) **Date: _____**

Signature of witness, if applicable.

Witnessed by: (print name): _____

Signature of Witness: _____ Date _____

Surrogate informed consent form for the association of hypercoagulability state with ischaemic stroke in HIV+ve study

1. My patient has been invited to take part in a research project being conducted at the University Teaching Hospital by Dr Stanley Zimba, Dr Patrice Mukomena Ntanda and Professor Atadhzanov department of medicine, UTH, Lusaka, Zambia;Tel:+260977437082

   Email: **stanpaulzimba@yahoo.co.uk**, **patricemuko@yahoo.fr**, **masharip.atadzhanov@gmail.com**
2. The study is being supervised by professor Atadzhanov and Dr Patrice Mukomena Ntanda, Department of medicine, School of Medicine, UNZA. Tel 00260977775662.
3. I, on behalf of my patient, have been told the purposes of this research and understand the processes involved. I understand the potential distress that may occur.
4. I have been given a list of names and addresses of people and institutions I may contact in relation to this research. I have read the information in the **association of hypercoagulability with ischaemic stroke in HIV+ve information sheet** or have had it read or explained to me.
5. I have had the opportunity to ask questions and have had these answered satisfactorily.
6. I, on behalf of my patient, understand that I have the right to refuse participation in this study or withdraw from the study at any time. I understand that refusing to take part or withdrawing from the study will in no way compromise my patient's clinical care.
7. I agree on behalf of my patient to take part in the study

Surrogate's information:

Signature (or fingerprint): _____

Name: _____ (please print) **Date:** _____

The person who conducts the informed consent discussion must also sign and date this form.

Signature: _____

Name: _____ (please print) **Date:** _____

Witnessed by: (print name): _____

Signature of Witness: _____ **Date:** _____

Chivumekedzo

1. Nda itanidwa kutengako mbali mupunzilori lili ku tenga mbali ku UTH ndi a Dr. Stanley Zimba, Dr Patrice Mukomena Ntanda or Professor Masharip Atadzhanov
   Email: **stanpaulzimba@yahoo.co.uk**, **patricemuko@yahoo.fr**, **masharip.atadzhanov@gmail.com**
2. Oyanganila a dotolo Zimba ndi Dr Patrice Mukomena Ntanda and Professor Atadhzanov department of medicine, UTH, Lusaka, Zambia Tel 00260977775662
3. Na kutila lingalilo la punzilo ndu nvesesetsa zofunika
4. Na pasidwa na zdina ndi ma lamya ya anthu ndi vigawo bamene ninga kambe nawo ngati ndiri ndi mafunso
   Ba ndi masulila zofunika, ndi tengako mbali mu punzilori
5. Na funsa mafunso na ku kutira ndi mayanko
6. Ndi zdzi wa kuti ndine omasuka ku kana kutengako mbali kapena ku leka ntawi iliyonse ndipo kuti kuleka ketengako mbali si ku za lesta chichilisidwa kwanga ndi a dololo
7. Na bvumela kutengako mbali mu punzilo iri

Otengako mbali:

Signature kufwatika)): _____

Zdina:_____ Date: _____

Wo masula punzilo ndi chivumekedzo

Signature olo kufwatika _____

Zdina: _____ Date: _____

Zdina:_____

Signature olo kufwatika _____ Date_____

C. QUESTIONNAIRE

THE UNIVERSITY OF ZAMBIA

DEPARTMENT OF INTERNAL MEDICINE

Study title: <u>The association of hypercoagulability state markers with ischaemic stroke in adult HIV+ve patients at UTH</u>

DATE……

I. Demographic characteristics

File No_____ ID _____ Phone/address_____

1. How old where you on your last birthday? …….Years

2 Sex: M☐ F☐

3. Where you in formal employment before this admission?

☐ Yes ☐ No ☐ Unknown

4. What is your level of education?

☐ None ☐Primary ☐ Secondary ☐College ☐University

5. Residency:

Urban ☐

Rural ☐

What is your tribe?

☐Tonga ☐Bemba ☐ Lozi ☐Ngoni

☐ Luvale ☐ Lunda ☐Kaonde ☐Others

6. What is your marital status?

☐ Married ☐ Single ☐ Divorced ☐ Widow ☐ Widower ☐ Cohabiting

7. Date of stroke:_____

8. Date of enrolment:_____

9. Date of blood draw (hypercoagulability markers):_____

10. Who signed consent form?

☐Subject only

☐Surrogate and subject

☐Surrogate

If surrogate signed, what is the relationship of the surrogate to subject?

☐ Spouse or partner ☐Sibling

☐

☐ Child ☐ Other

☐ Parent ☐ Unknown relationship to subject

II. Risk factors

The questionnaire for verifying stroke – free status

Instructions: Questionnaire items are to be read aloud exactly as written and in numerical order. The interviewer is permitted to rephrase or repeat questions if necessary. A response must be recorded for all eight items in every interviewee who can give history.

	YES	NO	UNKNOWN
1. Where you ever told by a medical practitioner that you had a stroke?	☐	☐	☐
2. Where you ever told by a medical practitioner that you had a mini-stroke, TIA or transient ischaemic attack?	☐	☐	☐
3. Have you ever had sudden, painless weakness on one side of your body?	☐	☐	☐
4. Have you ever had sudden numbness or a dead feeling on one side of your body?	☐	☐	☐
5. Have you ever had sudden, painless loss of vision in one or both eyes?	☐	☐	☐
6. Have you ever suddenly lost one half of your vision?	☐	☐	☐
7. Have you ever suddenly lost the ability to under-stand what people were saying?	☐	☐	☐
8. Have you ever suddenly lost the ability to express yourself verbally or in writing?	☐	☐	☐

Inclusion criteria

a) Is ischaemic stroke confirmed by history, physical examination, neurologic examination and head imaging (CT or MRI)? ☐ Yes ☐ No

b) Is onset of stroke symptoms within 30 days of enrolment? ☐ Yes ☐ No

c) Achieved 18th birthday by the date of enrolment? ☐ Yes ☐ No

d) Did the patient have an HIV test done? ☐ Yes ☐ No

e) Did patient or surrogate provide written informed consent? ☐ Yes ☐ No

Exclusion criteria

a) Did the stroke symptoms begin within 30 days after intracranial haemorrhage? ☐ Yes ☐ No

b) Is the patient pregnant? ☐ Yes ☐ No

c) Does the patient have a history of liver disease? ☐ Yes ☐ No

d) Is the patient taking anticoagulant drugs, contraceptive drugs or hormonal replacement therapy? ☐ Yes ☐ No

e) Is the focal neurological deficit of non-vascular origin (head injury, space occupying lesion, CNS infection or neurodegenerative disorders)? ☐ Yes ☐ No

f) Does the patient have a neurological condition other than stroke that is likely to influence NIH stroke scale? ☐ Yes ☐ No

III. Medical history

Does the subject have any history of the following? (check all that apply)

Transient Ischaemic attack	☐ Yes	☐ No	☐ Unknown
Congestive heart failure	☐ Yes	☐ No	☐ Unknown
Heart disease	☐ Yes	☐ No	☐ Unknown
Myocardial infarction	☐ Yes	☐ No	☐ Unknown
Peripheral vascular disease	☐ Yes	☐ No	☐ Unknown
Atrial fibrillation	☐ Yes	☐ No	☐ Unknown
Hypertension	☐ Yes	☐ No	☐ Unknown
Deep vein thrombosis	☐ Yes	☐ No	☐ Unknown
Hyperlipidaemia	☐ Yes	☐ No	☐ Unknown
Diabetes mellitus	☐ Yes	☐ No	☐ Unknown
Cancer	☐ Yes	☐ No	☐ Unknown
Recurrent miscarriages	☐ Yes	☐ No	☐ Unknown
Epilepsy	☐ Yes	☐ No	☐ Unknown
Sickle cell disease	☐ Yes	☐ No	☐ Unknown
Permanent cardiac pacemaker	☐ Yes	☐ No	☐ Unknown

IV. Drug inventory

Has the participant taken any of the following medication prior to or after the stroke, but before enrolment?

	Yes	No	Unknown
Aspirin	☐	☐	☐
Heparin	☐	☐	☐
Clopidogrel	☐	☐	☐
Warfarin	☐	☐	☐

Oral contraceptives	☐	☐	☐
Hormone replacement therapy	☐	☐	☐
Other anticoagulants	☐	☐	☐

Was the patient taking antihypertensive drugs at the time of stroke?

☐ Yes ☐ No ☐ Unknown

Was the patient taking lipid-lowering drugs at the time of stroke?

☐ Yes ☐ No ☐ Unknown

Was the participant taking antiretroviral therapy prior to or at the time of stroke?

☐ Yes ☐ No ☐ Unknown

If yes, state duration.

☐ < 3 months

☐ 3 – 6 months

☐ > 6 months

Which drugs (NRTI & PI)? (check all that apply)

☐ AZT ☐ D4T ☐ 3TC ☐ TDF ☐ FTC ☐ ABC

☐ ddI ☐ LPV/r ☐ EFV ☐ ATV ☐ DRV ☐ NVP

Source of family history (check all that apply)

☐ Proband/patient

☐ Spouse

☐ Medical records

☐ Friend or relative other than spouse

V. Family history of stroke

_____ Total number of full siblings

_____ Total number of full siblings with fatal or non-fatal stroke

_____ Total number of biological children

_____ Total number of biological children with fatal or non-fatal stroke

_____ Total number of biological parents with a history of stroke

Family history details:

Do any of the subject's family members have history of the following? (check all that apply)

Biological grandparents: ☐ Unknown

Heart disease ☐ Yes ☐ No ☐ Unknown

Hypertension	☐ Yes	☐ No	☐ Unknown
Stroke	☐ Yes	☐ No	☐ Unknown
Diabetes mellitus	☐ Yes	☐ No	☐ Unknown
Cancer	☐ Yes	☐ No	☐ Unknown
Migraine	☐ Yes	☐ No	☐ Unknown
Epilepsy	☐ Yes	☐ No	☐ Unknown
Sudden death	☐ Yes	☐ No	☐ Unknown

Biological parents: ☐ Unknown

Heart disease	☐ Yes	☐ No	☐ Unknown
Hypertension	☐ Yes	☐ No	☐ Unknown
Stroke	☐ Yes	☐ No	☐ Unknown
Diabetes mellitus	☐ Yes	☐ No	☐ Unknown
Cancer	☐ Yes	☐ No	☐ Unknown
Migraine	☐ Yes	☐ No	☐ Unknown
Epilepsy	☐ Yes	☐ No	☐ Unknown
Sudden death	☐ Yes	☐ No	☐ Unknown

Biological aunts/uncles: ☐ Unknown

☐ Participant has no living or dead aunts or uncles

Heart disease	☐ Yes	☐ No	☐ Unknown
Hypertension	☐ Yes	☐ No	☐ Unknown
Stroke	☐ Yes	☐ No	☐ Unknown
Diabetes mellitus	☐ Yes	☐ No	☐ Unknown
Cancer	☐ Yes	☐ No	☐ Unknown
Migraine	☐ Yes	☐ No	☐ Unknown
Epilepsy	☐ Yes	☐ No	☐ Unknown
Sudden death	☐ Yes	☐ No	☐ Unknown

Biological siblings: ☐ Unknown

☐ Patient has no living or dead full brothers or sisters living or dead

Heart disease	☐ Yes	☐ No	☐ Unknown
Hypertension	☐ Yes	☐ No	☐ Unknown
Stroke	☐ Yes	☐ No	☐ Unknown
Diabetes mellitus	☐ Yes	☐ No	☐ Unknown
Cancer	☐ Yes	☐ No	☐ Unknown
Migraine	☐ Yes	☐ No	☐ Unknown
	☐	☐	☐

Epilepsy	Yes	No	Unknown
Sudden death	☐ Yes	☐ No	☐ Unknown

Biological first cousins: ☐ Unknown

Heart disease	☐ Yes	☐ No	☐ Unknown
Hypertension	☐ Yes	☐ No	☐ Unknown
Stroke	☐ Yes	☐ No	☐ Unknown
Diabetes mellitus	☐ Yes	☐ No	☐ Unknown
Cancer	☐ Yes	☐ No	☐ Unknown
Migraine	☐ Yes	☐ No	☐ Unknown
Epilepsy	☐ Yes	☐ No	☐ Unknown
Sudden death	☐ Yes	☐ No	☐ Unknown

Biological children: ☐ Unknown

☐ Patient has no living or dead biological children

Heart disease	☐ Yes	☐ No	☐ Unknown
Hypertension	☐ Yes	☐ No	☐ Unknown
Stroke	☐ Yes	☐ No	☐ Unknown
Diabetes mellitus	☐ Yes	☐ No	☐ Unknown
Cancer	☐ Yes	☐ No	☐ Unknown
Migraine	☐ Yes	☐ No	☐ Unknown
Epilepsy	☐ Yes	☐ No	☐ Unknown
Sudden death	☐ Yes	☐ No	☐ Unknown

Stroke pedigree to capture 3 or 4 generations

Legend

	Male	Female	Sex unknown
Individual			
Affected individuals With > 2 conditions	 	 	 
Multiple individuals number known			
Multiple individuals number unknown (n = ?)			
Deceased individual			

VI. social:

Personal cigarette smoking history

Smoking habits

Which item best describes the subjects smoking habits (check one)?

- ☐ Never smoked
- ☐ Prolonged abstainer (abstained for > 1 year)
- ☐ Recent abstainer (abstained for > 30 days, but < 1 year)
- ☐ Active smoker (any cigarette within 30 days)

Pack – years

Instructions: Pack – years = Number of packs of cigarettes smoked multiplied by the number of years smoked. For example, if a person smoked one and a half packs of cigarettes for six years, the patient would have smoked 9 pack – years.

Pack – years of cigarette smoking:_____

Second – hand cigarette exposure

Has the patient been with a household member who regularly smoked in his/her presence for more than 1 year during the past 10 years?

☐ Yes ☐ No ☐ Unknown

Has the patient been with a co-worker who smoked in the same indoor room in his/her presence for more than 1 year during the past 10 years?

☐ Yes ☐ No ☐ Unknown

Alcohol use

Instructions: Consider a "drink" to be a can or bottle of beer, a glass of wine, a wine cooler, or one shot of hard liquor (like scotch, gin or vodka). Select one.

How often did the patient have a drink containing alcohol in the past year?

☐ Rare/never
☐ 1 drink per week
☐ 2 to 4 drinks per week
☐ 5 to 6 drinks per week
☐ 1 drink per day
☐ 2 or more drinks per day

Physical activity

Instructions: Select the one category which best describes the subjects physical activity level for the past one years.

☐ Vigorous activity sufficient to break a sweat or noticeably raise heart rate < 1 time/week
☐ Vigorous activity sufficient to break a sweat or noticeably raise heart rate 1 – 3 times/week
☐ Vigorous activity sufficient to break a sweat or noticeably raise heart rate 4 or more times/week

VII. Clinical information:

D. Oxford Handicap Scale

1. <u>Oxford Handicap Scale at enrolment</u>

Instructions: Rate any limitations in study subject's social role after stroke.

Grade	**Description**
☐ 0	No symptoms
☐ 1	Minor symptoms that do not interfere with lifestyle
☐ 2	Minor handicap; symptoms that lead to some restriction in lifestyle, but do not interfere with the patient's capacity to look after him/herself
☐ 3	Moderate handicap; symptoms that significantly restrict lifestyle and prevent totally independent existence
☐ 4	Moderate severe handicap; symptoms that clearly prevent independent existence, though not needing constant attention
☐ 5	Severe handicap; totally dependent patient requiring constant attention night and day

E. NIH Stroke Scale

2. <u>NIH Stroke Scale</u>

1. (a) Level of consciousness:

Alert	0	☐
Drowsy	1	☐
Stuporous	2	☐
Coma	3	☐

(b) Level of consciousness questions:

Answers both questions correctly	0	☐
Answers one correctly	1	☐
Answers none correctly	2	☐

(c) Level of consciousness commands:

Obeys both correctly	0	☐
Obeys one correctly	1	☐
Obeys neither	2	☐

2. Best gaze:

Normal	0	☐
Partial gaze palsy	1	☐
Forced deviation	2	☐

3. Best visual:

No visual loss	0	☐
Partial hemianopia	1	☐
Complete hemianopia	2	☐
Bilateral hemianopia	3	☐

4. Facial palsy:

Normal	0	☐
Minor	1	☐

Partial 2 ☐

Complete 3 ☐

5. Best motor arm right:

No drift 0 ☐

Drift at 10 seconds 1 ☐

Cannot resist gravity 2 ☐

No effort against gravity 3 ☐

No movement 4 ☐

6. Best motor arm left:

No drift 0 ☐

Drift at 10 seconds 1 ☐

Cannot resist gravity 2 ☐

No effort against gravity 3 ☐

No movement 4 ☐

7. Best motor leg right:

No drift 0 ☐

Drift at 10 seconds 1 ☐

Cannot resist gravity 2 ☐

No effort against gravity 3 ☐

No movement 4 ☐

8. Best motor leg left:

No drift 0 ☐

Drift at 10 seconds 1 ☐

Cannot resist gravity 2 ☐

No effort against gravity 3 ☐

No movement 4 ☐

9. Limb ataxia:

Absent 0 ☐

Present in either upper or lower limb 1 ☐

Present in both upper and lower limb 2 ☐

10. Sensory:

Normal to pin	0	☐
Partial loss	1	☐
Dense loss	2	☐

11. Neglect (extinction):

No neglect	0	☐
Partial neglect (vision or touch)	1	☐
Complete neglect (vision or touch)	2	☐

12. Dysarthria:

Normal articulation	0	☐
Mild to moderate dysarthria	1	☐
Near unintelligible or worse	2	☐

13. Best language

No aphasia	0	☐
Mild to moderate aphasia	1	☐
Severe aphasia	2	☐
Mute	3	☐

Total NIH Stroke Score:_ _ _ _ _

3. <u>Vital signs</u>

Height; ___ ___ ___cm

Weight; ___ ___ ___kg

Pulse; ___ ___ ___beats/minutes

Temperature; ___ ___ ____degrees Celsius

Systolic blood pressure; ____ ____ ____mmHg

Diastolic blood pressure; ____ _____ ____mmHg

4. <u>Laboratory data</u>

Plasma glucose……..mmol/l; Date done…….. Not done

Urinalysis: Blood…….

Protein……

Glucose…… Date done…… Not done

WBC……..; Date done…….. Not done

Platelet count…….; Date done…….. Not done

Haemoglobin……..; Date done…….. Not done

Total cholesterol……..; Date done…….. Not done

Alt……; Date done…….. Not done

Albumin……; Date done…….. Not done

Creatinine……..; Date done……... Not done

RVT……; Date done…….

RPR…….; Date done…….. Not done

CD_4 count……; Date done……..

Pregnancy test (where applicable)……..; Date done…….

Homocysteine…….; Date done…….

Protein S level…….; Date done……

Protein C function……...; Date done…….

Antithrombin III function……; Date done…….

Anticardiolipin antibodies (IgG)……..; Date done……..

5. <u>Neuroimaging</u>

Head CT scan

Date of scan: _____

What is the maximum diameter of the symptomatic infarct?

☐ < 1.5 cm ☐ > 3.0 cm

☐ 1.5 – 3.0 cm ☐ Not seen

Where is the symptomatic infarct located?

☐ Supratentorial left ☐ Supratentorial right

☐ Infratentorial (Brainstem or Cerebellum) ☐ Not seen

Is intracranial haemorrhage present?

☐ Yes ☐ No

If yes, what type?

☐ HT1 (small petechiae along the margins of the infarct)

☐ HT2 (confluent petechiae within the infarcted area with some slight space occupying effect)

☐ PH1 (blood clots in ≤ 30% of the infarcted area with some slight space occupying effect)

☐ PH2 (blood clots in > 30% of the infarcted area with a substantial space occupying effect)

Does the infarct involve cerebral cortex?

☐ Yes ☐ No ☐ Infarct not seen

Is there noticeable lateral shift (>2mm) of pineal?

☐ Yes

☐ No

Brain MRI

Date of scan: _____

What is the maximum diameter of the symptomatic infarct?

☐ < 1.5 cm ☐ > 3.0 cm

☐ 1.5 – 3.0 cm ☐ Not seen

Where is the symptomatic infarct located?

☐ Supratentorial left ☐ Supratentorial right

☐ Infratentorial (Brainstem or Cerebellum) ☐ Not seen

Is intracranial haemorrhage present?

☐ Yes ☐ No

If yes, what type?

☐ HT1 (small petechiae along the margins of the infarct)

☐ HT2 (confluent petechiae within the infarcted area with some slight space occupying effect)

☐ PH1 (blood clots in ≤ 30% of the infarcted area with some slight space occupying effect)

☐ PH2 (blood clots in > 30% of the infarcted area with a substantial space occupying effect)

Does the infarct involve cerebral cortex?

☐ Yes ☐ No ☐ Infarct not seen

Is there noticeable lateral shift (>2mm) of pineal?

☐ Yes ☐ No

6. <u>Electrocardiogram</u>

Date performed……. ☐ Not done

Ventricular rate:…….BPM

Rhythm (select one):

☐ Sinus rhythm ☐ Atrial fibrillation

☐ Atrial flutter ☐ Atrioventricular (AV) block

☐ Atrial tachycardia ☐ Ventricular tachycardia

☐ Artificially paced (pacemaker) ☐ Other

☐ Unknown

Is there left ventricular hypertrophy (LVH) by voltage criteria?

☐ Yes ☐ No ☐ Unknown

QT (msec):…….. ☐ Unknown ☐ Not applicable

Echocardiogram, transthoracic

☐ Yes ☐ No

Report obtained (+ EF):…….....

……

……

F. TOAST classification

7. <u>TOAST classification</u>

Instructions: Select the single diagnostic category that best describes the ischaemic stroke based on review of the clinical evaluation and all relevant cardiac, laboratory and radiological studies.

☐ Large – artery atherosclerosis, probable

☐ Large – artery atherosclerosis, possible

☐ Cardioembolism, probable

☐ Cardioembolism, possible

☐ Small – vessel occlusion (lacune), probable

☐ Small – vessel occlusion (lacune), possible

☐ Stroke of other determined aetiology, probable

☐ Stroke of other determined aetiology, possible

☐ Stroke of undetermined aetiology due to the presence of two or more causes

☐ Stroke of undetermined aetiology due to negative evaluation

☐ Stroke of undetermined aetiology due to incomplete evaluation

G. Ethical Approval Letter

ERES CONVERGE

33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

16th February, 2014

Ref. No. 2013-Dec-009

The Principal Investigator
Dr. Stanley Zimba
University Teaching Hospital
Dept. of Internal Medicine
LUSAKA.

Dear Dr. Zimba,

RE: The association of Hypercoagulability state markers with ischaemic stroke in adult Zambian HIV patients at UTH.

Reference is made to your corrections. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	Ordinary	Approval No. **2013-Dec-009**
Approval and Expiry Date	Approval Date: 16th February, 2014	Expiry Date: 15th February, 2015
Protocol Version and Date	Version 31/0114.	15th February, 2015
Information Sheet, Consent Forms and Dates	• English, Nyanja.	15th February, 2015
Consent form ID and Date	Version-Nil	15th February, 2015
Recruitment Materials	Nil	15th February, 2015
Other Study Documents	Data Collection Tool.	15th February, 2015
Number of participants approved for study	213	15th February, 2015

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON