HIV infection and Stroke in Malawian Adults

A thesis submitted in accordance with the requirements and regulations of the University of Liverpool for

A Degree of Doctor of Philosophy

By

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Declaration

Except for the assistance outlined in the acknowledgements, the work described is my own work and has not been submitted for a degree or other qualification to this or any other university.

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Abstract

There is an increased incidence of young people with stroke (age ≤45years) in Human immunodeficiency virus (HIV) endemic countries; this has been largely attributed to hypertension. However, hospital based surveys in countries like Malawi and South Africa have shown that the prevalence of hypertension in these young people is lower than expected, but HIV infection is substantially higher, implicating HIV as a risk factor.

For many years a link between HIV and stroke has been postulated, but the relationship is uncertain. Whilst HIV may be a risk factor for stroke directly through mechanisms linked with HIV-associated vasculopathy, or indirectly through opportunistic infections, the drugs that treat HIV infection may also increase the risk of stroke because of their metabolic effects. Many studies, almost all retrospective, have failed to separate the direct effect of HIV infection from the indirect effects, including combined antiretroviral therapy, on cerebrovascular risk.

HIV infection increases the risk of stroke mimics such as intracranial toxoplasma infection. The Recognition of Stroke in the Emergency Room (ROSIER) score is commonly used to screen for a stroke and triage patients for computer tomography (CT) of the brain. However, the accuracy of the ROSIER score and CT brain to reliably differentiate a stroke diagnosis from those with a stroke mimic in people with HIV infection is uncertain. I found that the ROSIER score and CT brain imaging had poor diagnostic accuracy in an HIV positive population. Therefore, in my thesis, every patient with an acute neurological

symptom was fully assessed for a stroke as part of the screening process and confirmation was by magnetic resonance brain imaging.

I subsequently investigated the risk factors and aetiology of stroke through a prospective case-control study in an HIV endemic country. Through this work, I showed that HIV infection is associated with cerebrovascular disease. Although hypertension was the leading risk factor in the population overall, HIV infection and its treatment was the second most important, and the most important in younger patients. Unexpectedly, I found that starting combined antiretroviral therapy in a subgroup of people living with HIV infection independently increased the risk of stroke. In this cohort, ischaemic stroke was the predominant stroke type and opportunistic infections only accounted for less than a third of these cases.

The heterogeneity of HIV stroke with respect to risk factors for stroke, the degree of immunosuppression and HIV activity, and prior or current opportunistic infection has made it difficult to generalise epidemiological findings in some studies to populations at large. My study, to some extent unravels some of this ambiguity. I speculate that HIV related strokes evolves through the introduction of cART and then transitions into an aging population, accelerating atherosclerotic stroke and potentially contributing to an anticipated stroke epidemic in countries like Malawi.

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Abbreviations and Notations

3TC	**Lamuvudine**
ACE	**Angiotensin Converting Enzyme**
ACL	**Anti-cardiolipin antibody**
aHR	**Adjusted Hazard Ratio**
AIDS	**Acquired Immunodeficiency Syndrome**
ANA	**Antinuclear Antibody**
ANCA	**Anti-neutrophil Cytoplasm Antibody**
aOR	**Adjusted Odds Ratio**
APS	**Anti-phospholipid Syndrome**
ART	**Antiretroviral therapy**
A-S-C-O	**Atherosclerosis-Small vessel disease-Cardiac cause-Other cause**
AZT	**Zidovudine**
BLAST	**Basic Local Alignment Search Tool**
C3/4	**Complement C3/4**
cART	**Combined Antiretroviral Therapy**
CCL2	**Chemokine Ligand 2**
CI	**Confidence Interval**
cIMT	**Carotid Intimal Medial Thickness**
CMV	**Cytomegalovirus**

CNS	**Central Nervous System**
CSF	**Cerebrospinal Fluid**
CT	**Computed Tomography**
CXR	**Chest Radiograph**
d4T	**Stavudine**
DNA	**Deoxyribonucleic Acid**
EIA	**Enzyme Immunoassay**
ELISA	**Enzyme Linked Immunosorbant Assay**
ENA	**Extractable Nuclear Antigen**
ESR	**Erythrocyte Sedimentary Rate**
FBC	**Full Blood Count**
FTA	**Fluorescent Treponemal Antibody**
FTA-ABS	**Fluorescent Treponemal Antibody-Absorbed**
GCS	**Glasgow Coma Scale**
GDP	**Gross Domestic Product**
HAND	**HIV Associated Neurocognitive Disorder**
HBMECs	**Human Brain Microvascular Endothelial Cells**
HDSS	**Health and Demographic Surveillance Sight**
HIV	**Human Immunodeficiency Virus**
HR	**Hazard Ratio**
hsCRP	**High-sensitivity C-reactive Protein**
HUVECS	**Human Umbilical Vascular Endothelial Cells**

ICAM-1	**Intracellular Adhesion Molecule 1**
IDU	**Intravenous Drug Use**
Ig	**Immunoglobulin**
IHD	**Ischaemic Heart disease**
IL6	**Interleukin 6**
IQR	**Interquartile Range**
IRIS	**Immune Reconstitution Inflammatory Syndrome**
JCV	**John Cunningham Virus**
LA	**Lupus Anticoagulant**
LACI	**Lacunar Infarct**
LACS	**Lacunar Syndrome**
MCP-1	**Monocyte Chemotatic Protein 1**
MI	**Myocardial Infarction**
MLW	**Malawi Liverpool Wellcome Trust Clinical Research Programme**
MRI	**Magnetic Resonance Imaging**
MTB	***Mycobacterium* Tuberculosis**
NIHSS	**National Institutes of Health Stroke Scale**
NNRTI	**Non-nucleotide Reverse Transcriptase Inhibitor**
NRTI	**Nucleotide Reverse Transcriptase Inhibitor**
NVP	**Nevirapine**
OCSP	**Oxfordshire Community Stroke Project**
OR	**Odds Ratio**

PACI	**Partial Anterior Circulation Infarct**
PACS	**Partial Anterior Circulation Syndrome**
PAF	**Population Attributable Fraction**
PCR	**Polymerase Chain Reaction**
PI	**Protease Inhibitors**
PML	**Progressive Multifocal Leukoencephalopathy**
POCI	**Posterior Circulation Infarct**
POCS	**Posterior Circulation Syndrome**
QECH	**Queen Elizabeth Central Hospital**
RCC	**Red Cell Count**
ROSIER	**Recognition of Stroke in the Emergency Room**
RPR	**Rapid Plasma Reagin**
Std. Err	**Standard Error**
TACI	**Total Anterior Circulation Infarct**
TACS	**Total Anterior Circulation Syndrome**
TB	**Tuberculosis**
TBM	**Tuberculous Meningitis**
TOAST	**Trial of Org 10172 in Acute Stroke Treatment**
TPPA	**Treponema Pallidum Particle Agglutination**
Tp	**Treponema**
UK	**United Kingdom**
UN	**United Nations**

USA	**United States of America**
VCAM-1	**Vascular Cell Adhesion Molecule 1**
VDRL	**Venereal Disease Research Laboratory**
VZV	**Varicella Zoster Virus**
WCC	**White Cell Count**
WHO	**World Health Organisation**
β_2-GP1	**β_2 Glycoprotein 1**

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1 Introduction

1.1 Overview

Stroke is a common and devastating human disease. It is one of the leading causes of premature death and disability worldwide (Lozano et al. 2012; Murray et al. 2012).

Stroke is clinically defined as “rapidly developing clinical signs or symptoms of focal (at times global) disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than vascular” (Hatano 1976). Once diagnosed a stroke is typed (e.g. ischaemic or haemorrhagic) using brain imaging. Ischaemic arterial stroke is the predominant type worldwide and accounts for approximately 80% of acute stroke events (C. Warlow 2007). The clinical presentation can be diverse such as dizziness or unilateral weakness. This can make the diagnosis challenging and delay treatment.

The subtype of stroke is determined by the vascular territory involved and relates to outcome Figure 1:1. There are several causes of arterial ischaemic stroke and these are categorised by different aetiological classifications (Adams et al. 1993; Amarenco et al. 2009). A commonly used example is the Trial of Org 10172 in Acute Stroke Treatment (TOAST) and includes the following: 1) large atherosclerosis 2) cardioembolism 3) small vessel disease 4) determined aetiology (e.g. infective disorders and coagulopathy), and 5) undetermined aetiology (Adams et al. 1993). Atherosclerosis is the

commonest cause of stroke worldwide and is strongly associated with established vascular risk factors such as hypertension, diabetes, and smoking (Warlow et al. 2003).

Oxfordshire Community Stroke Project (OCSP) classification: syndromes and imaging examples

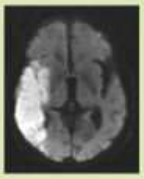
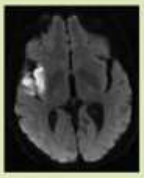
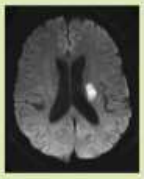
OCSP term	Clinical features	Vascular basis	Example CT	Example MRI
Total Anterior Circulation Syndrome (TACS)	• Hemiparesis **AND** • Higher cortical dysfunction (dysphasia or visuospatial neglect) **AND** • Homonymous hemianopia	Usually proximal MCA or ICA occlusion		
Partial Anterior Circulation Syndrome (PACS)	• Isolated higher cortical dysfunction **OR** • Any 2 of hemiparesis, higher cortical dysfunction, hemianopia	Usually branch MCA occlusion		
Posterior Circulation Syndrome (POCS)	• Isolated hemianopia (PCA), brainstem or cerebellar syndromes	Occlusion of vertebral, basilar, cerebellar or PCA vessels		
Lacunar Syndrome (LACS)	• Pure motor stroke **OR** • Pure sensory stroke **OR** • Sensorimotor stroke **OR** • Ataxic hemiparesis **OR** • Clumsy hand-dysarthria	Usually small vessel occlusion e.g. lenticiulostriate arteries.		

Figure 1:1: Subtype of stroke using the Oxfordshire Community Stroke Project (OCSP) classification: syndromes and imaging examples. Modified from Muir 2009

Identification of causal stroke risk factors and implementing preventative therapies to reduce these risk factors at a population level can dramatically reduce stroke incidence. Rothwell and colleagues demonstrated a 40% decrease in stroke incidence in a UK community over a 10 year period; there were several speculations why this may have been the case, one important observation was the increased uptake of anti-hypertensive medication during this timeframe (Rothwell et al. 2004).

1.2 Epidemiology of stroke

In 2005 16 million individuals were estimated to have had a first-ever stroke and in the absence of public health interventions to reduce risk factors, this is expected to rise to 23 million by 2030 (Strong et al. 2007). After ischaemic heart disease, stroke is the 2nd most important diagnosis of non-communicable diseases (Lozano et al. 2012). Deaths from non-communicable diseases, rose by just under 8 million between 1990 and 2010, accounting for two of every three deaths (34·5 million) worldwide (Lozano et al. 2012). On the basis of a systematic review of studies published between 1970-2008, there was a 42% decrease in stroke incidence rates in high-income countries, due to better recognition of stroke and improved primary and secondary interventions. However, an increase in stroke incidence rates of over 100% was reported in low-middle income countries (Feigin et al. 2009). As low-middle income countries develop economically, they will evolve through the 'health transition', this involves improved health services, better sanitation, reduced prevalence of infectious diseases and rural to urban migration, as a result, individuals will live longer. Because age is a strong predictor of stroke, stroke incidence will increase as the population ages. Established stroke risk factors are also associated with aging and urbanisation, and partly explains why emerging economies now suffer from the highest burden of stroke (Yusuf S 2001; Feigin et al. 2009).

Although stroke studies from sub-Saharan Africa are limited, the few that exist suggests that the incidence of young stroke (≤ 45 years-old) is more frequent compared to an industrialised country like the UK Table 1:1 (Connor et al. 2007; O'Donnell et al. 2010). This interesting trend is thought to be largely explained by hypertension (O'Donnell et al. 2010). However, hospital based data from Malawi and South Africa shows that hypertension is less important (Kumwenda et al. 2005; Tipping et al. 2007; Heikinheimo et al. 2012). The disparity in the attribution of risk factors of young stroke, even within a continent, highlights the gaps in our knowledge (O'Donnell et al. 2010).

	Age							
	20–24	25–34	35–44	45–54	55–64	65–74	>75	Total*
Men								
South Africa (1986)[23]	8	9	37	107	249	643	445	84
Zimbabwe (1997)[22]	..	7	23	126	174	402	619	20
OXVASC (2004)[17]	..	..	27	73	177	646	..	134
Women								
South Africa (1986)[23]	..	10	43	109	239	583	611	90
Zimbabwe (1997)[22]	..	13	66	102	237	563	957	32
OXVASC (2004)[17]	..	..	16	54	175	408	..	156
Total								
South Africa (1986)[23]	..	..	..	..	280	837	726	101
Zimbabwe (1997)[22]	..	10	41	118	194	469	788	31
NMSS (1998)[18]	19	0	54	184	366	636	..	223
OXVASC (2004)[17]	..	..	22	64	176	526	..	145

OXVASC=Oxford Vascular Study; NMSS=North of Manhattan Stroke Study. *For total population over age 20 years.

Table 1:1: Age specific incidence of stroke per 100 000 population (Connor, Walker et al. 2007)

Figure 1:2 describes the distribution of important risk factors of common diseases, ranked globally and for each country. Specifically for stroke, risk factors included tobacco smoking, alcohol use, high fasting glucose, high total cholesterol, high blood pressure, high body mass index, abnormal diet (low in fruit, vegetable, whole grain or high in sugar, sodium) and physical inactivity. The variation in the ranking of

these risk factors reflects the 'health transition' across the globe. Whilst hypertension was ranked first globally and in many industralised countries it took a lesser rank in the poorer African countries.

Sub-Saharan Africa also faces an enormous burden of infectious diseases, the impact of acute and chronic infection on the increasing burden of stroke is uncertain (Emsley et al. 2008; Lim et al. 2012). A better understanding of the risk factor profile in sub-Saharan African countries is needed to facilitate appropriate intervention strategies.

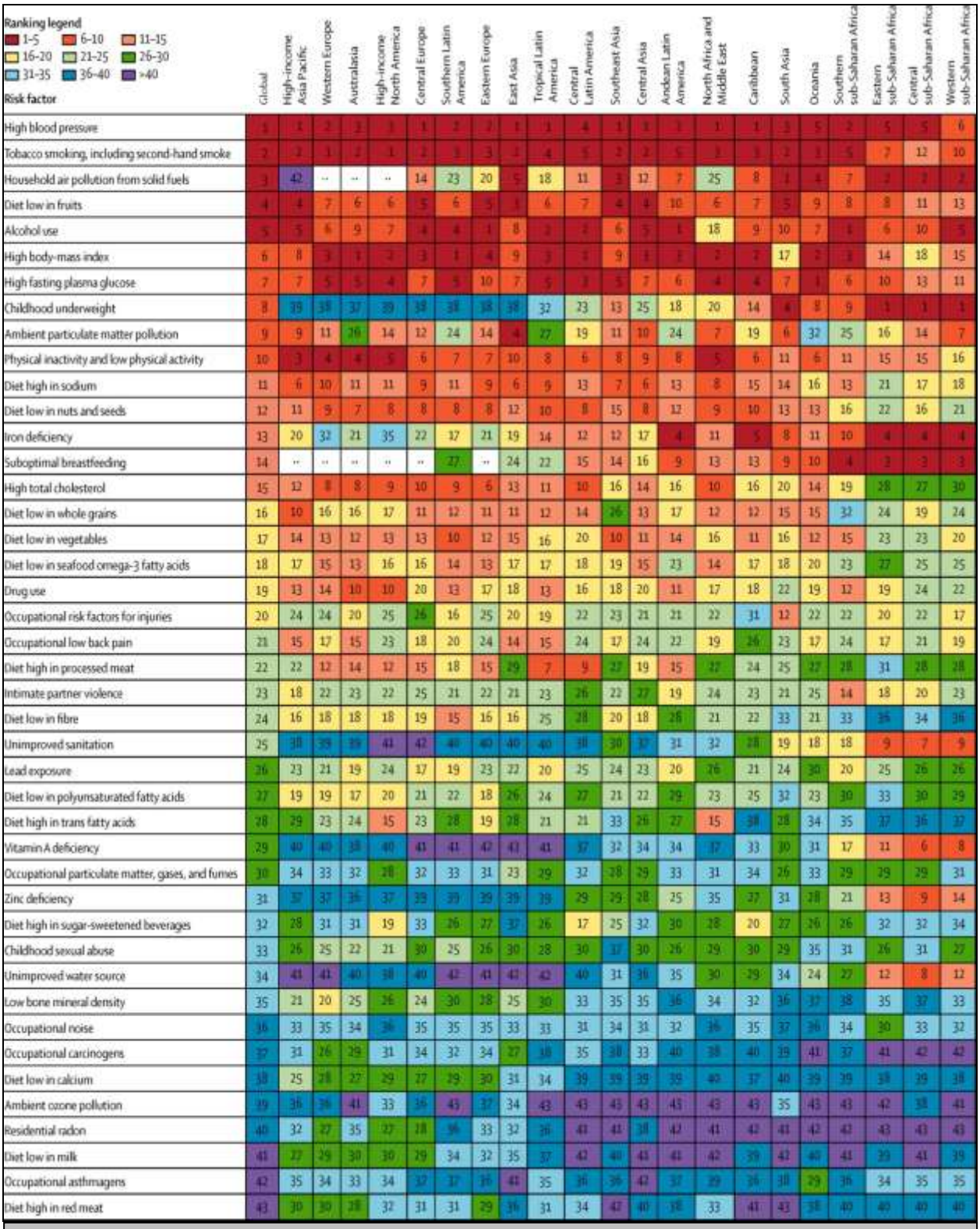


Figure 1:2: Risk factors ranked by attributable burden of disease, 2010. Regions are ordered by mean life expectancy

No data=attributable disability-adjusted life-years were not quantified (Lim, Vos et al. 2012).

1.3 Clinical features of stroke

Stroke is usually characterised by acute focal neurological deficits, such as hemiparesis, aphasia, hemi-inattention or hemianopia, depending on the part of the brain that is affected. Figure 1:1 describes the subtypes of stroke (Oxford community stroke project classification) and relates this to the clinical presentation and brain imaging (Muir 2009). Almost any neurological symptom can be indicative of a stroke but when the symptoms are non-localising (e.g. loss of consciousness, psychiatric symptoms) and without clear-cut focal deficits, which is infrequent, the diagnosis then becomes challenging (Edlow et al. 2011). There are also diagnoses that present like stroke but of non-vascular origin, these are referred to as stroke mimics and examples include migraine and focal epilepsy (C. Warlow 2007).

1.4 Aetiology of stroke and mechanism of atherosclerosis

Fifty percent of stroke is attributable to atherosclerosis, followed by small vessel disease (25%), cardioembolism (20%) and rare causes (5%); these proportions are typical of older (>45 years old) stroke patients (Warlow et al. 2003). Rarer causes of stroke, for example, primary vasculitis, infective vasculitis (e.g. varicella zoster) genetic disorders (e.g. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) and coagulopathies (e.g. antiphopholipid syndrome) feature more in younger stroke patients but can still occur in the older cohort (Dichgans 2007; Gilden et al. 2009; Putaala et al. 2009; Urbanus et al. 2009).

Atherosclerosis is the main cause of ischaemic stroke. When the arterial wall is irritated or injured by stimuli (such as dyslipidaemia, hypertension, diabetes, smoking or pro-inflammatory mediators) the cell biology of the arterial wall mal-adapts and this leads to plaque formation, narrowing of the luminal wall,

plaque instability, thrombus formation and ultimately ischaemia Figure 1:3. Distal embolisation of the ruptured plaque or thrombus is also likely to play an important role. If this arose in the brain, the clinical manifestation would be an ischaemic stroke (Libby et al. 2011). Alternatively, hypertension associated arterial wall tension could give rise to microaneurysm formation and rupture, leading to a haemorrhagic stroke; there are many other causes of haemorrhagic stroke but this is the most common.

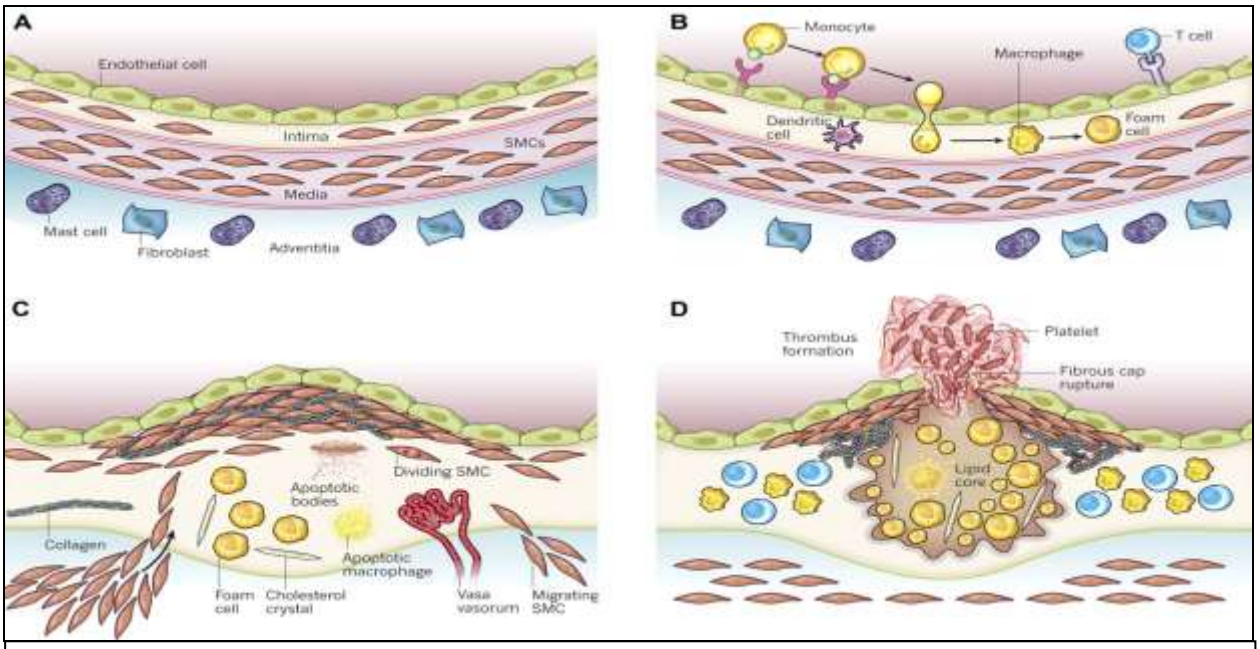


Figure 1:3: Stages in the development of atherosclerosis

The normal muscular artery and the cell changes that occur during disease progression to thrombosis are shown. (**Image A**) The normal artery contains three layers. The inner layer, the tunica intima, is lined by a monolayer of endothelial cells that is in contact with blood overlying a basement membrane. In contrast to many animal species used for atherosclerosis experiments, the human intima contains resident smooth muscle cells (SMCs). The middle layer, or tunica media, contains SMCs embedded in a complex extracellular matrix. Arteries affected by obstructive atherosclerosis generally have the structure of muscular arteries. The arteries often studied in experimental atherosclerosis are elastic arteries, which have clearly demarcated laminae in the tunica media, where layers of elastin lie between strata of SMCs. The adventitia, the outer layer of arteries, contains mast cells, nerve endings and microvessels. (**Image B**) The initial steps of atherosclerosis include adhesion of blood leukocytes to the activated endothelial monolayer, directed migration of the bound leukocytes into the intima, maturation of monocytes (the most numerous of the leukocytes recruited) into macrophages, and their uptake of lipid, yielding foam cells. (**Image C**), Lesion progression involves the migration of SMCs from the media to the intima, the proliferation of resident intimal SMCs and media-derived SMCs, and the heightened synthesis of extracellular matrix macromolecules such as collagen, elastin and proteoglycans. Plaque macrophages and SMCs can die in advancing lesions, some by apoptosis. Extracellular lipid derived from dead and dying cells can accumulate in the central region of a plaque, often denoted the lipid or necrotic core. Advancing plaques also contain cholesterol crystals and microvessels. **(Image D)**, Thrombosis, the ultimate complication of atherosclerosis, often complicates a physical disruption of the atherosclerotic plaque. Shown is a fracture of the plaque's fibrous cap, which has enabled blood coagulation components to come into contact with tissue factors in the plaque's interior, triggering the thrombus that extends into the vessel lumen, where it can impede blood flow (Libby et al. 2011).

1.5 Management and outcome

There are several established risk factors for ischaemic stroke where data have shown reduction in stroke risk following intervention; this includes management of hypertension, and high cholesterol (Goldstein et al. 2006). Risk-assessment tools like the Framingham risk score or equivalent, are important for assessing potential stroke risk to direct optimal management (Goldstein et al. 2006; D'Agostino et al. 2008).

Following an acute stroke, secondary prevention such as aspirin (for those with ischaemic stroke) plus dipyridamole or clopidogrel, anticoagulation (for those that are high risk of a cardio-embolic stroke), carotid endarterectomy (for those with >70% carotid stenosis) and administering blood pressure and cholesterol lowering drugs, have all been proven to reduce the incidence of recurrent stroke (Howells et al. 2010). The risk of dying within the first 7 days, 1 month and 6 months after a first ever stroke are about 11%, 15% and 22% respectively, this is higher than the general population of equivalent age (C. Warlow 2007). Patients with haemorrhagic strokes, have a much higher early risk of dying than those with ischaemic strokes (C. Warlow 2007).

Life saving and disability sparing interventions such as stroke units and thrombolysis for ischaemic stroke, have revolutionised stroke care in the 21st century (Howells et al. 2010). Stroke units are now seen as an effective component of stroke services. A stroke unit is characterised by the presence of a multidisciplinary team (comprising medical, nursing, physiotherapy, occupational therapy, speech therapy, and social-work staff) who coordinate their work through regular meetings; in this forum they identify problems and set short and long-term recovery goals (Langhorne et al. 2002).

In contrast, the management of stroke in low-middle income countries provides several challenges including, poorer access to care and limited availability of secondary prevention strategies (Mendis et al. 2005). Furthermore, health systems are already stretched and specialist input scarce. Prioritising interventions that are resource appropriate combined with identifying vascular risk factors that are region/country specific are essential steps needed to overcome some of these difficulties.

1.6 Malawi

1.6.1 Demography and health indicators

Malawi is situated in central southern Africa. In 2012 it had an estimated population of 13 million and this is predicted to rise to 26 million by 2030 (UNAIDS 2012). The average life expectancy at birth for males is 57 years and females 58 years (UNAIDS 2012). Malawi has a predominately young population with a median age of 17, 46% are less than 15 years and 6% greater than 60 years (UNAIDS 2012). As one of the poorest countries in the world, it has a gross domestic product (GDP) of US$357 per capita per year and is ranked 170/181 according to the human development index (UNdata).

Blantyre, the commercial capital, is situated in the South of Malawi at an altitude of 1065m, it has a population of 1 million and two-thirds of these reside in urban dwellings. Queen Elizabeth Central Hospital (QECH) is the main hospital for Blantyre district, as well as being the southern regional referral hospital. In 1.5 years, QECH had in excess of 7000 adult medical admissions; median age was 37 years-old, 50% were male, 26% were HIV positive (21% undiagnosed), the median stay in hospital was 5 days and in-hospital fatality 15% (Miguel A. SanJoaquin 2013).

The national adult HIV prevalence is 10.6%, however in urban Blantyre this is reported to be as high as 18%; this makes it the top 10 of countries hardest hit by HIV/AIDS infection (Choko et al. 2011; UNAIDS 2012). Heterosexual contact is the principal mode of HIV transmission in Malawi, while mother-to-child transmission of HIV accounts for approximately 25% of all new HIV infections (UNAIDS 2012). The Malawi demographic and health survey results shows that from 2004 to 2010 there was an upward shift in the HIV epidemic, suggesting that the HIV population was aging, there was also a gender variation Figure 1:4 (UNAIDS 2012). Since 2004 Malawi has had a free national programme to provide combined antiretroviral therapy (cART) for those living with HIV infection; up until 2011, the first-line treatment was a combination of nevirapine, stavudine and lamivudine but was later updated to three first line options; 1) nevirapine, stavudine and lamivudine 2) nevirapine, zidovudine and lamivudine 3) tenofovir, lamivudine and efavirenz (for pregnant, breastfeeding mothers and those already on TB treatment) (Malawi 2008; Malawi 2011).

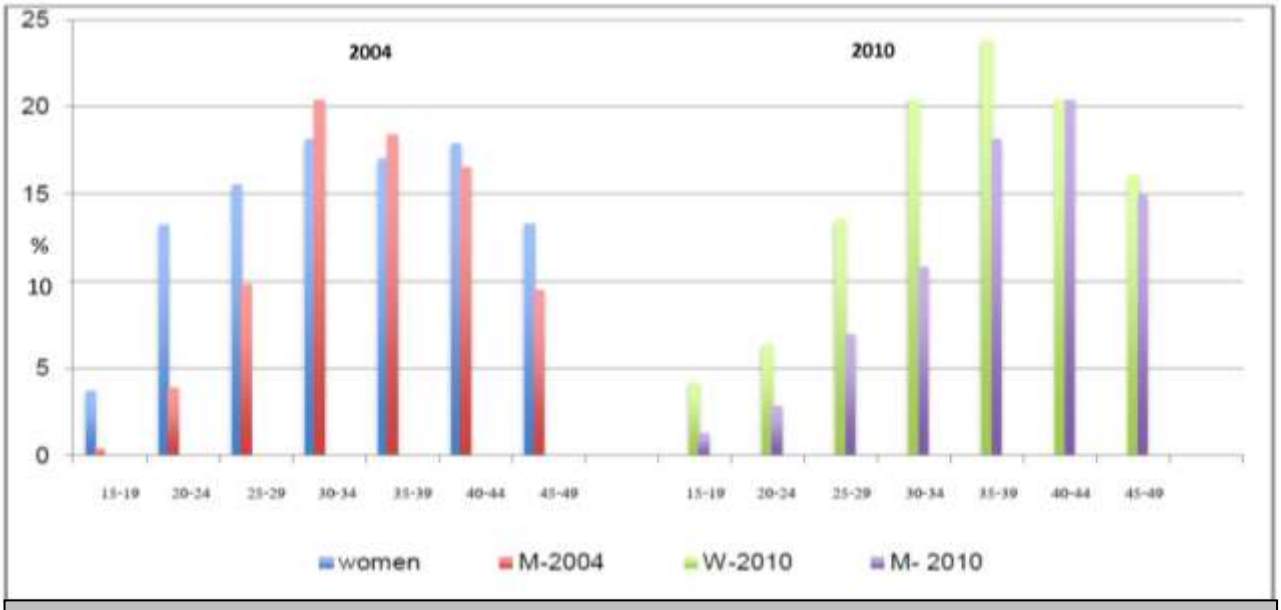


Figure 1:4: Prevalence of HIV infection by 5 years age bands and by sex, for 2004 and 2010
W – woman, M – Male (Government 2012).

1.6.2 Stroke

Stroke is among the top 10 diagnoses in the adult medical wards in Malawi (Miguel A. SanJoaquin 2013). Although the true incidence of stroke in this population is not clearly defined, neighboring countries like Tanzania show that the age adjusted incidence of stroke is substantially higher than the equivalent black American population (Walker et al. 2010). Young stroke patients (≤45 years-old) account for approximately one-third of adult stroke admissions at QECH (Kumwenda et al. 2005; Heikinheimo et al. 2012). A high proportion of young patients with stroke has also been described in other sub-Saharan African countries (Kumwenda et al. 2005; Connor et al. 2007; Walker et al. 2010; Heikinheimo et al. 2012).

Cumulative hospital mortality post-stroke at 6 months and 1 year is 39% and 45% respectively; these rates are almost double those reported in high income countries (Bravata et al. 2003; C. Warlow 2007; Heikinheimo et al. 2012). It is likely that these differences are related to design methodology (i.e. hospital versus community based study) but there is also the possibility of this being due to an increased incidence of stroke or poor quality of care post stroke.

The WHO STEPS survey described the distribution of vascular risk factors in the general Malawian population. They showed that within the 25-64 years age category, hypertension and smoking were more frequent (32.9% and 14.9% respectively) than diabetes, hypercholesterolaemia, and obesity (5.6%, 8.7% and 4.6% respectively) (Msyamboza et al. 2011). Furthermore, the frequency of smoking, diabetes and hypercholesterolaemia was substantially lower compared to high income countries (O'Donnell et al. 2010).

Figure 1:5 shows the distribution of stroke admissions by age at QECH. The early peak in stroke hospital admissions in the 25-45 years age-band mirrors the HIV epidemic in this country Figure 1:4. Furthermore, the prevalence of established vascular risk factors in this young stroke population is low and does not fully explain this peak, thus possibly implicating HIV infection as a stroke risk factor (Kumwenda et al. 2005; Heikinheimo et al. 2012).

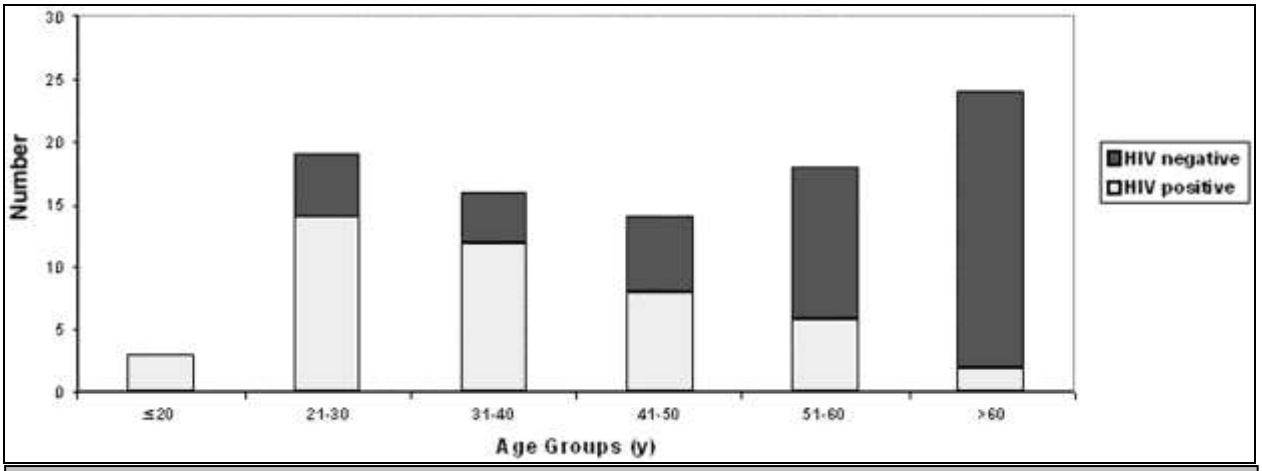


Figure 1:5: Distribution in age groups with HIV prevalence in 98 patients admitted with a recent stroke syndrome (Kumwenda, Mateyu et al. 2005)

Long-term treatment with antiretroviral therapy has been linked to a high prevalence of hypertension and hypercholesterolaemia in Malawi (Muronya et al. 2011; van Oosterhout et al. 2012) . A better understanding of the risks associated with HIV infection and its treatment would give an insight into the drivers of stroke in Malawi.

1.7 Trends in HIV infection

Sub-Saharan Africa suffers from the bulk of the world's HIV epidemic and the incidence remains high in Malawi (Nel et al. 2012; UNAIDS 2012). The anticipated decline in HIV-associated mortality as observed in industrialised countries, owing to the widespread use of cART, will shift the landscape of HIV infection into an aging population. This will be accompanied by an expansion of age-related diseases like stroke (Hasse et al. 2011). Furthermore, HIV infected individuals on long-term cART, who are virologically suppressed and immunocompetent, still suffer from premature death (Lewden et al. 2007). The concern being that accelerated vascular disease due to HIV infection may be the cause (Bozzette et al. 2003; Hasse et al. 2011).

The transition of HIV infection from a terminal illness to a chronic disease is likely to dominate the HIV landscape for the foreseeable future. Thus, dissecting the relationship of HIV infection, its treatment and stroke risk is of urgent priority.

1.8 HIV and stroke; current perspective

1.8.1 Overview

The incidence of stroke has increased by 100% in low-middle income countries in the last ten years (Feigin 2007; Feigin et al. 2009). While much of this increase is likely related to the health transition driven by the increasing burden of vascular risk factors and aging of the population, infectious causes of stroke may also add to the burden (Emsley et al. 2008; Walker et al. 2010). It is in these countries that the bulk of HIV infection is found (Quinn 1996). The two conditions may therefore be coincidental.

However, HIV infection potentially influences stroke risk and cause, and current HIV treatment results in vascular damage perhaps conferring an additional risk (Dobbs et al. 2009; Sen et al. 2012).

Between 1–5% of HIV infected patients develop stroke in clinical series though a higher proportion have cerebral ischaemic lesions at autopsy (4–34%) (Moskowitz et al. 1984; Sharer et al. 1985; Anders et al. 1986; Mizusawa et al. 1988; Berger et al. 1990; Kieburtz et al. 1993; Connor et al. 2000). There was little correlation between pathological evidence of cerebral ischaemic lesions and clinical manifestations prior to death in series that assessed this (Connor et al. 2000; Pinto 2005). In the United States, admissions of stroke patients with concurrent HIV infection has increased by 43% over nine years (Ovbiagele et al. 2011). Despite this apparent association, surprisingly little research has assessed the impact of HIV infection on the burden and nature of stroke, the extent to which HIV increases stroke risk and the pathogenesis of stroke in HIV infected individuals (Berger 2004; Dobbs et al. 2009).

The current mainstay of HIV treatment, combination antiretroviral therapy (cART), may also contribute to the risk of stroke directly by accelerating atherosclerosis and indirectly by increasing life expectancy (de Gaetano Donati et al. 2004). Inevitably, there will continue to be an increased exposure to conventional vascular risk factors (e.g. ageing, hypertension, diabetes mellitus, hypercholesterolaemia and cigarette smoking) as the HIV population lives longer. Furthermore, the continuous exposure to HIV, albeit at lower viral titre, and low-grade chronic systemic inflammation, may additionally add to the risk of stroke (Emsley et al. 2002; Lindsberg et al. 2003).

Quantifying the impact of HIV infection upon stroke has public health relevance, particularly when attempts are made to address the increased frequency of stroke in regions of high HIV prevalence. From a practical clinical perspective, when physicians in all regions are faced with an HIV infected patient who has had a stroke, they need to know the extent to which HIV infection and its treatment may impact upon the aetiology, clinical presentation and management of the stroke. Futhermore, the physician will need to consider a stroke presentation being the presenting feature of HIV infection.

This review describes what is known about the risk of stroke in HIV infected individuals, the emerging theories of the mechanism of stroke in HIV infection, the impact of HIV on clinical stroke syndromes and implications for management.

1.8.2 HIV infection and the risk of stroke

Both HIV infection and cART may potentially increase an individual's risk of stroke, therefore any assessment of the risk attributable to HIV infection must ideally have been assessed in an untreated population. The majority of studies have been retrospective or compared unmatched groups or cohorts, or the prevalence of HIV infection in stroke series compared to the general population of a similar age. Most were hospital based series, while some studies compared cerebral infarction (rather than clinical stroke) in HIV infected and unaffected brains at autopsy. No case controlled or cohort studies have prospectively assessed the risk of stroke in HIV infected populations and so the evidence to support the notion of an increased risk with HIV infection is based on very limited data.

Pre cART era

A retrospective hospital based case-control study compared 113 young (19-44 years) stroke patients with 113 age and sex matched asthma patients with known HIV status in South Florida, US, between 1990-94. The study found that HIV infection was associated with a doubling of the risk of stroke (OR 2.3; 95% confidence interval [CI], 1.0-5.3). The risk was higher for cerebral infarction (OR 3.4; 95% CI, 1.1-8.9) than cerebral haemorrhage (OR 1.3; 95% CI, 0.3-6.4). Eleven of the 25 HIV infected stroke patients (44%) had a coagulopathy (protein S deficiency) or meningitis (Qureshi 2005).

A population-based study from Baltimore, US, from 1988-1991 found that AIDS conferred an adjusted relative risk of 13.7 (95% CI, 6.1 to 30.8) for ischaemic stroke and 25.5 (95% CI, 11.2 to 58.0) for cerebral haemorrhage (Cole et al. 2004). This study included a small cohort of stroke patients with acquired immunodeficiency syndrome (AIDS) (n=12), ascertained retrospectively using discharge coding from multiple institutions. Several shortcomings may have resulted in surprisingly high relative risk ratios, including the selection of (by definition) patients with advanced HIV infection and therefore at particularly high risk of stroke, and under-reporting of AIDS in the study population (Berger 2004).

Not all studies have found an association between HIV infection and stroke. An autopsy study from Florida (1986-87), retrospectively compared the brains of AIDS cases and non-AIDS cases between 20 and 50 years of age. Cerebrovascular disease was present in 13 (8%) of 154 AIDS cases, and in a higher proportion of 25 of 111 (23%) of controls which suggested that stroke was not more common in AIDS cases at autopsy (Berger et al. 1990).

A hospital based study in Kwazulu Natal, South Africa found that the prevalence of HIV infection in a series of young patients with stroke was 16%, similar to the prevalence of HIV in the non-stroke population of a similar age (Hoffmann et al. 2000). However, it is questionable how generalisable this was to the population of Kwazulu Natal as the Durban Stroke Data Bank included around 12% black African stroke patients compared to a community proportion of 85% (Hoffman 1998).

In a retrospective hospital-based study of 293 black African stroke patients aged between 15 and 44 years, from the same region, HIV infection (present in 56) increased the risk of ischaemic stroke (OR 2.3 CI 0.8 to 7.7; p = 0.09) though this finding was not statistically significant (Patel et al. 2005). Patients with opportunistic infection were excluded from the study. However, almost a third of patients did not have HIV serology assessed.

Post cART era

No studies have prospectively assessed the impact of HIV infection on stroke risk since the introduction of cART (Dobbs et al. 2009; Sen et al. 2012).

In a recent retrospective analysis of hospital admission data for all HIV infected individuals in Denmark (January 1995 to January 2010), the rate of cerebrovascular events was determined and compared to a sex and age matched general population, and stratified by Intravenous Drug Use (IDU) and conventional vascular risk factors. HIV-infected individuals had an increased risk of cerebrovascular events compared with controls (non-IDU HIV adjusted Incidence Rate Ratio (IRR) 1.60; 95% CI, 1.32–1.94). Risk of cerebrovascular events was greater when associated with intravenous drug use, low CD4+ T-lymphocyte

count pre-cART and treatment with abacavir (Rasmussen et al. 2011). Although arguably, this study provides the best evidence of risk of HIV for stroke, the retrospective nature of the study and lack of detailed prospective clinical assessment might have led to misclassification of cerebrovascular events in a population where stroke mimics are common.

In the large US study that assessed population wide hospital stroke discharge diagnoses over nine years (1997-2006), there was a 43% rise in the number of HIV positive patients admitted with stroke, after adjustment for the population size. This increase was associated with a total increase in the proportion of ischaemic strokes in the HIV infected population and also coincided with the introduction of combination antiretroviral drugs in the mid-1990s (Ovbiagele et al. 2011). It is impossible to tease out how much of this rise reflected an increase in the incidence of HIV infection, the impact of cART use on stroke risk in people with HIV infection, improved survival or indeed better recognition of stroke symptoms. However, the increase in HIV associated stroke admissions occurred simultaneously with an overall reduction in all stroke admissions (7%), suggesting but not confirming an association between HIV infection and stroke.

1.8.3 Clinical presentation of stroke in patients with HIV infection

Age

Individuals with HIV infection and stroke are reported to be younger than non-HIV infected stroke patients. This could be a reflection of the population at risk of HIV infection or a sign that the mechanism of stroke in HIV is largely independent of classical vascular risk factors. Ovbiagele *et al* described a median age of 42.9 years in 1997 and 48.4 years in 2006 of HIV infected stroke individuals in

the previously described retrospective analysis of hospital discharges in the US, and Ortiz *et al* found a median age of 42 years in a similar setting between 1997-2002 ((Ortiz et al. 2007; Ovbiagele et al. 2011). However, in lower income countries such as South Africa and Malawi HIV infected stroke patients are younger; a median age of 33.4 (n=61) years and 39.8 years (n=26) was described in Cape Town, South Africa (2000-2006) and Blantyre, Malawi (2008-2009) respectively (Tipping et al. 2007; Heikinheimo et al. 2012). One possible explanation for this global variation and the increase in median age seen in the US over a decade may be the use of cART, perhaps by delaying the time to stroke onset.

Other Clinical Considerations

Clinical descriptions of stroke in HIV infected and non-HIV infected patients are similar (Kumwenda et al. 2005; Tipping et al. 2006; Bermel et al. 2009). While the sudden onset of a focal neurological deficit is typical, atypical stroke presentations are common in the context of HIV infection e.g. acute confusion, fever, acute loss of consciousness and stepwise focal neurological presentation over hours to days (Sharfstein et al. 2007; Bhagavati et al. 2008; Melica et al. 2009). An alternative cause or mimic of stroke should be excluded before a final diagnosis of HIV associated stroke is made.

Stroke type and subtype

Ischaemic stroke appears to be more frequent than cerebral haemorrhage in HIV infected patients, at least in hospital based series from sub-Saharan Africa, where it is reported in over 90% of HIV strokes (Mochan et al. 2005; Tipping et al. 2006; Ortiz et al. 2007). This trend was previously not supported by the literature (during the pre-cART era) in the US; with near equal proportions of cerebral haemorrhage and ischaemic infarction in the Baltimore study described previously, but this is likely the reflection of

illicit drug use or hospital admission bias (Cole et al. 2004). However, Ovbiagele *et al* confirmed ischaemic stroke as the predominant pathological stroke type. This study found that the proportion of ischaemic stroke in HIV infected persons doubled between 1997 and 2006 (Ovbiagele et al. 2011). Whether this reflects the impact of cART or methodological factors is not clear.

In a South African hospital based study, the subtype of ischaemic stroke using the Oxfordshire Community Stroke Project (OCSP) classification in the HIV positive group (n= 64), comprised lacunar stroke (n=13), partial anterior circulation stroke (n=33), total anterior circulation stroke (n=11) and posterior circulation stroke (n=7) (Tipping et al. 2007). The proportions of these subtypes follow similar trends to the original OCSP classification but posterior circulation stroke was less frequent in this HIV population though this is based on small patient numbers (Bamford et al. 1991). However, I will be able to accurately determine the proportions of stroke type and subtype in a community based HIV stroke population to avoid hospital admission bias that favours cerebral haemorrhage.

1.8.4 Causes of stroke in people with HIV infection

HIV infection can potentially cause stroke in many ways; indirectly through cardioembolism, coagulopathy, non-HIV infective vasculitis, or directly through HIV-associated vasculopathy. Table 1:2 lists the possible mechanisms that may result in an ischaemic or haemorrhagic stroke.

Coagulopathy

HIV infection may predispose to both arterial and venous thrombosis; however, it is not clear to what extent this is due to a coagulopathy. Protein C and S deficiency are sometimes associated with intracranial venous thrombosis but rarely with arterial stroke in non-HIV infected adults (Kenet et al. 2010; Morris et al. 2010). Although Protein C and S deficiency have been described in HIV infected stroke patients, it is not clear whether this is an epiphenomenon or causal. In one case series comparing unmatched stroke patients (n=33) with and without (n=33) HIV infection from South Africa, there was no significant difference in the proportion of patients with Protein S deficiency in the two groups (Mochan et al. 2005).

Antiphospholipid antibodies have been infrequently described in people with stroke and HIV infection, but as transient elevated antiphospholipid antibody titres are commonly found with viral infection, elevated levels should only be considered significant in patients fulfilling the International Haematology Consensus statement criteria for antiphospholipid syndrome (Miyakis et al. 2006). This has only rarely been applied in studies investigating stroke and HIV infection and the true frequency of any association remains unclear (Mochan et al. 2003; Tipping et al. 2006; Giannakopoulos et al. 2009). Factor V Leiden is not associated with HIV related stroke.

Cardioembolism

Cardioembolism accounts for 4-15% of ischaemic strokes in an HIV infected population (Hoffmann et al. 2000; Mochan et al. 2003; Ortiz et al. 2007). HIV-associated dilated cardiomyopathy is a commonly reported cause of cardiac disease particularly in sub-Saharan Africa (Magula et al. 2003; Ntsekhe et al.

2009; Sliwa et al. 2012). It may be associated with opportunistic infection and has been attributed to HIV itself although its pathogenesis is uncertain. I have therefore used a deliberately vague term 'HIV associated cardiac dysfunction' in Table 1:2. Assessment of cardioembolic causes of stroke in patients with HIV in studies from sub-Saharan Africa is further complicated by the relatively high prevalence of non-ischaemic cardiomyopathy and rheumatic heart disease in the non-HIV infected population (Ntusi et al. 2009). Other potential causes of cardioembolism include bacterial and marantic endocarditis, and ischaemic heart disease (Barbaro et al. 2001; Barbaro 2002).

Opportunistic infections

Some infections are well-established causes of stroke. Mycobacterium tuberculosis (TB), syphilis and Varicella zoster Virus (VZV) cause stroke in non-HIV infected patients, but immunosuppression due to HIV increases susceptibility to acquiring or reactivating these infections (Timmermans et al. 2004; Gilden et al. 2009; Lammie et al. 2009). TB related stroke is thought to be a complication of tuberculous meningitis and acute focal signs presenting as stroke may be the first manifestation (Berenguer et al. 1992; Lammie et al. 2009). Therefore, actively looking for tuberculosis in HIV infected stroke patients, who are more at risk, is important. VZV infection may cause cerebral vasculitis and stroke in the immunosupressed and the skin manifestation can be absent in approximately a third of stroke cases at the time of presentation, making the diagnosis less obvious (Nagel et al. 2008; Gilden et al. 2009; Gutierrez et al. 2011). The increased incidence of syphilis in HIV infected individuals could be related to one or more of the following factors; 1) immunosuppresion or 2) share the same mode of acquisition because both are sexually transmitted infections. Co-infection with HIV compounds the difficulty in diagnosing neurosyphilis (Timmermans et al. 2004; Zetola et al. 2007). Furthermore, an increase in meningovascular complications has been described in the HIV population (Timmermans et al. 2004; Zetola et al. 2007; Chahine et al. 2011). HSV-2 is another sexually transmitted infection that is known to infect the brain. However, it causes meningitis rather than stroke syndromes.

Cytomegalovirus and Candida ablicans infections have been associated with HIV infection and stroke in a few case series and further evidence is needed to confirm this (Kieburtz et al. 1993; Anderson et al. 2010). cART may unmask occult opportunistic infections that consequently cause a stroke. This should

be considered in all patients with an acute stroke or worsening of stroke symptoms following the initiation of cART (Lucas et al. 2008; Newsome et al. 2009; Pepper et al. 2009; Anderson et al. 2010).

Even in high-income countries, opportunistic infection still accounts for significant mortality despite the use of cART and optimal diagnostic facilities (Lucas et al. 2008). Furthermore, infections outside the central nervous system may contribute to the development of a prothrombotic state potentially increasing the risk of ischaemic stroke (Emsley et al. 2008).

HIV- associated vasculopathy

HIV-associated vasculopathy is a relatively new and evolving term used to describe several arterial changes associated with HIV infection Figure 1:6. Some authors have distinguished aneurysmal arterial changes from vasculitis and others have suggested that given the uncertainty regarding pathogenesis of vasculitis, all arterial abnormalities thought to be associated with stroke are considered part of an HIV-associated vasculopathy. A working definition would, in my opinion, be useful to both clinicians and pathologists. Based on the current literature, I propose that the term HIV-associated vasculopathy should include any abnormality of the intra or extracranial cerebral blood vessels that results directly or indirectly from HIV infection, but excludes opportunistic infection associated vasculitis or neoplastic involvement of the vessels Table 1:2. In the table, I suggest a classification of HIV-associated vasculopathy to include large and small vessel abnormality associated with aneurysm formation, vasculitis, accelerated atherosclerosis and other abnormalities including altered vascular reactivity and small vessel disease. Aneurysmal dilatation in HIV infected patients may be extracranial involving the carotid, aorta, iliac and other large arteries, or intracranial involving branches of the circle of Willis (Nair et al. 1999; Chetty et al. 2000; Modi et al. 2008). In a series of HIV positive stroke patients in Cape Town

(n=64), seven patients had an extracranial non-aneurysmal vasculopathy which manifested as either stenosis or occlusion of the internal carotid artery. Autopsy of one of the individuals who died from complications associated with their stroke had microscopic evidence of neovascularisation and vessel wall inflammation with a thrombus occluding the lumen. Six patients had radiological evidence of intracranial vasculopathy, typically involving the medium sized vessels with or without fusiform aneurysm or stenosis. One patient's histology showed luminal thrombosis, concentric intimal hyperplasia with hyalinization and fragmentation of the elastic lamina (Tipping et al. 2007). In this case series, patients with an extracranial vasculopathy had a significantly higher CD4+ T lymphocyte count compared with those with intracranial vasculopathy (Tipping et al. 2007). Patients in this study were young and did not have evidence of atherosclerosis.

Table 1:2: Possible HIV related causes of stroke	
Ischaemic	**HIV-associated vasculopathy (abnormality of the cerebral blood vessels result directly or indirectly from HIV infection but excluding opportunistic infection vasculitis)** ➢ Associated with aneurysm formation - Intracranial - Extracranial ➢ Vasculitis (where this is the direct result of HIV infection and opportunistic infections have been excluded) ➢ Accelerated atherosclerosis ➢ Other disease of cerebral blood vessels associated with HIV infection (including small vessel disease changes and altered vasoreactivity)
	Opportunistic infection / neoplasia ➢ Opportunistic infection causing stroke e.g. tuberculous meningitis, Varicella Zoster Virus vasculitis, meningovascular syphilis ➢ Neoplasia such as lymphoma involving cerebral blood vessels
	Cardioembolism ➢ Bacterial endocarditis ➢ Marantic endocarditis ➢ HIV-associated cardiac dysfunction ➢ Ischaemic Heart Disease
	Other determined cause ➢ Coagulopathy (e.g. antiphospholipid syndrome) ➢ HIV associated hyperviscosity
Haemorrhagic	➢ HIV-associated vasculopathy (aneurysm or vasculitis associated) ➢ HIV associated thrombocytopenia ➢ Mycotic aneurysm (secondary to bacterial endocarditis

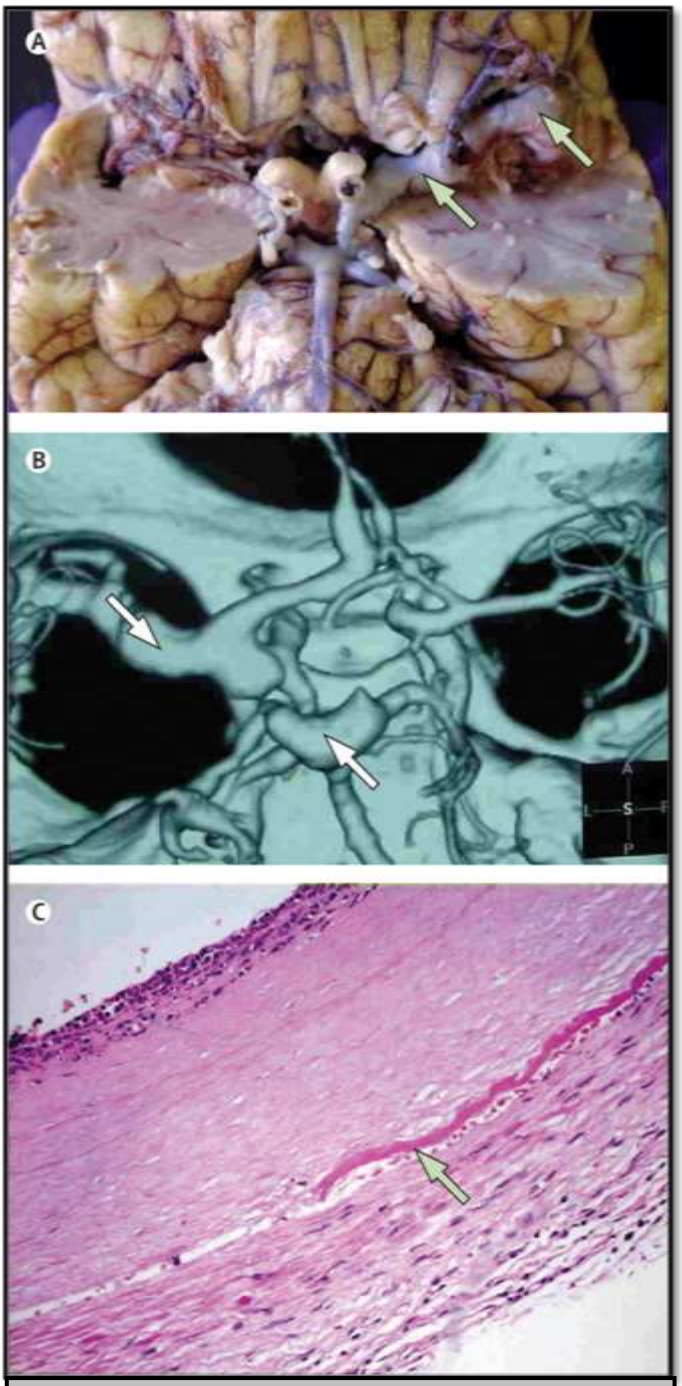


Figure 1:6: HIV-associated vasculopathy

Aneurysmal HIV-associated vasculopathy (Image A) View of the ventral surface of the brain showing a thrombotic occlusion of the left internal carotid artery and evidence of vasculopathy of the left middle cerebral artery (arrows). (Image B) CT angiogram showing several fusiform aneurysms affecting the circle of Willis. (Image C) Haematoxylin and eosin stain of a cross-section through an aneurysmal cerebral artery showing evidence of intimal hyperplasia, fragmentation of internal elastic lamina (arrow), and neutrophil infiltrate; note the absence of atherosclerosis.

In other studies, HIV infected patients with extracranial carotid aneurysms but without stroke, had evidence of a leucocytoclastic vasculitis of the vasa vasorum and periadventitial vessels without evidence of atherosclerosis or infection on culture of the blood or aneurysm wall (Nair et al. 1999; Chetty 2001). Whether this form of vasculitis is an initiating or pivotal event in the development of extracranial carotid artery aneurysm formation in HIV infection or one of several factors responsible is not clear. Fusiform intracranial cerebral aneurysms have been described in children usually as incidental findings but in some, associated with subarachnoid haemorrhage or 'hemiparesis' (Mazzoni et al. 2000; Bulsara et al. 2005).

The term 'vasculitis' should be reserved for the histologically confirmed appearance of inflammatory cells in the blood vessel wall together with associated wall damage. In early pathological studies that did not exclude all potential causes of infective vasculitis other than HIV, cerebral vasculitis attributed to HIV was probably overestimated (Connor et al. 2000; Nogueras et al. 2002). However, a heterogenous array of extra-cerebral vasculitis of small, medium and large arteries has been described in HIV-infected patients (Chetty 2001). Although HIV antigen and particles have been described in perivascular cells in two patients and HIV-like particles in another suggesting direct HIV infection at least of perivascular tissue, this is far from certain (Gherardi et al. 1993; Chetty 2001). Other postulated mechanisms for vasculitis include immune deposition and indirect damage caused by T cell derived growth factors and cytokines (Chetty 2001). In clinical practice, histology is seldom available and the diagnosis of cerebral vasculitis is usually based on clinical and radiological findings and exclusion of other possible causes. In recent clinical case series HIV-associated arterial vasculitis (attributable to opportunistic infection or other known cause), accounted for 13-28% of ischaemic stroke patients (Mochan et al. 2003; Ortiz et al. 2007; Tipping et al. 2007). In a patient presenting with typical clinical and radiological features of

vasculitis, in whom all other likely causes have been excluded, HIV-associated vasculitis is the most likely diagnosis.

HIV infection may be associated with acceleration of large vessel atherosclerosis, potentially as a result of cART and associated metabolic complications such as dyslipidaemia, insulin resistance or diabetes mellitus, via low grade chronic systemic inflammation, or as a result of co-infections such as hepatitis C or cytomegalovirus (CMV) (Maniar et al. 2012; Sen et al. 2012). Interactions between co-infections and cART in the context of HIV may be more complicated than currently appreciated. For example, one recent study suggested mitochondrial genomics may play a significant role in metabolic disorders and cardiovascular diseases in HIV/hepatitis C virus-coinfected patients on cART (Micheloud et al. 2011). Relatively little is known currently about the role of specific co-infections in atherogenesis in HIV infected individuals. It might even be relevant to consider infectious burden of a number of infectious microorganisms (for example Chlamydia pneumonia, Helicobacter pylori, CMV, herpes viruses 1 and 2) contributing to atherosclerosis rather than individual co-infections, as it appears to be in non-HIV infected individuals (Elkind et al. 2010).

Other abnormalities of cerebral blood vessels such as altered vasoreactivity and small vessel abnormalities have been described (Connor et al. 2000; Sen et al. 2012). The significance of these abnormalities in the context of clinical stroke is not clear.

Other causes

Although arterial changes in HIV infected patients may be associated with HIV or opportunistic infection (e.g. varicella zoster virus or tuberculosis), other unrelated aetiologies such as connective tissue disease and an arterial dissection should also be considered in this young population. Mycotic aneurysm resulting from bacterial endocarditis, HIV induced thrombocytopenia and complications of aneurysmal and vasculitic HIV-associated vasculopathy may result in cerebral or subarachnoid haemorrhage Table 1:2.

1.8.5 Pathogenesis of vasculopathy and atherogenesis in HIV (in the absence of cART)

Much of our understanding of the potential pathogenesis of HIV infection and stroke comes from experimental studies using *in vitro* human brain microvascular endothelial cells (HBMECs), human umbilical vascular endothelial cells (HUVECs) models and HIV-transgenic animal models (Tinkle et al. 1997; Chi et al. 2000; Hag et al. 2009). *In vivo* studies in humans have utilised various imaging modalities (e.g. carotid and transcranial-Doppler, magnetic resonance imaging and angiography) to demonstrate endothelial integrity indirectly. In addition, the use of circulating biomarkers has given us some insight into endothelial dysfunction in HIV infection (de Gaetano Donati et al. 2004).

The vascular endothelium is a protective barrier, preventing arterial wall inflammation, coagulation, remodelling and ultimately, in some instances, stroke. Dysfunction of the endothelium is pivotal to the initiation and progression of vascular disease and may lead to occlusive thrombotic events mediated by leucocyte recruitment, platelet adhesion and aggregation, blood clotting activation, and fibrinolysis

derangement (Chi et al. 2000; Kline et al. 2008). This is believed to underpin the inflammatory process of atherosclerosis and similarities have been seen in HIV experimental models and individuals with stroke Figure 1:7 (Kline et al. 2008; Lo et al. 2012). I will explore the impact of HIV on the endothelium and its potential impact on chronic vascular inflammation and vessel wall remodelling.

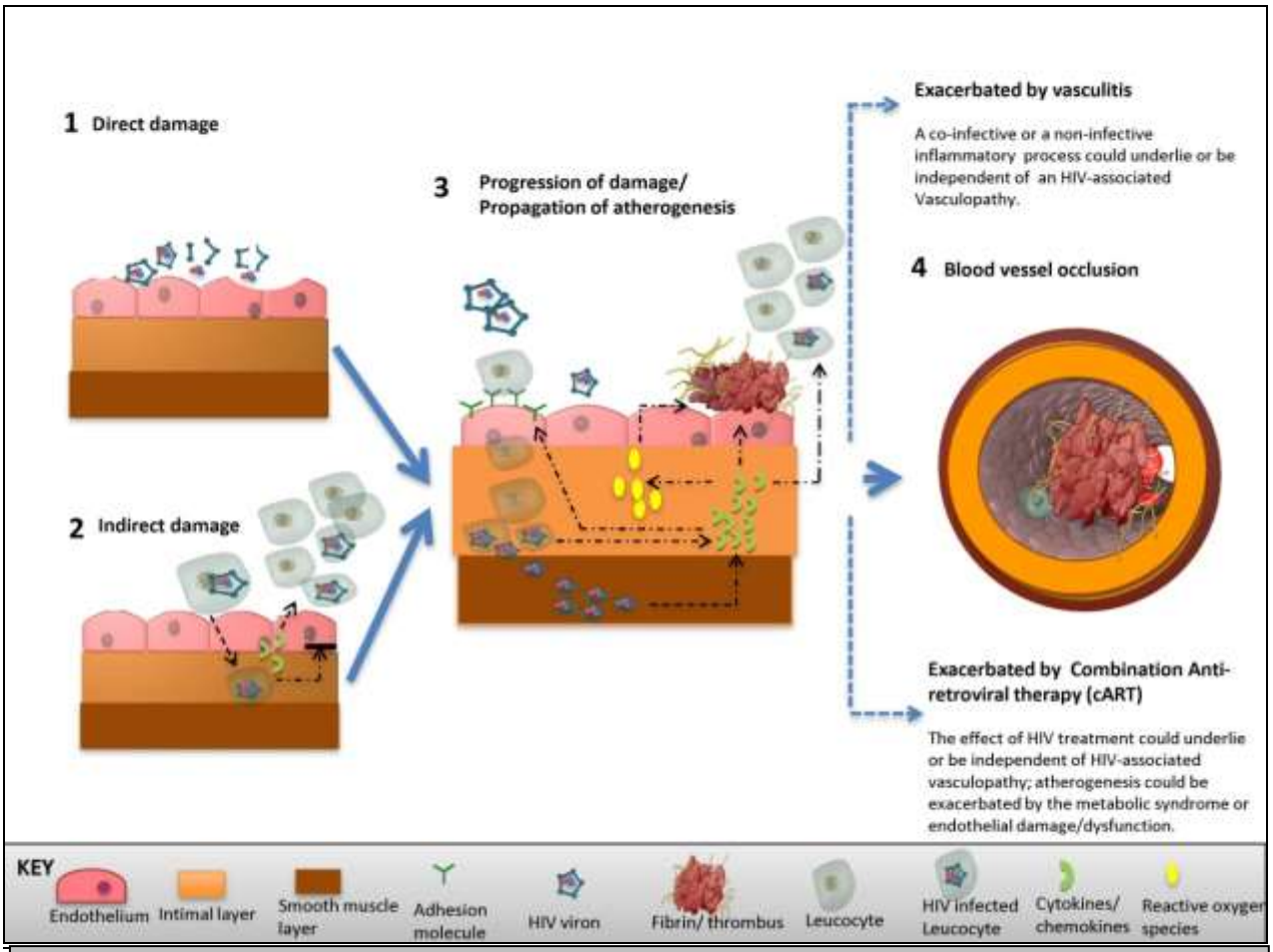


Figure 1:7: Hypotheses on the mechanism of HIV-associated vasculopathy

Mechanisms are specific to atheroma and potentially applicable to the other forms of HIV-associated vasculopathy. **(1)** Direct damage can occur through continuous exposure of the endothelium to HIV virion or viral particles (eg, GP120 or TAT) leading to endothelial dysfunction. **(2)** Indirect damage can arise from circulating infected monocytes freely transmigrating the endothelium as part of normal surveillance, with an impaired reverse transmigration, thus increasing the subendothelial population of HIV infected monocytes. The release of chemokines such as CCL2 from infected leucocytes attracts more leucocytes. **(3)** Several events lead to the progression of damage and propagation of atherogenesis: upregulation of cell adhesion molecules (eg, selectins), leading to increased adhesion of infected or non-infected leucocytes; release of HIV virions into the arterial smooth muscle and continued active replication of the virus in smooth muscle cells; inflammatory cytokine release from HIV-infected cells, leading to further recruitment and adhesion of leucocytes, increased production of reactive oxygen species, and derangement of the coagulation system, favouring a prothrombotic state. Underlying this continuing process is the remodelling of the vessel wall, involving intimal hyperplasia and fragmentation of the elastic lamina. **(4)** Thrombotic occlusion of the vessel wall lumen is one of the outcomes of this process.

The role of inflammation

Although HIV-1 itself is unlikely to be vasculotropic, the virus affects endothelial homeostasis and function in important ways that could initiate and propagate atherogenesis. The vascular endothelium is continually exposed to stimuli such as HIV-1-infected cells ($CD4^+$ T cells, monocytes, and macrophages), freely circulating HIV-1 viruses; HIV-1 proteins (i.e. Tat and gp120) that are released with host cell lysis or actively secreted, and viral-induced pro-inflammatory cytokines (Chi et al. 2000; Kuller et al. 2008). All these factors potentially activate the endothelium, damage and increase its permeability, facilitating leucocyte invasion into the vessel wall and chronic inflammation.

HIV-1-induced cytokine endothelial activation may result in production of reactive oxygen species (ROS), expression of cell adhesion molecules (CAMs), and the release of chemoattractants at localised areas of vascular inflammation. Akin to the pathogenesis of atherosclerosis, chemoattractants such as chemokine ligand 2 (CCL2) may recruit leucocytes to the brain during HIV infection (Park et al. 2001; Eugenin et al. 2008). CCL2 is also associated with an increase in HIV viral burden (Floris-Moore et al. 2009). Plasma concentration of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and E-selectin, in addition to other markers of inflammation (high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6 and cystatin) are significantly elevated in HIV-1-positive patients compared with controls (Schved et al. 1992; Chi et al. 2000; Ross et al. 2009; Neuhaus et al. 2010). The activation of these biomarkers implicates HIV-1 virus in dysfunction of the endothelium and inflammation. Endothelial cell molecules involved in coagulation such as von Willebrand factor, thrombomodulin, plasminogen activator inhibitor-1 antigen, tissue factor and d-dimer are deranged in HIV infection, favouring a prothrombotic state (Schved et al. 1992; Neuhaus et al. 2010). Furthermore, some of these biomarkers of endothelial dysfunction and inflammation positively correlate with anti-

p24 antibody levels and disease severity (Schved et al. 1992; Triant et al. 2011). This propagated inflammatory process could potentially accelerate atherosclerosis or independently initiate vascular disease.

Monocytes continually migrate from the bloodstream across the vascular endothelium for systemic immune surveillance and maintenance of macrophage populations in the brain. CD163 is a novel marker of activated monocyte; it is elevated and associated with noncalcified coronary plaque in HIV-infected patients; implicating a role in the pathogenesis of HIV-associated atherogenesis (Burdo et al. 2011).

Monocytes can perform reverse transendothelial migration across the endothelium, necessary for the movement of tissue monocytes/macrophages back into the bloodstream. HIV-1 infected macrophages have a reduced capacity to emigrate from the subendothelial extracellular matrix *in vitro*, indicating that the infected monocyte can easily move from the blood stream to the brain tissue but not the reverse (Westhorpe et al. 2009). Clinically, such an adaptation could promote establishment of viral reservoirs and increase the exposure of HIV-1 and its viral proteins to the vessel wall. Since the vascular bed serves as the interface between the entry and exit of these monocytes, it is conceivable that this is where the greatest damage occurs. In the context of vascular disease, it has been demonstrated that once the virus is subendothelial it can then infect coronary arterial smooth muscle in a CD4+ T cell, chemokine and endocytosis dependent manner and actively replicate in its new niche (Schecter et al. 2001; Eugenin et al. 2008).

Evidence of vessel wall remodelling

Insights from animal models have shown a plausible link between endothelial dysfunction and vasculopathy. The well-characterised "murine AIDS" model of retroviral infection time-dependently decreased maximum aortic contractile responses and impaired endothelium-dependent relaxation (Clark et al. 2001). Isolated aortas from infected mice also expressed higher levels of ICAM-1 and VCAM-1 (Hag et al. 2009). These models also demonstrated independent HIV-associated vasculopathy (Tinkle et al. 1997; Baliga et al. 2005). Thus, the "murine AIDS" model strongly suggests that retroviral infection, similar to that of HIV-1 in humans, is capable of causing vasculopathy and endothelial dysregulation in mice.

Some of these experimental findings have been corroborated in humans; brachial endothelial-dependent flow mediated dilatation and carotid intimal medial thickness (cIMT) are functional and structural indicators of endothelial integrity. Impaired brachial endothelial-dependent flow mediated dilatation and increased cIMT are both associated with immunosuppression or increasing HIV viral levels (Solages et al. 2006; Oliviero et al. 2009; Seaberg et al. 2010). Suppressed HIV replication is associated with less cIMT progression (a marker of subclinical atherosclesosis) (Baker et al. 2011). Furthermore, thinning of the arterial media layer, a possible preclinical stage of HIV-associated vasculopathy has been described (Gutierrez et al. 2012).

HIV-1 associated endothelial dysfunction likely compromises the central nervous system though whether or not this results in hard clinical outcomes such as stroke is unclear. It is plausible that HIV

initiates injury to the vascular tree or contributes to further injury to an already damaged vascular tree caused by atherosclerosis, predisposing to stroke.

1.8.6 Role of cART in the pathogenesis of cerebrovascular disease and stroke

More recently, the focus on stroke risk is not only on HIV infection but its treatment, cART**.** De Gaetano Donati and colleagues proposed that cART could cause both direct tissue injury to arteries resulting in elevated markers of endothelial dysfunction or indirectly cause injury through lipid modification. However, it is still uncertain how relevant this is to cerebrovascular disease (de Gaetano Donati et al. 2003). Elevation of biomarkers indicating endothelial dysfunction should be interpreted with caution, as this could arise as a consequence of other factors, namely autoreactive cell destruction by an improved autoimmune system and/or the efficacy of the therapy destroying cells.

In the short term cART might reduce the risk of ischaemic stroke and transient ischaemic attack, though this is based on retrospective data from only one study (Corral et al. 2009). However, there is increasing evidence suggesting that long-term cART results in endothelial toxicity and vascular dysfunction (Corral et al. 2009; Ross et al. 2009). Non nucleotide reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) but not nucleotide reverse transcriptase inhibitors (NRTI) are thought to cause inflammation and may increase cardiovascular risk. Again, little is known about the impact on cerebrovascular risk. Abacavir, a NRTI has been associated with an increased 'high sensitivity' (hs) CRP, IL-6 levels and in a retrospective cohort study, an increase in cerebrovascular events in an HIV infected population (Kuller et al. 2008; Rasmussen et al. 2011). However, a recent meta-analysis of published and unpublished Randomised Controlled Trials did not confirm an increased risk of cardiovascular events (mostly

myocardial infarction) with abacavir-containing cART regimens compared with other cART. How much of this extrapolates to cerebrovascular disease is unknown (Cruciani et al. 2011). More research is needed; specifically with cerebrovascular events as an endpoint.

HIV related endothelial dysfunction and inflammation continues on cART; Ross and colleagues demonstrated enhanced endothelial activation, inflammation, and increased cIMT in HIV-infected patients despite cART; concluding that although cART is reducing the virulence of HIV, it has little anti-inflammatory potential on the endothelium (Ross et al. 2009). Therefore, HIV infected individuals receiving cART are likely to live longer with prolonged endothelial and metabolic challenges that increase stroke risk.

1.8.7 Management

Stroke mimics are not uncommon in the HIV population. Of the ninety-eight consecutive patients presenting with an acute focal neurological deficit in Malawi, eleven were found to have another cause for their presentation (e.g. toxoplasmosis, neurocysticercosis, tuberculoma, brain tumour) (Kumwenda et al. 2005). Brain imaging in HIV infected patients presenting with sudden onset focal signs and features suggestive of a stroke is therefore essential to the diagnosis as well as distinguishing between cerebral haemorrhage and ischaemic stroke.

Once the diagnosis of stroke is confirmed, management should be directed towards acute stroke treatment, establishing the cause of the stroke, management of HIV infection and secondary prevention of stroke. Concomitant HIV infection raises doubt about extrapolating from evidence based treatment of

non-HIV related stroke. The role of intra-venous thrombolysis is uncertain in the absence of randomised controlled studies in HIV related stroke. Often, the patient's HIV status is not known at the time of presentation of the acute stroke and the decision whether or not to administer thrombolysis must be taken within a short time. Although there is no clear evidence of harm and HIV infected individuals may well have a stroke unrelated to HIV infection, the pathogenesis of stroke may include HIV-associated vasculopathy, infective vasculitis, infective meningitis and other causes that may increase bleeding risk Table 1:2. Reassuringly, there are isolated reports of successful use of thrombolysis to treat myocardial infarction in HIV infected individuals (Boccara et al. 2003). However, how generalisable this is to patients with potentially diseased cerebral vessels and higher bleeding risk with thrombolysis is unclear. Until such data become available, acute therapy including the use of thrombolysis will have to be individualised and based on clinical judgment and of course patient choice.

Investigation should be directed both at assessing for conventional causes of stroke and those associated with HIV (Table 1:2, with particular emphasis on finding treatable causes). Figure 1:8 provides an approach to management but in many areas where HIV infection is prevalent this will not be possible. In low resourced settings, investigation and treatment should be directed at finding treatable causes of stroke or stroke mimics such as tuberculosis, cryptococcus, toxoplasmosis, varicella zoster and herpes virus infection, perhaps by combining CT brain scan, chest x-ray, lumbar puncture (if not contraindicated and in the absence of an alternative cause e.g. an obvious cardioembolic source) and selected blood tests. Figure 1:8. Ancillary tests may be needed to establish the cause of the stroke e.g. sputum and cerebrospinal fluid (CSF) samples for tuberculosis (Kent et al. 1993; Nelson et al. 2011). Measuring intrathecal IgG against varicella zoster virus together with CSF DNA PCR improves the likelihood of identifying this potential cause (Nagel et al. 2008). The ideal test for neurosyphilis is controversial,

however CSF VDRL and when this is negative in a HIV positive patient, CSF fluorescent treponemal antibody (FTA) seems a reasonable approach (Marra et al. 2004; Timmermans et al. 2004).

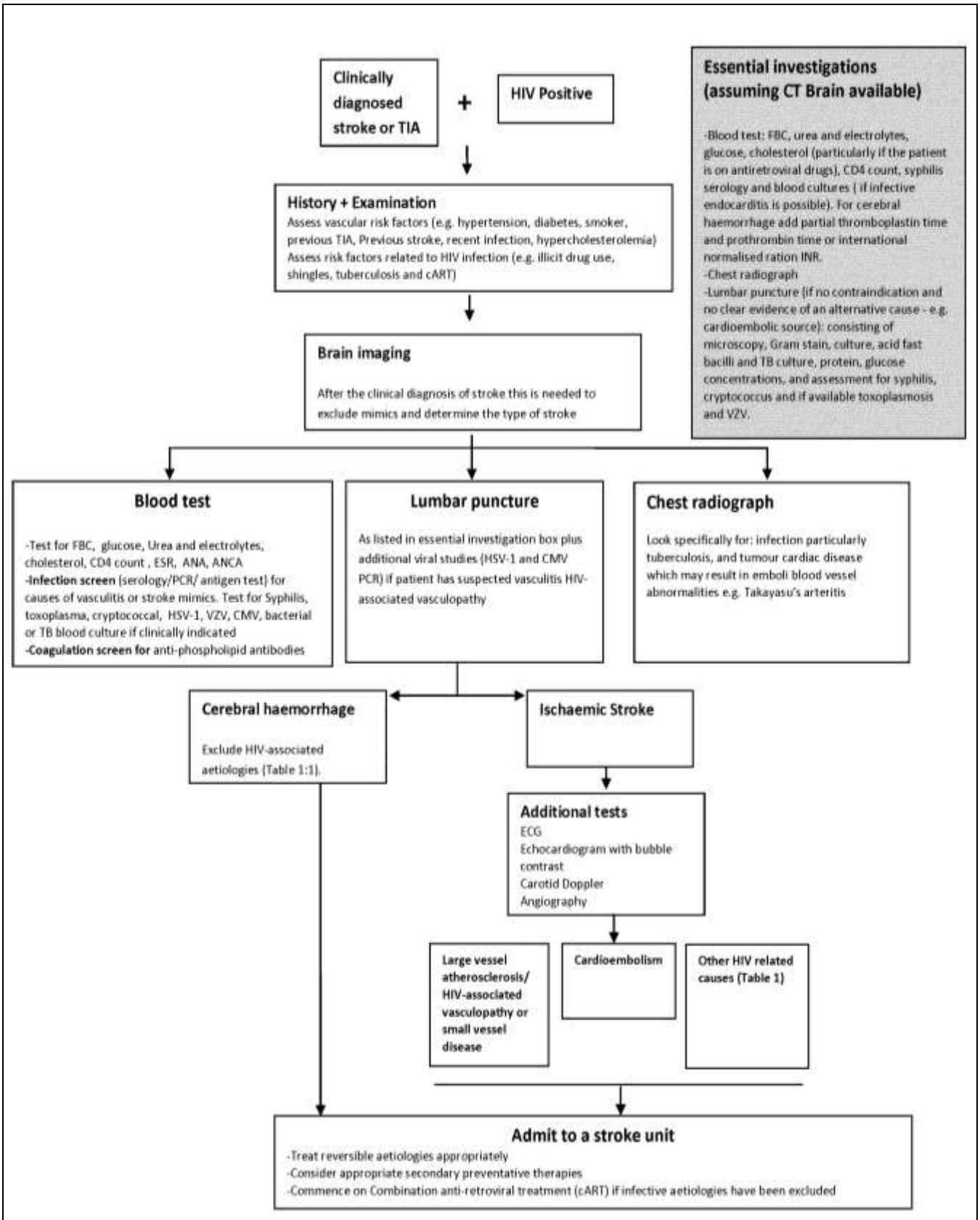


Figure 1:8: Management approach for HIV infected patients with stroke

TIA=transient ischaemic attack. cART=combined antiretroviral therapy. FBC=full blood count. ESR=erythrocyte sedimentation rate. ANA=antinuclear antibodies. ANCA=antineutrophil cytoplasmic antibodies. HSV-1=herpes simplex virus type 1. VZV=varicella zoster virus. CMV=cytomegalovirus. TB=tuberculosis. ECG=electrocardiograph.

Antineutrophil cytoplasmic antibodies (ANCA) assessed by immunofluorescence and enzyme-linked immunosorbent assay have been described in HIV infection but not necessarily in patients with vasculitis, autoimmune disease or specific opportunistic infection (Savige et al. 1993; Cornely et al. 1999; Jansen et al. 2005). A diagnosis of cerebral vasculitis in individuals with HIV infection should be based on the results of appropriate radiological and if possible histological features in a patient without any other potential cause of vasculitis (e.g. opportunistic infection). In this setting a positive ANCA may strengthen the diagnosis.

In high resourced settings, the investigation should include detailed assessment of cerebral arteries including carotid doppler and CT or MR angiography depending on local expertise. In selected patients, perhaps those who have further events or comorbidity suggesting autoimmune or other disease, conventional angiography and or brain biopsy may be indicated. In low resourced settings without access to brain imaging, I advise basic stroke care using the approach outlined in the South African stroke guideline (Bryer et al. 2011).

The role of immunosuppression with corticosteroids is far from clear (Bhagavati et al. 2008; Bermel et al. 2009). In the absence of any evidence to guide management, it may be useful to follow this approach; if vasculitic HIV-associated vasculopathy is suspected and other potential autoimmune or infectious causes have been excluded, then the introduction of cART and addition of corticosteroid, if the patient has a poor response, seems reasonable.

There is convincing evidence that the introduction of cART results in reduction of all cause mortality in HIV infected patients (Bozzette et al. 2003; Bozzette 2011). Far less certain is whether cART, particularly exposure to protease inhibitors, increases the risk of stroke and myocardial infarction in the long term as a result of metabolic effects e.g. hypercholesterolaemia already described, and prolonged survival (aging is a risk factor for stroke and some HIV infected groups have a high prevalence of cigarette smoking) (Currier et al. 2008; Bedimo et al. 2011; Bozzette 2011). The risk-benefit ratio of cART on the basis of current knowledge appears favourable. However, given the concern about long-term stroke and cardiovascular disease risk, a pragmatic approach seems reasonable; identifying and managing risk factors, perhaps changing the class of cART regimen or considering a cholesterol lowering drug if appropriate (Holmberg et al. 2002; Bozzette et al. 2003; Kwong et al. 2006; Bozzette 2011; Islam et al. 2012).

None of the studies that currently guide the use of secondary prevention for stroke including use of antiplatelets, statins and blood pressure lowering therapy, can be directly extrapolated to the care of the HIV-infected stroke patients. However, general lifestyle factors and aiming for optimal reduction of vascular risk factors seems sensible.

Finally, it is important to consider the mode of HIV infection relevant to the patient as this may influence the underlying stroke risk factors, cause and management. In sub-Saharan Africa the major mode of HIV transmission is heterosexual, intravenous drug use is rare (Ocama et al. 2011; Wamai et al. 2011). However, intravenous drug use is more common elsewhere and may be associated with several potential stroke causes including use of specific drugs (cocaine, amphetamines, sympathomimetic

drugs), infective endocarditis, and embolisation of particulate matter (C. Warlow 2007). In many regions, cigarette smoking is more common in people with HIV infection than the general population (Shurtleff et al. 2012).

1.8.8 Conclusion

The incidence of stroke is increasing in low and middle income countries and HIV infection, often prevalent in the same regions, may add to the risk, though data are very limited. While cART may decrease stroke risk in the short term, the impact of cART on the vasculature and long term stroke risk is unknown but may be significant. Certainly, cART has increased the lifespan of those infected with HIV infection but paradoxically this may increase their risk of stroke in the long term, particularly in the context of endothelial and metabolic side effects of current medication. The risk of stroke in HIV infection and further research to clarify burden, causes, pathogenesis, and management, is needed in areas with little or inadequate antiretroviral therapy. Equally, the need for further research exists in high income regions with populations of patients on long term antiretroviral therapy. The increased burden of stroke to patients and health services may only be realised in future decades.

There are very little high calibre community based studies assessing the burden and nature of HIV related stroke. Epidemiological studies with detailed clinical assessment of stroke patients are needed both in high and low income regions. Ideally, these should include early imaging, clearly documented stroke types, subtypes, risk factors and causes, and importantly autopsy confirmation or exclusion of stroke. Improved knowledge about the mechanisms and causes of stroke should lead to improved investigation and treatment of patients.

1.9 Scope of thesis

This thesis sets out to examine the relationship of HIV infection and stroke. HIV also increases the risk of stroke mimics such as intracranial toxoplasma infection. To determine whether routine stroke screening tools such as the Recognition of Stroke in the Emergency Room (ROSIER) score and computer tomography of the brain reliably differentiate those with a stroke diagnosis from those with stroke mimics, I explored the diagnostic accuracy of both clinical tools in an HIV positive population. Following the validation of these methods I was then able to answer the following questions:

Is HIV infection a risk factor for stroke?

Is there consensus on the aetiological case definitions of HIV infection and stroke?

What is the aetiological spectrum and outcome of HIV related stroke and is this different from the non-HIV stroke population?

2 Diagnostic accuracy of the Recognition of Stroke in the Emergency Room (ROSIER) score and CT brain scanning of people with HIV infection and suspected stroke

2.1 Abstract

Background

HIV increases the risk of stroke mimics such as intracranial toxoplasma infection. To determine whether routine stroke screening tools such as the Recognition of Stroke in the Emergency Room (ROSIER) score and computer tomography of the brain reliably differentiate those with a stroke diagnosis from those with stroke mimics, I explored the diagnostic accuracy of both clinical tools in an HIV positive population.

Method

In this retrospective diagnostic accuracy study, 56 HIV infected patients with stroke-like symptoms presented to the Infectious Disease Units in Liverpool and Manchester Hospitals, UK, during the study period of January 2007 to December 2009. Eighteen had a stroke and 38 had a stroke mimic. The clinical characteristics and diagnostic accuracy of routine clinical tools used to discriminate strokes from their mimics was studied.

Results

The median age of patients with stroke and stroke mimics were 41-years and 38-years respectively. HIV status was unknown at presentation for 6/18 (33%) stroke patients and 18/38 (47%) stroke mimic patients. The sensitivity and specificity of combining ROSIER score and CT brain scan were 67% (95% CI 30-92) and 90% (95% CI 68-98) respectively.

Conclusion

The ROSIER stroke score and CT brain imaging have a poor diagnostic accuracy in people with HIV infection. A high proportion of undiagnosed HIV infection in this population adds to the difficulty in directing appropriate care. This study highlights the need for rapid HIV testing in young patients with a suspected stroke. I therefore decided not to use ROSIER to help classify stroke in my study, and to focus on MRI scanning of the brain to help determine the aetiology of stroke.

2.2 Introduction

The management of ischaemic stroke was revolutionised in the mid 1990s when thrombolytic therapy became available (Hacke et al. 1995; Hacke et al. 2008). One urgent step in managing such patients is the computed tomography (CT) brain scan which excludes haemorrhagic stroke and some stroke mimics, both contraindications to thrombolysis. The recognition of stroke in the emergency room (ROSIER) stroke score was devised in 2005 to allow patients with suspected stroke to be triaged rapidly for a CT brain scan. The score is based on easily accessible components from the history and physical examination. The presence of sudden asymmetric face, arm and leg weakness, speech disturbance and visual field deficit scores one point each; a point is subtracted if there is any seizure activity or loss of consciousness. Patients with scores greater than zero are highly likely to have had a stroke (Nor et al. 2005). Administration of the score takes less than 5 minutes. In the general UK population, where this score was validated, it had a sensitivity of 92% and a specificity of 86% and a positive predictive value of 86% (Nor et al. 2005). The ROSIER score is the most sensitive and specific rapid screening tool when compared with others and therefore, the most widely recommended (Whiteley et al. 2011) (NICE 2008). Although the ROSIER score is commonly used in industrialised countries, it has not been validated in many sub-Saharan African countries, including Malawi. Furthermore, little is known about the performance of this clinical tool in people with HIV infection.

As discussed in chapter 1, HIV infection and its treatment, are thought to increase the risk of stroke, especially ischaemic stroke in young adults. However, HIV also increases the risk of stroke mimics such as intracranial toxoplasma infection (Kumwenda et al. 2005; Alkali et al. 2013). Correctly identifying such mimics, especially infections, is essential, because they have different management and are potentially treatable. However, the reliability of CT scan in identifying strokes and its mimics in people with HIV

infection is also uncertain. Thus, for people with HIV infection who present with a suspected stroke, both the role of the ROSIER stroke score in determining who needs an urgent CT, and the ability of the CT to identify stroke or its mimics are unclear.

The objective of this study was to determine the diagnostic accuracy for recognition of stroke in the emergency room (ROSIER) score and computed tomography (CT) brain imaging in stroke and stroke mimic patients with HIV infection.

2.3 Methods

2.3.1 Clinical methods

This retrospective study was conducted at the Infectious Disease units at the Royal Liverpool University Hospital and North Manchester General Hospital, which serve a combined HIV positive population of approximately 2600. I screened the hospital electronic databases for HIV positive patients from Jan 2007 to Dec 2009 for patients with any neurological diagnosis. Admission and discharge databases, discharge letters and clinic letters were explored. The medical records of patients with a neurological diagnosis were extracted and those that presented with acute stroke-like symptoms within 48 hours of symptom onset were included. Stroke-like symptom was defined as unilateral or bilateral motor impairment (including lack of coordination), unilateral or bilateral sensory loss, speech disturbance (aphasia/dysphasia), visual disturbance (hemianopia/forced gaze), perception deficit, dysphagia, confusion, personality change and seizure (WHO 2005). A definitive diagnosis was defined as a consultant Infectious diseases or neurology physician's final opinion, after an MRI head had been performed. An MRI head was the gold standard in determining if the patient had a stroke or not, in

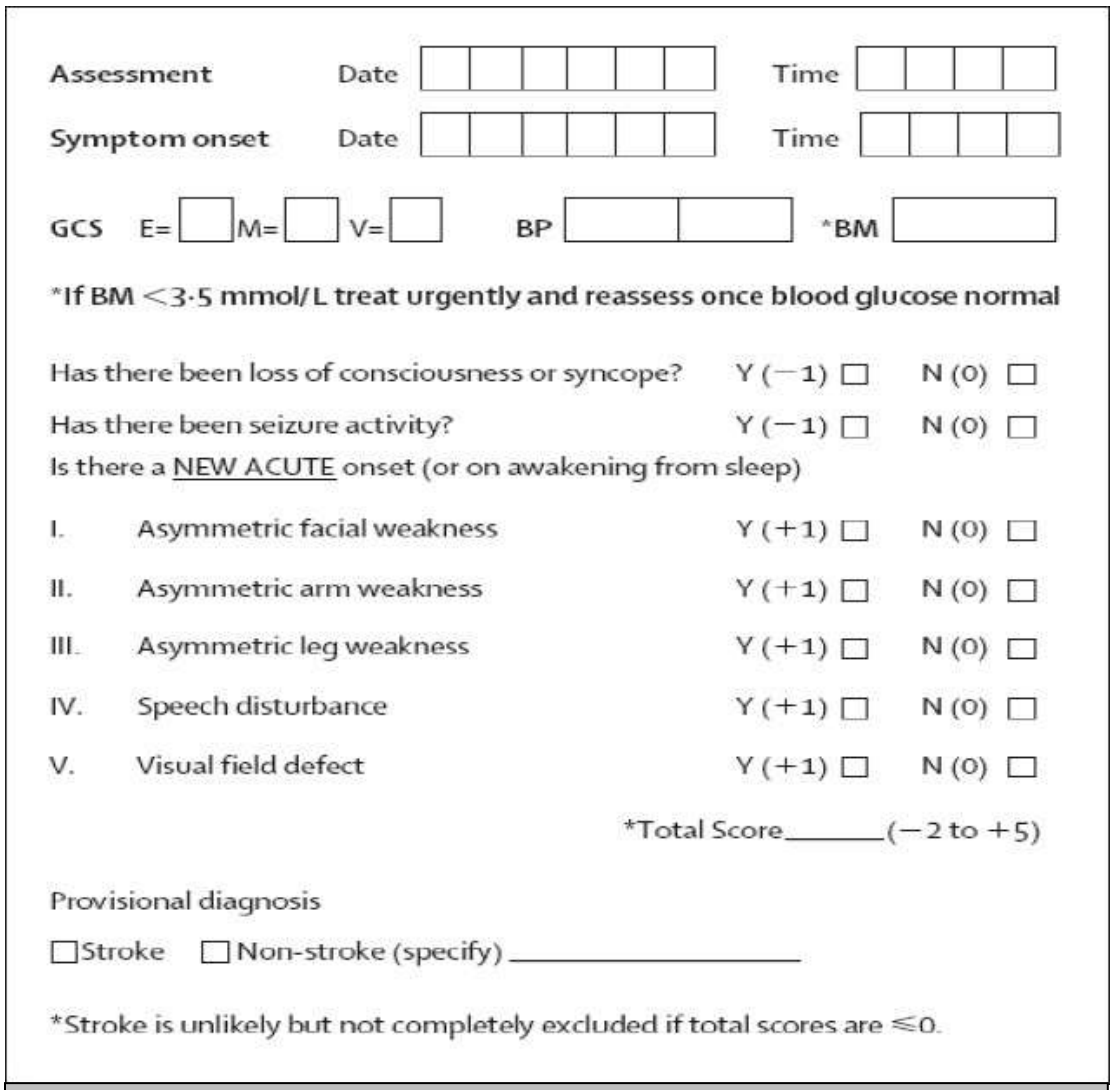
some cases, this was not performed (i.e. unable to tolerate the scan, died before imaging etc). In such cases the clinical history and examination and other gold standard tests (e.g. microbiologically confirmed blood and/or cerebrospinal fluid (CSF) infection, brain biopsy) were used.

From this cohort, I was then able to determine definitive stroke group and a definitive stroke mimic group. I excluded patients without a clear diagnosis.

This study was ethically approved by the Liverpool Northwest 4 Research Ethics Committee (10/H1005/9).

2.3.2 Clinical and radiological tools

Age, gender, presence of vascular risk factors (hypertension, diabetes, hyperlipidaemia (defined as those on lipid lowering treatment on discharge), smoking, alcohol excess, ischaemic heart disease, HIV characteristics (i.e. duration of HIV infection, cART use, CD4+ T-lymphocyte count) and evidence of systemic inflammation (elevated peripheral white cell count and C-reactive protein) were collated from the case notes and recorded in the case record form. Each case patient was then scored by an independent (registrar level) physician, blinded to the definitive diagnosis, using the ROSIER scale; a cut off score > 0 was used to define a ROSIER diagnosis of stroke; if the score was ≤ 0, a ROSIER diagnosis of stroke mimic was made Figure 2:1 (Nor et al. 2005). The diagnosis made from the ROSIER score was then compared to the definitive diagnosis.



Assessment Date ☐☐☐☐☐☐ Time ☐☐☐☐

Symptom onset Date ☐☐☐☐☐☐ Time ☐☐☐☐

GCS E=☐ M=☐ V=☐ BP ☐☐☐ *BM ☐☐☐

*If BM <3·5 mmol/L treat urgently and reassess once blood glucose normal

Has there been loss of consciousness or syncope? Y (−1) ☐ N (0) ☐

Has there been seizure activity? Y (−1) ☐ N (0) ☐

Is there a NEW ACUTE onset (or on awakening from sleep)

I. Asymmetric facial weakness Y (+1) ☐ N (0) ☐

II. Asymmetric arm weakness Y (+1) ☐ N (0) ☐

III. Asymmetric leg weakness Y (+1) ☐ N (0) ☐

IV. Speech disturbance Y (+1) ☐ N (0) ☐

V. Visual field defect Y (+1) ☐ N (0) ☐

*Total Score_____(−2 to +5)

Provisional diagnosis

☐Stroke ☐Non-stroke (specify) _____

*Stroke is unlikely but not completely excluded if total scores are ≤0.

Figure 2:1: The Recognition of Stroke in the Emergency Room scale

GCS=Glasgow Coma Scale; E=eye, M=motor; V=verbal component; BM=blood glucose; BP=blood pressure (mmHg) (Nor et al. 2005).

A list of CT brain characteristics that are associated with stroke or stroke mimics were compiled into a questionnaire; these variables included hypodensity, hyperdensity, cytotoxic oedema, vasogenic oedema, evidence of mass effect (presence of any of the following; loss of prominence of sulci/gyri, effacement of the lateral ventricles, loss of grey/white matter distinction or loss of patency of the basal cistern), multiple lesions and a normal image. The presence or absence of these variables were evaluated and recorded independently by two senior radiologists who then each made a final diagnosis of stroke, stroke mimic or normal scan. The radiologists were blinded to the definitive diagnosis but had some clinical information to simulate a real-life setting and facilitate the interpretation of the brain scan (e.g. symptom type and affected side). One radiologist was a neuroradiologist working in a specialist neurology centre, the other was a general radiologist based in one of the teaching hospitals with the infectious diseases unit. When there was disagreement in diagnosis between the two radiologists, the diagnosis from the original CT report was used.

2.3.3 Statistical methods

Data were entered into a Microsoft Excel database, and statistical analysis was done using STATA 11.2. Categorical data were represented as proportions and continuous data as medians with interquartile ranges (IQR). Univariate analysis was used on all variables and presented as proportions or odds ratios (OR) with 95% Confidence Intervals (CI). Significance levels were taken at $p \leq 0{\cdot}05$. I calculated the sensitivity and specificity with 95% CI for the ROSIER score alone and in combination with a CT brain scan. Missing observations were included in the analysis by creating missing value categories.

An inter-rater reliability analysis using unweighted Kappa statistic was used to determine the level of agreement between both radiologists (Brennan et al. 1992).

2.4 Results

2.4.1 Participants

Among the population served by the two hospitals of approximately 2600 people with HIV infection, 152 presented with acute stroke-like symptoms between January 2007 and December 2009. Clinical records were evaluated between January-March 2010. Of the 152 records 56 had complete data and therefore a definite diagnosis was made; 18 with a stroke, and 38 with a stroke mimic Figure 2:2. In the remaining 90, although there was a working diagnosis in some cases, it did not fulfil our strictly defined criteria. A definitive diagnosis was confirmed by MRI in 46 (82%) of the 56, by microbiological analysis of the blood/or cerebrospinal fluid (CSF) for all the infective aetiologies in 27 (48%), by brain biopsy in 2 (4% - this confirmed the diagnoses of lymphoma and HIV tumefactive demyelination), and by response to

treatment in 20 (35%). The subsequent clinical course of disease was consistent with the diagnosis for all 56 patients.

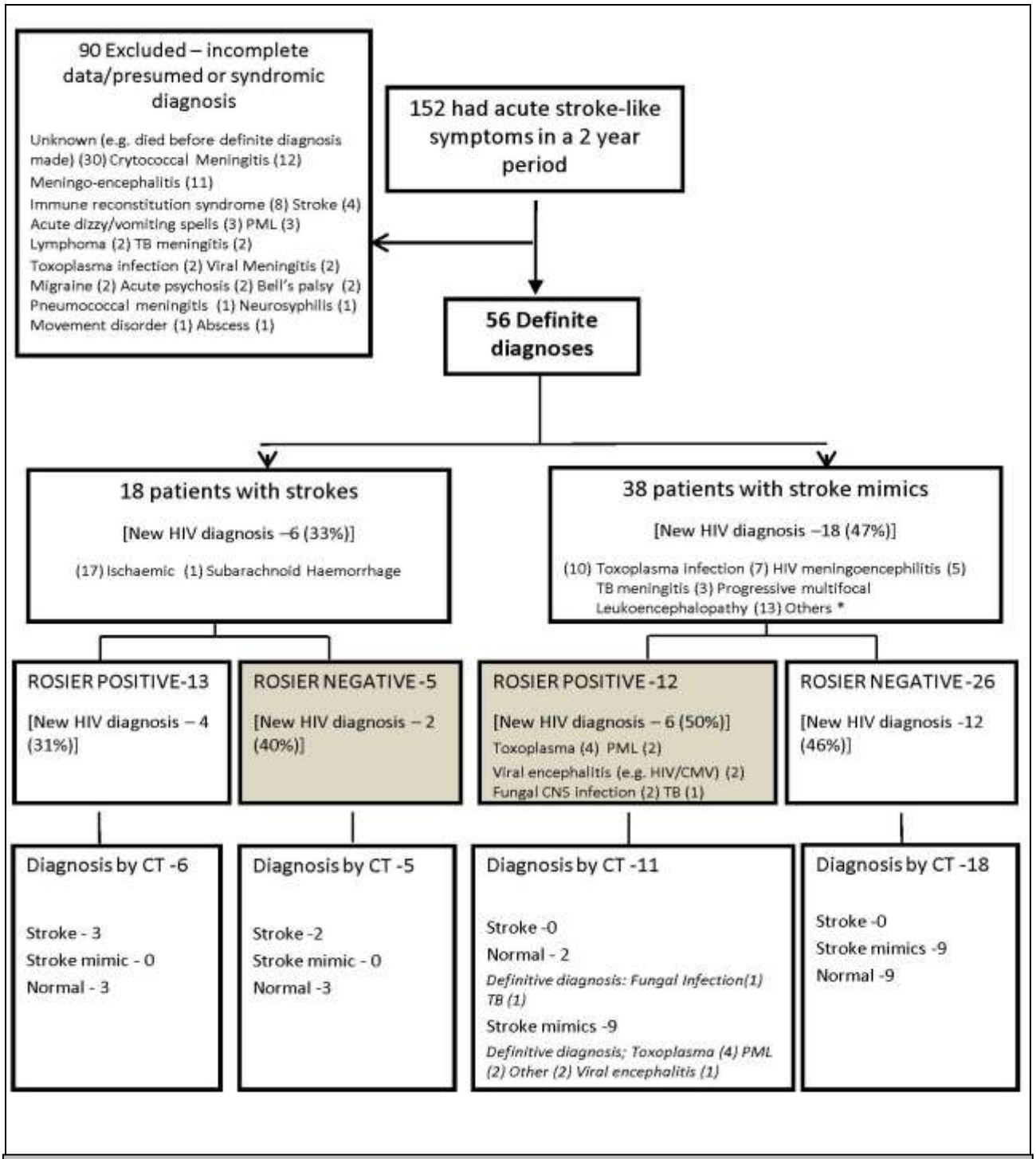


Figure 2:2: Flow diagram detailing the selection process of patients

***included varicella zoster encephalitis, neurocysticercosis, neurosyphilis, HIV tumefactive demyelination, aspergillus encephalitis, lymphoma, HIV dementia and cryptogenic epilepsy. The shaded box highlights the false negatives and positives respectively for the ROSIER score.**

2.4.2 Clinical characteristics

The age at presentation and gender were similar for patients with strokes and stroke mimics. Patients with strokes were more likely to have had a known HIV diagnosis for 12 months or more Table 2:1. The prevalence of common vascular risk factors was similar between the two groups, though there was a non-significant trend towards hyperlipidemia being more common in patients with stroke. Twenty-six patients were diagnosed HIV-infected as a result of their acute stroke-like presentation: 6/18 (33%) had a stroke and 18/38 (47%) had a stroke mimic Figure 2:2.

Patients with stroke were more likely to have had a CD4+ T lymphocyte count >200cells/mm^3. There was no significant difference with cART use between the groups. Neither peripheral white cell count nor CRP (when requested) differentiated between a stroke or a stroke mimic Table 2:1.

Table 2:1: The demography and clinical characteristics of all patients categorised into strokes and stroke mimics*

Definitive Diagnosis	Stroke (n=18)	Stroke Mimic (n=38)	(OR, 95% Confidence Interval)	P value
Demographics				
Age (Median, IQR)	40.5 (32,46)	38 (33,46)	1 (0.95-1.05)	0.970
Male (%)	13 (72)	20 (53)	2.34 (0.70-7.86)	0.169
HIV characteristics				
≥ 12 months duration of HIV positive diagnosis (%)	10 (63)	12 (32)	3.61 (1.06-12.25)	0.039
On cART (%)	11 (61)	16 (42)	2.16 (0.68-6.79)	0.188
CD4+ >200cells/mm3 (%)	13 (72)	10 (29)	6.5 (1.83-23.04)	0.004
Vascular risk factors				
Current Smoker (%)	2 (12)	5 (17)	0.64 (0.11 – 3.73)	0.620
Diabetes (%)	0	2 (6)	-	-
Hypertension (%)	1 (5)	2 (6)	0.91 (0.08-10.79)	0.938
Hyperlipidaemia (%)	5 (29)	3 (9)	4.49 (0.94-21.49)	0.060
Evidence of inflammation				
Serum white cell count >5x10^9/L (%)	8 (72)	14 (41)	3.81 (0.87-16.94)	0.079
Serum CRP >5 (%)	1 (25)	14 (58)	0.23 (0.02-2.63)	0.242

***Data were missing for some individuals; two for duration of HIV positive infection, 3 for CD4+ T-lymphocyte count, 10 for smoking, 8 diabetes, 8 for hypertension, 6 hyperlipidaemia, 11 peripheral white cell count, 28 for CRP (this was not requested in all of the cases); these individuals were excluded from the denominator in percentage calculations. CD4+ - CD4+ T lymphocyte count.**

2.4.3 Diagnostic performance of the ROSIER score and CT scan

CT scans were reviewed for 40 (71%) of the 56 patients; 11 of these were from patients with stroke and 29 were from patients with stroke mimics. For the 16 remaining patients either the scan was not available for review, or they had proceeded directly to MRI scan, and so no CT was performed. There was substantial agreement between the two radiologists for an overall diagnosis of stroke/stroke mimic/normal CT (88%). The overall unweighted Kappa (K) was 0.75 (Std. Err +/-0.16 p <0.0001).

Both the sensitivity and specificity of the ROSIER stroke score alone was 68% Table 2:2. When the ROSIER score was combined with a CT brain scan, the sensitivity was 67% and specificity 90%. The standard algorithm of suspected stroke patients is to firstly screen by the ROSIER score and a positive score then determines the need for an urgent CT brain scan; I found that the ROSIER incorrectly categorised 12/38 (32%) stroke mimics as strokes. Of the 12, 11 had CT brain imaging available, 2/11 (18%) would have had a confirmed diagnosis of stroke after the CT scan; both of these had treatable infectious aetiologies Figure 2:2. The ROSIER also incorrectly categorised 5/18 (28%) definite strokes as stroke mimics; all had CT brain scans and in 2/5 (40%), a stroke diagnosis was confirmed, but 3/5 (60%) had a normal scan. The presence of vasogenic oedema was the only CT brain characteristic, of the eight evaluated, that helped discriminate strokes from its mimics OR 0.11 95% CI 0.01-1.14 p=0.0045, this was consistent for both radiologists Table 2:3.

Table 2:2: Sensitivity and specificity of the Recognition of Stroke in the Emergency Room Score (ROSIER) alone and in combination with a CT brain scan

	Sensitivity*		Specificity*	
Test	**(%)**	**95% CI**	**%**	**95% CI**
ROSIER	68	43-87	68	51-82
ROSIER and CT	67	30-92	90	68-98

***A normal CT brain in a patient with an acute stroke-like symptom and ROSIER score positive was treated as a stroke in the sensitivity analysis. However, if the working diagnosis was not a stroke (i.e. acute stroke-like symptom and ROSIER negative) then a normal CT brain scan was not considered a stroke.**

Table 2:3: CT brain scan characteristics in HIV positive strokes and stroke mimics

CT characteristics	Stroke (n=11)	Stroke Mimic (n=29)	OR, 95% Confidence Interval	P value
Hypodensity	4 (36)	20 (69)	0.26 (0.05-1.21)	0.064
Hyperdensity	3 (27)	4 (14)	2.34 (0.41-13.34)	0.323
Vasogenic oedema	1 (9)	14 (48)	0.11 (0.01-0.94)	0.045*
Cytotoxic oedema	1 (9)	3 (10)	0.87 (0.08-9.34)	0.906
Evidence of mass effect	1 (9)	13 (45)	0.12 (0.01-1.09)	0.060
Evidence of enhancement	1 (20)	7 (33)	0.50 (0.04-5.36)	0.567
Multiple lesions (>2)	3 (27)	11 (38)	0.61 (1.13-2.81)	0.530
Normal scan	4 (36)	7 (24)	1.79 (0.40-8.00)	0.442

2.5 Discussion

The early distinction between a stroke and a stroke mimic is important with the increasing use of thrombolytic therapy. In an HIV positive population the proportion of stroke mimics presenting with acute stroke-like symptom is high (Kumwenda et al. 2005; Tipping et al. 2007). An accurate and timely diagnosis is crucial for establishing appropriate treatment plans.

In this retrospective study, I showed that the ROSIER score had a sensitivity and specificity of 68%; the score assigned a stroke diagnosis in three quarters of those who actually had one, but also one third of those with a stroke mimic. A quarter of patients with definite strokes would not have had an urgent CT brain scan and therefore these individuals would have missed the narrow window of opportunity for thrombolysis. Furthermore, one third of people with stroke mimics classified as strokes would have had a delay in appropriate investigations, such as an MRI scan, and cerebrospinal fluid analysis; this delay would have been compounded by the fact that almost 50% of this group had an unknown HIV diagnosis.

Overall, the combination of ROSIER and CT brain had a sensitivity of 67% for detecting stroke, which is inadequate for a screening tool. Historically, CT brain imaging is known for its poor sensitivity in diagnosing strokes (Chalela et al. 2007; Brazzelli et al. 2009). I found that of the eight characteristics evaluated, vasogenic oedema was the only one that helped discriminate a stroke from its mimics and this was only present in half of the mimics and probably linked to the number of patients with toxoplasmosis. Nonetheless, a CT is easier to operate, cost-effective and therefore widely used as part of the initial evaluation of patients presenting with acute stroke-like symptoms (Wardlaw et al. 2004). In patients with HIV infection, an MRI brain especially when diffusion weighted magnetic resonance imaging is used, is superior to a CT scan in detecting strokes and their mimics but this will only be requested rapidly if the patient's HIV status is known (Maschke et al. 2004; Kidwell et al. 2010).

Another concern is that investigations that would normally indicate an alternative diagnosis to a stroke (e.g. peripheral white cell count and CRP (when requested), were also non-discriminatory in this population. As in this study, HIV positive patients with stroke are younger than the general population with stroke, and young age at presentation should be a trigger for HIV testing (Ovbiagele et al. 2011). The British HIV Association guideline recommends HIV testing in patient with neurological symptoms in local populations where the HIV prevalence exceeds 2 in 1000 but this is often not adhered to (British HIV Association 2008; Palfreeman et al. 2009). MRI or CT brain scans are usually not accessible in resource poor settings, in such circumstances, the patients HIV status alone may be the only useful guide to appropriately managing their stroke syndrome.

In the UK over 90,000 people are infected with HIV, a quarter of whom are unaware of their diagnosis, and this number continues to rise (HPA 2012). In this study, stroke was a HIV-presenting condition; 33% of the definite stroke patients had a new HIV positive diagnosis. Other studies have reported rates as high as 42% (Tipping et al. 2007). In most cases the decision over thrombolysis is taken without knowledge of a patient's HIV status and the consequence of this, is yet to be realised.

The burden of stroke in an HIV positive population is still a problem as HIV infection not only increases the risk of ischaemic stroke through different mechanisms (e.g. vasculopathy and coagulopathy) but also, an aging population due to the success of cART, will further expose these individuals to other established vascular risk factors and thus increase their risk of stroke (Chapter 1).

There is also the theoretical risk of patients with stroke mimics (e.g. toxoplasma, PML etc) receiving thrombolysis at the expense of appropriate therapy. Reassuringly, this would have only been applicable to 2/26 (6%).

There are two studies that have validated the ROSIER score in a low HIV-endemic population, both demonstrated a sensitivity of over 80% (Jackson et al. 2008; Whiteley et al. 2011). However, this is the first study describing the diagnostic accuracy of routine triaging tools (i.e. ROSIER score and CT brain) for suspected stroke patients in an HIV population. The study also utilised two Infectious Disease Units with a combined cohort of 2600 HIV infected individuals. The retrospective nature lends itself to coding bias especially since a portion of our cohort did not have a definitive diagnosis. However, this study generates hypotheses that would need to be followed up by a prospective study. The study was also limited by the sample size; this was largely due to the low prevalence of HIV infection in this population and our strict criteria for establishing a definitive diagnosis. Nonetheless, establishing a definitive diagnosis using gold standard tests from which comparisons can then be made is easier to achieve in high income settings, where a lower HIV prevalence is often reported. Misclassification of diagnoses was a concern in this study, however this bias was reduced by; 1) using a battery of gold standard diagnostic tests to help establish the definitive diagnosis (i.e. serological techniques, CSF PCR for pathogens, MRI imaging and in some cases brain biopsy), 2) blinding the scorer of the ROSIER stroke score/radiologists from the definitive diagnosis, and 3) having two radiologists report the CT imaging.

In conclusion, the ROSIER stroke score and CT brain imaging had poor diagnostic accuracy in HIV infected patients presenting with stroke-like symptoms. For a large proportion of patients, this was their HIV-defining presentation and therefore knowledge of their status would direct the physician to rapidly request non-standard stroke investigations like MRI of the brain and CSF analysis. This study also suggests that HIV testing in a young cohort presenting with acute focal neurological deficit could be helpful. I therefore decided not to use ROSIER to screen for the diagnosis of a stroke; instead, I fully assessed every patient with an acute stroke symptom as part of the screening process in my study. I

also decided to focus on MRI scanning of the brain to help determine the aetiology of stroke, rather than CT scanning, especially as there was no scope for thrombolysis in Malawi.

3 Untreated HIV infection, antiretroviral treatment and stroke in Malawian adults: a case control study

3.1 Abstract

Background

An increasing incidence of strokes, especially in young adults, has been noted in HIV endemic countries. This is postulated to relate to HIV infection. I investigated HIV, its treatment and other risk factors for stroke in Malawi.

Methods

I performed a case-control study of 222 adults with acute stroke, confirmed by MRI in 86%, and 503 population controls, frequency-matched for age, sex and place of residence, using Global Positioning System for random selection. Multivariate logistic regression models were used for case–control comparisons.

Results

HIV infection (Population Attributable Fraction [PAF] 15%) and hypertension (PAF 46%) were strongly linked to stroke. HIV was the predominant risk factor for young stroke (≤45 years), with a prevalence of

67% and an adjusted odds ratio [aOR, 95%CI] of 5.57 [2.43,12.8]; PAF 42%. There was an increased risk of a stroke in patients with untreated HIV infection (aOR 4.48 [2.44,8.24 p<0.001]), but the highest risk was in the first 6 months after starting combined antiretroviral therapy (cART) (aOR 15.6 [4.21,46.6] p<0.001). Stroke risk increased with declining CD4+ T-lymphocyte count (p=0.023). In older participants (HIV prevalence 17%) HIV was associated with stroke, but with a lower PAF (5%) than hypertension (PAF 68%).

Conclusion

HIV infection is associated with an increased risk of stroke especially ischaemic stroke in young people; this also relates to the degree of immunosuppression. Although treatment is beneficial overall, the highest stroke risk is in the first 6 months of starting cART. A better understanding of this risk is urgently needed in order to try and reduce it.

3.2 Introduction

Stroke is one of the leading causes of premature adult death and disability worldwide (Lozano et al. 2012; Murray et al. 2012). Identifying and treating risk factors for stroke is key to reducing the disease burden (Rothwell et al. 2004). Across most of sub-Saharan Africa, the incidence of stroke is increasing (Feigin et al. 2013). Much of this has been attributed to hypertension but the observation that in some countries, such as Malawi and South Africa, a substantial proportion of stroke patients are young (i.e. age ≤45 years), and have a low prevalence of established risk factors such as hypertension, suggests other risk factors may be important (Kumwenda et al. 2005; Tipping et al. 2007; Heikinheimo et al. 2012).

HIV is postulated to be one such risk factor (Rasmussen et al. 2011). The virus potentially causes stroke directly, for example through HIV-associated vasculopathy, or indirectly through opportunistic infections such as varicella zoster virus (VZV) (Chapter 1). In addition, some drugs used in combined antiretroviral therapy (cART) for HIV are associated with metabolic syndromes, therefore increasing stroke risk with prolonged use (Friis-Moller et al. 2003).

The recently published study by Walker et al made important observations on HIV infection and stroke (Walker et al. 2013). However, this study, like many that have preceded it, failed to separate the independent effects of HIV infection and its treatment on cerebrovascular risk (Chapter 1). I therefore conducted a case-control study examining the role and impact of HIV and its treatment as risk factors for stroke in Malawian adults, together with other risk factors prominent in this setting.

3.3 Methods

3.3.1 Study site

Queen Elizabeth Central Hospital is the main hospital for Blantyre district (population 1 million), as well as being the referral hospital for the southern region of Malawi, which has an estimated adult HIV prevalence of 18.5% (Choko et al. 2011).

3.3.2 Study design

Adult (age >17 years) residents of the Blantyre district who presented to the hospital within seven days of the onset of symptoms that met the WHO case definition of stroke (Hatano 1976), were recruited between February 2011 and April 2012. Participants were initially screened for the presence of an acute neurological symptom by the study nurse using a screening questionnaire, before I performed a detailed screening. Scans were performed on a GE 0.35 Tesla Signa Ovation Excite Magnetic Resonance Imaging (MRI) scanner (Milwaukee, Wisconsin) within seven days of admission. MRI sequences included a midline sagittal localising view of the brain - T1-weighted, axial diffusion-weighted, gradient echo, axial T2-weighted and a fluid attenuated inversion recovery sequence. The images were reported at the time by a local radiologist and subsequently reviewed by a neuroradiologist and an infectious diseases radiologist. Patients with recurrent stroke were eligible for inclusion, provided they had not already participated in the study.

Population controls were recruited from the local population in predefined residential neighbourhoods within the district of Blantyre, between January and November 2012. Two community controls were

selected at random for every case, using a modification of a previously described approach (Crampin et al. 2001). The distribution of age (5 year age-bands), gender, and place of residence of the first 100 cases was used to guide stratified recruitment, frequency-matching for age, gender and residential neighbourhood. Individuals were included if they were > 17 years of age, patients with a past history of stroke were not excluded. I had rationalised not to exclude these patients, as reported stroke prevalence rates in similar settings to Malawi were low [e.g. 300/100 000 (95% CI, 250 to 357]) (Connor et al. 2004). Therefore, the likelihood of picking up a patient with a past history of stroke was also low. Other large case-control stroke studies in western populations have also used similar methods (Urbanus et al. 2009).

Random starting points and direction were overlaid onto high resolution satellite maps (March 2010 images: Geo Eye-1/Eurimage SpA) using Google Earth pro software Figure 3:1. Global Positioning System coordinates from the first dwelling intersected by any given randomly generated vector were recorded. After sensitiziting the community about the project, dwellings were visited and all eligible potential control participants were identified. The control recruitment was undertaken by two qualified nurses. Both had undergone a period of training by myself prior to recruitment. Standardized operating protocols for collecting data were used and their performance in adhering to this was regularly checked. If no one was home, dwellings were visited up to three times. Where multiple individuals were eligible, the oldest individual was recruited. If no eligible individual was identified, the next dwelling intersected was visited. Recruitment continued in each residential neighbourhood until the pre-specified numbers of individuals in each age-gender category had been met.

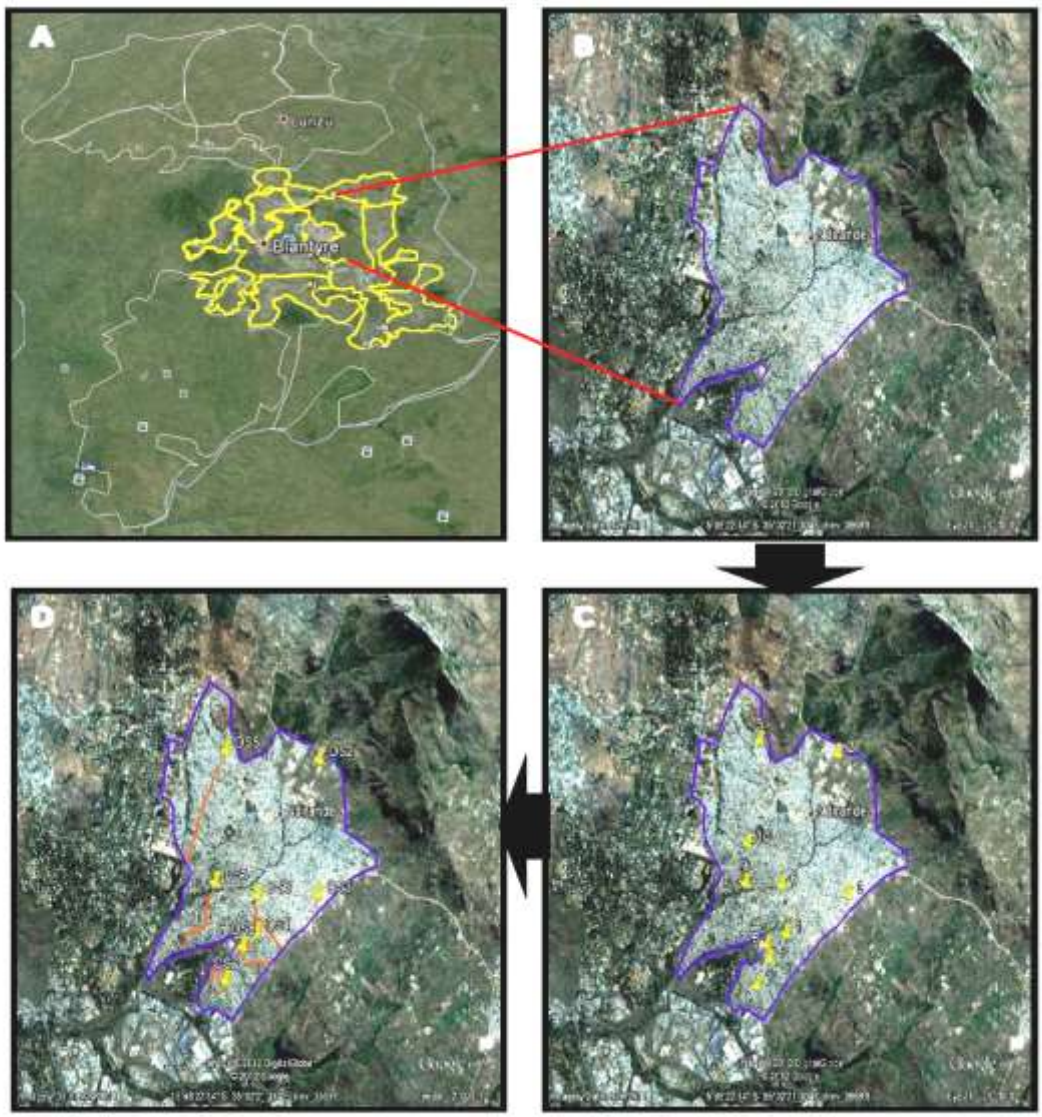


Figure 3:1: Random sampling using Google Earth Pro

Blantyre was defined by 16 residential neighbourhood; 9 urban and 7 rural (Image A). The boundaries defined a neighbourhood. Image B was an example of the neighbourhood Ndirande. Random starting points (Image C) and directions (Image D) were generated and exported into a Global Positioning System to facilitate sampling of the study participants.

3.3.3 Procedures

For cases, the clinical subtype and severity of stroke were recorded using the Oxfordshire Community Stroke Project classification and National Institutes of Health Stroke Scale (NIHSS) criteria respectively (Brott et al. 1989; Bamford et al. 1991). I determined the stroke diagnosis after a detailed history and examination. Ischaemic and haemorrhagic stroke types were determined by MRI brain scan findings. Patients were dual managed by myself and the admitting medical consultant using standard hospital protocols (Zijlstra 2007).

Demographic information and exposure to risk factors were obtained from the cases and controls using the same structured questionnaire (appendix). Non-modifiable risk factors (i.e. age, sex, socioeconomic status and family history of stroke) and modifiable risk factors (HIV infection, HIV treatment and its duration, hypertension, diabetes, hypercholesterolaemia, acute infection, smoking, alcohol use, pregnancy and substance use [cannabis, cocaine and heroin]) were recorded. Socioeconomic status was defined by education, occupation, place of residence and housing type (Cox et al. 2006; de Villiers et al. 2011). If the patient was dysphasic or unconscious, risk factors that could not be determined by examination or investigation were extrapolated from the guardian; if this was unclear this was marked as unknown. In sub-Saharan Africa ascertaining the exact age can sometimes prove difficult, in such circumstances, age was confirmed by memory prompts (Paraiso et al. 2010). Examination was performed to determine the waist-hip-ratio, a marker of abdominal obesity and blood pressure. The admitting blood pressure for the cases was chosen for comparison with the international multi-site case-control study; this explored the association of established vascular risk factors and stroke (O'Donnell et al. 2010). Cases and controls were screened for diabetes, hypercholesterolaemia, and HIV infection, if

positive, the CD4+ T lymphocyte count was measured (BD FACS Count System, Becton, Dickinson, San Jose, CA).

3.3.4 Definition of criteria used

Stroke severity was classified as non-severe or severe using the NIHSS (Brott et al. 1989). For cases and controls, HIV diagnosis was made from two rapid tests in parallel (Unigold, trinity Biotech, Ireland; Determine, Alere Medical Co Ltd, Japan and SD Bioline, Standard Diagnostics, Korea, was used as a tiebreak). CD4+ T lymphocyte count was classified as <200 cell/mm^3, 200-349 cell/mm^3, 350-500 cell/mm^3, >500 cell/mm^3, which also determined cART eligibility (WHO 2010). To explore the possible role of immune reconstitution syndrome, a cut-off of 6 months was used to classify the duration on cART (Shelburne et al. 2005). The timing of cART was cross-checked with an electronic medical records system at QECH when there was ambiguity (Douglas et al. 2010). Blood pressure was recorded at Day 0 for both cases and controls, using clinically validated automated Omron M5-I upper arm monitors (El Assaad et al. 2003). Three sequential readings were recorded and an average taken. Hypertension was defined as a composite of blood pressure >140/90mmHg or use of anti-hypertensive medication (Go et al. 2013). Diabetes mellitus was defined as a non-fasting blood glucose of ≥11.1 mmol/L or use of glucose-lowering medication (American Diabetes Association 2010). Patients were classified as having hypercholesterolaemia if they used lipid-lowering medication or had a non-fasting serum cholesterol concentration ≥6.2 mmol/L (NCEP 2001). Waist-hip-ratio was calculated as tertiles from the control cohort (O'Donnell et al. 2010). Recent infection was defined as a fever or treated infection within 14 days of the stroke (cases) or interview (controls) (Emsley et al. 2008). Young stroke was defined as ≤ 45 years (Jacobs et al. 2002).

3.3.5 Ethical consideration

The study was approved by the Liverpool School of Tropical Medicine, UK and the College of Medicine Research Ethics Committee, University of Malawi. All participants or guardians gave written informed consent. Individuals with newly diagnosed risk factors in the community (e.g. HIV infection, hypertension) were counselled by qualified fieldworkers and referred to an appropriate outpatient clinic.

3.3.6 Statistical analysis

The prevalence of potential risk factors was compared between cases and controls. Continuous variables were summarised using means and medians and compared using a Student independent-samples t-test or Mann Whitney U test as appropriate for the distribution properties of each variable.

A sample size of 750 individuals (250 case-control sets each with one case and two controls) was estimated as providing 95% power to detect an odds ratio of 2 or greater (i.e. to detect a doubling of the risk of stroke) for HIV prevalence, assuming that 18% of the control individuals would be found to have HIV infection (Choko et al. 2011).

A conceptual framework was used to divide all other potential risk factors into proximal determinants which have direct effects on stroke, and distal determinants which have indirect effects. Distal determinants were defined as age, gender, family history, socioeconomic status and season. Proximal determinants were HIV status (also expanded or substituted by HIV treatment status and level of

immunosuppression in some of the analysis), hypertension, diabetes, hypercholesterolaemia, acute infection, abdominal obesity, alcohol and substance use. The proximal determinants were initially evaluated individually ("univariate" model approach) after adjustment for age, sex and urban location (the variables used for frequency matching). Unconditional "multivariate" logistic regression models were then constructed based on this conceptual framework describing the hierarchical relationships between the matching, proximal and distal determinants. The role of each distal determinant was further assessed by comparing the model with or without, using the likelihood ratio test. Subtracting those from the model that were not significant (Victora et al. 1997). This was repeated after stratifying by age and type of stroke. Missing observations were included in the analysis by creating missing value categories. The findings from these models are reported as odds ratios with their 95% CIs. Population Attributable Faction (PAF) is defined as the proportion of disease cases over a specified time that would be prevented following elimination of the exposures, assuming the exposures were causal (Rockhill et al. 1998). PAF was calculated using methods defined by Greenland and Drescher and it depended on the prevalence of the risk factor and the rate ratio (Greenland et al. 1993). PAF presented are adjusted for confounders in a similar manner to the corresponding logistic regression models for odds ratio estimates and, where indicated, are stratified by subgroups of interest. The data were analysed with STATA version 11.2.

3.4 Results

I screened 553 patients with suspected stroke and 1686 households to obtain 222 stroke cases and 503 controls Figure 3:2. An MRI brain scan was performed in 190/222 (86%) cases, with findings consistent with 149 (78%) ischaemic and 41 (22%) haemorrhagic strokes. Ischaemic stroke subtype and severity are

summarised in Table 3:1. Cases and controls were well matched for age, sex, socioeconomic status and season of admission to the study Table 3:1.

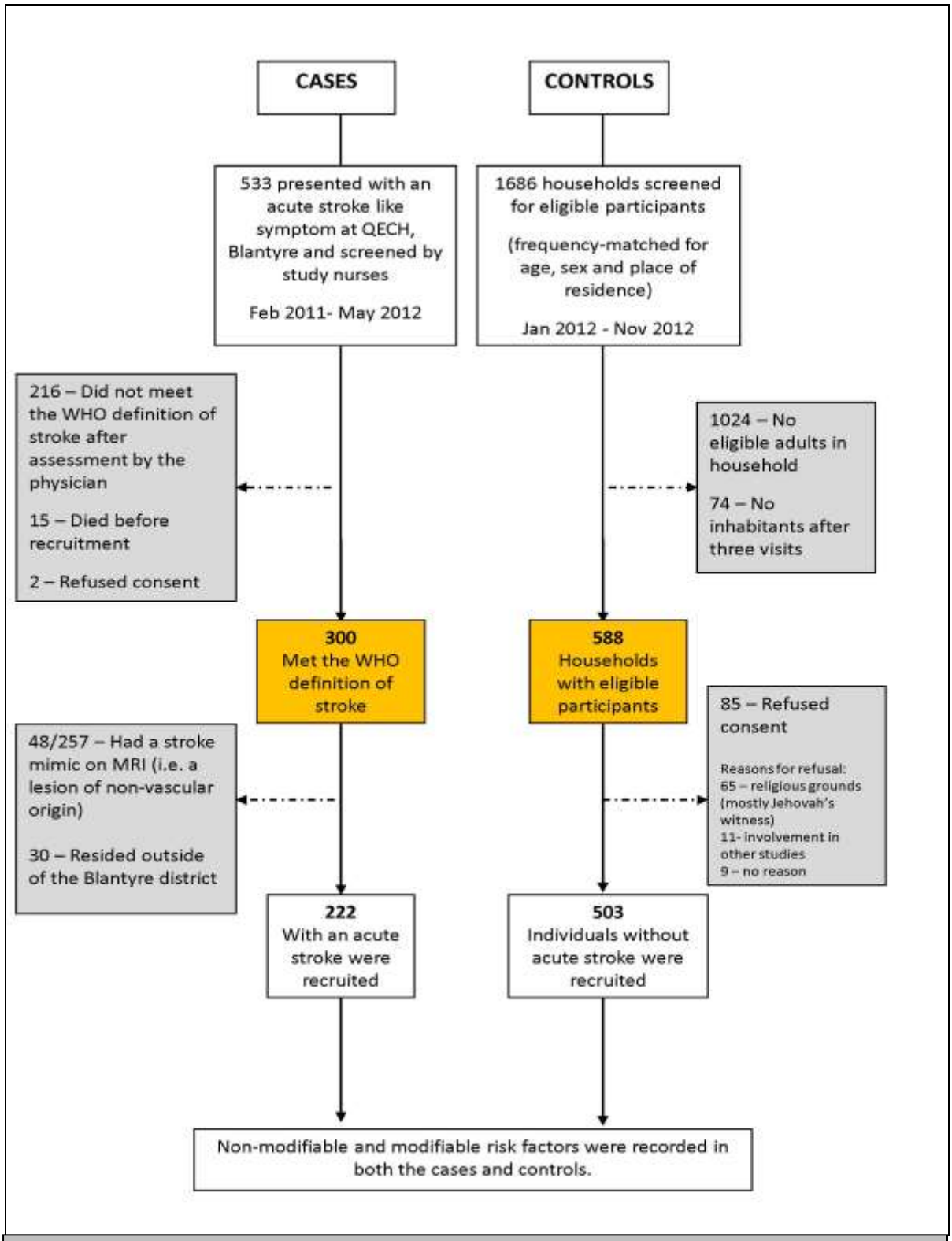


Figure 3:2: Flow diagram of case and control recruitment

QECH – Queen Elizabeth Central Hospital

Table 3:1: Baseline characteristics of cases and controls[¶] **

	Case-All Stroke (WHO defined) n=222 (%)	Control n=503 (%)
Median Age (IQR)*	60 (42,70)	57 (42,67)
Sex (M)	107 (48)	249 (50)
Urban location‡	143 (64)	343 (68)
HIV prevalence	69 (31)	95 (19)
In formal employment	69 (31)	163 (32)
Education		
Less than primary school	136 (61)	309 (61)
Primary education	53 (24)	125 (25)
Secondary education	25 (11)	51 (10)
Tertiary	4 (2)	18 (4)
Type of housing		
Employers accommodation	0	8 (1)
House/flat	23 (10)	48 (10)
Shack	189 (85)	447 (89)
Family history of stroke	37 (17)	67 (13)
Season		
Jan-Mar	56 (25)	137 (27)
Apr-Jun	54 (24)	120 (24)
Jul-Sept	64 (29)	129 (26)
Oct-Dec	48 (22)	117 (23)
Subtype of Ischaemic stroke[†](n=149)		
Total Anterior circulation Infarct	37 (25)	NA
Partial Anterior circulation Infarct	46 (31)	
Posterior circulation Infarct	6 (4)	
Lacunar Infarct	60 (40)	
Severity of Ischaemic stroke[¤] (n=149)		
Non-severe (<14)	93 (62)	NA
Severe (≥14)	55 (37)	

***IQR=inter-quartile range**
****NA denotes not performed**
[†]Severity of stroke was defined by the National Institutes of Health Stroke Scale (NIHSS).18
[¤]Subtype of stroke was defined by Oxfordshire Community Stroke (OCSP) classification.17
¶ Data were missing for the following cases and controls: one for HIV prevalence, 4 Education, 10 housing type and 1 stroke severity.

3.4.1 HIV and cART

Stroke was strongly associated with HIV infection (aOR 3.28 [2.05,5.25] p<0.001; PAF 15%). Timing of cART was important: starting ART in the first 6 months (aOR 15.6 [4.21,46.6] p<0.001) posed the greatest risk of strokeTable 3:2.

The association of HIV infection and stroke was more evident in younger (≤45 years) individuals (PAF 42%) compared to older individuals (combined PAF of 6%;Table 3:3) Although the prevalence of HIV was lower in the older participants (67% versus 18%) there was still a strong association with untreated HIV infection and early initiation of cART Table 3:3.

To explore the role of CD4+ T-lymphocyte cell count and its interplay with HIV treatment status, we performed a subgroup analysis of the HIV positive population. There was an independent relationship between the likelihood of stroke risk and decreasing CD4+ T-lymphocyte cell count Table 3:4. Although not significant, there was a trend towards a reduced stroke risk in the untreated HIV cohort after adjusting for CD4+ T-lymphocyte cell count Table 3:4. The stroke risk was partly reduced after adjusting for CD4+ T-lymphocyte cell count among those who initiated cART in the first 6 months. However, this still remained an independent risk factor of stroke (aOR 5.52 [1.22,25.0] p=0.026) Table 3:4.

HIV infection was significantly associated with ischaemic stroke (aOR 4.36 [2.58,7.36] p<0.001) but not with haemorrhagic stroke (aOR 1.79 [0.63,5.05] p=0.274) Table 3:5.

There were 32 patients who did not have an MRI brain scan. When they were excluded from the analysis, the association between HIV infection and stroke remained significant (aOR of 4.36 [2.58,7.36] p<0.001).

The national cART programme in Malawi uses standardised protocols, the 2008 edition was in use during the study period. First line therapy for HIV infection was Stavudine[d4T], Lamivudine[3TC] and Nevirapine[NVP]) and alternative first line was Zidovudine [AZT],3TC and NVP) (Malawi 2008). The prevalence of the Stavudine based regimen in cases and controls was 23 and 13 (64% and 36%; p <0.001) respectively.

Table 3:2: Univariate and multivariate analysis for modifiable risk factors in all patients with stroke¶

	Prevalence Case n=222 (%)	Controls n=503 (%)	All Stroke (WHO definition) Univariate* OR (95% CI) p value		Multivariate OR** (95% CI) p value		PAF† (95% CI)
HIV positive status	69 (31)	95 (19)	2.33	(1.58,3.48) <0.001	3.28	(2.05,5.25) <0.001	15 (9,21)
HIV treatment status							
HIV negative	151 (68)	408 (81)	1		1		
Untreated	38 (17)	47 (9)	2.85	(1.70,4.77) <0.001	4.48	(2.44,8.24) <0.001	
Had ART for <6 months	16 (7)	7 (1)	8.86	(3.42,22.9) <0.001	15.6	(4.21,46.6) <0.001	-
Had ART for ≥6 months	14 (6)	38 (8)	1.14	(0.59,2.02) 0.249	1.49	(0.72,3.07) 0.280	
Hypertension	165 (74)	273 (54)	3.13	(2.10,4.66) <0.001	5.01	(3.02,8.29) <0.001	46 (35,56)
Other vascular risk factors							
Diabetes	19 (9)	11 (2)	4.42	(2.05,9.53) <0.001	3.41	(1.45,8.01) 0.005	3 (1,6)
Hypercholesterolaemia	18 (8)	28 (6)	1.69	(0.90,3.15) 0.102	1.54	(0.77,3.08) 0.219	2 (-1,5)
Recent infection	25 (11)	37 (7)	1.67	(0.98,2.87) 0.062	1.38	(1.45,8.01) 0.005	3 (0.3,5)
Current smoker	41 (18)	63 (13)	1.66	(1.05,2.63) 0.029	2.36	(1.34,4.13) 0.003	6 (2,11)
Cannabis use¤	6 (3)	7 (1)	2.03	(0.66,6.23) 0.215	1.23	(0.72,2.12) 0.447	0.5 (-0.8,2)
Current alcohol drinker	34 (15)	92 (18)	0.79	(0.50,1.25) 0.322	0.73	(0.52,1.02) 0.067	-4 (-8,0.2)
Pregnancy	4 (2)	8 (2)	1.02	(0.29,3.64) 0.965	1.67	(0.78,3.62) 0.194	1 (0.1,2)
Abdominal obesity ∞							
T1	49 (22)	150 (30)	1		1		
T2	75 (34)	211 (42)	1.10	(0.72,1.67) 0.661	0.83	(0.51,1.35) 0.459	-6 (-17,3)
T3	93 (42)	141 (28)	2.07	(1.35,3.16) 0.001	1.36	(0.84,2.20) 0.208	5 (-5,14)

Table 3:2

*Adjusted for frequency matched variables; age, sex and urban location.

**Adjusted for hypertension, recent infection, abdominal obesity, HIV positive status, smoking, current alcohol drinker, hypercholesterolaemia, cannabis use, age, sex, type of housing and urban location.

† PAF= Population Attributable Fraction (%), When PAF was negative this was considered protective. For variables with more than 2 categories (i.e. HIV status and waist-hip-ratio) PAF was calculated from the reference category (e.g. T1 versus T2 and T1 versus T3).

¶ Data were missing for the following cases and controls: one for HIV status, 6 HIV treatment status, 24 hypercholesterolaemia, 9 recent infection, 5 cannabis use, 13 alcohol use, 3 pregnancy and 6 abdominal obesity; these individuals were included in the analysis by creating missing value categories.

¤ There was no exposure to other substance use such as heroin or cocaine.

3.4.2 Hypertension

Hypertension was strongly associated with stroke overall (aOR 5.01 [3.02,8.29] p<0.001); PAF 46%;Table 3:2). The risk was stronger for haemorrhagic stroke (aOR 9.35 [2.89,30.3] p<0.001) than for ischaemic stroke (aOR 3.66 [2.15,6.22] p<0.001). The effect of hypertension was more evident in those >45 years old than those ≤45 years (PAF 68% versus 11%;Table 3:3. Of the 438 participants with hypertension, only 118 (27%) were on treatment.

3.4.3 Other vascular risk factors

Diabetes was associated with a small proportion of strokes overall (aOR 3.41 [1.45,8.01] p=0.005; PAF 3%;Table 3:2). This association was specifically with ischaemic and not haemorrhagic stroke (Table 3:5). Smoking (PAF 6%) and recent infection (PAF 3%) were also associated with stroke (Table 3:2). Waist-hip-ratio cut-offs of 0.86 and 0.9 were used to divide participants into thirds. At the highest tertile, there was an increased risk of ischaemic (aOR 2.01 [1.15,3.49] p=0.014) but not haemorrhagic stroke (Table 3:5). There was no significant association between hypercholesterolaemia and stroke (Table 3:2, Table 3:3, Table 3:5), and none of the case or control participants were on treatment for hypercholesterolaemia. Waist-hip-ratio, a marker of abdominal obesity, was calculated as tertiles (T) on the basis of the overall control data. Cutoffs of 0.86 and 0.9 were used to divide participants into thirds. At the highest tertile (T3), there was an increased risk of ischaemic (aOR 2.01 [1.15,3.49] p=0.014) but not haemorrhagic stroke Table 3:5. Smoking (PAF 6%), recent infection (PAF 3%) were also associated with stroke overall Table 3:2.

Table 3:3: Multivariate analysis for modifiable risk factors in younger and older stroke patients

	Prevalence		Younger (≤45years) stroke[¶] (WHO definition)			Prevalence		Older (>45years) stroke[‡] (WHO definition)		
	Cases n=61 (%)	Controls n=151 (%)	Multivariate* OR (95% CI) p value		PAF[†] (95% CI)	Cases n=161 (%)	Controls n=352 (%)	Multivariate** OR (95% CI) p value		PAF[†] (95% CI)
HIV positive status	41 (67)	41 (31)	5.57	(2.43,12.8) <0.001	42 (20,58)	28 (18)	48 (14)	2.10	(1.10,4.01) 0.024	6 (0.3,10)
HIV treatment status										
HIV negative	20 (33)	104(69)	1			131 (81)	304 (86)	1		
Untreated	24 (39)	29 (19)	5.04	(1.99,12.8) 0.001		14 (9)	18 (5)	2.93	(1.18,7.27) 0.020	
Had ART for <6 months	12 (20)	4 (3)	22.8	(4.91,106) <0.001		4 (2)	3 (1)	15.9	(2.03,124) 0.008	
Had ART for ≥6 months	5 (8)	13 (9)	3.27	(0.88,12.1) 0.077		9 (6)	25 (7)	1.31	(0.52,3.28) 0.591	
Hypertension	21 (34)	46 (31)	1.92	(0.84,4.36) 0.119	11(-4,24)	144 (89)	227 (64)	8.57	(4.31,17.0) <0.001	68 (52,79)
Other vascular risk factors										
Diabetes	1 (2)	1 (1)	4.83	(0.23,100) 0.308	1 (-2,4)	18 (11)	10 (2)	2.79	(1.17,6.65) 0.021	4 (1,7)
Hypercholesterolaemia	4 (7)	3 (2)	4.66	(0.71,30.8) 0.110	3 (-1,7)	14 (9)	25 (7)	1.32	(0.62,2.80) 0.465	1 (-3,4)
Recent infection	14 (23)	11 (7)	2.87	(0.98,8.42) 0.054	9 (-1,17)	11 (7)	26 (7)	1.36	(0.97,1.91) 0.077	2 (0.2,5)
Current smoker	8 (13)	16 (11)	0.73	(0.18,2.87) 0.655	-2(-11,6)	33 (21)	47 (13)	2.65	(1.40,5.03) 0.003	8 (3,13)
***Abdominal obesity*∞**										
T1	13 (21)	63 (42)	1			36 (22)	87 (25)	1		
T2	26 (43)	60 (40)	1.29	(0.52,3.21) 0.579	3 (-18,22)	49 (30)	151 (43)	0.59	(0.33,1.05) 0.072	-13 (-25,-1)
T3	21 (34)	28 (19)	3.05	(1.14,8.18) 0.026	15 (-0.6,28)	72 (45)	113 (32)	1.04	(0.59,1.83) 0.886	-1 (-14,11)

Table 3:3

*****Adjusted for hypertension, recent infection, abdominal obesity, HIV positive status, smoking, current alcohol drinker, hypercholesterolemia, cannabis use, age, sex and urban location.

****** Adjusted for hypertension, recent infection, abdominal obesity, HIV positive status, smoking, current alcohol drinker, hypercholesterolemia, cannabis use, age, sex, type of housing and urban location.

† PAF= Population Attributable Fraction (%), when PAF was negative this was considered protective. For variables with more than 2 categories. (i.e.waist-hip-ratio) PAF was calculated from the reference category (e.g.T1 versus T2 and T1 versus T3).

¤ There was no exposure to other substance use such as heroin or cocaine.

¶For the younger stroke patient's analysis, data were missing in the following cases and controls: One HIV treatment status, 4 hypercholesterolaemia, 2 cannabis use, 3 alcohol use and 1 abdominal obesity; these individuals were included in the analysis by creating missing value categories.

‡ For the older stroke analysis, data were missing in the following cases and controls: One HIV positive status, 5 for HIV treatment status, 20 hypercholesterolaemia, 9 recent infections, 3 cannabis use, 10 alcohol use, 3 pregnancy and 5 abdominal obesity. Missing observations were included in the analysis by creating missing value categories.

∞ Abdominal obesity (Waist-hip-ratio) tertiles (T) was calculated as tertiles from the control cohort. Cutoffs of 0.86 and 0.9 were used to divide participants into thirds

Table 3:4: Exploring the interplay of HIV treatment status and immunosuppression in an HIV subpopulation

	Prevalence		All Stroke (WHO definition)					
	HIV+ Cases n=69 (%)	HIV- Controls n=95 (%)	Univariate* OR (95% CI)	p value		Multivariate** OR (95%CI)		p value
HIV treatment status								
Had ART for ≥6 months	14(20)	38 (40)	1			1		
Untreated	38 (55)	47 (49)	2.01	(0.90,4.50)	0.091	1.56	(0.55,4.45)	0.409
Had ART for <6 months	16 (23)	7 (7)	6.89	(2.11,22.5)	0.001	5.52	(1.22,25.0)	0.026
CD4+ T-lymphocyte cell count‡								
>500 cell cells/mm^3	4 (6)	27 (28)	1			1		
350-500 cells/mm^3	11 (16)	23 (24)	3.69	(0.97,14.0)		1.92	(0.42,8.75)	
200-350 cells/mm^3	17 (25)	18 (19)	7.95	(2.14,29.5)	<0.001	6.06	(1.42,25.8)	0.023
< 200 cells/mm^3	31 (45)	21 (22)	11.47	(3.27,40.3)		6.97	(1.63,29.8)	

*Adjusted for frequency matched variables; age, sex and urban location.

** Adjusted for hypertension, recent infection, abdominal obesity, HIV treatment status, smoking, current alcohol drinker, CD4+ T-lymphocyte count, hypercholesterolaemia, cannabis use, age, sex, type of housing and urban location.

‡ A combined p-value was calculated using a likelihood ratio test for variables with > 3 categories.

¶ Data were missing for the following cases and controls: Four HIV treatment status, 12 CD4+ T-lymphocyte cell count, 2 recent infection, 2 Waist-hip-ratio, 1 ETOH, 8 pregnant, 1 substance use. Missing observations were included in the analysis by creating missing value categories.

Table 3:5: Multivariate analysis for modifiable risk factors in ischaemic and hemorrhagic stroke patients

	Prevalence		Ischaemic stroke * (WHO definition)			Prevalence		Haemorrhagic stroke ** (WHO definition)		
	Cases n=149 (%)	Controls n=503 (%)	Adjusted OR	(95% CI)	p value	Cases n=41 (%)	Controls n=503 (%)	Adjusted OR	(95% CI)	p value
HIV positive status	57 (38)	95 (19)	4.36	(2.58,7.36)	<0.001	8 (20)	95 (19)	1.79	(0.63,5.05)	0.274
HIV treatment status‡										
HIV negative	92 (62)	408 (81)	1			32(78)	408 (81)	1		
Untreated	34 (23)	47 (9)	6.31	(3.27,12.1)	<0.001	3 (7)	47 (9)	2.27	(0.45,11.4)	0.410
Had ART for <6 months	13 (9)	7 (1)	20.2	(6.31,64.4)	<0.001	2 (5)	7 (1)	5.17	(0.56,48.0)	0.119
Had ART for ≥6 months	9 (6)	38 (8)	1.56	(0.65,3.69)	0.314	3 (7)	38 (8)	1.42	(0.36,5.66)	0.200
Hypertension¶	103 (69)	273 (54)	3.66	(2.15,6.22)	<0.001	35 (85)	273 (54)	9.35	(2.89,30.3)	<0.001
Other vascular risk factors										
Diabetes	12 (8)	11 (2)	3.70	(1.42,9.63)	0.007	1 (2)	11 (2)	0.33	(0.03,3.59)	0.365
Hypercholesterolaemia	12 (8)	28 (6)	1.65	(0.76,3.61)	0.208	5 (12)	28 (5)	1.62	(0.47,5.57)	0.441
Recent infection	18(12)	37 (7)	1.53	(1.07,2.19)	0.020	6 (15)	37 (7)	2.02	(1.28,3.19)	0.002
Current smoker¥	30 (20)	63 (13)	2.48	(1.34,4.61)	0.004	6 (15)	63 (13)	3.12	(0.85,11.5)	0.087
Abdominal obesity ∞										
T1	29(19)	150 (30)	1			15 (36)	150 (30)	1		
T2	52 (35)	211 (42)	0.93	(0.53,1.61)	0.788	9 (22)	211 (42)	0.36	(0.13,0.97)	0.044
T3	67 (45)	141 (28)	2.01	(1.15,3.49)	0.014	17 (41)	141 (28)	0.73	(0.30,1.80)	0.500

Table 3:5

*****Adjusted for hypertension, recent infection, abdominal obesity, HIV positive status, smoking, current alcohol drinker, hypercholesterolaemia, cannabis use, age, sex, type of housing and urban location.

******Adjusted for hypertension, recent infection, abdominal obesity, HIV positive status, smoking, current alcohol drinker, hypercholesterolaemia, cannabis use, age, sex, type of housing, level of schooling and urban location.

†For ischaemic stroke analysis - data were missing in the following cases and controls: One HIV positive status, 4 HIV treatment status, 18 hypercholesterolaemia, 6 recent infection and 2 abdominal obesity.

‡For haemorrhagic stroke - data were missing in the following cases and controls: Four for HIV treatment status, 10 hypercholesterolaemia, 5 recent infections and 1 abdominal obesity. Missing observations were included in the analysis by creating missing value categories.

∞Waist-to-hip ratio tertiles (T) was calculated as tertiles from the control cohort. Cutoffs of 0.86 and 0.9 were used to divide participants into thirds.

3.5 Discussion

This large prospective case-control study has confirmed that HIV infection is an independent risk factor for all strokes in Malawian adults (adjusted odds ratio of 3.2). Surprisingly, I also found that for patients who had started HIV treatment in the previous 6 months, the risk of stroke was even higher (adjusted odds ratio of 15.6). Although hypertension was the leading risk factor in the population overall (PAF 46%), HIV infection and its treatment were the second most important risk factor (PAF 15%), and the most important in younger patients (PAF 42%). In the older cohort, HIV was associated with stroke but was less important (PAF 6%) than hypertension (PAF 68%).

Whereas most previous studies of HIV infection and stroke have been retrospective (Chapter 1) one recent prospective study made important observations in HIV infection and stroke (Walker et al. 2013) but it was limited by missing data and lacked a definitive case definition of stroke (O'Donnell et al. 2013). As a consequence, there is still considerable uncertainty over the relationship between HIV infection and stroke. My prospective study, with well defined cases, carefully selected population controls, and 99% ascertainment of HIV status, provides the clearest data yet that HIV is indeed an important risk factor for stroke. We found that HIV infection was associated with ischaemic but not haemorrhagic stroke. A variety of mechanisms might be implicated: HIV infection is known to cause endothelial dysfunction resulting in a vasculopathy which can manifest in several forms (e.g. accelerated atherosclerosis, aneurysmal or non-aneurysmal disease and small vessel disease); in addition, opportunistic infections such as VZV, which are more common in HIV infection, can also cause a vasculopathy (Chapter 1).

Importantly, I found that that the risk of stroke was much higher in the first 6 months of cART. This is a new finding, not reported in any previous studies (Walker et al. 2013) (Chapter 1). Clinical deterioration in the first 6 months of starting cART suggests an immune reconstitution inflammatory syndrome (IRIS)-like process (Martin-Blondel et al. 2011). IRIS is thought to be the consequence of an over-whelming pathogen-specific, cell-mediated immune response; arising either through unmasking of an occult infection or, a paradoxical deterioration following HIV treatment (Martin-Blondel et al. 2011). In the latter, the recovering immune response is thought to target persisting pathogen-derived antigens or, possibly, self-antigens, causing tissue damage (Martin-Blondel et al. 2011). Although immunosuppression is a risk factor for IRIS, I found that the risk of stroke in the first 6 months of starting cART was independent of CD4+ T-lymphocyte count. However, monitoring the number of circulating CD4 +T-lymphocyte cells after initiating cART is not always reliable at predicting IRIS and thus immune dysregulation is still an important mechanism to consider (Martin-Blondel et al. 2011). There is also the possibility of other simultaneous or independent mechanisms occurring, such as stavudine toxicity. Insights from the large prospective D:A:D study (Data Collection on Adverse Events of anti-HIV Drugs) support my findings; in that study there was an increased risk of cardio- and cerebrovascular disease events for 5 years after starting cART; although it was not highlighted at the time, the data indicate the rate of vascular events was greatest in the first year, which we suspect may have been related to initiation of ART (d'Arminio et al. 2004).

Ideally, HIV viral load would have been a better correlate of HIV disease activity however; I was limited by the study budget to pursue this routinely. Instead, I have used CD4+T-lymphocyte cell count as a surrogate marker of HIV disease activity. I found that a higher CD4+T-lymphocyte cell counts (a function of cART) was associated with reduced stroke risk, suggesting that HIV treatment is indeed beneficial overall. Whilst initiating cART is necessary for improved HIV related outcome, the apparent increase in

early stroke risk needs investigating further; a better understanding of the mechanisms may point to empirical antimicrobial treatment of occult infection and/or initiation of anti-inflammatory agents at the time of starting cART.

Hypertension exceeded all risk factors and should not be neglected. Of highest concern is the double burden of two epidemics as HIV infection and hypertension overlap. Lessons should be learnt from the success of cART programs and surveillance and treatment of hypertension should be integrated into these services to reduce the burden of stroke (Ibrahim et al. 2012). In Malawi, hypertension was an important risk factor in older, but not younger, stroke patients. This finding is different to the multi-national inter-stroke study which attributed most young strokes in low-middle income countries to hypertension; however in that study only one fifth of the patients were from sub-Saharan Africa, and most of these were from wealthier African countries (O'Donnell et al. 2010). Malawi is one of the poorest countries in the world, and a low prevalence of diabetes and hypercholesterolaemia has been reported from other studies (Msyamboza et al. 2011; Muronya et al. 2011).

My study had several limitations. I cannot completely exclude recall bias, but the prospective nature and objective measurement for most of the important risk factors has considerably reduced this possibility. Equally, ascertainment bias was kept to a minimum by training the study team to use standard operating procedures, measuring clearly defined outcomes and managing the patients using standardised hospital protocols (Zijlstra 2007). I was able to perform an MRI head scan in 86% of suspected stroke patients. This helped to further define my cases and reduced the possibility of misclassifying mimics of stroke.

Recruiting from the hospital alone could have selected for known HIV positive patients but over 50% were newly diagnosed. Selection bias could also be argued for those who were untreated HIV positive individuals or who recently started cART. There are two possible scenarios which could have arisen: 1) untreated HIV infected individuals or those recently starting cART had mild or severe stroke and thus did not present to hospital. This could have potentially underestimated this stroke risk, assuming that the control prevalence was accurate 2) the cohort that refused to enter the study were untreated HIV positive individuals or had recently started treatment, potentially overestimating stroke risk associated with these factors. However, the refusal rate for entering the study was low (14%) and most of these were based on religious beliefs. Importantly, the HIV prevalence in my control cohort was consistent with a previous study in this setting (Choko et al. 2011); this consistency inadvertently validated my control selection process. Therefore, the latter scenario was less likely to have occurred.

Because age, sex and urban location are important confounders of stroke and HIV infection, we adopted a stratified approach for these variables. This was especially important in our setting because the age of stroke patients was considerably greater than the median age of Blantyre residents. This stratification minimised selection bias but also limited our ability to comment on these factors.

The population attributable fraction can be calculated from using the distribution of risk factors among the cases alone, independent of the control population (Bruzzi et al. 1985). The formula assumes that 1) the estimation of the PAF is unbiased; 2) the exposure is causal; and 3) elimination of the risk factor has to have no effect on the distribution of other risk factors. Although the evidence associating HIV infection and stroke is increasing this is not definitive and therefore the assumption of causality limits our interpretation of PAF.

Access to health care is a problem in Malawi, diabetes and hypertension are often poorly controlled, even for patients on treatment (Cohen et al. 2007). Therefore, we assumed that treatment did not modify stroke risk for these conditions.

This study has important public health implications, particularly demonstrating the dangers of extrapolating findings from high income countries, even within the same continent, to inform policy on stroke risk reduction in lower income countries. Whereas hypercholesterolaemia, diabetes and abdominal obesity are each important risk factors for stroke in industrialised countries, I found population attributable fractions to be very low in this setting, with far greater risk attributable to HIV infection and starting cART.

In conclusion, HIV infection is an important risk factor for stroke in Malawi, especially ischaemic stroke in young people. Importantly, although being on cART reduced the risk of stroke overall, there was a markedly increased risk of ischaemic stroke in the first 6 months after starting treatment. A better understanding of the mechanism of stroke in the first 6 months of cART is urgently needed to guide appropriate interventions.

4 Ischaemic stroke in HIV infected individuals: defining the aetiologies using a hierarchal approach for research studies.

4.1 Abstract

Background

In chapter 3 I showed that untreated HIV infection and recent combined antiretroviral therapy (cART) initiation were associated with an acute ischaemic stroke; this reinforces the global priority to improve our understanding of HIV infection and vascular complications in an era of an aging HIV infected population. There are several potential aetiologies related to HIV infection and stroke (e.g. opportunistic infection and HIV-associated vasculopathy), many of which are treatable. However before I could examine the causes of stroke in my cohort I needed a robust classification framework to help me ascribe aetiologies. The development of such a framework forms the subject of this chapter.

Methods

To address these issues I convened an international working group of experts in the field and related field to develop a consensus statement for ischaemic stroke in HIV infected individuals. I developed a working template to enable discussions between experts. Ideas and proposals were discussed, deliberated, clarified, and modified based on published literature. When published evidence was limited, expert opinion was used.

Results

Case definitions for the major aetiologies in HIV related strokes were refined in the context of HIV infection and in some cases new case definitions were described. The level of certainty of a definition was confirmed or probable depending on the quality of diagnostic modality used. These case definitions provided a framework for a hierarchical algorithm to help assign a final diagnosis.

Conclusions

I provided a framework of case definitions for the different aetiologies to facilitate standardised reporting by using a hierarchical approach. This chapter contains a pragmatic way forward as a starting point to guide the evolution of this disease. Importantly, it will provide guidance in determining the aetiologies in the large cohort of HIV infected patients with stroke that I studied in Malawi.

4.2 Introduction

In chapter 3 I showed that untreated HIV infection and recent combined antiretroviral therapy (cART) initiation was associated with acute ischaemic stroke. These risk factors will undoubtedly contribute to the increasing reports of vascular complications in an aging HIV-infected population (Bozzette et al. 2003; Ovbiagele et al. 2011; Freiberg et al. 2013). There are several potential aetiologies related to HIV infection and stroke (e.g. opportunistic infection and HIV-associated vasculopathy), many of which are treatable. However, before I could examine the causes of stroke in my cohort I needed a robust classification framework to help me ascribe aetiologies. The development of such a framework forms the subject of this chapter.

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Stroke is a complex syndrome with multiple aetiologies and mechanisms. The Trial of Org 10172 in Acute Stroke Treatment (TOAST) and Atherosclerosis-Small vessel disease-Cardiac source-Other cause (A-S-C-O) are widely used stroke classifications, but they were not defined with HIV infection in mind. Under these classifications many potentially treatable aetiologies (e.g. HIV-associated vasculopathy, coagulopathy, opportunistic infection etc), fall into the generic categories of *other determined*, *other cause* and *undetermined*. Furthermore, multiple aetiologies frequently occur in HIV related strokes and under the current stroke classification it would be assigned as underdetermined. Ideally, researchers (and clinicians) should have guidance in classifying these vague categories and thus identify; 1) recognisable established causes and 2) patterns and phenotypes which we recognise as occurring but with as yet uncertain evidence to support the mechanism.

Furthermore, causes of stroke that occur more frequently in HIV infected individuals need to be considered in the context of HIV infection; for example, diagnosing VZV vasculopathy with CSF Immunoglobulin G (IgG) or PCR can be complicated by HIV infection. A positive result from either test could reflect blood brain barrier breakdown, caused by HIV infection, with leakage from blood to CSF, subclinical reactivation secondary to HIV infection without causing disease or pathological disease (Reiber et al. 1991; Cinque et al. 1997).

While a non-uniform approach may be acceptable for clinical practice, research studies should strive for standardised diagnoses to obtain greater consistency and accuracy across studies. A hierarchical approach of diagnostic tests has proved useful recently for defining another microbial related neurological syndrome (encephalitis) and thus facilitating large prospective cohort studies (Granerod et al. 2010; Granerod et al. 2010).

With international experts in the field I therefore developed a pragmatic approach to defining the different causes of stroke in an HIV infected stroke population that will facilitate standardised reporting by using a hierarchical approach, allow for comparison of data and ultimately improve the standard of care of patients with HIV infection and stroke.

4.3 Methods

HIV related stroke classifications were primarily defined based on informal discussions with my supervisory and collaborator team to produce Table 1:2.

However it was clear that further refinement was needed and therefore involved multidisciplinary experts. With the assistance of one of my supervisors I identified and invited experts in the field or related field of HIV infection and stroke to contribute to this document. The working group included specialist in neurology, stroke, neuroradiology, pathology, infectious disease, general medicine, haematology, microbiology and virology. The collaboration also encompassed those working in high, middle and low income countries. The first step was to determine the main aetiologies of interest for HIV related ischaemic stroke; this was largely based on the classification described in chapter one (i.e. opportunistic infections, HIV-associated vasculopathy, cardio-thromboembolism and coagulopathy) Table 1:2. I then determined the level of certainty of each case definition based on the accuracy of the diagnostic test used. A confirmed definition was defined as direct evidence of disease based on gold standard diagnostic tests. A probable definition was defined as indirect evidence of disease based on less sensitive tests or clinical information. The case definitions where then built into a hierarchical algorithm by ranking them on the following criteria 1) prognosis 2) understanding of disease mechanism 3) availability of definitive treatment. A minimum workup of investigation was defined to allow for classification and an optimum work-up gave the best chance of classifying any aetiology. I developed a working template of case definitions and a hierarchical algorithm to enable discussions between the group members. For 6 months, ideas and proposals were discussed in group e-mails received by members, and were deliberated, clarified, and modified based on e-mail input from all participants. Each of the experts were asked to comment on the working template presenting their evidence based on the published literature and also to provide expert opinion when there was no published evidence. This formed the basis of a document outlining the case definitions with probable and confirmed certainty and a hierarchical algorithm for the different aetiologies in HIV related stroke.

4.4 Rationale behind the case definitions for the different aetiologies of HIV stroke

Stroke is a clinical diagnosis that is usually based on history and examination, confirmed by brain imaging and the exclusion of stroke mimics. Aetiological case definitions, as derived by myself and the panel of experts, for the different mechanisms of stroke in HIV infected individuals are displayed in Table 4:1, Table 4:2, Table 4:3 and Table 4:4. These definitions apply to all cases of HIV infected arterial pre-cerebral and cerebral ischaemic stroke. The rationale for each of the case definition used is discussed in turn.

4.4.1 Opportunistic infections

As discussed in chapter 1 TBM, VZV and syphilis are key opportunistic infections that are closely linked to stroke in people with HIV infection, thus we recommend routinely screening for these infections.

TBM

TBM is common in many HIV endemic countries. Early diagnosis remains a challenge and suspicion of the diagnosis is often based on clinical symptoms (sub-acute presentation: feeling generally unwell, tired and irritable, gradually worsening headache, vomiting, neck stiffness and fever) and CSF abnormalities (lymphocytosis, elevated protein, and reduced glucose levels). In an immunosuppressed HIV infected individual the clinical symptoms and CSF abnormalities may be subtle but it is more the difficulty in isolating tubercle bacilli in the brain/CSF, that makes diagnosing TBM challenging (Lammie et al. 2009;

Marais et al. 2010). Marias and colleagues have attempted to overcome this diagnostic challenge by developing and validating a scoring algorithm based on clinical, CSF, imaging and evidence of TB elsewhere. This scoring algorithm does not rely on isolating the tubercle bacilli and can also be applied to HIV infected stroke patients (Marais et al. 2010). It is worth noting that this scoring algorithm was validated in TB endemic regions and will need to be further validated in countries where TBM is less common (Marais et al. 2011).

Syphilis

When syphilis causes stroke this is often because of the meningovascular complications of infection. A positive blood result using treponemal or non-treponemal methods can be a challenge to interpret, especially in the context of HIV infection; for example, both HIV and antiphospholipid syndrome (APS) can give false positive results using non-treponemal methods (Chahine et al. 2011). The Centre for Disease Control recommends that a screening treponemal test (e.g. serum syphilis IgG), followed by a confirmatory test (e.g. Venereal Disease Research Laboratory [VDRL] or Rapid plasma reagin [RPR]) in the blood should be used in combination with an elevated CSF protein or white cell count, and a positive CSF VDRL/RPR to confirm the diagnosis of neurosyphilis (Centers for Disease Control and Prevention (CDC) 1997). CSF VDRL/RPR can be negative during the early stages of neurosyphilis but one study showed that the diagnostic sensitivity can be improved by testing CSF FTA-ABS (fluorescent treponemal antibody-absorbed) or CSF-FTA (fluorescent treponemal antibody) and the specificity can be improved by combining this with CSF B lymphocyte cell count in fresh and cryopreserved samples, using flow cytometry (Marra et al. 2004). However the validated performance of CSF-FTA-ABS/CSF-FTA has been variable, depending largely on the choice of controls used and few studies included subjects with HIV infection (Harding et al. 2012). HIV and other opportunistic infections can independently cause CSF

abnormalities (e.g. low glucose, raised white cell count), therefore, in the absence of a positive CSF VDRL/RPR, neurosyphilis is still probable but exclusion of an alternative infectious diagnosis is especially important. While there are no clear recommendations regarding the interpretation of CSF white cell count in HIV-infected individuals, some series have suggested a cut-off of ≥20 CSF white cells as opposed to ≥5 cells in non-HIV infected patients (Marra et al. 2004).

VZV

One third of patients with VZV vasculopathy do not have a rash at the time of presentation, this often leads to missed diagnosis (Nagel et al. 2008). Currently a diagnosis is confirmed by CSF VZV IgG or PCR (Nagel et al. 2008; Gutierrez et al. 2011). Although VZV PCR testing is still commonly used, recent evidence shows that IgG detection in the CSF is more sensitive (Nagel et al. 2008). In the context of HIV infection, caution should be taken in measuring CSF VZV IgG, as elevated levels may also reflect an impaired blood brain barrier with leakage of blood antibody into the CSF rather than intrathecal VZV IgG production (Reiber et al. 1991). We therefore recommended that in HIV infected individuals, screening for VZV vasculopathy should involve calculating the antibody index by comparing the serum/CSF ratio of anti-VZV IgG antibody to the serum/CSF ratio of albumin and total IgG, thus discriminating between blood derived and pathology brain-derived antibody (Reiber et al. 1991; Winchester et al. 2011). The specificity of CSF VZV PCR alone in HIV infected individuals is also questionable, because a positive result may also be associated with subclinical activation, reaffirming the utility of CSF VZV IgG index in HIV infected individuals (Cinque et al. 1997). Therefore, a positive CSF VZV PCR should confirm rather than determine the diagnosis of VZV vasculopathy in an HIV infected individual.

Table 4:1: Diagnostic criteria for opportunistic infection of the central nervous system found in arterial ischaemic stroke in HIV infected individuals

Aetiology	Confirmed	Probable
Varicella-zoster virus (VZV)	Ischaemic stroke And 1 out of 2 pathology/laboratory criteria: 1) Brain histopathology evidence of vasculitis and isolation of VZV virus particle by in situ hydribidisation, PCR or antigen detection using immunohistochemistry, 2) Intrathecal VZV IgG production +/- DNA VZV PCR in the CSF *(Nagel et al. 2008; Gilden et al. 2009).* And Exclusion of alternative diagnosis.	Ischaemic stroke And 1 out of 2 clinical/laboratory criteria: 1)Trigeminal or cervical zoster distribution within the 6 weeks prior to the onset of stroke, in the absence of histology or laboratory confirmation *(Gilden et al. 2009)*, 2) Negative Intrathecal VZV IgG production and positive DNA VZV PCR in the CSF. And Exclusion of alternative diagnosis.
Tuberculosis infection	Ischaemic stroke And Brain histopathology or CSF AFB, culture or PCR positive for MTB with associated endarteritis obliterans *(Marais et al. 2010)*. And Exclusion of alternative diagnosis.	Ischaemic stroke And Score ≥ 10 (without CT/MRI brain) or ≥ 12 (with CT/MRI brain) based on the uniform case definition for tuberculous meningitis. Case definition encompasses clinical, CSF, evidence of MTB elsewhere +/- imaging (Marais et al. 2010). And Exclusion of alternative diagnosis.

Table 4:1: Diagnostic criteria for opportunistic infection of the central nervous system found in arterial ischaemic stroke in HIV infected individuals.

Aetiology	Confirmed	Probable
Syphilis	Ischaemic stroke And 3 out of 3 Laboratory criteria; 1) Reactive non-treponemal test (i.e., VDRL or RPR), and a reactive treponemal test (i.e. Tp EIA, TPPA) in the blood (Centers for Disease Control and Prevention (CDC) 1997), 2) Positive CSF RPR/VDRL or FTA-ABS /FTA +/- elevated CSF B-cell count (in the absence of substantial contamination of CSF with blood) *(Centers for Disease Control and Prevention (CDC) 1997; Marra et al. 2004)*, 3) CSF leucocyte >20 cells/mm^3 or CSF protein >0.45 g/l, or IgG index >0.6 *(Centers for Disease Control and Prevention (CDC) 1997; Timmermans et al. 2004; Chahine et al. 2011)*. Or Brain histopathology confirmation of Tp spirochaetes by immunohistochemistry with associated endarteritis obliterans. And Exclusion of alternative diagnosis	Ischaemic stroke And 3 out of 3 Laboratory criteria; 1) Reactive non-treponemal test (i.e. VDRL or RPR), and a reactive treponemal test (i.e. Tp EIA, TPPA) in the blood, 2) Negative CSF confirmation of syphilis (or substantial contamination of CSF with blood), 3) CSF leucocyte >20 cells/mm^3 or CSF protein >0.45 g/l, or IgG index >0.6 *(Centers for Disease Control and Prevention (CDC) 1997).* Or 3 out of 3 Laboratory criteria; 1) Reactive non-treponemal test (i.e. VDRL or RPR), and a reactive treponemal test (i.e.Tp EIA, TPPA) in blood, 2) Positive CSF EIA/TPPA confirmation of syphilis (or substantial contamination of CSF with blood), 3) CSF leucocyte >20 cells/mm^3 or CSF protein >0.45 g/l, or IgG index >0.6 *(Centers for Disease Control and Prevention (CDC) 1997).* Or 3 out of 3 Laboratory criteria; 1) Reactive non-treponemal test (i.e. VDRL or RPR), and a reactive treponemal test (i.e. Tp EIA, TPPA) in blood, 2) Positive CSF RPR/VDRL or FTA-ABS/FTA +/- elevated CSF B-cell count (in the absence of substantial contamination of CSF with blood), 3) CSF leucocyte ≤20 cells/mm^3 or CSF protein ≤0.45 g/l, or IgG index ≤0.6 (Brown et al. 2006). And Exclusion of alternative diagnosis.

Table 4:1: Diagnostic criteria for opportunistic infection of the central nervous system found in arterial ischaemic stroke in HIV infected individuals

Opportunistic infections	**Minimum work-up** *TB*: CSF microscopy and biochemistry or unenhanced CT/MRI and chest x-ray *Syphilis*: non-treponemal and a treponemal blood test, CSF microscopy and biochemistry *VZV*: nil **Optimum work-up** *TB*: CSF/brain histopathology/sputum - TB culture or AFB stain or TB PCR and CSF microscopy and biochemistry and CT/MRI with contrast and CXR Syphilis: non-treponemal and a treponemal blood test, CSF microscopy and biochemistry and CSF VDRL/RPR, FTA-ABS/FTA and B cell count. Brain histopathology *VZV*: CSF VZV IgG index and PCR, brain histopathology.

Case definitions refer exclusively to the brain; retinal and spinal cord infarction fall out with the scope of these definitions. CSF – Cerebrospinal fluid, VDRL – Venereal Disease Research Laboratory, RPR – Rapid Plasma Reagin, Tp EIA – Treoinema Enzyme immunoassay, TPPA – Treponema pallidum particle agglutination, FTA-ABS - Fluorescent Treponemal Antibody-Absorbed), FTA - Fluorescent Treponemal, MTB- *Mycobacterium* Tuberculosis, TB-Tuberculosis, AFB – Acid fast bacilli, CXR – chest radiograph.

4.4.2 HIV-associated vasculopathy

Vasculopathy is defined as significant thickening of the intima, usually from intimal hyperplasia, more than expected for age. In chapter 1, HIV-associated vasculopathy was defined as an abnormality of the cerebral blood vessels that result directly or indirectly from HIV infection but excluding opportunistic infection, vasculitis and lymphoma. This definition was deliberately inclusive, in an attempt to encompass the several cerebrovascular changes described in HIV infection. Each of these will now be discussed in turn.

Atherosclerotic and non-atherosclerotic vasculopathy

Intimal hyperplasia is the hallmark of atherosclerosis. It is usually typified by plaque formation and consists of form cells, a lipid core and a fibrous cap (Libby et al. 2011). While the mechanism of accelerated atherosclerosis by chronic inflammation owing to HIV infection or from dyslipidaemia associated with HIV treatment is more certain, the mechanism of a non-atherosclerotic group is not. The latter is typically found in young stroke patients and these patients usually have evidence of aneurysmal/ non-aneurysmal intracranial/extracranial asssociated strokes (Friis-Moller et al. 2003; Tipping et al. 2006; Tipping et al. 2007; Gutierrez et al. 2011; Ovbiagele et al. 2011; Rasmussen et al. 2011) (Chapter 1).

The mechanism of non-atherosclerotic vasculopathy may differ, however their phenotypes overlap and are sometimes difficult to differentiate without histopathology and optimal imaging; neither of which are readily available in resource-poor settings where the highest burden of HIV infection exists. Young age is the most important predictor of this phenotype and was therefore used to define this non-atherosclerotic group; a cut-off of 45 years was chosen to discriminate from the typical atherosclerotic

related strokes (Tipping et al. 2006; Tipping et al. 2007) (Chapter 1). This cut-off was based on the age distribution of histologically supported case series (Nair et al. 1999; Tipping et al. 2006; Tipping et al. 2007; Delgado Almandoz et al. 2013). Although we envisage that our knowledge will expand in this field and thus guide a refined classification of this non-atherosclerotic group, we suggest this pragmatic definition as a starting point to guide the evolution of this emerging syndrome.

Table 4:2: Diagnostic criteria for HIV-associated vasculopathy found in arterial ischaemic stroke in HIV infected individuals

Aetiology	Confirmed	Probable
Non-atherosclerotic Stroke	Ischaemic stroke And 1 out of 4 pathology/radiology criteria: 1) Brain histopathology evidence (macro- and microscopic demonstration) of vasculopathy (significant thickening of the intima, usually from intimal hyperplasia, more than expected for age) of the arterial wall in the absence of atherosclerosis and vasculitis, 2) Patients with stenosis (or complete occlusion) ≥50% in an intra-/or extracranial artery supplying the ischaemic field +/- luminal thrombus, 3) a mobile thrombus in the aortic arch; 4) occlusion with imaging evidence of vasculopathy in an intra-/or extracranial artery supplying the ischaemic field *(Tipping et al. 2006; Tipping et al. 2007; Gutierrez et al. 2011).* And Age ≤45 years And Exclusion of alternative diagnosis.	Ischaemic stroke And A history of intermittent claudication, Transient Ischaemic Attacks (TIAs) in the same vascular territory, a carotid bruit, or diminished peripheral pulses. And Age ≤45 years And Exclusion of alternative diagnosis.†
	Minimum work up: nil **Optimum work up**: Carotid/vertebral duplex ultrasound/echography. CT-angiography or MR-angiography or Digital-subtraction angiography (Adams et al. 1993; Amarenco et al. 2009) . Brain histopathology. †There should be careful consideration for a psycogenic cause of symptoms in those without brain imaging or a normal unenhanced CT within 24hours of index stroke.	

Table 4:2: Diagnostic criteria HIV-associated vasculopathy found in arterial ischaemic stroke in HIV infected individuals

Aetiology	Confirmed	Probable
Atherosclerotic Stroke	Ischaemic stroke And 1 out of 4 pathology/radiology criteria: 1) Brain histopathology evidence (macro- and microscopic demonstration) of atherosclerotic disease of the arterial wall, with or without local thrombus, 2) Patients with stenosis (or complete occlusion) ≥50% in an intra-/or extracranial artery supplying the ischaemic field +/- luminal thrombus, 3) A mobile thrombus in the aortic arch; 4) occlusion with imaging evidence of atherosclerosis in an intra-/or extracranial artery supplying the ischaemic field *(Adams et al. 1993; Amarenco et al. 2009).* And Age >45 years And Exclusion of alternative diagnosis.	Ischaemic stroke And A history of intermittent claudication, Transient Ischaemic Attacks (TIAs) in the same vascular territory, a carotid bruit on auscultation or diminished peripheral pulses *(Adams et al. 1993; Amarenco et al. 2009).* And Age >45 years And Exclusion of alternative diagnosis.†
	Minimum work up: nil **Optimum work up**: Carotid/vertebral duplex ultrasound/echography. CT-angiography or MR-angiography or Digital-subtraction angiography (Adams et al. 1993; Amarenco et al. 2009). Brain histopathology. †There should be careful consideration for a psycogenic cause of symptoms in those without brain imaging or a normal unenhanced CT within 24hours of index stroke.	

Case definitions refer exclusively to the brain; retinal and spinal cord infarction fall out with the scope of these definitions. CT – Computer tomography.

Vasculitis

The association of HIV and cerebral vasculitis has been reported in a few case series (Nogueras et al. 2002; Taylor et al. 2008; Bermel et al. 2009; Melica et al. 2009). Vasculitis can either be caused by infection, where direct invasion of the pathogen leads to proliferation and inflammation of the vessel wall (e.g. VZV) or by non-infectious mechanisms (Jennette et al. 2013). Although a pathogen may be involved in the latter, this type of vasculitis does not have direct vessel wall invasion; this is suspected to be the likely mechanism for HIV-associated vasculitis. Most of the case series that describes HIV-associated vasculitis meets the Chapel Hill criteria of single organ vasculitis (i.e. the vasculitis is limited to the brain) (Jennette et al. 2013). Diagnoses of single organ vasculitis is usually one of deduction, firstly by identifying the characteristic clinical and radiological features and then excluding alternative aetiologies (); neoplasia (e.g. lymphoma, leukaemia, lung cancer), infections (VZV, syphilis, TB, neuroborreliosis, fungal infection - aspergillosis, nocardiosis, cryptococcus, histoplasmosis, hepatitis B and C) and inflammatory disorders (e.g. Behcet's disease, scleroderma, polyarteritis nodosa, Sjogren's syndrome, antiphospholipid antibody syndrome, Wegener's granulomatosis, systemic lupus erythematosis, Crohn's disease, Kohlmeier-Degos disease, Cogan'syndrome, sarcoid granulomatosis and angiitis, urticarial hypocomplementemic) (Hajj-Ali et al. 2011) Table 4:2. Investigations to exclude these alternative diagnoses should be directed by clinical history and in some cases, the regional endemicity of the condition.

Table 4:2: Diagnostic criteria HIV-associated vasculopathy found in arterial ischaemic stroke in HIV infected individuals

Aetiology	Confirmed	Probable
Vasculitis	Ischaemic stroke And The presence of either classic angiographic or histopathology features of angiitis within the central nervous system *(Chetty 2001; Nogueras et al. 2002; Kuker 2007; Melica et al. 2009; Hajj-Ali et al. 2011).* And Exclusion of alternative diagnosis[+]	Ischaemic stroke And CT/MRI confirmation of acute and/or chronic ischeamic changes in more than one vascular territory, involving any or all of cortical, subcortical and deep white matter distribution. In the absence of an embolic source *(Kuker 2007; Hajj-Ali et al. 2011).* And Exclusion of alternative diagnosis
	Minimum work up: unenhanced CT/ MRI (T1, T2, FLAIR and DWI sequences) and exclude VZV and TB and syphilis and CXR *(Wardlaw et al. 2013).* **Optimum work up**: CT (with contrast)/ MRI (minimum T2, T2*and DWI sequences) and CT-angiography or MR-angiography or Digital-subtraction angiography. Histopathology examination. †Alternative diagnosis should be excluded; for inflammatory disorders, ANA antibodies (if positive ENA, DNA antibodies), or C3/C4, or ANCA, or cryoglobulins or rheumatoid factor or serum/CSF angiotensin-converting enzyme (ACE) and APS screen (as above) and	

Case definitions refer exclusively to the brain; retinal and spinal cord infarction fall out with the scope of these definitions. CT – Computer tomography, MRI – Magnetic resonance imaging, VZV – varicella zoster virus, TB – mycobacterium tuberculosis, CXR – chest radiograph, ANA –Antinuclear Antibody, ENA – Extractable Nuclear Antigen, C3/4 – Complement C3/4, ANCA – Anti-neutrophil cytoplasm antibody.

Small vessel disease

Small vessel disease is a type of stroke. As a term, small vessel disease covers many pathologies, the main causes of small vessel disease described in pathology series are those thought to be associated with chronic hypertension, hyaline arteriolosclerosis and lipohyalinosis, and cerebral amyloid angiopathy (Pantoni 2010). Lacunar infarcts and white matter lesions are easily detected by neuroimaging but small vessel disease due to vessel wall alteration is not. Therefore, neuroimaging classification of recent infarct (<20mm in size) restricts the definition to ischaemic lesions and might be misleading (Pantoni 2010; Wardlaw et al. 2013). The Edinburgh cohort of 183 case autopsy series showed that SVD does indeed exist in an HIV infected population however, these were largely asymptomatic cases, characterised by hyaline small vessel wall thickening, perivascular space dilatation, rarefaction and pigment deposition with vessel wall mineralization and occasional perivascular inflammatory cell infiltrates and associated with microinfarcts (Connor et al. 2000). Further work is needed to confirm if stroke syndromes are the only entity of small vessel disease in patients with HIV infection. Especially as small vessel disease may be implicated in neurological disorders such as cognitive impairment (Wright et al. 2010).

Table 4:2: Diagnostic criteria HIV-associated vasculopathy found in arterial ischaemic stroke in HIV infected individuals

Aetiology	Confirmed	Probable
Small Vessel Disease	Ischaemic stroke And 1 out of 2 pathology/clinical/radiology criteria. 1) Brain histopathology evidence of small vessel disease characterised by hyaline arteriolosclerosis, lipohyalinosis or amyloid angiopathy *(Connor et al. 2000; Wardlaw et al. 2013)*, 2) At least one traditional clinical lacunar syndromes* in the absence of cortical involvement** and CT/MRI demonstration of a relevant brainstem or subcortical lesion with a diameter of less ≤20mm *(Bamford et al. 1987; Adams et al. 1993; Wardlaw et al. 2013)*. In the absence of cardio-thromboembolic source and/or ≥50% ipsilateral carotid stenosis. And Exclusion of alternative diagnosis.	Ischaemic stroke And A least one traditional clinical lacunar syndromes* in the absence of cortical involvement**, in the absence of cardio-thromboembolic source and/or ≥50% ipsilateral carotid stenosis *(Adams et al. 1993; Amarenco et al. 2009).* Or A least one traditional clinical lacunar syndromes* in the absence of cortical involvement** and normal unenhanced CT within 24 hours of index stroke, in the absence of cardio-thromboembolic source and/or ≥50% ipsilateral carotid stenosis *(Adams et al. 1993; Amarenco et al. 2009)*. And Exclusion of alternative diagnosis.†
	Minimum work up: nil **Optimum work up**: MRI (T1, T2, FLAIR, T2*GRE, DWI) *(Wardlaw et al. 2013)*. *The following 4 syndromes were recognised: pure motor stroke, pure sensory stroke, sensorimotor stroke, and ataxic hemiparesis. **The presence of a visual field defect, evidence of higher cerebral dysfunction (e.g. dysphasia, visuospatial disturbance, predominantly proprioceptive sensory loss) on standard clinical testing, or features that clearly localize the lesion in the vertebrobasilar distribution (e.g. gaze palsies or crossed deficits, though not nystagmus or dysarthria) exclude the diagnosis of lacunar syndrome *(Bamford et al. 1987).* †There should be careful consideration for a psycogenic cause of symptoms in those without brain imaging or a normal unenhanced CT within 24hours of index stroke.	

Case definitions refer exclusively to the brain; retinal and spinal cord infarction fall out with the scope of these definitions. CT – Computer tomography, MRI – Magnetic resonance imaging, T1- T1 weighted sequence T2 – T2 weighted sequence, DWI – diffusion weighted imaging, GRE- gradient ECHO, FLAIR – fluid attenuated inversion recovery.

4.4.3 Cardioembolism

The criteria we devised for cardio-thromboembolism were based largely on the TOAST classification (Adams et al. 1993). We modified this to also include marantic endocarditis in the high risk group Table 4:3 (Berger et al. 1990).

Table 4:3: Diagnostic criteria for cardio-thromboembolism found in arterial ischaemic stroke in HIV infected individuals

Aetiology	Confirmed	Probable
Cardio-thromboembolism	Ischaemic stroke And Identification of *high-risk* cardio-thromboembolic lesions (Mechanical prosthetic valve, Mitral stenosis with atrial fibrillation, Atrial fibrillation (other than lone atrial fibrillation), Left atrial/atrial appendage thrombus, Sick sinus syndrome, Recent myocardial infarction (<4 weeks), Left ventricular thrombus, Dilated cardiomyopathy, Akinetic left ventricular segment, Atrial myxoma, infective and marantaric endocarditis) (Adams et al. 1993). And Exclusion of alternative diagnosis	Ischaemic stroke And Identification of *medium-risk* cardio-thromboembolic lesions (Mitral valve prolapsed, Mitral annulus calcification, Mitral stenosis without atrial fibrillation, Left atrial turbulence (smoke), Atrial septal aneurysm, Patent foramen ovale, Atrial flutter Lone atrial fibrillation, Bioprosthetic cardiac valve, Nonbacterial thrombotic endocarditis, Congestive heart failure, Hypokinetic left ventricular segment, Myocardial infarction [>4 weeks, <6 months of stroke event]) *(Adams et al. 1993).* And Exclusion of alternative diagnosis†
	Minimum work-up ECG and auscultation by a cardiologist **Optimum work-up** ECG/telemetry/Holter ECG and Trans-thoracic echography or Trans-oesophageal echography or cardiac CT/MRI (Amarenco et al. 2009). †There should be careful consideration for a psycogenic cause of symptoms in those without brain imaging or a normal unenhanced CT within 24hours of index stroke.	

Case definitions refer exclusively to the brain; retinal and spinal cord infarction fall out with the scope of these definitions. ECG – electrocardiogram.

4.4.4 Coagulopathy

Antiphospholid syndrome (APS) is a prothrombotic disorder. Greater than 20% of cases can present as a stroke (Ruiz-Irastorza et al. 2010). The revised classification criteria for APS (2006) specify that at least one of the clinical criteria and laboratory criteria are met (Miyakis et al. 2006). A patient presenting with an ischaemic stroke (confirmed by brain imaging) automatically meets the clinical criteria but they will also need to have anti–β2-glycoprotein I (β_2GP1) or anticardiolipin (ACL) antibodies or lupus-anticoagulant (LA), detected by enzyme-linked immunosorbant assay (ELISA) in their blood and persistence of medium to high titers of these antibodies for >12 weeks Table 4:4. More recent evidence shows that the anti–β_2GP1 and LA are strongly associated with incident stroke (Urbanus et al. 2009). However, the evidence demonstrating the utility of ACL as a predictor of APS and stroke is conflicting (Brey et al. 2003; Urbanus et al. 2009). HIV infection is associated with ACL and LA but not anti– β_2GP1 (Petrovas et al. 1999). As anti–β_2GP1 appears to be specific in stroke individuals with APS in both HIV positive and negative populations, the consensus was to refine the laboratory definition to include the detection of anti- β_2GP1 in combination with ACL or LA in those with HIV infection.

The definition of a confirmed APS is defined by time therefore precluding a diagnosis at the acute stage of the patient's presentation. Part of the definition requires a repeat blood test at 12 weeks to confirm or refute this diagnosis. Other coagulopathies like Protein C and S deficiency have been implicated in HIV infection but these are associated with venous and not arterial strokes.

Table 4:4: Diagnostic criteria anti-phospholipid syndrome found in arterial ischaemic stroke in HIV infected individuals

Aetiology	Confirmed	Probable
Coagulopathy		
Anti-phospholipid syndrome	Ischaemic stroke.[†] And 1 out of 2 laboratory criteria: 1) Lupus anticoagulant present in plasma 2) Anticardiolipin antibody of IgG or IgM isotype, present in serum or plasma, in medium or high titer (i.e. >40 GPL or MPL units, or > 99th percentile) (*Miyakis et al. 2006)*. And Anti-β2-glycoprotein 1 antibody of IgG or IgM isotype, present in serum or plasma, in titer >99th percentile *(Petrovas et al. 1999).* All present on two or more occasions, at least 12 weeks apart. And Exclusion of alternative diagnosis.	Ischaemic stroke And 1 out of 2 laboratory criteria: 1) Lupus anticoagulant present in plasma 2) Anticardiolipin antibody of IgG or IgM isotype, present in serum or plasma, in medium or high titer (i.e. >40 GPL or MPL units, or > 99th percentile) *(Miyakis et al. 2006).* And Anti-β2-glycoprotein 1 antibody of IgG or IgM isotype, present in serum or plasma, in titer >99th percentile *(Petrovas et al. 1999)*. Present *at one time-point,* or on two occasions separated by less than 12 weeks. And Exclusion of alternative diagnosis.†
	Minimum work-up: Lupus anticoagulant or Anticardiolipin antibody and Anti-β2-glycoprotein 1 **Optimum work-up**: MRI (T1, T2, FLAIR sequences); CT-angiography plus CT-venography or MR-angiography plus MR-venography *(Hajj-Ali et al. 2011; Kaichi et al. 2013)*. Lupus anticoagulant or Anticardiolipin antibody and Anti-β2-glycoprotein 1. Brain histopathology examination. [†]histopathology confirmation is not essential for a confirmed diagnosis but if used, thrombosis should be present without significant evidence of inflammation in the vessel wall *(Miyakis et al. 2006).* †There should be careful consideration for a psycogenic cause of symptoms in those without brain imaging or a normal unenhanced CT within 24hours of index stroke.	

Case definitions refer exclusively to the brain; retinal and spinal cord infarction fall out with the scope of these definitions. CT – Computer tomography, MRI – Magnetic resonance imaging, T1- T1 weighted sequence T2 – T2 weighted sequence, DWI – diffusion weighted imaging, FLAIR – fluid attenuated inversion recovery, IgG-immunoglobulin G, IgM – immunoglobulin M.

4.4.5 Other determined aetiology

Although we have described the common aetiologies encountered in HIV related stroke, this is by no means an exhaustive list. There are aetiologies where the mechanism of HIV stroke may be biologically plausible but there was minimal evidence in the literature to support an association (e.g. systemic vasculitidies and precerebral/cerebral arterial dissection, hyperviscosity syndrome). There are also aetiologies described in young populations that may co-occur in HIV infected individuals (e.g. hereditary causes of stroke and drug-induced vasculopathy). A thorough clinical history and examination, with diagnostic studies such as blood tests and brain imaging should reveal these less frequently described causes of stroke.

4.4.6 Stroke mimics

In an HIV endemic population one quarter of patients presenting with an acute focal neurological deficit will have a stroke mimic (Kumwenda et al. 2005). Frequently occurring mimics include toxoplasma infection, progressive multifocal leukoencephalopathy, viral encephalitidies (e.g. HSV-1, CMV) and fungal infections (Chapter 2). The selection of appropriate imaging (at least a CT scan with contrast or preferably an MRI with a minimum sequence of T1, T2, FLAIR and DWI) is essential for the exclusion of stroke mimics (Chapter 2).

4.5 Building the hierarchical framework

The starting point of this framework was defined as:

1) Stroke - rapidly developing clinical symptoms lasting more than 24 hours or leading to death, with no apparent cause other than a vascular one (Hatano 1976).
2) HIV infection – based on a positive antibody test which either involves HIV enzyme immunoassay (EIA) or rapid HIV antibody test, a positive result requires confirmation with western blot, antigen test or PCR test. However, in resource-poor settings confirmation is usually with a second antibody test using a different manufacturer system.

When possible, this was accompanied by brain imaging to type the stroke and exclude stroke mimics. The aetiological definition was based on clinical, laboratory, radiology and brain histopathology findings; a minimum work-up of investigations (in addition to clinical history and examination) was needed for a diagnosis to be established. The level of certainty “confirmed” or “probable” was based on the quality of diagnostic evidence.

A complete assessment of all the potential causes preceded the use of our proposed algorithm. The selected aetiologies were based on those with good evidence or plausible mechanisms that linked HIV infection and stroke; this included opportunistic infection (i.e. VZV, syphilis, TB), coagulopathy (i.e. antiphospholipid syndrome [APS]), cardio-thromboembolism and HIV-associated vasculopathy (Chapter 1).

The hierarchical placement of the different aetiologies was based on several factors 1) poor prognosis 2) our confidence of the underlying mechanism of the disease in the context of HIV infection (based on current evidence) 3) availability of definitive treatment. Those that satisfied all three criteria were placed higher up in the algorithm. The highest aetiology in the hierarchy with a "confirmed diagnosis" was taken as the final diagnosis even if there were multiple confirmed aetiologies. However, a "probable diagnosis" was only allocated as the final diagnosis if there was one option; otherwise this was defined as undetermined Figure 4:1. We considered several scenarios to justify the rationale behind the placement of the different aetiologies; 1) if after an assessment of all the possible causes, the patient had a confirmed CNS VZV infection and confirmed vasculitis; it was likely that VZV caused the vasculitis and without treatment of the VZV infection the patient would have a worse prognosis therefore CNS VZV infection was placed higher than vasculitis in the hierarchy: 2) If after an assessment of all the possible causes, the patient was found to have both confirmed cardio-thromboembolic and atherosclerotic stroke, those with cardio-thromboembolic stroke were more likely to have recurrent events thus a poorer prognosis (Grau et al. 2001; Gilden et al. 2009). In the latter scenario, the timely initiation of long-term anti-coagulation is an important consideration. In the era of thrombolysis, a non-infectious cardio-thromboembolic stroke would be considered for treatment, as long as there were no contraindications therefore, short-term management does not necessarily differ between the two aetiologies but long-term management does, justifying the higher placement of cardio-thromboembolic stroke; 3) although antiphosholipid syndrome is well characterised in the non-HIV population as a cause of young stroke, and has a plausible mechanism of a pro-thrombotic state in HIV infected individuals, there is little evidence that demonstrates an association with APS and HIV stroke (Maclean et al. 1990; Urbanus et al. 2009) (Chapter 1). Furthermore, treatment interventions in the non-HIV and HIV infected populations are uncertain (Brey et al. 2003). For this reason, this was placed lower down in the hierarchy

but ahead of small vessel disease. Small vessel disease requires the exclusion of APS for a diagnosis to be made and does not have a definitive treatment (Pantoni 2010) Figure 4:1.

A diagnosis was defined as "undetermined" when; 1) more than two probable cause was identified at the same level of "probable" diagnostic certainty 2) the diagnosis was "uncertain" (following a minimum work-up for all aetiologies) 3) the diagnosis was "incomplete" (this arose when one or more of the aetiologies did not have a minimum work-up) or classified as "other determined" when rarer causes of HIV stroke were found (Amarenco et al. 2009).

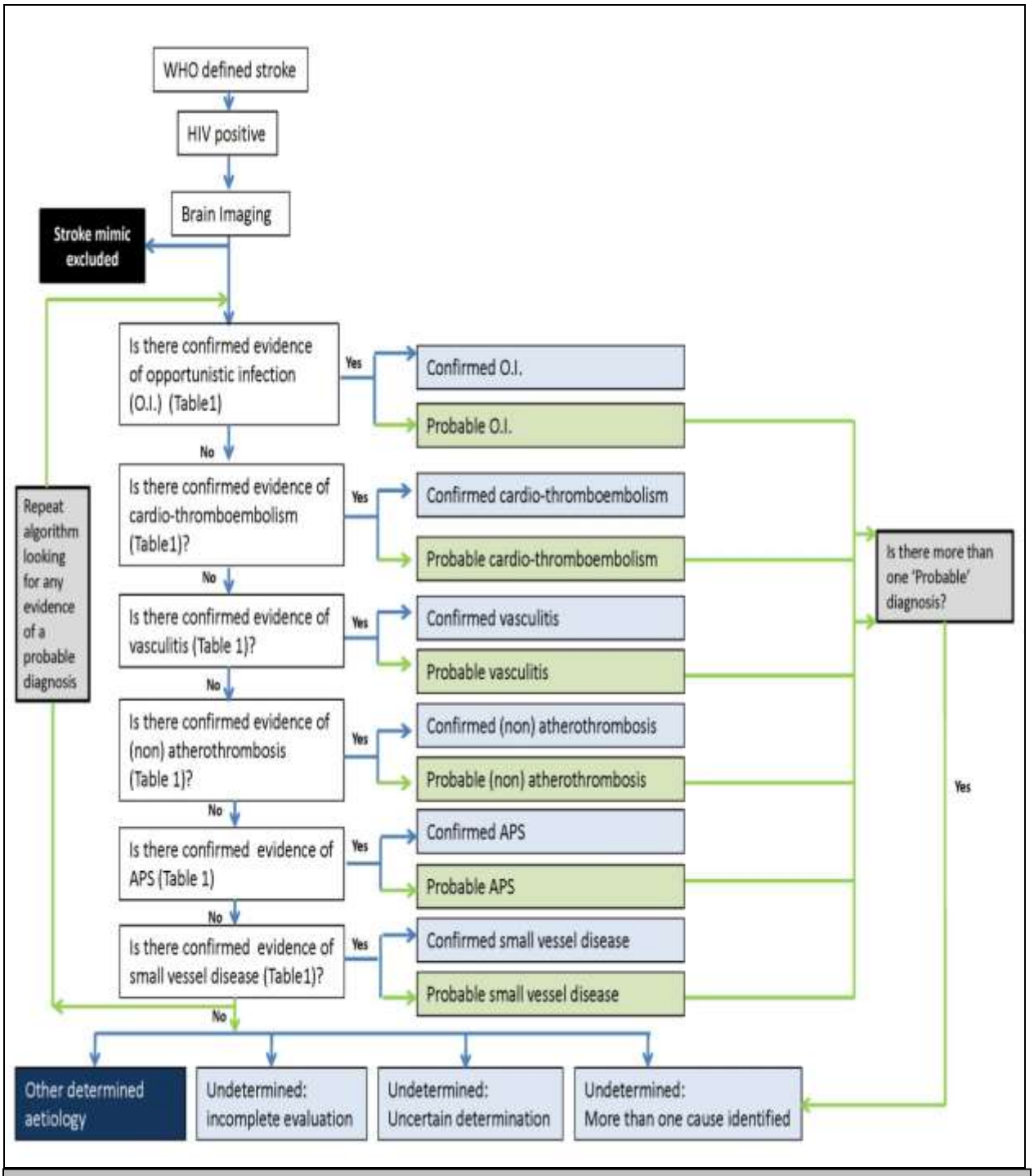


Figure 4:1: Hierarchical approach to defining the aetiology of arterial ischaemic stroke in HIV infected individuals

APS: Anti-phospholipid syndrome

4.6 Discussion

The preliminary classification of HIV related stroke in chapter one was based on several phenotypes reported in the literature. In this chapter I further refined definitions in the context of HIV infection and developed new definitions as in the case of non-atherosclerotic stroke, a form of HIV-associated vasculopathy.

These case definitions and hierarchical algorithm are timely and underscores the global priority to focus on HIV infection and vascular diseases (Ullrich et al. 2011). A better understanding of the pathogenesis and interventions to reduce this stroke burden is of top priority.

We intentionally deviated from the horizontal type algorithm seen in past stroke classifications, largely to enforce a diagnosis (Adams et al. 1993; Amarenco et al. 2009). The vertical hierarchical algorithm assumes that the top aetiology is the likely causative agent/ mechanism and this is deduced from the systematic investigation of common aetiologies found in HIV related stroke. This concept is straightforward except for when multiple aetiologies arise which may or may not have the same underlying mechanism. For infective organisms such as VZV, TB, syphilis it can be convincingly argued that all these infections can potentially cause vasculitis, vasculopathy, cardioembolic stroke (especially for syphilis) and small vessel disease and therefore, can be placed higher in the algorithm. However, aetiologies like atherosclerotic stroke, cardio-thromboembolism, vasculitis, small vessel disease may coexist and have related mechanisms or be entirely exclusive. In this instance, we adopted a logical approach i.e. what would be useful for the clinician? The consensus was that the prognosis and availability of definitive treatment were important components in managing any patient with a stroke

thus, we priortised those with poorer prognosis and availability of definitive treatment above those without. We were also cautious not to prioritise aetiologies which logically should be higher up in the algorithm but limited evidence supporting the mechanism in HIV related stroke prevented this (e.g. APS).

Most studies will benefit from this stringent approach which maximises on the specificity but at the expense of sensitivity. Validation studies in existing and prospective cohorts will advance this approach and optimise its sensitivity, specificity, and accuracy. We also envisage that once validated that this can also be used in a clinical setting.

The intention of this algorithm was not to stop investigating at the point of diagnosis but rather, to perform a complete assessment of all the frequently occurring aetiologies in HIV related stroke. We developed a minimum assessment which collectively included (blood tests – ACL, LA, B_2GP1, syphilis IgG or Tp EIA, VDRL or RPR, CXR, CSF – microscopy, biochemistry, bacterial culture and unenhanced CT); if a probable diagnosis occurred, these investigations, which are mostly resource appropriate, should capture the diagnosis. The optimum work-up, when resource allows will confirm a definitive diagnosis if it existed. This is in addition to the standard work-up of stroke patients (i.e. fasting glucose, fasting cholesterol, full blood count and urea and electrolyte) (Chapter 1).

The heterogeneous nature of HIV stroke and the limited number of studies to extrapolate evidence from made the development of this consensus statement challenging. The number of specialties needed to develop this document also highlighted the diversity of aetiologies in HIV infected stroke patients.

Within the working group we had contributors from Malawi and Cape Town; authors from these centers were working on probably the largest prospective cohort of HIV stroke in Sub-Saharan Africa at the time of writing and insight from these databases helped in bringing this document together. Furthermore, contribution from these regions also ensured that poorer countries, which suffer from the highest burden of HIV infection, were sensitively considered with regards to diagnostic modalities.

HIV infection and stroke is an emerging field and we have some way to go in understanding the different causes of stroke and their pathogenesis in this population. However, this chapter contains a pragmatic way forward as a starting point to guide the evolution of this disease. My next step, covered in the next chapter, was to use these case definitions to determine the aetiologies in the large cohort of HIV infected patients with stroke that I studied in Malawi.

5 Aetiology and HIV predictors of outcome at six months in HIV related stroke in Malawi; a prospective case series

5.1 Abstract

Background

Untreated HIV infection and recently starting combined antiretroviral therapy (cART) are important risk factors for stroke in HIV endemic regions. However there is uncertainty about the aetiology, clinico-pathological presentation and the predictors of a poor outcome in HIV related stroke.

Methods

The cohort of adults (age >17 years) with acute stroke described in chapter 3 were investigated by MRI brain imaging, cardiac and carotid ultrasound scan, electrocardiogram, blood and CSF screening for infectious and inflammatory aetiologies, and histopathology examination of the brain when possible. HIV positive ischaemic stroke cases were classified according to the aetiologies defined in chapter 4. Survivors of stroke were followed up for 6 months.

Results

Seventy-eight of the 252 (31%) confirmed stroke patients were HIV positive (64 had an ischaemic stroke). Their median age and CD4+ T-lymphocyte count was 41 years (IQR;32,54) and 192 cells/mm^3 (IQR; 66,342) respectively. Among those with an Ischaemic stroke: 34/64 (53%) had HIV-associated vasculopathy (32% were undetermined), 18/64 (28%) had opportunistic infections, 7/64 (11%) had anti-phospholipid syndrome and 5/64 (8%) had cardioembolism. Thirty-four (44%) were on cART, of these, 19 had recently started within 6 months (15 had an ischaemic stroke). Among those starting cART within 6 months of an ischaemic stroke only 4/15 (27%) had an opportunistic infection and 11/15 (73%) were dead or missing by 6 months. One case with a histologically-proven diagnosis had evidence of HIV-associated vasculitis after starting cART. Advanced immnuosuppression (adjusted hazard ratio [aHR] 8.22 (1.85, 36.48) was associated with a poor outcome but initiating cART within the previous 6 months prior to a stroke (aHR 1.33(0.30, 6.01) was not.

Conclusion

Less than a third of HIV related strokes are due to opportunistic infections. Although HIV-associated vasculopathy was the predominant aetiology, this cohort needs to be further characterised. It is possible that in a subpopulation of HIV infected individuals, cART initiation exacerbates an ongoing pro-inflammatory state however; a better understanding of this subpopulation is urgently needed to guide appropriate interventions.

5.2 Introduction

As discussed in chapter 3, HIV infection is an important risk factor for young stroke in HIV endemic regions. Several aetiologies have been described which are directly (e.g. HIV-associated vasculopathy) or indirectly (e.g. opportunistic infection) related to HIV infection (Chapter 1).

HIV-associated vasculopathy is a term that encompasses the different HIV-associated cerebrovascular diseases in the absence of opportunistic infection vasculitis and lymphoma. Accelerated atherosclerosis in HIV infected individuals is a more accepted cause of HIV-associated vasculopathy (Chapter 1). However, the mechanism of non-atherosclerotic stroke, found especially in younger HIV infected patients, is less clear (Chapter 4). Furthermore, age is a strong predictor of stroke, and as the HIV positive population ages on treatment it is uncertain if accelerated atherosclerosis will become the dominant mechanism of HIV related stroke.

In chapter 3, I showed that untreated HIV infection is associated with ischaemic stroke which is mostly mediated by immunosuppression but more importantly, that there was an independent risk of developing a stroke within 6 months of starting combined antiretroviral therapy (cART). The characteristics of this subgroup and their underlying aetiology are undefined.

Because of the clinico-pathological uncertainties about HIV related stroke, especially in those recently starting antiretroviral treatment, I prospectively investigated the clinical, radiological and pathological characteristics of patients presenting with HIV related stroke in Malawi. I determined the aetiology of ischaemic stroke, using the definitions described in chapter 4. I also examined the variation in

phenotype compared to stroke patients who did not have HIV infection as well as determine the HIV characteristics that predicted a poor outcome at 6 months.

5.3 Methods

5.3.1 Patients

This study was conducted at the Queen Elizabeth Central Hospital in the cohort of adults (age >17 years) with acute stroke described in chapter 3. The 30 patients that were excluded for residing outside the Blantyre district were included in this analysis (Chapter 3). All patients that met the WHO criteria of a stroke and presented to hospital between February 2011 and April 2012 were included in this case series, the eligibility criteria were previously described (Chapter 3). The study was approved by the Liverpool School of Tropical Medicine, UK and the College of Medicine Research Ethics Committee, University of Malawi. All participants or guardians gave written informed consent.

5.3.2 *Procedures*

I took a full history, including details of clinical demographics, clinical features of the stroke presentation, exposure to potential vascular risk factors and drug history from each patient. I also performed a detailed clinical examination, including a full neurological examination. Stroke severity at baseline was assessed with the National Institutes of Health Stroke Scale (NIHSS) and classified as non-severe (NIHSS<14) or severe (NIHSS≥14) and stroke subtype was assessed using the Oxfordshire Community Stroke Project classification, both were performed within 7 days of the index stroke (Brott et al. 1989; Bamford et al. 1991). Blood pressure and waist-hip-ratio were assessed as previously described

(Chapter 3). Ankle brachial index was measured using HI Dop Vascular Doppler (Ana Wiz Ltd UK), peripheral vascular disease was defined as an ankle brachial index <0.9 (Hirsch et al. 2006). Brain imaging was performed on a GE 0.35 Tesla Signa Ovation Excite Magnetic-resonance (MRI) scanner (Milwaukee, Wisconsin) within seven days of admission. Routine MRI brain sequences included a midline sagittal localising view of the brain - T1-weighted, axial diffusion-weighted images, gradient echo, axial T2-weighted and a fluid attenuated inversion recovery sequence. The images were immediately reviewed by a radiologist and subsequently validated by two other radiologists (one neuroradiologist and one Infectious disease radiologist). Electrocardiogram was performed and interpreted by the physician and a 2D-mode echocardiogram and duplex carotid doppler (Philips HD3) were performed and interpreted by a radiologist. When possible, blood samples and cerebrospinal fluid (CSF) were taken soon after admission. The blood samples were tested for full blood count, random blood glucose, random cholesterol and HIV infection at Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW) diagnostic facilities; previously described cut-off parameters were used (Chapter 3). Specialist tests for Anti-phospholipid syndrome (APS) included, anti-cardiolipin antibody and β2-glycoprotein, these were performed in batches at the Royal Liverpool diagnostic laboratory using a commercial ELISA kit (Cambridge Life Sciences Ltd, Cambridgeshire, UK). Rapid plasma reagin (RPR), Treponema (Tp) enzyme immunoassay (EIA), Treponema Pallidum Particle Agglutination (TPPA) were tested for syphilis at the Public Health of England (PHE) clinical diagnostic laboratory, London. Each patient's CSF had microscopy for white and red cells, and CSF gram stain. CSF bacterial culture, protein and glucose were also performed. Additional tests such as indian ink stain and cryptoccocal antigen were performed in all CSFs from HIV positive patients. Staining and microscopy for acid fast bacilli and culture for *Mycobacterium Tuberculosis* (MTB) were performed in the CSF of those with white cell count ≥5cells/mm^3. RPR, TPPA and Tp EIA were tested in the CSF of those with a positive serum and VZV intrathecal index was determined by comparing the serum/CSF ratio of anti-VZV IgG antibody to the

serum/CSF ratio of albumin and total IgG at the PHE clinical diagnostic laboratory, London (Reiber et al. 1991; Winchester et al. 2011). Limited brain autopsy was performed in deceased HIV positive patients (when possible) by a local pathologist. Brain tissue was stored in 10% formalin and shipped to the University of Edinburgh for processing. The tissue sections were stained with hematoxylin and eosin and Zeihl-Neelsen stain. Additional staining to assess for evidence of HIV-associated encephalitis included CD8, CD68 and CR3. The results were interpreted by a neuropathologist and general pathologist with an interest in HIV infection.

Patients were managed by the admitting medical team according to standard hospital protocols, which included starting anti-hypertension treatment if hypertension was present after day 5 post-stroke and commencing on anti-glycaemic agents, if diabetes was diagnosed. All patients with ischaemic stroke were commenced on aspirin if no contra-indications. On discharge, patients were referred to the appropriate ART, diabetes or hypertension out-patient clinic if indicated.

5.3.3 Aetiological diagnosis

All patients were classified using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification. For ischaemic HIV positive patients the case definitions for opportunistic infections (VZV, TB, syphilis), cardio-thromboembolism, HIV-associated vasculopathy (i.e. atherosclerosis, non-atherosclerosis, vasculitis and small vessel disease) and anti-phospholipid syndrome were applied and the final diagnoses was then determined using the hierarchical algorithm I developed in Chapter 4.

5.3.4 Follow-up

At the point of in-patient death or discharge, at 3 months and at 6 months, the modified Rankin score [mRS] was recorded using validated structured questions (Janssen et al. 2010). The modified rankin scale classifies the level of disability and death in stroke patients where 0=no symptoms, 1=no significant disability, 2=slight disability, 3=moderate disability, 4=moderately severe disability 5=severe disability and 6=death (Janssen et al. 2010). The assessment was performed over the phone at 3 months and in person at 6 months. Because mobile phone use was widespread in Malawi I used this as a way to communicate with the patients. I obtained two numbers (the second usually being another family member or neighbour) to maximise ascertainment at follow-up. Patients were called up to 5 times on a weekly basis with text message communication at the point of follow-up. If unsuccessful, they were classified as lost-to-follow-up after this intensive period of ascertainment. Death was confirmed in hospital or by verbal notification (over the phone) by the patient's guardian, relative or neighbour.

5.3.5 Statistical analysis

This case series was largely descriptive. Clinico-pathological information was described in detail for 2 patients and clinico-radiological for 3. Univariate analysis was performed to identify significant differences between the clinical characteristics and outcome of ischaemic HIV and non-HIV patients, stratified by age and the different aetiologies. Continuous variables were compared using the Student t-test or Mann-Whitney U test and categorical variables were compared by Pearson Chi-Squared and Fisher Exact tests. After checking that the assumption of proportionality was satisfied, a cox proportional hazard model was used to assess the association of HIV characteristics (untreated infection, <6 months treatment, ≥6 months treatment and advanced immunosuppression; <200cells/mm^3) and death within 6 months for the survivors of stroke patients post-discharge. The following variables were included in the model: HIV characteristics, NIHSS classification, gender and age at stroke onset. All patients were censored at 6 months of follow-up or 13th October 2012 for those that were missing. Missing covariates were included in the analysis by creating missing value categories. I reported hazard ratios with 95% CIs. The data were analysed with STATA version 11.2 and GraphPad Prism version 6, GraphPad Software Inc., California, USA. Two-sided values of $P \leq 0.05$ was considered statistically significant.

5.4 Results

I identified 300 suspected stroke patients. Two-hundred and sixty out of 300 (87%) had an MRI scan. I excluded 48 (16%) patients with a stroke mimic, the aetiology was unknown in 27 (56%), other causes found included brain tumours 7 (15%), old strokes 5 (10%), toxoplasma 3 (6%), subdural haemorrhage 2 (4%), meningitis 2 (4%), tuberculoma 1 (2%) and an inflammatory disorder 1 (2%). For the overall stroke cohort; an MRI confirmed 172 ischaemic strokes, 45 haemorrhagic and 35 unknowns. The ischaemic

stroke subtype was predominantly lacunar infarct (72; 42%), followed by partial anterior circulation infarct (51; 30%), total anterior circulation infarct (42; 24%) and posterior circulation infarct (8; 4%). Sixty-three (37%) of the ischaemic strokes were severe (NIHSS ≥14). The median age was 60 years (Interquartile range; 43, 70) and 117/252 (46%) were male. In-patient mortality was 43/252 (17%) and the cumulative mortality for survivors post discharge was 51/209 (24%) at 6 months. Thirty-nine out of 209 (19%) were lost to follow-up.

Seventy-eight of the 252 (31%) confirmed stroke patients were HIV positive. Of these MRI brain confirmed 64 ischaemic strokes,9 haemorrhagic and 5 did not have a scan. The median age was 41 years (IQR; 32, 54) and 36 (46%) were male. Thirty-four (44%) were on cART, of these, 19 had started within 6 months. The median CD4+ T-lymphocyte count was 192 cells/mm^3 (IQR; 66,342). The ischaemic stroke subtype was predominantly lacunar infarct (26; 40%), followed by partial anterior circulation infarct (19; 29%), total anterior circulation infarct (16; 25%) and posterior circulation infarct (4; 6%). Twenty out of 64 (31%) of the ischaemic stroke were severe (NIHSS ≥14). In-patient mortality was 13/78 (17%), the cumulative mortality for survivors post discharge was 13/65 (20%) at 6 months. Seventeen out of 65 (26%) were lost to follow-up.

5.4.1 Phenotype of HIV related stroke

Being a young stroke patient was strongly associated with HIV infection (median 41 years versus 65 years in the HIV negative cohort; p <0.001). Ischaemic stroke type was predominant in HIV infected individuals compared with HIV negatives (p=0.025). Although haemorrhagic stroke was less frequent, the burden increased with age and had similar proportions to the non-HIV participants from 55 years

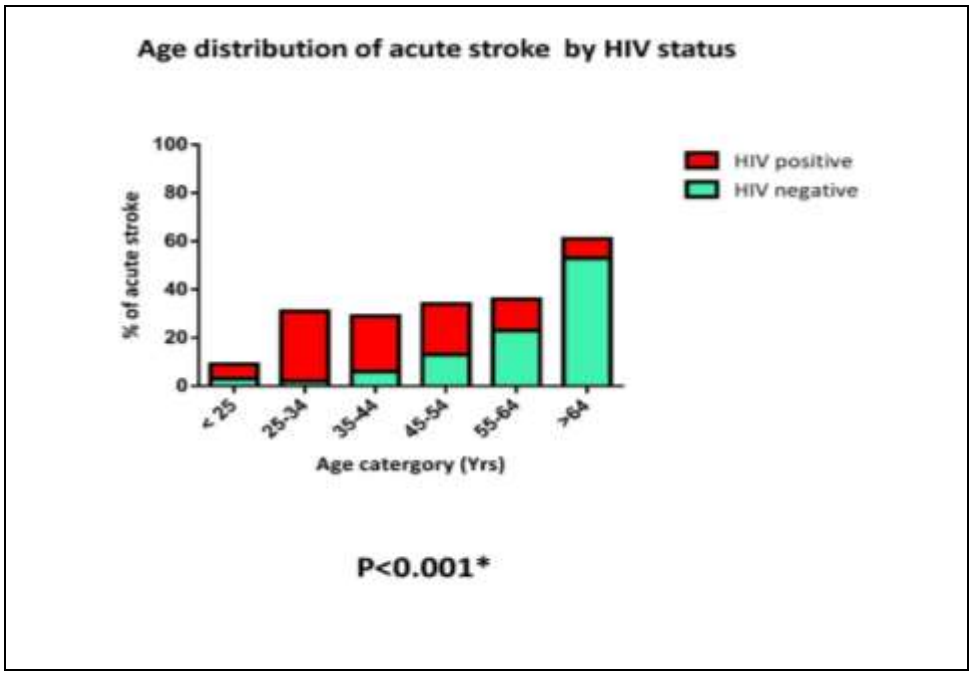
Figure 5:1. There were no significant differences in subtype and severity of HIV reated ishcaemic stroke compared to the equivalent HIV negative cohort. The aetiology differed substantially between the HIV positive and negative stroke patients (p<0.001). The causes of HIV stroke was defined by the TOAST classification. The aetiologies were less diverse in the younger age-bands (e.g. other determined cause predominated). This transitioned to a more heterogeneous spectrum from age 45 years and included large atherosclerosis, cardio-thromboembolism, small vessel disease and undetermined cause. In the non-HIV stroke cohort, undetermined aetiology predominated Figure 5:1. The prevalence of cardiovascular risk factors in HIV related stroke participants were hypertension 28 (42%), diabetes 2 (3%), hypercholesterolaemia 5 (8%), smoking 6 (9%), acute infection 12 (18%), peripheral vascular disease 8 (12%), heating houses with wood 10 (15%) and abdominal obesity 26 (40%). Only heating houses with wood was significantly associated with HIV related stroke (15% versus 10%; 0.027), while hypertension, diabetes and smoking were significantly associated with non-HIV stroke. There were no significant differences in the prevalence of peripheral vascular disease in HIV positive and negative participants (12% versus 13%; p=0.384).

5.4.2 Aetiology of ischaemic HIV related stroke

The aetiology of HIV related ischaemic stroke was based on case definitions of confirmed or probable certainty Table 5:1. I described several aetiologies; HIV-associated vasculopathy 34 (53%), opportunistic infection 18 (28%), anti-phospholipid 7 (10%) and cardio-thromboembolism 5 (8%) Table 5:2. There was a trend towards an association in the age distribution for the different aetiologies (p=0.066); those with opportunistic infection were younger (median age 35) than those with HIV-associated vasculopathy, anti-phospholipid syndrome and cardio-thromboembolism (median age 41, 43 and 54 respectively). There was no association between the different ischaemic aetiologies and gender, having additional

symptoms to a focal neurological deficit, severity of stroke, full blood count, in-patient mortality and death within 6 months Table 5:2. APS and opportunistic infections were associated with a lower CD4+ T-lymphocyte count compared with the other aetiologies, and a significantly higher proportion were untreated (71% and 72% respectively) compared to those with HIV-associated vasculopathy and cardio-thromboembolism (47% and 60% respectively) Table 5:2.

A



B

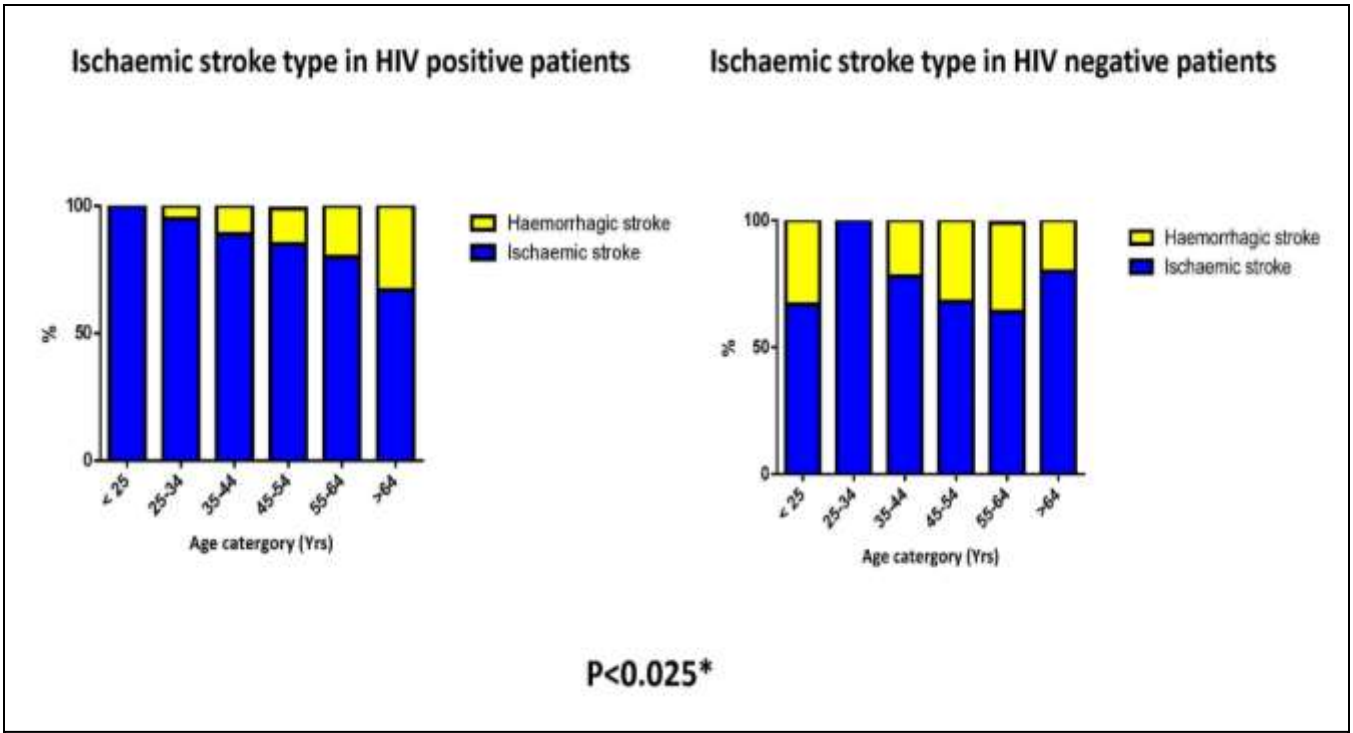
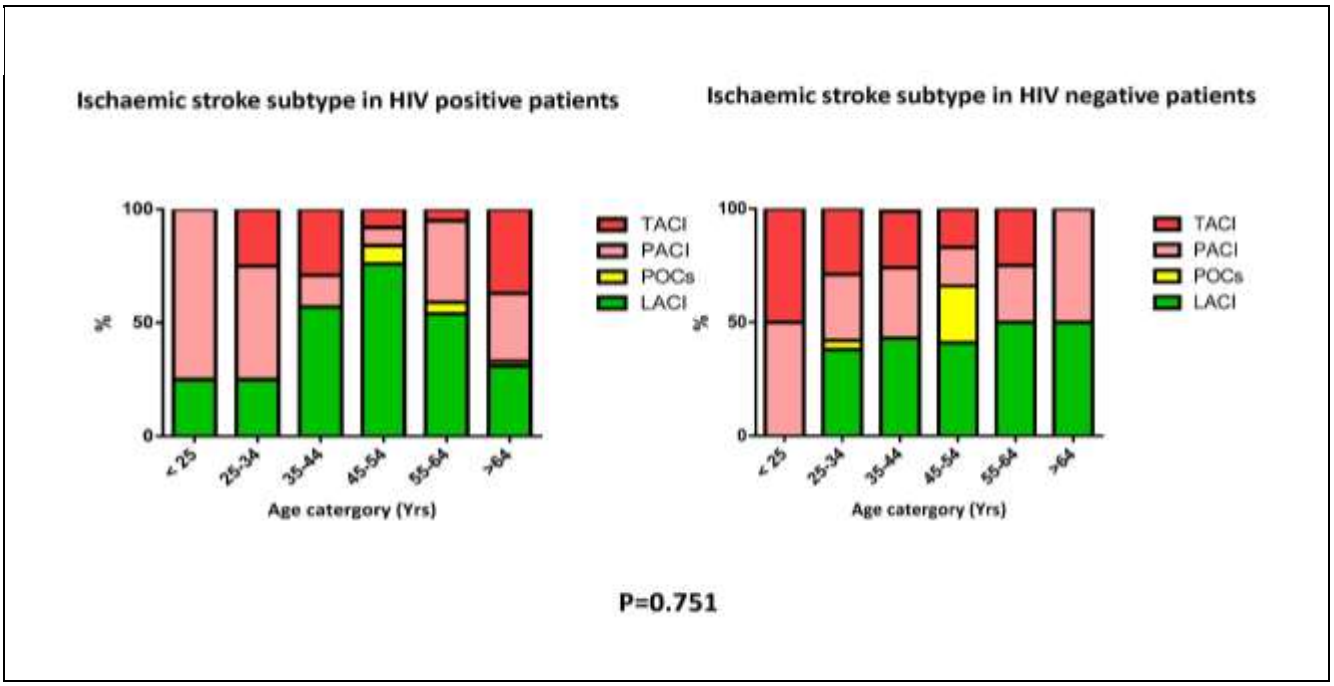


Figure 5:1: Describes the age distribution (Image A) and stroke type stratified by HIV positive status (Image B)

A



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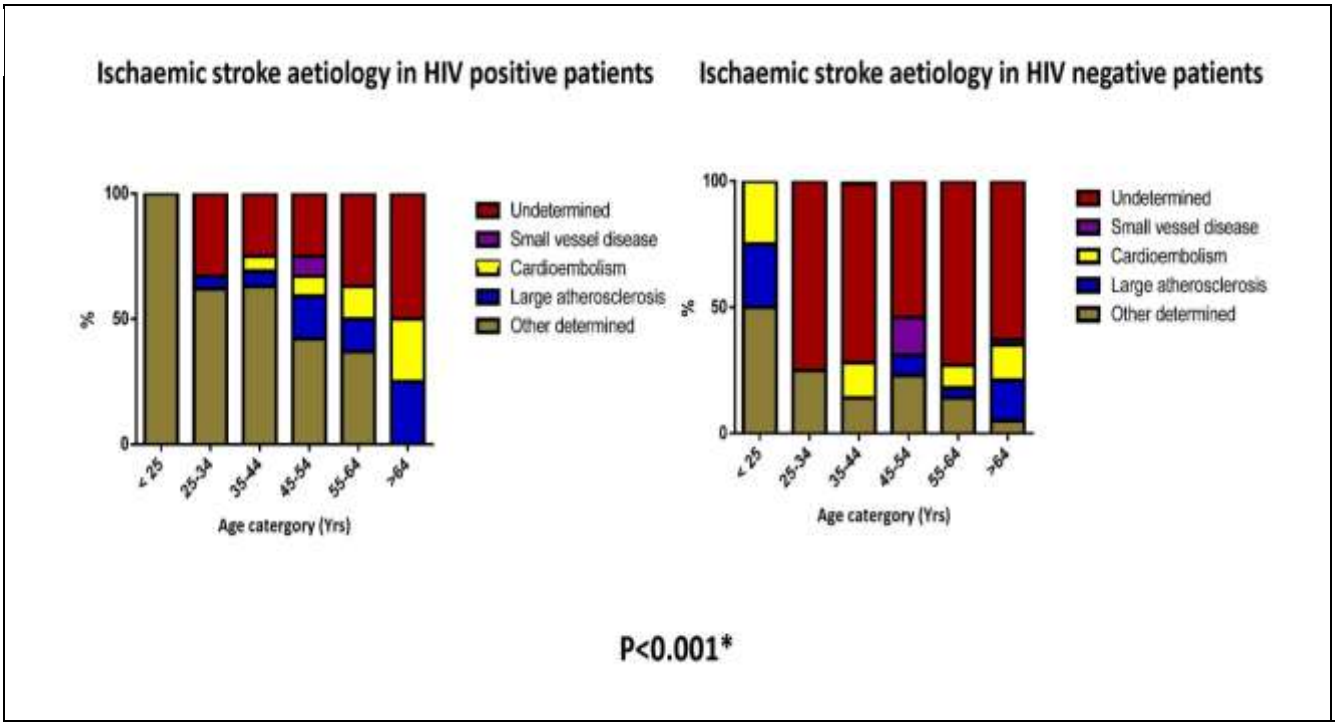


Figure 5:2: Describes the subtype (image A) and aetiology (Image B) of ischaemic stroke stratified by HIV positive status.

Stroke subtype was defined by the Oxford Community Stroke Project classification. Aetiology of stroke was defined by TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification. TACI – Total anterior circulation infarct, PACI – Partial anterior circulation infarct, POC – Posterior circulation infarct, LACI – Lacunar infarct.

Table 5:1: Aetiologies of HIV ischaemic stroke and their degree of certainty (n=64)

Aetiological classification	Sub-classification	No.	Certainty of diagnosis
HIV-associated vasculopathy	Atherosclerosis [AS] (6) Non-Atherosclerosis [Non-AS](7) Small vessel disease [SVD] (2) Vasculitis [VA] (8) Undetermined disease [UD](11)	34	Confirmed AS -3, Non-AS-4, SVD-2, Probable AS-3, Non-AS-3, VA-8
Opportunistic infection	Varicella Zoster Virus [VZV] (9) *Mycobacterium* Tuberculosis [MTB] (5) Syphilis (4)	18	Confirmed VZV-9, MTB-1 Probable MTB-4, Syphilis-4
Cardioembolism	-	5	Confirmed -5
APS	-	7	Probable-7

AS-Atherosclerosis, Non-AS - Non-Atherosclerosis, SVD - Small vessel disease, VA – Vasculitis, UD - Undetermined disease. (5) Inconclusive aetiology, (9) Haemorrhagic stroke.

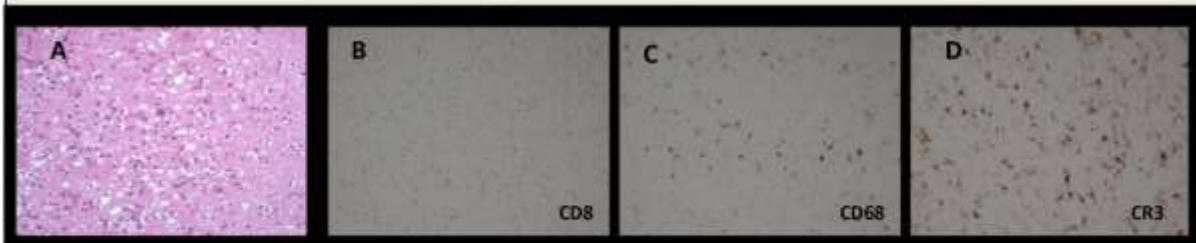
Table 5:2: Clinical features and outcome for the different aetiologies of HIV positive iscahemic strokes

	HIV-associated vasculopathy (n=34)	Opportunistic infection (n=18)	Anti-phospholipid syndrome (n=7)	Cardio-thromboembolism (n=5)	P value
Median age (IQR)	41 (31,50)	35 (28,40)	43 (32,60)	54 (42,62)	0.066
Male (%)	18 (53)	7 (39)	4 (57)	2 (40)	0.732
Not on cART (%)	16 (47)	13 (72)	5 (71)	3 (60)	0.019*
CD4+ T-lymphocyte count	221 (90,323)	133 (55,266)	89 (63,120)	302 (240,558)	0.038*
Additional symptoms to focal neurological deficit:					
Seizure	0	0	0	0	-
GCS ≤ 8	8 (25)	3 (17)	1 (14)	3 (60)	0.218
Headache	15 (48)	9 (52)	5 (71)	2 (40)	0.680
Fever	5 (15)	5 (28)	1 (17)	0	0.479
Severity of stroke (Median NIHSS)	12 (8,16)	13 (8,16)	10 (7,12)	9 (8,11)	0.369
Blood result					
Median white cell count	5 (4,6)	6 (4,6)	5 (4,5)	5 (5,6)	0.735
Median Haemaglobin	13 (10,14)	12 (10,13)	10 (9,12)	13 (12,14)	0.299
Median Platelet	257 (183,317)	223 (182,297)	258 (211,299)	281 (203,333)	0.702
CSF result					
Median white cell count	0 (0,2)	0	0	0 (0,2)	0.458
Median red cell count	3 (0,23)	12 (0,200)	0 (0,80)	45 (7,5800)	0.141
Median glucose	3.1 (2.8,3.9)	2.6 (1.6,3.2)	2.6 (2.3,3.1)	3.2 (2.9,3.7)	0.035*
Median protein	0.7 (0.4,0.9)	0.8 (0.5,2.5)	0.6 (0.6,1.4)	0.4 (0.3,0.7)	0.212
Outcome of stroke					
In-patient mortality	5 (15)	3 (17)	1 (14)	1 (20)	0.990
Survivors post discharge					
Cumulative mortality at 6 months	11 (32)	7 (39)	3 (43)	2 (40)	0.661
Missing data	7 (21)	6 (33)	1 (14)	0	

5.4.3 HIV-associated vasculopathy

This term encompasses several mechanisms and the frequency of the different types among those with ischaemic stroke were atherosclerosis 6/34 (18%), non-atherosclerosis 7/34 (20%), vasculitis 8/34 (24%), small vessel disease 2/34 (6%), undetermined 11/34 (32%). I had limited access to HIV stroke deceased brain for histopathological examination. However, those that were available showed distinct mechanisms of HIV-associated vasculopathy. Some illustrative material from individual patients is described here Figure 5:4, Figure 5:5, Figure 5:6. For example Patient 218 was found at autopsy to have a diagnosis of extensive atherosclerosis, despite his relatively young age (age 50 years). He also had borderline elevated hypercholesterolaemia Figure 5:4. For Patient 219 the cause of her stroke, which was exacebated 5 days after starting cART, was unknown; based on her admission clinical features and progress. However at autopsy, she was found to have an extensive HIV-associated vasculitis in the absence of background HIV encephalitis or other infections on histopathological examination Figure 5:5. In three patients, one with undetermined and two with a non-atherosclerosis diagnoses, I demonstrated evidence of extra-cranial with or without intra-cranial disease from MRI imaging and duplex carotid doppler Figure 5:6.

Patient 218: HIV-associated vasculopathy (Atherosclerosis)

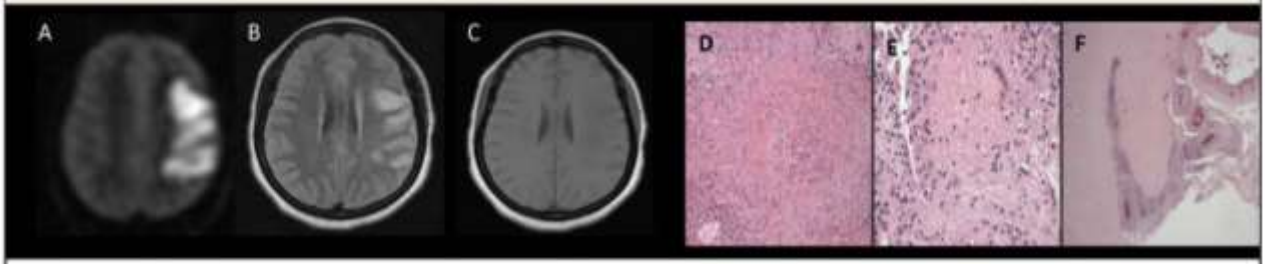


A 50-year old male presented with an acute right hemiparesis and dysphasia. He was diagnosed with HIV infection 1 year ago and had been on cART for 7 months. He was a non-smoker. His NIHSS on admission was 28. His vital signs were within the normal ranges. There was no evidence of diabetes, hypertension or peripheral vascular disease. He had a normal full blood count and a CD4+ T-lymphocyte count of 192cells/mm^3 on admission. His random cholesterol was 6.0mmol/L. Blood screen for syphilis was negative and there was no evidence of anti-phospholipid syndrome. CSF examination revealed no CSF white or red cells, CSF glucose was 4.23 mg/L, and he had an elevated CSF protein of 0.9mg/L. CSF bacterial, TB culture, VZV IgG index and crytococcal antigen were negative. On day 4 of admission he became drowsy and febrile. He had an increased respiratory rate and was producing copious secretions. He was started on antibiotics for a presumed aspiration pneumonia. He died on day 5 (before an MRI scan). He had an acute infarction on histopathology examination (Image A) and evidence of arteriosclerosis in all sized vessels , in both left and right cerebral hemisphere. There was no evidence of underlying encephalitis, CD8, CD68 and CR3 were negative (Images B-C).

Figure 5:3: Patient 218 case report

NIHSS – National Institutes of Health Stroke Scale, CXR – chest radiograph, MRI – magnetic resonance imaging, CSF – cerebrospinal fluid, TB – Mycobacterium Tuberculosis VZV – varicella zoster virus, CNS cART – combined antiretroviral therapy, IgG – immunoglobulin.

Patient 219: HIV-associated vasculopathy (undermined → vasculitis after histopathology examination)

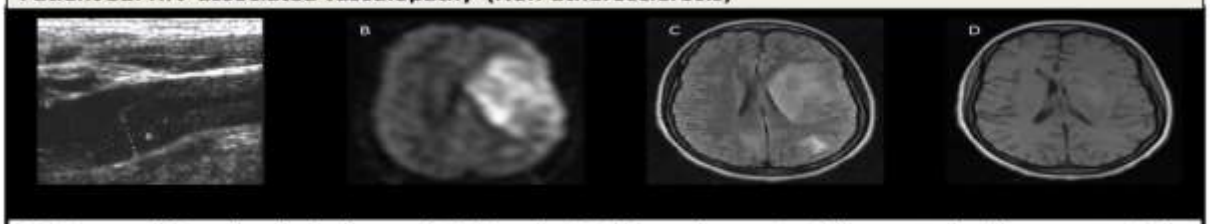


A 32-year old HIV untreated pregnant (5 months) female had a sudden right arm monoparesis and dysphasia. She had a 3 month history of progressive headache but no associated vomiting, neck stiffness or fever. She was not on cART. She had an NIHSS of 12 on admission. There was no evidence of hypertension, diabetes, high cholesterol or peripheral vascular disease. She had a normal CXR, normal full blood count and her CD4+ T-lymphocyte count was 175 cells/mm^3. Blood screen for syphilis was negative and there was no evidence of anti-phospholipid syndrome. MRI images were taken on day 6 and included: DWI (Image A), T2 axial (Image B) and T1 axial (Image C) sequences, all images confirmed an acute left temporal and parietal lobe infarct. Examination of her CSF revealed the following; 10 white cells/mm^3 , 290 red cells/mm^3, glucose 2.38 mg/L and protein 1.6mg/L. CSF bacterial, TB culture, VZV IgG index and crytococcal antigen were negative. There was no cardioembolism or carotid stenosis. She was started on TB therapy with high dose steroids on day 5 of admission on the basis of the high CSF protein. Her arm weakness improved but on day 10, she developed new weakness of her left leg and was unable to walk, the dose of steroid was increased (for a presumed paradoxical worsening of CNS TB infection), she responded immediately to this and by day 24 her symptoms had resolved and she was mobile. She was started on cART on day 25. On day 30 she became apathetic and on day 31 she had a seizure and died. Histopathology examination showed florid vasculitis (Image D – low power) with associated giant cells (Image E – high power) and enteritis obliterans (Image F- high power) involving large medium and small vessels. There was no evidence of TB infection (Ziehl-Neelsen stain was negative) or atherosclerosis. CD68, CR3/43 and CD8 stains were negative.

Figure 5:4: Patient 219 case report

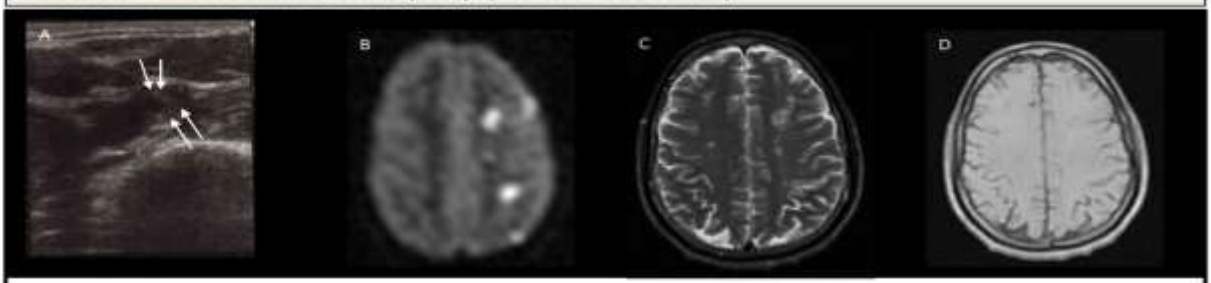
NIHSS – National Institutes of Health Stroke Scale, CXR – chest radiograph, MRI – magnetic resonance imaging, DWI-diffusion weighted imaging, T1 – T1 weighted imaging, T2 – T2 weighted imaging, CSF – cerebrospinal fluid, TB – Mycobacterium Tuberculosis VZV – varicella zoster virus, CNS – central nervous system, cART – combined antiretroviral treatment, IgG – immunoglobulin.

Patient 32: HIV-associated vasculopathy (Non-atherosclerosis)



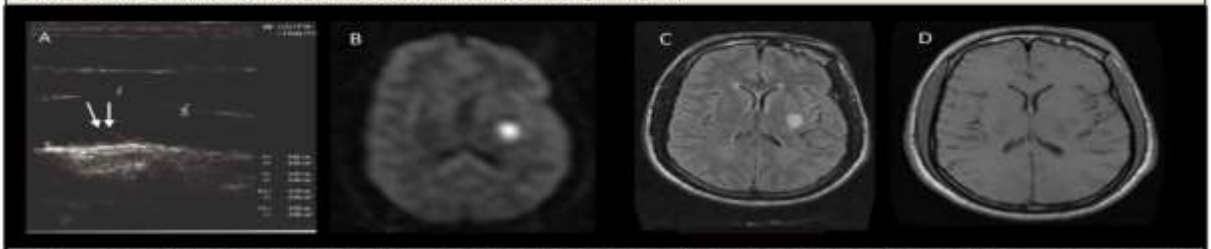
A 17-year old female who had recently initiated cART (3 weeks previously) presented with an acute right hemiparesis, dysphasia and an homonymous hemianopia. There was no associated head, neck or facial pain. She had an NIHSS of 22 on admission. There was no evidence of hypertension, diabetes, high cholesterol or peripheral vascular disease. She had a normal CXR, normal full blood count and her CD4+ T-lymphocyte count was 305 cells/mm^3. Blood screen was negative for syphilis but positive for ACL and β2-GP1. Examination of her CSF revealed the following; 0 white cells/mm^3 , 0 red cells/mm^3, glucose 2.48 mg/L and protein 0.2mg/L. CSF bacterial, VZV IgG index, TB culture and crytococcal antigen were negative. Duplex carotid doppler (Image A) showed concentric stenosis (≥70% stenosis) of the left common carotid artery extending into the bulb (longitudinal view). MRI images were taken on day 7 of the index stroke and included the following sequences, DWI (Image B), T2-axial (Image C) and T1-axial (Image D), these confirmed an acute left cortical infarct. She was discharged walking independently with an mRS of 2 on day 20 of her presentation, she continued with her cART regimen and started on aspirin. Using the hierarchical algorithm she was assigned a diagnosis of non-atherosclerosis HIV-associated vasculopathy. She died within 3 months.

Patient 82: HIV-associated vasculopathy (Non-atherosclerosis)



A 37-year-old untreated HIV positive male presented with an acute right hemiparesis and headache but no neck or facial pain. He had an NIHSS 3 on admission. His CD4+ T-lymphocyte count was 271cells/mm^3. His full blood count and CSF examination was unremarkable. There no was evidence of TB, VZV and syphilis nor anti-phospholipid syndrome. ECG and cardiac ECHO were normal. Duplex carotid doppler showed concentric stenosis of ≥50% stenosis, involving the left internal carotid artery (Image A- transverse view). MRI images were taken on day 6 of the index stroke and included the following sequences, DWI (Image B), T2-axial (Image C) and T1-axial (Image D), these confirmed acute left hemisphere multi-focal infarcts. He was discharged with an mRS of 1 and started on cART within one month of discharge. His mRS at 3 months and 6 months was 0 and 1 respectively.

Patient 200: HIV-associated vasculopathy (Undetermined)



A 42-year-old untreated HIV positive male had an acute right hemiparesis and dysarthria, and was newly diagnosed with hypertension. He had an NIHSS 9 of on admission. His CD4+ T-lymphocyte count was 224cells/mm^3 . Full blood count and CSF examination was unremarkable. There was no evidence of opportunistic infection or anti-phospholipid syndrome. Duplex carotid doppler (image A) showed eccentric wall thickening (arrow – the stenosis was <50%) of the left common carotid artery (longitudinal view). MRI images were taken on day 5 of the index stroke and included the following sequences, DWI (Image B), T2 axial (Image C) and T1 axial (Image D), these confirmed an acute left subcortical infarct. He was walking independently with mild symptoms and had an mRS of 1 at discharge. He was started on cART within one month of discharge. His mRS was stable at 3 and 6 months of follow-up.

Figure 5:5: Case reports of HIV-associated vasculopathy with extra-cranial manifestation
NIHSS – National Institutes of Health Stroke Scale, CXR – chest radiograph, MRI – magnetic resonance imaging, DWI-diffusion weighted imaging, T1 – T1 weighted imaging, T2 – T2 weighted imaging, CSF – cerebrospinal fluid, TB – Mycobacterium Tuberculosis VZV – varicella zoster virus, CNS – central nervous system, cART – combined antiretroviral therapy, mRS – modified rankin score, ACL – anticardiolipin antibody, β2 –GP1 – Beta 2 glycoprotein 1.

5.4.4 Clinical features in patients who recently initiated cART

Although the spectrum of the final diagnoses in those who recently initiated cART was diverse, the majority 8/15 (53%) had HIV-associated vasculopathy. Only 4/15 (27%) had opportunistic infections. Twelve out of 15 (80%) were on stavudine based cART regimens. Arguably, the level of immunosuppression was variable but the majority were immunosupressed; the median CD4+ T-lymphocyte count was 113 (Interquartile range; 63, 218). Ten out of 15 (67%) had initiated cART within a month and most, 11/15 (73%) died (8) or were missing (3) by six months Table 5:3.

Table 5:3: Clinical features of 15 patients with an ischaemic stroke that recently initiated cART

ID	Age	Sex	Approximate date of stroke	cART initiated within a month of index stroke	On Stavdine based regimen	CD4+ count	NIHSS score on admission	CSF characteristics	Final diagnosis	Outcome
21	50	M	Mar 2011	Yes	Yes	15	27	WCC 0 cells/mm3, RCC 30 cells/mm3, glucose 2.89mg/L, protein 0.75mg/L	HIV-associated vasculopathy – small vessel disease	Died in hospital
32	17	F	Apr 2011	Yes	Yes	305	22	WCC 0 cells/mm3, RCC 0 cells/mm3, glucose 2.48 mg/L, protein 0.2mg/L	HIV-associated vasculopathy – confirmed non-atherothrombosis	mRS 4 at discharge, died at 3 months
46	42	M	Apr 2011	Yes	Yes	12	18	WCC 26 cells/mm3, RCC 15 cells/mm3, glucose 3.07 mg/L, protein 0.69 mg/L	HIV-associated vasculopathy – probable vasculitis	mRS 3 at discharge, died at 3 months
50	52	F	Apr 2011	Yes	Yes	133	12	WCC 0 cells/mm3, RCC 0 cells/mm3, glucose 3.04 mg/L, protein 0.84 mg/L	Opportunistic infection – confirmed VZV	mRS 4 at discharge, died at 3 months
75	30	M	May 2011	Yes	Yes	50	13	WCC 640 cells/mm3, RCC 0 cells/mm3, glucose 1.37mg/L, protein 1.55mg/L	Opportunistic infection – probable TB	mRS 4 at discharge, mRS 3 at 3 months, missing at 6 months
85	31	M	Jun 2011	Yes	Yes	82	13	WCC 0 cells/mm3, RCC 15 cells/mm3, glucose 2.90mg/L, protein 0.95mg/L	HIV-associated vasculopathy – probable vasculitis	mRS 3 at discharge, missing at 3 and 6 months
87	42	F	Jun 2011	No	Yes	362	12	WCC 0 cells/mm3, RCC 10 cells/mm3, glucose 4.13 mg/L, protein 0.36 mg/L	Cardio-thromboembolism	mRS 4 at discharge and mRS 1 at 6 months
118	62	F	Aug 2011	Yes	-	63	10	WCC 0 cells/mm3, RCC 20 cells/mm3, glucose 2.65mg/L, protein 0.9 mg/L	Antiphospholipid syndrome	mRS 3 at discharge, died at 3 months

ID	Age	Sex	Approximate date of stroke	cART initiated within a month of index stroke	On Stavdine based regime	CD4+ count	NIHSS score on admission	CSF characteristics	Final diagnosis	Outcome
129	34	F	Aug 2011	Yes	Yes	128	27	WCC 0 cells/mm3, RCC 8 cells/mm3, glucose 2.54 mg/L, protein 16mg/L	Opportunistic infection – confirmed TB	Died in hospital
190	29	F	Nov 2011	Yes	Yes	88	12	WCC 0 cells/mm3, RCC 3 cells/mm3, glucose 2.49 mg/L, protein 0.98mg/L	HIV-associated vasculopathy – probable vasculitis	mRS 3 at discharge, died at 6 months
215	39	M	Dec 2011	No	Yes	92	13	WCC 15 cells/mm3, RCC 44 cells/mm3, glucose 1.91 mg/L, protein 1.38 mg/L	HIV-associated vasculopathy – probable vasculitis	mRS 2 at discharge and 0 at 6 months
220	32	F	Dec 2011	No	Yes	156	12	WCC 0 cells/mm3, RCC 0 cells/mm3, glucose 3.21mg/L, protein 0.37 mg/L	HIV-associated vasculopathy – undetermined	mRS 3 at discharge and 0 at 6 months
268	52	M	Mar 2012	No	Yes	113	7	WCC 0 cells/mm3, RCC 80 cells/mm3, glucose 0.56 mg/L, protein 1.35 mg/L	Anti-phospholipid syndrome	mRS 3 at discharge, died at 3 months
278	45	M	Mar 2012	Yes	-	218	17	WCC 0 cells/mm3, RCC 0 cells/mm3, glucose 3.7mg/L, protein 0.51mg/L	HIV-associated vasculopathy – confirmed non-atherothrombosis	mRS 4 at discharge and 4 at 6 months
293	27	F	Apr 2012	No	-	623	14	WCC 0 cells/mm3, RCC 800 cells/mm3, glucose 3.08 mg/L, protein 0.7 mg/L	Opportunistic infection – probable syphilis	mRS 3 at discharge, missing at 3 and 6 months

M – male, F – female, ID – patient identifier, WCC – white cell count, RCC – red cell count TB – Mycobacterium Tuberculosis VZV – varicella zoster virus, , cART – combined antiretroviral treatment, mRS – modified rankin score, CD4+ count – CD4+ T-lymphocyte count, "-" missing.

5.4.5 HIV characteristics that are predictors of mortality within 6 months

After adjusting for age, sex and severity of disease, advanced immunosuppression (CD4+ T-lymphocyte count <200cells/mm3) was a significant predictor of death within 6 months in those with ischaemic stroke (adjusted hazard ratio [aHR] 8.22 [1.85, 36.5]. Although being on cART for ≥6 months showed trends towards predicting death within 6 months, it was not significant Figure 5:7. Over 85% of newly diagnosed HIV positive stroke survivors were initiated on cART within a month of discharge. Interestingly, untreated HIV positive patients who survived a stroke, showed trends towards a favorable outcome at 6 months (HR 0.37 [0.06, 2.14]). However, ten out of 32 (31%) were missing at 6 months. The aetiology of this missing individuals included (4) HIV-associated vasculpathy, (4) opportunistic infection (1) antiphospholipid syndrome (1) inconclusive Figure 5:7.

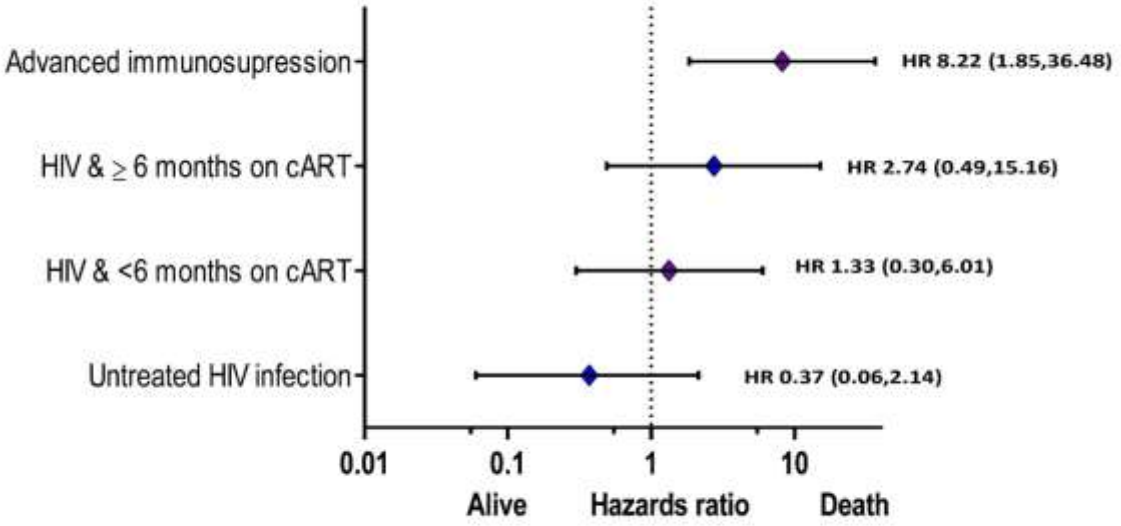


Figure 5:6: HIV characteristics that predict death among discharged survivors of ischaemic stroke

Advanced immunosuppression was defined as a CD4+ T-lymphocyte count <200 cells/mm^3. Loss to follow-up was found in the following categories: 6/25 advanced immunosuppression, 1/8 among participants on cART for ≥ 6 months, 3/13 among those on cART for < 6 months, and 10/32 untreated HIV infected participants.

5.5 Discussion

HIV related stroke is an emerging disease and threatens to worsen the increasing burden of stroke in low and middle income countries. Current knowledge about the mechanisms of disease in those initiating cART and the transition of the different phenotypes as the HIV population ages is uncertain. Importantly, I have shown that only a third of stroke patients that had been initiated on cART in the first 6 months prior to developing a stroke had opportunistic infections, most were on stavudine based therapy and most died within 6 months of their stroke. My work further characterises the spectrum of the different aetiologies in HIV positive and negative stroke patients and shows that the aetiologies are distinct in the young but converges from the age of 45 years.

In chapter 3, I described the association with recent cART initiation and ischaemic stroke. The data in this chapter gave some understanding about the possible mechanisms, and prognosis: Opportunistic infections occurred in less than a third and although HIV-associated vasculopathy was the most common aetiology, this phenotype needs better characterisation. Furthermore, most of these patients died within 6 months of their stroke. A large cohort study in Uganda evaluating adverse events in those initiating cART reported a high mortality of 18% in the first year, but without routine neuroimaging of these participants, it was not clear how much of this can be attributable to stroke (Castelnuovo et al. 2009). I also found that the timeframe from starting cART to developing a stroke was within one month. Uncontrolled or interrupted HIV infection is associated with increased levels of tissue factor, interlukin-6, D-dimer and nonclassic [CD14(+) CD16(++)] and classic [(CD14++) CD16(+) monocyte activation. Non-HIV infected individuals with acute vascular events also have similar activation profiles (Kuller et al. 2008; Funderburg et al. 2012). It is conceivable that starting cART in a subpopulation of HIV positive

individuals exacerbates this process. There is also the possibility of other simultaneous or independent mechanisms; namely nucleotide reverse transcriptase inhibitor (i.e. stavudine) toxicity.

The concept that starting cART exacerbates an ongoing pro-inflammatory state in a subgroup of individuals was supported by the case report of the young woman who improved from a stroke whilst on anti-inflammatory therapy. She developed worsening symptoms and consequently died soon after starting cART, histopathological examination of the brain confirmed a florid HIV-associated vasculitis, her MRI prior to commencing HIV treatment showed a uni-focal lesion. It is possible that a previously localised HIV-associated vasculitis was exacerbated by starting cART. Whereas the case of the 50-year-old man on HIV treatment for ≥ 6 months, supports the concept of an accelerated atherosclerosis induced by chronic HIV infection. He also had borderline hypercholesterolaemia. Progressive hypercholestrolaemia can occur rapidly on stavudine based cART, as shown by the 9% increase in prevalence between 12-24 months of routine surveillance in Malawi and thus an additive risk of stroke in this population (van Oosterhout et al. 2012).

Stroke is not the only vascular entity that has been linked with HIV infection. Recently, in a large USA based cohort, HIV infection was associated with a 50% increased risk of acute myocardial infarction after taking account of recognised vascular risk factors (Freiberg et al. 2013). Peripheral vascular disease was not uncommon in the HIV positive participants. I would have expected a significantly higher prevalence in the older non-HIV stroke cohort but instead, I showed no significant difference compared to the young HIV stroke patients; this may indicate a link with HIV infection.

As this study was hospital based, our data were limited for individuals with mild strokes and silent infarcts, who were less likely to come to hospital. The latter could be especially relevant to HIV infection,

as a previous review of a large series of autopsies on brains of HIV positive people showed evidence of widespread small vessel ischaemia in an asymptomatic cohort (Connor et al. 2000). Mild strokes and silent infarcts are associated with subtle physical and cognitive deficits; the latter may be relevant to the increased prevalence of mild to moderate HIV-associated neurocognitive impairment in the cART era (Vermeer et al. 2007; Schouten et al. 2011). I was unable to explore the role of HIV co-infection with other chronic infections such as hepatitis B and C and although hepatitis C is associated with stroke risk, hepatitis B is not (Sung et al. 2007; Lee et al. 2010). However, HIV and hepatitis C co-infection rates are low in Malawi (0.1%) and thus unlikely to have had an impact on our findings (Chasela et al. 2012). Although we showed a favourable trend between untreated infected stroke patients and outcome at 6 months, the high loss to follow-up in this cohort, could have substantially underestimated this risk.

In conclusion, in this chapter I found a variety of aetiologies associated with HIV infection. Importantly, less than a third of HIV related strokes were due to opportunistic infections. Although HIV-associated vasculopathy was the predominant aetiology, this cohort needs to be further characterised. HIV infection may accelerate the typical causes of stroke found in the non-HIV infected population. Furthermore, the potential impact of HIV treatment and the additive effect of other vascular risk factors as the HIV population ages is yet to be realised. It is possible that in a subpopulation of HIV infected individuals, initiating cART exacerbates an ongoing pro-inflammatory state however; a better understanding of this subpopulation is urgently needed to guide appropriate interventions.

6 Overall discussion

6.1 The epidemiology of HIV infection and stroke

The high incidence of young stroke (age ≤45years) in HIV endemic countries has been largely attributed to hypertension but my work has shown that HIV infection also has a role in this population. Because HIV infection is frequently omitted when assessing the global burden of stroke, it has been challenging to quantify the true impact of HIV infection on cerebrovascular disease risk (O'Donnell et al. 2010; Feigin et al. 2013). This also occurs at the hospital level, for example, in a UK based hospital, a 22 year-old man was extensively investigated for cryptogenic stroke and it was only on day 12 that a positive HIV result was realised (Benjamin et al. 2009). A concerted effort from both the physician and the researcher is essential to address the burden of HIV related stroke. Work from my thesis supports the idea of routine HIV testing in young stroke patients, especially in regions with a prevalence of more than 2 in 1000. Furthermore, requesting appropriate brain imaging (e.g. MRI brain) to confidently exclude mimics of stroke, and including HIV infection as a measurable index for stroke surveillance studies will also help (British HIV Association 2008) (Chapter 2). Without these simple changes to medical practice the true extent of HIV related stroke will not be fully realised.

My findings are consistent with large retrospective datasets in Europe and USA linking HIV infection with stroke (Ovbiagele et al. 2011; Rasmussen et al. 2011). Although the association of HIV infection and stroke is not restricted to sub-Saharan Africa, other possible exacerbating factors like the global variation in HIV subtype (e.g. predominantly subtype A,C, D in Africa and B in USA and Europe) and the effect of other prevalent infectious diseases that are known to exacerbate atherogenesis, cannot be

completely ruled out of contributing to the increased burden of stroke in sub-Saharan Africa (Elkind et al. 2010).

The phenotype of HIV related stroke in industrialised countries is distinct from my cohort; patients are less likely to be immunosuppressed, older than our described cohort but younger than the non-HIV stroke population and most are on cART (Ovbiagele et al. 2011; Rasmussen et al. 2011) (Chapter 1). This global variation is likely to reflect a shift in the landscape of HIV related stroke; involving a transition from non-atherosclerosis HIV-associated vasculopathy, HIV-associated vasculitis and an opportunistic infection vasculitis in the young, to an accelerated atherosclerosis in an aging population. Exposure to metabolic disorders associated with cART and the overlap of established vascular risk factors in the older populations may also exacerbate the latter. In the USA, the rate of older people living with HIV infection is rapidly increasing and is estimated to be double by 2015; recent modeling using South African data suggests that the number of those over 50 years of age will nearly double in the next 30 years (Hontelez et al. 2011; Greene et al. 2013).

6.2 Clinical implication of cART and stroke

Whilst I have shown an association between commencing cART and having a stroke, I have also demonstrated that increasing CD4+ T-lymphocyte count (a function of being on cART) is associated with a reduction in stroke risk. This emphasises the overall benefit of cART in reducing the risk of stroke (Rasmussen et al. 2011). However, there could potentially be public health implications with the subpopulation that are at an increased risk of stroke when initiating cART. In this cohort, ischaemic stroke was the predominant stroke type and opportunistic infections only accounted for less than a third of cases. One possible mechanism of stroke after starting cART, as revealed in the histopathology supported case report, is that of an underlying HIV-associated vasculitis. Further characterisation of this subpopulation is urgently needed, as these patients could potentially benefit from anti-inflammatory treatment.

Because 30% of the untreated HIV positive stroke patients were lost to follow-up at 6 months, I was unable to fully comment on the outcome of this group. However, with knowledge of their aetiology it is plausible that they had died. This also questions the exacerbating role of starting cART in this cohort.

Overall, the risk of stroke with longer-term (≥ 6 months) cART use was substantially lower overall (aOR 1.49) and not significantly different from HIV-uninfected individuals. However, there was suggestion of an increased risk of stroke in the younger population (aOR 3.27); this cohort was more likely to survive a stroke. A few studies have reported a link between cART and a high prevalence of dyslipidaemia (Behrens et al. 1999; Friis-Moller et al. 2003). In my study, although the association with stroke was still

present after adjusting for hypercholesterolaemia , there is still the possibility of ongoing chronic vessel wall inflammation due to HIV infection.

6.3 Other clinical implications of HIV infection and cerebrovascular disease

People with small vessel disease constituted a small component of our cohort presenting with stroke. However, stroke syndromes only form part of the clinical manifestation of small vessel disease, another major clinical presentation is neurocognitive impairment (Wardlaw et al. 2013). Although I did not assess my cohort for evidence of HIV associated neurocognitive disorder (HAND), there is growing evidence to suggest that this is on the rise (Cruse et al. 2012). The definition of HAND is based on the consensus opinion of experts in the field and relies on a battery of neuropsychological (NP) tests (Antinori et al. 2007). The classification of HAND includes asymptomatic neurocognitive impairment, mild neurocognitive disorder, and HIV-associated dementia (Antinori et al. 2007). It is specifically asymptomatic neurocognitive impairment and mild neurocognitive disorders that account for the increasing burden of HAND in the treated HIV infected population (Heaton et al. 2010). HIV encephalitis has been closely implicated in HIV-associated dementia but since the introduction of cART, HIV-associated dementia has diminished and the neuropathology of asymptomatic neurocognitive impairment and mild neurocognitive disorders is less certain. Recent evidence suggests that cerebrovascular disease has an important role (Wright et al. 2010).

The converging impact of HIV infection, cART and advancing age could potentially increase the burden of not only stroke but HAND.

6.4 Scope for interventions

In order for patients and clinicians to make the most informed choices, it is important to risk stratify, at several time-points to facilitate primary and secondary prevention Figure 6:1:

1) ***People with untreated HIV positive stroke***

Opportunistic infection and anti-phospholipid syndrome were the typical aetiology in this cohort. The role for empirical interventions with anti-viral and/or anti-bacterial agents, especially in resource-poor settings needs to be explored. Identifying biomarkers with a high sensitivity for those with opportunistic infections could also be used to guide the use of such empirical interventions. Initiating cART obviously with the caveat of excluding opportunistic infections appears to be beneficial. The optimal time point of when to start cART in those with proven CNS infection is less clear. Torok and colleagues showed that in TBM, immediate cART initiation did not improve outcome and was associated with adverse events (Torok et al. 2011) . However, delaying cART initiation by 1 month did no harm (Torok et al. 2011). For APS, the priority should be based on a better understanding of the mechanism in HIV positive individuals.

2) ***People living with HIV infection about to start cART***

Although initiating cART increases the risk of a stroke in a subgroup of individuals, the overall benefit still outweighs this risk especially as increasing CD4+ T-lymphocyte count (a function of cART) reduces the risk of stroke. Furthermore, advanced HIV infection can be improved if on appropriate cART and thus reduces the likelihood of a poor outcome at 6 months.

HIV-associated vasculopathy is the most frequently occurring aetiology in those initiating cART. Developing clinical biomarkers that can predict those at risk of HIV-associated vasculopathy would facilitate risk stratification. Adjunctive interventions such as anti-inflammatory agents (e.g. statins or steriods) could have a role in those initiating cART but needs to be evaluated through clinical trials. Furthermore, once a stroke has occurred, the role of high dose corticosteroids among those without opportunistic infection should also be explored.

3) People living with HIV infection without cART and without a stroke

The World Health Organisation's timely agenda to reduce transmission of HIV and HIV related mortality will theoretically reduce the burden of cerebrovascular disease. However, I believe that starting cART irrespective of CD4+ T-lymphocyte count will further delay the chronic inflammatory state induced by HIV infection and thus delay the anticipated stroke epidemic owing to accelerated atherosclerosis (Mills et al. 2012).

4) People on longer term cART with a stroke

These patients are generally older and thus likely to overlap with other established vascular risk factors (e.g hypertension, hypercholesterolaemia and diabetes). Furthermore, HIV infection and its treatment could exacerbate the burden of these established vascular risk factors e.g. hypercholesterolaemia, by independently increasing their prevalence (Friis-Moller et al. 2003). The risk of recurrent stroke is therefore high. Regular surveillance and aggressive management of these risk factors should be explored in intervention trials. Cardio-thromboembolic stroke is also frequent in this cohort and the safety of long-term anticoagulation will need to be determined. In addition to treating the individual risk factors, long term anti-inflammatory agents could be of use in reducing HIV-associated chronic inflammation. Non-steriodals and

statins are the obvious choice but the novel use of an anti-inflammatory agent such as low-dose methotrexate, in secondary prevention of cardiovascular disease is being studied in a non-HIV population and could be beneficial in the HIV context (Everett et al. 2013).

5) People on long term treatment without a stroke

The surveillance and treatment of risk factors as part of routine HIV care is important at this stage. Metabolic complications (e.g. hypercholesterolaemia) can be related to the class of cART used; the phasing out of stavudine for zidovuine or tenofovir will also help (WHO 2013). However, the overall safety of the different classes will need to be explored in more detail. This is especially true for WHO recommend cART regimens in low-middle income countries (WHO 2013).

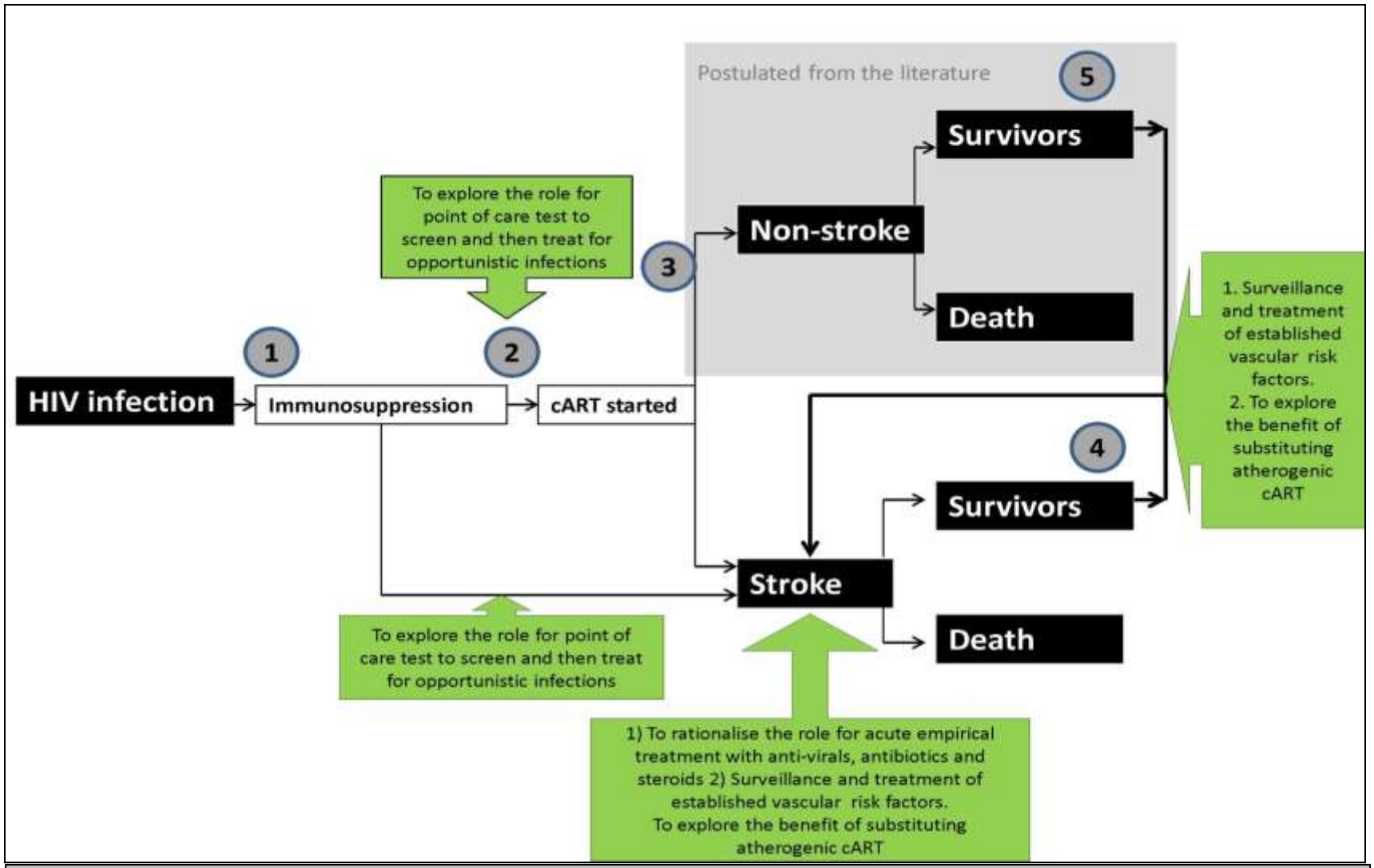


Figure 6:1: Scope for primary and secondary interventions to reduce the burden HIV related stroke

Information in the Grey box is postulated from the literature. 1) People with untreated HIV positive stroke, 2) People living with HIV infection about to start cART 3) People living with HIV infection without cART and without a stroke, 4) People on long term cART with a stroke 5) People on long term treatment without a stroke. The risks of stroke associated with each of these numbered stages are explained in section 7:4. cART – combined cART.

The overall high in-patient and 6 months mortality seen in my study and another study recently performed in Malawi could be related to the high prevalence of advanced HIV disease (Heikinheimo et al. 2012). However, the absence of integrated care for stroke patients (i.e. a stroke unit) could also play an important role in the poor outcomes. The benefit of such an intervention or variations of it (i.e. nurse-led or guardian-led) will need to be explored.

I have helped to expand our understanding of the mechanisms of HIV related stroke described in chapter 1. From the clinical characteristics in Chapter 5 I was able postulate the concept of HIV related stroke evolving through an aging population. A better understanding of these mechanisms will help to confirm or refute these concepts and thus direct the development of novel therapeutic strategies in HIV related stroke Figure 6:2.

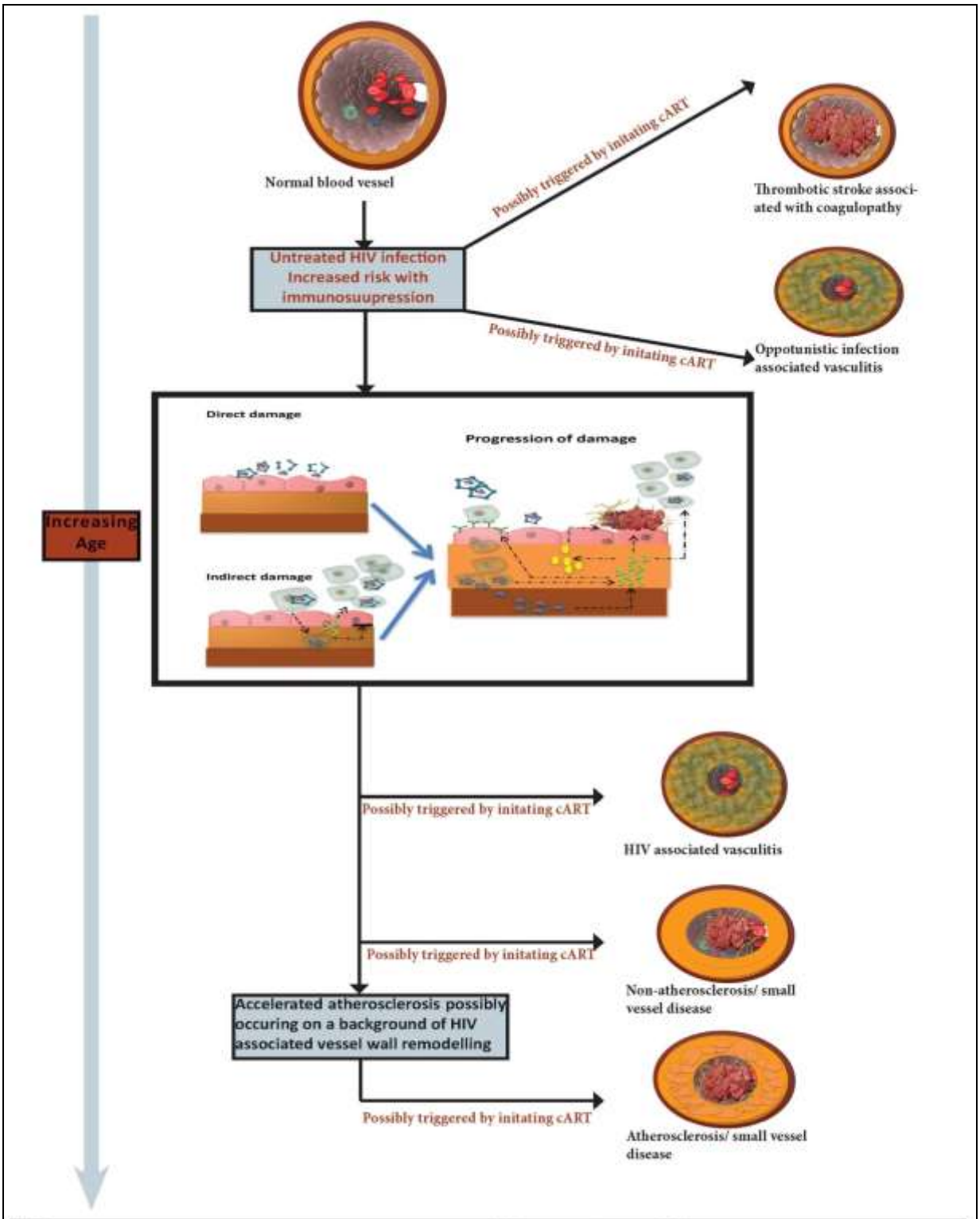


Figure 6:2: Mechanisms of HIV related ischaemic stroke; redefining the hypothetical model

cART – Combined anti-retroviral treatment.

6.5 Concluding remarks

I have shown that untreated HIV infection and more importantly, recently initiating cART are independent risk factors for stroke. The causes of stroke vary but HIV-associated vasculopathy, although an evolving aetiology, predominates, especially in those who have recently commenced cART. With the advent of effective cART, HIV infection is becoming a chronic disease. HIV positive individuals on cART can expect to live longer and, as a result, they are at risk of developing accelerated atherosclerotic strokes among other vascular complications such as HIV-associated neurocognitive impairment. The consequence is far reaching and is likely to increase death and disability in countries where health facilities are already at maximum capacity. I support the WHO initiative to start cART at a CD4+ T lymphocyte count >500 cells/mm^3 and the phasing-out of stavudine based cART regimens. I would recommend going a step further and initiating cART irrespective of CD4+ T-lymphocyte count to delay cerebrovascular complications. As access to cART continues to increase in Malawi, long-term cerebrovascular complications are anticipated as seen in the USA. A better understanding of the mechanisms in the high risk groups, such as those initiating cART will optimise early recognition and diagnosis, and will allow us to implement effective prevention strategies and/or disease interventions.

6.6 Future directions

Improved knowledge about the mechanisms of stroke, especially in those initiating cART and a better understanding of the different forms of HIV-associated vasculopathy should lead to improved investigations and treatment.

There is growing evidence that HIV related stroke is driven by a pro-inflammatory state. The benefit and safety of anti-inflammatory medications in people living with HIV infection such as aspirin, statins, steriods and low dose methotrexate will need to be explored through intervention trials (Everett et al. 2013). The choice of anti-inflammatory drug is especially important as HIV infection is different from other inflammatory conditions because it eliminates CD4+ T lymphocytes but activates other components of the immune system. Very specific immune modulatory treatments might be needed.

Appropriately designed, well powered longitudinal studies are arguably the most useful way of developing prediction models, similar to the Framingham Risk Score or the Veterans Aging Cohort Study index, for intervention strategies (D'Agostino et al. 1994; Justice et al. 2012). Such models will alert us to those most at risk and therefore, those most in need of an intervention.

6.7 What this study has added to the literature

My work contributes to the literature on HIV infection and stroke and highlighted an important association with starting cART and having a stroke. I have shown that a large proportion of individuals in

the UK and Malawi presenting with HIV related stroke are newly diagnosed with HIV infection, thus emphasising the need for routine HIV testing especially in the young stroke populations. The heterogeneity of HIV stroke with respect to risk factors for stroke, the degree of immunosuppression and HIV activity, and prior or current opportunistic infection has made it difficult to generalise epidemiological findings in some studies to populations at large. My study, to some extent unravels some of this ambiguity. I speculate that HIV related strokes evolves through the introduction of cART and then transitions into an aging population, accelerating atherosclerotic stroke and potentially contributing to an anticipated stroke epidemic in countries like Malawi.

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8 Appendices

8.1 Publications arising directly from PhD

8.1.1 Diagnostic accuracy of the Recognition of Stroke in the Emergency Room (ROSIER) score and CT brain in an HIV population

Benjamin LA, Joekes E, Das K, Beeching NJ, Wilkins E, Solomon T.*J Infect* 2013 Aug 28 [Epub ahead of print]

8.1.2 HIV infection and stroke: current perspective and future directions

Benjamin LA, Bryer A, Emsley HC, Khoo S, Solomon T, Connor MD. *Lancet Neurology.* 2012 Oct; 11 (10) 878-90

8.1.3 Detection of herpes viruses in the cerebrospinal fluid of adults with suspected viral meningitis in Malawi

Benjamin LA, Kelly M, Cohen D, Neuhann F, Galbraith S, Mallewa M, Hopkins M, Hart IJ, Guiver M, Lalloo DG, Heyderman RS, Solomon T. *Infection* 41(1) 2013

8.1.4 Parvovirus 4 in cerebrospinal fluid of children with encephalitis, India

Benjamin L, Lewthwaite P, Ravi V, Zhao G, Sharp C, Simmonds P, Virgin S, Wang D, Solomon T. *Emerg Infect Dis*. 2011 Aug; 17(8): 1484-7.

8.2 Publications arising indirectly from PhD

8.2.1 Epstein-Barr virus co-infection in the cerebrospinal fluid is associated with increased mortality in Malawian adults with bacterial meningitis.

Kelly MJ, **Benjamin LA**, Cartwright K, Ajdukiewicz KMB, Cohen DB, Menyere M, Galbraith S, Guiver M, Neuhann F, Solomon T, Lalloo DG, Heyderman S. *Journal of Infectious Diseases*, 205(1); 106-110

8.2.2 Surveillance Programme of IN-patients and Epidemiology (SPINE): Implementation of an Electronic Data Collection Tool within a Large Hospital in Malawi

Miguel A. SanJoaquin, Theresa J. Allain, Malcolm E. Molyneux, **Laura Benjamin**, Dean B. Everett, Oliver Gadabu, Camilla Rothe, Patrick Nguipdop, Moses Chilombe, Lawrence Kazembe, Servace Sakala, Andrew Gonani, Robert S. Heyderman. *PLoS Med* 10(3) 2013.

8.3 Clinical data collection sheet for cases and controls

8.3.1 Questionnaire for cases

8.3.2 Questionnaire for controls