The role of von Willebrand factor and its cleaving protease, ADAMTS13, in young patients with HIV-related stroke

Thesis submitted to the University of Cape Town in fulfilment of the requirements for the degree of Master of Science (Med) in Medicine – (MM095)

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ABSTRACT

Background: The Human Immunodeficiency Virus (HIV) is neuro-invasive and neurological complications of HIV infection occur frequently through a variety of possible mechanisms. Stroke in the setting of HIV is not uncommonly seen in young adults. High levels of von Willebrand factor (VWF), a protein with key roles in platelet adhesion and aggregation, and low levels of A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), the protease that cleaves ultra large VWF multimers into smaller less haemostatically active multimers, have been associated with an increased propensity for thrombosis. Stroke is a potential complication of the aberrant activity of these two proteins. HIV infection has also been associated with endothelial dysfunction, and VWF is a marker of the latter. The investigation of VWF and ADAMTS13 may therefore provide new insights into the pathogenesis of HIV-related stroke.

Aims: This study aimed to investigate the role of VWF and ADAMTS13 in the pathogenesis of HIV-related young stroke in South Africa.

Methods: I undertook a case-control study consisting of 100 participants. This was a pilot study, comprising three participant groups: HIV positive young strokes (n = 20), HIV negative young strokes (n = 40) and HIV positive non-stroke controls (n = 40). Plasma VWF (levels and activity) and ADAMTS13 (levels) were measured on samples collected at day 6 or 7 post-stroke for cases or on enrolment day for controls.

Results: HIV positive young strokes had significantly higher VWF levels than the HIV negative young strokes. They also tended to have higher levels of VWF than the HIV positive young non-stroke controls. HIV positive young strokes also had significantly lower levels of ADAMTS13 than the HIV positive young non-stroke controls. ADAMTS13 levels were notably low in both stroke groups, with no significant differences between HIV positive and HIV negative strokes. Assays of VWF activity were normal in all three groups with no significant between-group differences. In HIV positive participants, VWF levels and CD4 counts were significantly negatively correlated i.e. as CD4 counts decreased, VWF levels rose. No significant correlations were found between CD4 counts and ADAMTS13.

Conclusions: Stroke in HIV infection is associated with a pro-thrombotic coagulation profile. High VWF levels and low ADAMTS13 levels contribute to creating this pro-coagulant state. The association may be causal i.e. the pro-thrombotic state resulted in the stroke. Alternatively, the observed changes may be a post-stroke acute phase response. Our findings favour a causative explanation. A larger study is needed to verify these results.

THE ROLE OF VON WILLEBRAND FACTOR AND ITS CLEAVING PROTEASE, ADAMTS13, IN YOUNG PATIENTS WITH HIV-RELATED STROKE

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DECLARATION

I, Sameera Allie, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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LIST OF ABBREVIATIONS

ºC	degrees Celsius
α	alpha
µg/ml	microgram per millilitre
µl	microlitre
µl/well	microlitre per well
ADAM	A Disintegrin and Metalloproteinase
ADAMTS	A Disintegrin and Metalloproteinase with Thrombospondin motifs
ADAMTS13	A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13
ADAMTS13:Ag	ADAMTS13 antigen
ADP	adenosine diphosphate
AIDS	Acquired Immunodeficiency Syndrome
AMP	adenosine monophosphate
ANA	antinuclear antibody
ANCA	anti-neutrophil cytoplasmic antibody
Anti-DS DNA	anti-double stranded DNA
APL	antiphospholipid antibodies
APS	antiphospholipid syndrome
ARIC	Atherosclerosis Risk in Communities
ART	antiretroviral therapy
ARV	antiretroviral
A-S-C-O	Atherosclerosis - Small vessel disease - Cardiac source - Other
ATP	adenosine triphosphate
BMI	body mass index
BSA	bovine serum albumin
Ca^{2+}	calcium
CAD	coronary artery disease
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy
CD4	cluster of differentiation 4
CD39	cluster of differentiation 39
cells/mm^3	cells per cubic millimetre
CHC	Community Health Centre
CIMT	carotid intima-media thickness
CLAT	Cryptococcal Latex Agglutination Test
CMV	cytomegalovirus
CNS	central nervous system
CRP	C-reactive protein
CSF	cerebrospinal fluid
CT	computed tomography
CVD	cardiovascular disease
Cys	cysteine
cys-rich	cysteine-rich

DAD	Data Collection on Adverse Events of Anti-HIV Drugs
DALYs	disability-adjusted life-years
df	degrees of freedom
DIC	disseminated intravascular coagulation
dis-like	disintegrin-like
DVT	deep venous thrombosis
EC	Eastern Cape
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
F	factor
FBC	full blood count
FS	Free State
FTA	fluorescent treponemal antibody absorption
g/L	grams per litre
GFJ	GF Jooste hospital
GP	glycoprotein
GT	Gauteng
GSH	Groote Schuur Hospital
H1	Hypothesis 1
H2	Hypothesis 2
H3	Hypothesis 3
H4	Hypothesis 4
H5	Hypothesis 5
H6	Hypothesis 6
H7	Hypothesis 7
H1a	Hypothesis 1a
H3a	Hypothesis 3a
H5a	Hypothesis 5a
H7a	Hypothesis 7a
H7b	Hypothesis 7b
H7c	Hypothesis 7c
HAART	highly active antiretroviral therapy
HERNS	hereditary endotheliopathy with retinopathy, nephropathy, and stroke
HIV	Human Immunodeficiency Virus
HIV-1	HIV Type 1
HIV-2	HIV Type 2
HIV/AIDS	Human Immunodeficiency Virus infection and the Acquired Immunodeficiency Syndrome
HIV pos con	HIV positive young controls
HIV pos str	HIV positive young stroke
HIV neg str	HIV negative young stroke

HK	high molecular weight kininogen
HMGB1	high-mobility group box 1
HPT	hypertension
HREC	Human Research Ethics Committee
HRP	horseradish peroxidase
HSV	herpes simplex virus
ICAM	intercellular adhesion molecule
ICH	intracerebral haemorrhage
IgG	immunoglobulin G
IL-6	interleukin 6
INR	international normalised ratio
IQR	interquartile range
kDa	kilodalton
kg	kilograms
KZN	KwaZulu-Natal
LACI	lacunar infarct
LP	Limpopo
M	molar
Mdn	median
MELAS	mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
mg/L	milligram per litre
ml	millilitre
mm/hr	millimetres per hour
MP	Mpumalanga
MRI	magnetic resonance imaging
n	sample size
NA	not applicable
NC	Northern Cape
NHLS	National Health Laboratory Service
NIHSS	National Institutes of Health Stroke Scale
nm	nanometre
NNRTI	non-nucleoside reverse transcriptase inhibitor
No.	number
NO	nitric oxide
NSH	New Somerset Hospital
NW	North West
OCSP	Oxfordshire Community Stroke Project
OPD	ortho-phenylenediamine
p	p value

PACI	partial anterior circulation infarct
PAD	peripheral arterial disease
PAF	platelet-activating factor
PAI-1	plasminogen activator inhibitor-1
PAR	protease-activated receptor
PBS	phosphate buffered saline
PCNSL	primary central nervous system lymphoma
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PFO	patent foramen ovale
PI	protease inhibitor
PK	plasma kallikrein
PML	progressive multifocal leucoencephalopathy
PNS	peripheral nervous system
POCI	posterior circulation infarct
prim	primary
pro	propeptide
PTT	partial thromboplastin time
PVD	peripheral vascular disease
r	effect size
r_s	Spearman's rho
RNA	ribonucleic acid
rpm	revolutions per minute
RPR	rapid plasma reagin
RR	relative risk
SAHLSIS	Sahlgrenska Academy Study on Ischemic Stroke
SEMDSA	Society for Endocrinology, Metabolism and Diabetes of South Africa
SP	signal peptide
SPSS	Statistical Package for the Social Sciences
TACI	total anterior circulation infarct
TAFI	thrombin activatable fibrinolysis inhibitor
TB	tuberculosis
TBM	tuberculous meningitis
tech	technical
TFPI	tissue factor pathway inhibitor
TIA	transient ischaemic attack
TM	transmembrane
TNFα	tumour necrosis factor alpha
TOAST	Trial of Org 10172 in Acute Stroke Treatment
tPA	tissue plasminogen activator
TSP	thrombospondin type 1
TTP	thrombotic thrombocytopaenic purpura
TXA_2	thromboxane A2

U	U statistic
UCT	University of Cape Town
ULVWF	ultra large VWF
uPA	urokinase-type plasminogen activator
US	United States
VCAM	vascular cell adhesion molecule
VDRL	Venereal Disease Research Laboratory
VHW	Victoria hospital
vs.	versus
VWD	von Willebrand disease
VWF	von Willebrand factor
VWF:Ag	VWF antigen
VWF:CB	VWF collagen binding
VZV	varicella zoster virus
WC	Western Cape
WHO	World Health Organisation
WP	Weibel-Palade
χ^2	chi-squared statistic

INTRODUCTION

Human Immunodeficiency Virus infection and the Acquired Immunodeficiency Syndrome (HIV/AIDS) is a worldwide epidemic, with Sub-Saharan Africa, including South Africa, worst affected (UNAIDS 2011). HIV/AIDS results in multiple systemic complications. Neurological complications occur commonly due to the virus itself, to opportunistic infections related to immunosuppression, or to the complications associated with antiretroviral (ARV) drug therapy (Hogan *et al*. 2011; Mc Arthur *et al*. 2005; Boisse *et al*. 2008). An increased frequency of stroke in the setting of HIV has been reported and this has become a subject of increasing interest (Benjamin *et al*. 2012). Stroke unrelated to HIV occurs more frequently in an older population. However, HIV-related stroke affects mostly younger adults who form the bulk of the HIV-infected population (Tipping *et al*. 2007; Ortiz *et al*. 2007). Because it affects mostly young adults, it results in many years of disability and adversely affects the economy of already resource-poor countries.

Von Willebrand factor (VWF) and A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13) are two proteins involved in haemostasis. VWF is vital for platelet adhesion and aggregation. It initiates platelet plug formation at sites of vascular injury. ADAMTS13 cleaves the ultra large VWF (ULVWF) multimers into smaller multimers. The ULVWF multimers are haemostatically active and may promote active thrombosis if present in excess (Hassan *et al*. 2012; Denis *et al*. 2012). Thus ADAMTS13 maintains a balance between bleeding and thrombosis. There is evidence that high VWF and low ADAMTS13 levels are associated with an increased risk for thrombosis. The latter may play a role in stroke causation (Bongers *et al*. 2006; Lip *et al*. 2002). However, the data on stroke are limited and hardly any information is available on HIV-associated stroke. HIV infection is also associated with vascular endothelial dysfunction and VWF is considered to be a marker of endothelial dysfunction (Blann 1993).

I therefore undertook this study to determine the role of VWF and ADAMTS13 in HIV-related young stroke. A cohort of 100 participants from a larger HIV-related young stroke study that is currently still underway was selected to participate in this smaller case-control study. This pilot study is, to our knowledge, the first of its kind. I recruited three participant groups: HIV positive young strokes [sample size (n) = 20], HIV negative young strokes (n = 40) and HIV positive non-stroke controls (n = 40). Normal values for the markers of interest in this study were obtained from a recent South African study by Meiring *et al*. (2012).

VWF (levels and activity) and ADAMTS13 (levels), were measured on plasma samples collected within 7 days of stroke onset in cases or within 7 days following enrolment of controls. I assessed the levels and activity of VWF and ADAMTS13 in our three participant groups. Using post-hoc analyses, I addressed the question as to whether any abnormal findings could be causative i.e. pre-dated and predisposed to the stroke, or whether they were consequential upon the stroke e.g. due to an acute phase response.

CHAPTER 1 - REVIEW OF HIV/AIDS AND STROKE

INTRODUCTION

In this chapter, I shall provide a brief introduction to the Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS), together with a review of stroke. Thereafter, I shall discuss the relationship between stroke and HIV infection.

1.1 Review of HIV and AIDS

1.1.1 HIV and AIDS

In 1981, the United States (US) Centres for Disease Control first described the Acquired Immunodeficiency Syndrome (Centres for Disease Control and Prevention 1981). Two years later, a retrovirus was isolated from a patient who presented with prodromal clinical signs and symptoms that often preceded AIDS (Barre-Sinoussi *et al*. 1983). This was subsequently named the Human Immunodeficiency Virus. It has been divided into two types, HIV Type 1 (HIV-1) and HIV Type 2 (HIV-2). HIV-1 is known to be responsible for the global pandemic, and HIV-2, the less pathogenic of the two types, is found mainly in West Africa.

1.1.2 HIV burden of disease globally and locally in South Africa

At the end of 2010, it was estimated that approximately 34 million people were living with HIV worldwide. Of these, 68% resided in Sub-Saharan Africa. South Africa is home to the largest number of people living with HIV than any country worldwide - an estimated 5.6 million (UNAIDS 2011). Sadly, there are nine countries in Southern Africa that account for less than 2% of the world's population, but are known to represent approximately a third of global HIV infections (De Cock *et al*. 2012). The worldwide and African prevalence of HIV infection are shown in figures 1.1 and 1.2 respectively.

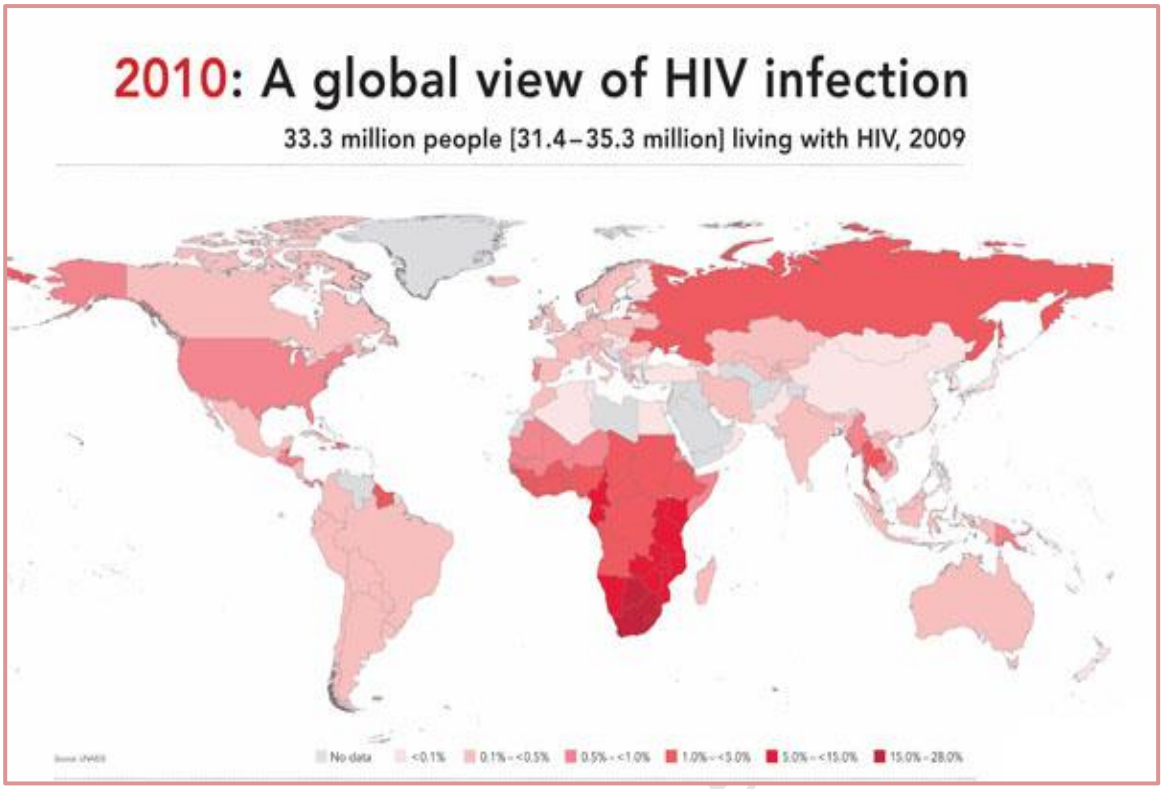


Figure 1.1 Prevalence of HIV worldwide (From: HIV: Geography of an Epidemic 2011)

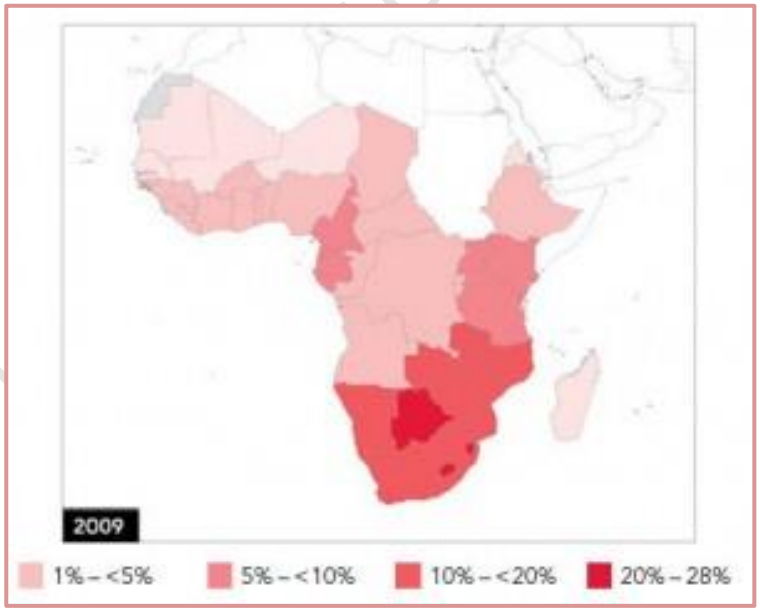


Figure 1.2 Prevalence of HIV in Africa (From: HIV: Geography of an Epidemic 2011)

HIV/AIDS undermines economic growth in South Africa, lowering the gross domestic product growth rate by 1.60% per year in the KwaZulu-Natal (KZN) province and 1.42% per year in the rest of South Africa (Thurlow *et al*. 2009). This can be attributed to its high prevalence amongst

the young economically active individuals of South Africa. A large national prevalence survey of HIV infection in South Africa conducted from 2008 to 2009 showed that HIV prevalence was higher amongst females than males across most of the age-group subdivisions used in the study. HIV prevalence peaked in females aged 25-29 years at 32.7% and in males aged 30-34 years at 25.8%. The prevalence rate was also reported to be highest in KZN and lowest in the Western Cape among the 15-49 year age group (Shisana *et al*. 2009) (see figures 1.3 and 1.4).

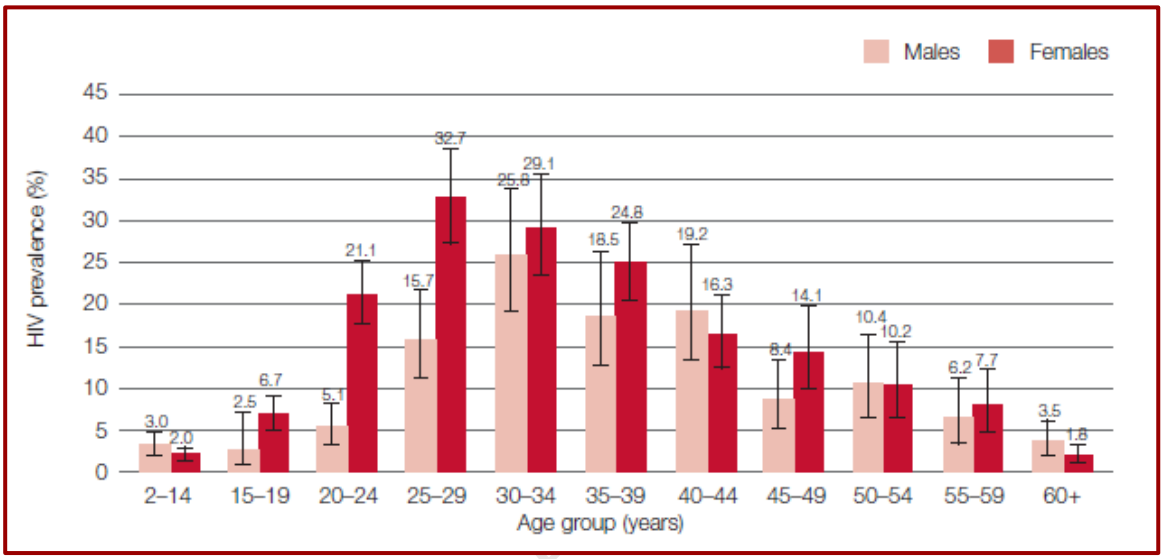


Figure 1.3 HIV prevalence in South Africa by sex and age group for 2008 (From: Shisana *et al*. 2009)

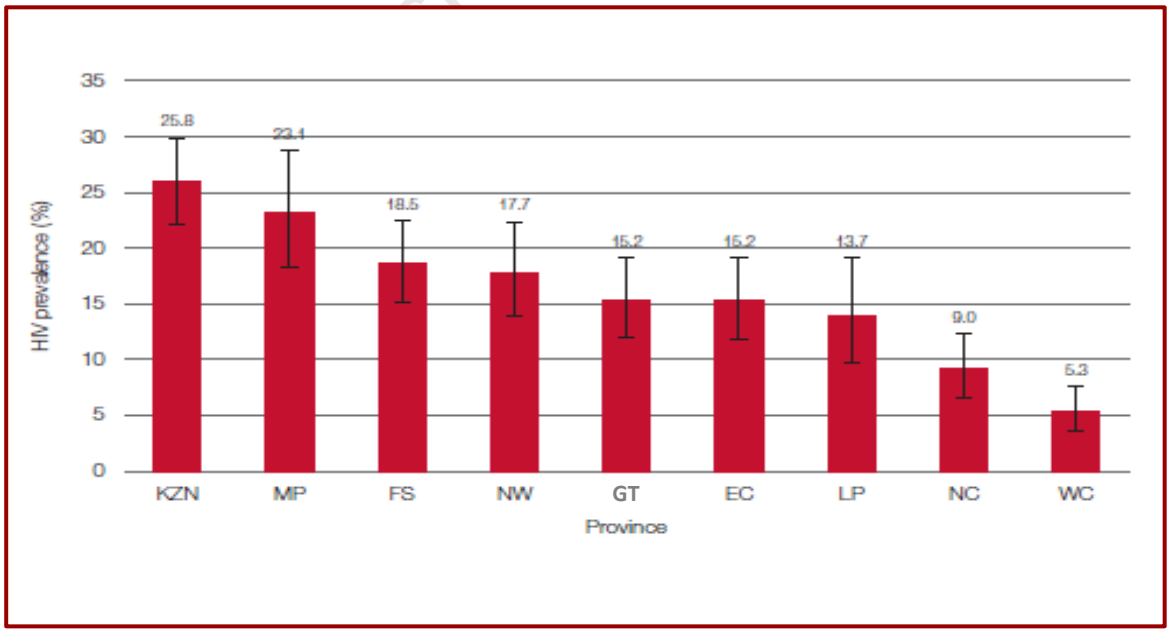


Figure 1.4 South African HIV prevalence among the 15-49 year age group by province for 2008
(From: Shisana *et al*. 2009) Abbreviations: KwaZulu-Natal (KZN), Mpumalanga (MP), Free State (FS), North West (NW), Gauteng (GT), Eastern Cape (EC), Limpopo (LP), Northern Cape (NC), Western Cape (WC).

1.1.3 Neurological complications of HIV

HIV is neuro-invasive and invasion of the nervous system occurs early in the course of the disease (Shapshak *et al*. 2011; Yao *et al*. 2010; Hogan *et al*. 2011). Both the central nervous system (CNS) and peripheral nervous system (PNS) may be affected, with the presentation of neurological symptoms occurring at any stage of infection. However, most neurologic disorders tend to manifest with advanced immunosuppression. Neurologic disease may be due to direct viral invasion which produces distinct neurological syndromes. It may also result from indirect immune-deficiency related opportunistic infections, AIDS-defining cancers, ARV drug therapy toxicity, and vascular events (Hogan *et al*. 2011; Mc Arthur *et al*. 2005; Boisse *et al*. 2008).

The complications may be classified on the basis of their neuroanatomical localisation (Price 1996). Table 1.1 lists the complications according to the CNS and PNS anatomy affected and further sub-classifies them temporally into early and late presentations of HIV/AIDS infection.

Central nervous system	Peripheral nervous system and muscle
Meninges and other structures surrounding the CNS	**Nerve and root**
Early and late-middle course	Early
'Aseptic' meningitis, and symptomless HIV infection	Brachial plexitis, focal neuropathy, and polyneuropathy
Late	Middle
Cryptococcal meningitis	Sub-acute and chronic demyelinating polyneuropathy
Tuberculous meningitis	Mononeuritis multiplex, benign
HIV headache	Late
	Distal predominantly sensory polyneuropathy
Brain	CMV polyradiculopathy
Focal-late	Mononeuritis multiplex: late CMV
Cerebral toxoplasmosis	Nucleoside polyneuropathy
PCNSL	
PML	**Muscle**
Diffuse-early	Late
Post-infectious encephalomyelitis	Inflammatory myopathy
Diffuse-late	Non-inflammatory myopathies
AIDS dementia complex	
CMV encephalitis	
Stroke	
Spinal cord	
Late	
Vacuolar myelopathy (part of AIDS dementia complex)	

Table 1.1 Neurological complications of HIV based on their neuroanatomical localisation
(Adapted from Table 2 in: Price 1996) Abbreviations: central nervous system (CNS), Human Immunodeficiency virus (HIV), Acquired Immunodeficiency Syndrome (AIDS), primary central nervous system lymphoma (PCNSL), progressive multifocal leucoencephalopathy (PML), cytomegalovirus (CMV).

Stroke is a less commonly described neurological manifestation of HIV. Cerebral infarction is reported as occurring in between 4 and 34% of AIDS cases in autopsy series (Benjamin *et al*. 2012). In the earlier years of the HIV epidemic, the specific association between AIDS and stroke was questioned (Pinto 1996). However, this has changed over the years. I shall review the literature on this question later in this chapter.

1.2 Review of stroke

1.2.1 Stroke versus TIA

The term stroke refers to a clinical syndrome of acute neurological deficit, usually focal, with an underlying vascular origin (Muir 2009). The World Health Organisation (WHO) defines stroke as,"rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, with symptoms lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin" (Hatano 1976). A transient ischaemic attack (TIA) is differentiated from stroke by the association of symptom resolution within 24 hours of onset (Muir 2009). However, there are reports of magnetic resonance imaging (MRI) detected acute infarction in many patients with neurologic deficits that resolve within 24 hours (Kidwell *et al*. 1999). The definition of TIA is thus undergoing revision.

1.2.2 Types of stroke, causes of stroke and stroke risk factors

A stroke may be either ischaemic or haemorrhagic in nature. Ischaemic stroke occurs as a result of occlusion of a cerebral artery by a thrombus or embolus. Haemorrhagic stroke is the result of blood vessel rupture, with subsequent leaking of blood into the brain (Bryer *et al*. 2010). Haemorrhagic stroke events will not be included in my study. Eighty-five to ninety percent of strokes are due to ischaemic causes, with 10-15% of strokes attributed to intracerebral haemorrhage (ICH) (Muir 2009). Interestingly, in South Africa, primary ICH accounts for a larger percentage of strokes, between 20-30%, as evidenced in a hospital based series (Connor *et al*. 2006).

The causes of either type of stroke are extremely varied and the range of commoner causes also differs with regard to age. Large vessel atherosclerosis giving rise to its thrombotic or embolic complications, small vessel disease and cardio-embolic stroke are the most common causes of ischaemic stroke in older persons. Other less common causes of ischaemic stroke include dissection of the cervical arteries, vasculitis, angiopathies, metabolic disorders, rare types of emboli (fat or gas), haematological disorders and intracranial vascular malformations. Strokes that are caused by an ICH may arise secondary to bleeds from vascular malformations, morphological

abnormalities of cerebral arteries, intracranial venous thrombosis, brain tumours and haemostatic disorders (Cordonnier *et al*. 2008).

The causes of stroke in younger persons will be discussed later in the text (see section 1.2.5). It is, however, important to note that the less common causes of stroke become more relevant in younger persons and that stroke in the young includes a long list of unusual causes. The role of HIV in the causation of stroke is also discussed as a separate entity later in this text and will be elaborated further on in the introduction (see section 1.3).

For ischaemic stroke, age (≥ 45 years), sex (men > female), race and ethnicity (black > white), genetic factors (single gene disorders that cause stroke and genetic disorders that include stroke as a manifestation) and other less well documented risk factors such as geographic location and socio-economic status are considered non-modifiable risk factors. The list of modifiable risk factors for ischaemic stroke is extensive. It includes: hypertension, diabetes mellitus, smoking, coronary heart disease, atrial fibrillation, left ventricular hypertrophy, abnormal waist to hip ratio (a reflection of abdominal obesity), high cholesterol, insulin resistance/metabolic syndrome and physical inactivity/sedentary behaviours. Other less common identified risk factors include: excessive alcohol consumption, elevated VWF levels, elevated white blood cell counts, variation in C-reactive protein (CRP) levels, elevated homocysteine levels, reactive oxygen species/oxidative stress, increased fibrinogen levels and elevated factor VIII (Elkind 2011; Allen *et al*. 2008). VWF, factor VIII and fibrinogen are all proteins that are essential for normal haemostasis and their roles will be illustrated in chapter 2. Hypertension, bleeding disorders, use of anticoagulant and antiplatelet drugs, pre-existing cerebral aneurysms and arteriovenous malformations are some of the risk factors for brain haemorrhage (Clark 2009).

The INTERSTROKE study, a large standardised case-control study of risk factors for stroke that included high, middle and low income countries (n = 3000), showed that hypertension, current smoking, abdominal obesity, diet and physical activity accounted for more than 80% of the global risk of all stroke (ischaemic and ICH) (O'Donnell *et al*. 2010). These findings are relevant to South Africa as it was one of the 22 countries that participated in the study.

1.2.3 Stroke classification systems and management of stroke

Many stroke classification systems have been devised over the years. The Oxfordshire Community Stroke Project (OCSP) classification is widely used and functions as an aid for the clinical localisation of stroke. It is also known as the Bamford or Oxford classification. It makes use of the

patient's presenting clinical features to classify acute ischaemic stroke into one of four distinct categories, viz. total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), lacunar infarct (LACI), and posterior circulation infarct (POCI). This classification system is obtained by dividing the blood supply into the anterior circulation (supplied by the internal carotid, middle and anterior cerebral arteries) and the posterior circulation (supplied by the vertebral and basilar arteries). In addition, these categories assist with predicting the extent of the stroke, the underlying cause of the stroke and the prognosis of the patient (Bamford *et al*. 1991).

The Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification classifies stroke into one of five categories according to its aetiology and underlying pathophysiological mechanism, using clinical findings and results of investigations performed. These categories include: large artery atherosclerosis, cardio-embolism, small blood vessel occlusion, stroke of other determined aetiology or stroke of undetermined aetiology (Adams *et al*. 1993).

The Atherosclerosis – Small vessel disease – Cardiac source – Other (A–S–C-O) classification is based on the stroke phenotypes. Stroke aetiology and the presence of all underlying disease are used in the evaluation. Patients are defined in terms of the four A-S-C-O phenotypes, with each phenotype assigned a grade (grade 1, 2, 3, 0 and 9) according to their contribution to the stroke causation (Amarenco *et al*. 2009). The advantage of the A-S-C-O classification for young strokes is that it highlights the uncertainty surrounding the role of factors such as the patent foramen ovale (PFO) in stroke causation. With this classification, many more young patients fall into the undetermined category (Cotter *et al*. 2012).

The mainstay of modern stroke management is prompt admission to a hospital, preferably with a dedicated stroke unit, and early administration of recombinant tissue-type plasminogen activator for ischaemic stroke. For haemorrhagic stroke, the therapeutic options depend on the size of the bleed and may be either a choice of haemostatic agents or surgical evacuation of the haematoma (Marsh *et al*. 2010).

1.2.4 Burden of stroke globally and locally in South Africa

Stroke accounts for 5.5 million deaths worldwide and the loss of 44 million disability-adjusted life-years (DALYs) annually (Mukherjee *et al*. 2011). In South Africa, heart disease, diabetes and stroke together constitute the second most important cause of death in adult South Africans, second only to HIV/AIDS (Mayosi *et al*. 2009; Bradshaw *et al*. 2003). In Sub-Saharan Africa, the

estimated stroke prevalence is 200 to 300 per 100 000 population, based on community surveys conducted in countries that included South Africa, Togo and Tanzania (Kengne *et al*. 2006).

In their review of stroke in South Africa, Connor and Bryer concluded that stroke mortality was high in South Africa. The impact of the growing burden of stroke in rural areas could be gauged by the increased prevalence of people requiring help with activities of daily living and the latter exceeded that of high-income countries (Connor *et al*. 2006). Most importantly, on the 2nd October 2006, stroke was declared a catastrophic disease in South Africa, at the Joint World Congress on Stroke held in Cape Town, South Africa, highlighting its deleterious impact on the South African population (Culebras 2006).

1.2.5 Stroke in the young

The term "young stroke" is usually used to refer to strokes in people aged <45 years. The overall incidence of stroke in young people ranges from 7-15 per 100 000 people/year for all types of stroke (ischaemic and haemorrhagic) and the incidence is thought to be greater in developing countries (Griffiths *et al*. 2011). A systematic review has shown that the incidence of stroke in young adults is not as rare as previously thought and requires a new focus on specific preventive programs in this group (Marini *et al*. 2010). In Sub-Saharan Africa most cases of stroke are reported to occur in people 10 to 15 years younger than those in developed countries (Kengne *et al*. 2006). In a review of young stroke, it was noted that even within the confines of "young stroke," the incidence does increase notably with age, especially with that of the age group above 34 years. Some population-based studies have, however, indicated greater stroke incidence in patients aged less than 30 years. In the same review article, the incidence rates of young stroke appeared greater in men than women in the 35-44 year old age group (Griffiths *et al*. 2011).

The aetiology of stroke in the young is much more diverse than that occurring in the older population, with varied ischaemic causes that include large or small artery disease, cardio-embolism, thrombophilias, infective and non-infective vasculitides, venous infarction, genetic and pregnancy-related causes (Naess *et al*. 2011; Martin *et al*. 1997). In young people, the identification of a PFO as a source of cardio-embolism is increasingly recognised, and therefore is an important abnormality to exclude during the investigation of young patients with stroke (Cerrato *et al*. 2004; Larrue *et al*. 2011). Diagnostic work-up should also include investigations for dissection of the carotid or vertebral arteries (Ferro *et al*. 2010). Nevertheless, despite extensive investigations, cryptogenic stroke – where no underlying cause can be established – accounts for up to 35%. Compared with the general stroke population, subarachnoid and intracranial haemorrhages account for a greater proportion of the underlying pathophysiological mechanism in

young strokes. However, cerebral infarction remains the commonest mechanism (Griffiths *et al*. 2011). The causes are listed in table 1.2.

Non-atherosclerotic angiopathies • Cervicocephalic arterial dissection • Fibromuscular dysplasia • Moyamoya disease • Angiitis • Genetic and hereditary diseases (Fabry's disease, CADASIL, MELAS, HERNS) • Reversible cerebral vasoconstriction syndrome • Susac's syndrome • Sneddon's syndrome
Cardio-embolism • Rheumatic valvular disease • Patent foramen ovale • Atrial septal aneurysm • Prosthetic valve • Infective endocarditis • Arrhythmia (atrial fibrillation) • Dilated cardiomyopathy (Chaga's disease) • Mitral valve prolapse • Atrial myxoma • Marantic and Libman-Sacks endocarditis
Large-artery atherosclerosis
Small-vessel disease
Haematologic conditions (pro-thrombotic states) • Antiphospholipid syndrome • Hyperhomocysteinaemia • Sickle cell disease • Myeloproliferative disorders • Factor V Leiden • Prothrombin 20210A mutation • Protein C, protein S deficiency • Antithrombin III deficiency
Migraine stroke
Cryptogenic stroke

Table 1.2 Aetiology of ischaemic stroke in young adults (Adapted from table 1 in: Yamamoto 2012) Abbreviations: cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS).

Smoking, hypertension, hyperlipidaemia and diabetes mellitus are frequently associated with the risk of ischaemic stroke in young adults (Chatzikonstantinou *et al*. 2012; Dharmasaroja *et al*. 2011; Balci *et al*. 2011; Spengos *et al*. 2010). In developing countries, urbanisation appears to play a role in the rise of these conventional vascular risk factors (Brainin *et al*. 2007). Certain risk factors, such as genetic disorders, cardiac abnormalities, pro-thrombotic states, migraine, oral contraceptive use and illicit drug use have a greater influence on stroke occurrence in the younger than the older adult (Marini *et al*. 2010; Naess *et al*. 2010).

Stroke morbidities are lower in the young, but these patients tend to live with their neurological deficits much longer, with the resultant loss of employment and diminished social interaction that accompanies this (Naess *et al*. 2011). Data on the risk factors and long-term prognosis in young stroke patients are needed. For this reason, the FUTURE study - a prospective cohort study investigating risk factors and assessing prognosis in a large cohort of young stroke patients - was set up in the hope of providing much more data than are currently available (Rutten-Jacobs *et al*. 2011).

Stroke prevalence in rural South Africa is higher than previously documented in other studies from Africa, with the prevalence of stroke survivors in the 35-44 year age category equal to 117/100 000 aged ≥ 15 years (Connor *et al*. 2004). Stroke in young South Africans appear to be commoner in Black Africans infected with HIV (Hoffmann 2000a). In a review investigating socio-economic status and stroke, the impact of stroke was noted to be 3-fold higher in low-income countries compared with high and middle-income countries, when DALYs and mortality rates were compared (Addo *et al*. 2012).

1.3 Stroke and HIV

1.3.1 Background history and current opinions

In 1990 a case-control study of autopsied patients who had died with AIDS concluded that stroke did not appear to be more common in patients with AIDS compared to those without AIDS at death. However, stroke needed to be considered in the differential diagnosis of neurological disease in these patients (Berger *et al*. 1990). Pinto (1996) reviewed the literature on the relationship between AIDS and stroke between mid-1976 and December 1994. Despite the extensive search period, no clear association between AIDS and stroke was identified (Pinto 1996). This concept has changed and currently it is believed that there is a specific association between

stroke and patients with HIV/AIDS (Dobbs *et al*. 2009; Burn 2006). I shall review this association in more detail.

There are two well-described theories with regards to stroke. Benjamin *et al*. (2012) summarised these recently in their review on HIV infection and stroke. They firstly pointed out that the occurrence of stroke and HIV infection in a patient could often be attributed to the coincidence of two diseases. This was because both stroke and HIV infection incidence have increased in low-middle income countries (Benjamin *et al*. 2012). The second opinion was that HIV infection is an independent *risk factor* for stroke, and additional vascular risk can be conferred due to the use of antiretroviral therapy (ART) (Dobbs *et al*. 2009; Sen *et al*. 2012; Benjamin *et al*. 2012).

Modi *et al*. (2008) concluded the following after reviewing the literature for the evidence of an association between stroke and HIV:

- Stroke occurs in association with HIV infection. However, this association appears to be mainly with advanced infection or AIDS.
- Cerebral ischaemic stroke is commoner than haemorrhagic stroke.
- Evidence of infarction at autopsy correlates poorly with clinical stroke.
- In the absence of infection or tumour, a cerebral vasculopathy is likely.
- The stroke is often attributable to an underlying treatable CNS infection with resultant vasculitis or due to embolism from a cardiac source.

1.3.2 Stroke rates, demographic characteristics, risk factors and stroke types in HIV-related stroke

Incidence and prevalence data on stroke in HIV-infected individuals are limited. More recent studies have estimated an annual incidence rate of 216 TIAs and strokes per 100 000 patient-years and 166 strokes per 100 000 patient-years in European studies (Evers *et al*. 2003; Corral *et al*. 2009). Chow *et al*. (2012) recently reported an incidence rate of 5.27 per 1000 person-years in HIV-infected patients compared to 3.75 per 1000 person-years in non HIV-infected patients in a US based study. The demographic characteristics of patients with HIV-related stroke compared to those with HIV infection sans stroke are similar, but advanced immunosuppression is more common in the stroke group (Dobbs *et al*. 2009).

A large observational cohort study conducted in the US, comparing ischaemic stroke incidence in HIV-infected and HIV negative patients, concluded that within their HIV cohort, atrial fibrillation,

age, a higher log-transformed viral load, and a history of CNS infections or malignancy, were significantly associated with increased risk of stroke. Significant factors that decreased the risk of stroke in the HIV-infected cohort in this study included: non-nucleoside reverse transcriptase inhibitor (NNRTI) use (as opposed to no NNRTI use), longer duration of any ART and an undetectable viral load, defined as HIV ribonucleic acid (RNA) $\leq$ 400 copies per millilitre (ml). The authors concluded that HIV was an independent predictor of ischaemic stroke, after they adjusted for known stroke risk factors (such as hypertension and smoking) and that an increase in stroke risk was most pronounced in HIV-infected women and younger age groups (18-49 years) (Chow *et al*. 2012). Brown *et al*. (2011) reported that risk factors traditionally associated with cardiovascular events were also associated with cardiovascular events in an HIV-infected population. Furthermore, smoking and hypertension were found to be highly prevalent among some HIV-infected cohorts and populations (Brown *et al*. 2011). Since cardiovascular risk and cerebrovascular risk cannot be viewed in isolation, this implies an association with stroke as well. Individuals with HIV are more likely to engage in high risk-taking behaviour and have a higher prevalence of smoking and illicit drug use (Burn 2006). Risk-taking behaviour is more common in the young and smoking and illicit drug use adds to the risk of stroke in these patients.

Early on, when the ideas around HIV and stroke were still being formulated, there appeared to be a greater propensity for the occurrence of ischaemic stroke compared to that of haemorrhagic stroke (Pinto 1996). This is in keeping with what is generally seen in patients that are not infected with HIV. Multiple studies following Pinto's initial review have reported a higher frequency of ischaemic stroke in their patient groups compared with haemorrhagic strokes (Mochan *et al*. 2003; Ortiz *et al*. 2007).

1.3.3 Mechanisms involved in HIV-related stroke

The mechanisms implicated in stroke causation in HIV-infected individuals are varied. Mechanisms include vasculopathy associated with opportunistic infections, opportunistic tumours, cardio-embolic sources and disorders of haemostasis. Mechanisms attributed to HIV itself such as HIV-associated cerebral vasculitis, HIV-associated vasculopathy, and the potentially toxic effect on the vascular endothelium that may occur with the use of long-term highly active antiretroviral therapy (HAART), have been described (Benjamin *et al*. 2012; Ovbiagele *et al*. 2011; Corral *et al*. 2009; Ross *et al*. 2009; Burn 2006; Connor *et al*. 2000). In many cases the causes remain unknown. These mechanisms will be discussed in more detail as many of them may play a contributory pathogenic role in VWF and ADAMTS13-related stroke.

a. Vasculopathy and HIV-related cerebral vasculitis

The term HIV-associated vasculopathy, when applied in the context of the cerebral circulation, is an umbrella term for a number of arterial disease pathologies occurring in HIV-infected individuals, not attributable to any cause other than the HIV infection itself. The exact pathogenetic mechanisms remain unclear (Connor 2009).

The evidence for HIV-associated small vessel vasculopathy is small: In a neuropathological study performed on patients with cerebral infarction in the Edinburgh HIV cohort, Connor and colleagues described the presence of a vasculopathy in ten patients, all characterised by the same histopathological features at autopsy (Connor *et al*. 2000). Similar histopathological changes were described in earlier research by Mizusawa and colleagues (Misuzawa *et al*. 1988). There is evidence for HIV-associated vasculopathy in large or medium extracranial and intracranial arteries in adults (Tipping *et al*. 2007). In a study conducted locally at the Groote Schuur Hospital (GSH), in Cape Town, South Africa, Tipping *et al*. (2007) reported an HIV-associated vasculopathy in 20% of the HIV positive patients (n = 67) in their cohort who presented with stroke.

Cerebral vasculopathies secondary to infection with viruses other than HIV have been associated with stroke. Varicella zoster virus (VZV) and cytomegalovirus (CMV) have been implicated (Nagel *et al*. 2010). Cerebral vasculitis solely due to HIV itself is a rare histological finding and its relationship with stroke causation is not clear (Nogueras *et al*. 2002; Hoffmann *et al*. 2000b). However, it remains to be seen how important this entity will become in stroke causation.

b. Opportunistic infections and tumours

Syphilis and HIV are both sexually transmitted illnesses and affect similar patient groups, thus leading to a greater propensity for co-infection (Karp *et al*. 2009; Lynn *et al*. 2004). Neurosyphilis manifesting as meningovascular syphilis is a known cause for stroke, with the vessel plaques in syphilitic arteritis usually longer and smoother than those seen in the vessels affected by atherosclerotic disease (Chahine *et al*. 2011).

Tuberculosis (TB) is the most common opportunistic infection in HIV-infected individuals. Tuberculous meningitis (TBM), an extra-pulmonary manifestation of infection with TB, occurs with greater frequency in persons infected with HIV. However, the exact incidence and prevalence is not known (Garg *et al*. 2011). Lammie *et al*. (2009) report that stroke in TBM is common, with approximately 20% of patients developing a neurological deficit clinically, 13-57% with neuro-imaging abnormalities and 22-56% with autopsy-proven cerebral infarction. TBM is characterised

by the presence of thick gelatinous exudates which display predominance for the basilar area of the brain, and cerebral infarcts may be a consequence of the entrapment of intracranial vessels within this exudate. However, the exudate is a thinner, minimal, serous type exudate, in HIV-infected individuals (Garg *et al*. 2011).

Immunosuppression arising from HIV results in multiple AIDS-associated malignancies (Wood *et al*. 2005; Bonnet *et al*. 2008). Cancers cause stroke through a variety of mechanisms. In ischaemic stroke this may be due to the direct tumour effect with tumour embolism and compressive effect of the tumour on the blood vessels, coagulation disorders, infection and therapy-related. Intraparenchymal bleeding, venous occlusion/venous infarction, haemorrhage into a primary brain tumour or a metastasis, ruptured neoplastic aneurysms and subdural haematomas are all mechanisms which may give rise to intracranial haemorrhage resulting in stroke (Grisold *et al*. 2009).

c. Cardio-embolic sources

In patients infected with HIV, cardio-embolism as a cause for ischaemic stroke varies from 9-18%. Potential causes for cardio-embolism include valvular heart disease, endocarditis, cardiomyopathy (HIV-related dilated or other cardiomyopathies), myocardial infarction and interstitial myocardial fibrosis (Ortiz *et al*. 2007; Tipping *et al*. 2007; Mochan *et al*. 2003; Hoffmann 2000a; Berger *et al*. 1990).

d. Disorders of haemostasis and HIV as a pro-thrombotic state

Antiphospholipid syndrome (APS), associated with the presence of antiphospholipid (APL) antibodies in the patient's blood, is a known cause for acquired thrombophilia and presents with thrombotic events (venous or arterial thromboembolism) and/or obstetric complications. These APL antibodies are acquired auto-antibodies directed against phospholipid-protein complexes in cell membranes (Sangle *et al*. 2011). Most reviews of HIV and APS identify the anticardiolipin antibodies (a type of APL antibody) as the more frequently detected antibody, yet APS and its manifestations are uncommon in HIV and the presence of APS in HIV does not necessarily correlate with the thrombosis risk or haematological manifestations of APS (Asherson *et al*. 2003; Sène *et al*. 2008; Hassoun *et al*. 2004). Ischaemic stroke is, however, one of the most common complications of APS. The association of stroke with people with APS is strongest in young adults (Panichpisal *et al*. 2012).

HIV infection is associated with a pro-thrombotic state. There is increasing evidence that thrombotic events occur in persons infected with HIV with greater frequency than in healthy non HIV-infected persons (Klein *et al*. 2005). These often manifest clinically with thrombosis as the first presentation of the disease (Basavanagowdappa *et al*. 2011; Witz *et al*. 2000; Louw *et al*. 2008; Modi *et al*. 2012). The association between HIV and a pro-thrombotic state may be attributed to a number of factors in persons infected with HIV, which include endothelial dysfunction/endothelial-related changes, as well as aberrant function of the coagulation system (Shen *et al*. 2004).

Thrombosis occurs in both the venous and arterial vasculature. The literature suggests that chronic HIV infection is associated with a two to tenfold increased risk of venous thrombosis (Klein *et al*. 2005). Reports on arterial thrombosis in HIV-infected patients frequently highlight that of coronary artery thrombosis/coronary artery disease, owing to the increased predisposition of these events in light of their association with combination ART (especially protease inhibitor) use in the treatment of HIV (Rickerts *et al*. 2000; Friis-Møller *et al*. 2003; D Ascenzo *et al*. 2012). Cerebrovascular arterial thrombosis presenting as stroke is especially important due to its increasing incidence in low-to-middle income countries and the morbidity and mortality with which it is associated (Feigin *et al*. 2011). The many causes for this pro-thrombotic state in HIV, including coagulation abnormalities and how they link with stroke causation, will be discussed in chapter 2 (see sections 2.2, 2.3 and 2.4).

e. Atherosclerosis in HIV

Atherosclerosis is a major known risk factor for cardiovascular disease and stroke. Atherosclerosis occurs as a result of the interplay between the biologically active endothelium, inflammation involving both innate and adaptive immune responses, and the adipocyte-mediated inflammatory drive seen in obesity. The development of atherosclerosis in persons infected with HIV is a relatively new focus of research and multiple pathways for its causation have been proposed. HIV is a state of chronic immune activation and the predisposition to other concomitant infections in persons infected with HIV multiplies the inflammatory contribution. Atherosclerosis is an inherently inflammatory mediated process, so HIV – a state of chronic inflammation - may predispose to the development of atherosclerosis (Lo *et al*. 2012). Endothelial dysfunction has been related to the pathogenesis of atherosclerosis (Halcox *et al*. 2009; Vita 2011). Endothelial dysfunction in HIV (see later, g. Endothelial dysfunction: its relationship to stroke and HIV) and some HIV viral proteins (HIV-1 Tat and HIV Nef protein) have been linked to atherosclerosis as well (Lo *et al*. 2012). HAART plays an important role in the mechanism of atherogenesis in HIV (Cruse *et al*. 2012).

f. HAART and stroke

Much has been written about the association between HAART and the occurrence of vascular events, especially myocardial infarction. There is a confirmed link between HAART use and increased cardiovascular risk (Friis-Møller *et al*. 2003; Zou *et al*. 2007), although the link between HAART and stroke is not yet as clearly proven. The Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study - a prospective, observational cohort study of 23 468 HIV positive patients - has shown that patients on HAART have an increased risk of cardio- and cerebrovascular disease events. Furthermore, the incidence of these events increased with longer exposure to HAART. These factors were still evident even after controlling for traditional vascular risk factors (d'Arminio *et al*. 2004). However, a retrospective review that included the d'Arminio *et al*. (2004) DAD study group report concluded that higher rates of stroke were evident in persons infected with HIV in the HAART era, but that no specific association between HAART and stroke could be established (Sen *et al*. 2012).

g. Endothelial dysfunction: its relationship to stroke and HIV

The normal healthy endothelium is involved in vasodilatation and vasoconstriction through its ability to regulate muscle tone and secrete vasoactive substances such as prostacyclin, thromboxane A_2, nitric oxide and endothelins. It also plays key roles in haemostasis and inflammation (Barrett *et al*. 2010; Subbarao *et al*. 2011).

Endothelial dysfunction has been observed in stroke (Roquer *et al*. 2009). It is linked to the pathogenesis of stroke, most notably lacunar stroke and is regarded as an important mechanism of cerebrovascular damage (Knotnerrus *et al*. 2009; Sierra *et al*. 2011). Blum *et al*. (2012) showed a rise in markers of endothelial activation in the systemic circulation and the presence of fewer endothelial progenitor cells (both indicators of severe endothelial dysfunction) in patients with acute ischaemic stroke in their small study. They questioned whether these results reflected an underlying primary disturbance of endothelial dysfunction in the pathogenesis of acute ischaemic stroke, or an acute phase response to cerebral injury. Furthermore, they concluded that severe endothelial dysfunction may be linked to the vascular instability that still exists in the early days following acute stroke, and places patients at risk for more vascular events during that time period (Blum *et al*. 2012).

Endothelial dysfunction is recognised as a vascular disease process occurring in HIV infection (Subbarao *et al*. 2011). Monsuez *et al*. (2009) summarised the most prevalent theories surrounding the aetiology of endothelial dysfunction in HIV-1 infection as follows:

1. Direct endothelial cell injury induced by the HIV infection itself and its viral proteins.
2. HIV-driven chronic inflammatory processes, aided by an increase in pro-inflammatory cytokine production.
3. HIV-induced dyslipidaemia and metabolic syndrome.
4. HAART-related mechanisms – HAART-induced dyslipidaemia and metabolic syndrome (indirect mechanism), and HAART causing direct endothelial injury (direct mechanism).

The common presence of endothelial dysfunction in both stroke and HIV leads to the idea that a link exists between HIV-infected patients presenting with acute ischaemic stroke and endothelial dysfunction. The question requiring further investigation is how stroke, HIV infection and endothelial dysfunction relate to one another, as well as the sequence in which the pathogenic events occur.

h. The ageing HIV population

The population of people living with HIV is getting older (Vance *et al*. 2011; Pratt *et al*. 2010). Age is itself a risk factor for stroke and HAART has converted HIV into a chronic condition for many people. Living longer thus increases the risk of age as a traditional risk factor for stroke irrespective of HAART use. Apart from HAART use, older people are at greater risk of contracting HIV due to biological changes (thinner mucosal membranes allowing for easier virus entry) and the use of sexual performance enhancers (increased sexual activity in patients who form new/multiple sexual relationships following divorce or death of a spouse) (Vance *et al*. 2011). With ageing also comes the increase in traditional older age-associated vascular risk factors for stroke.

i. Cryptogenic stroke

Often, despite extensive investigation, no obvious cause for the stroke can be identified, and the aetiology is thus unknown and labelled as cryptogenic. Cryptogenic stroke is recognised in HIV-related stroke (Hoffmann *et al*. 2000b; Burn 2006). However, the question always remains as to how extensive the investigations have been to identify a cause, before labelling the stroke "cryptogenic/idiopathic.''

1.3.4 HIV-related young stroke

HIV-infected individuals present with stroke at a younger age (Tipping *et al*. 2007; Ortiz *et al*. 2007). The association between HIV-related strokes occurring in younger persons may relate to the

fact that new HIV infections occur predominantly in the 15-24 year old age group (Napierala Mavedzenge *et al*. 2011).

Many studies conducted in African countries shed light on HIV-related young stroke. HIV was found to be an important risk factor for stroke in young Nigerians (Onwuchekwa *et al*. 2009). A recent Malawian study on stroke outcomes found that younger patients with strokes were often HIV-seropositive and that traditional vascular risk factors were uncommon in the same group. They concluded that HIV infection could be a risk factor for stroke for young people without other common stroke risk factors (Heikinheimo *et al*. 2012). Younger age has also been identified as an independent risk factor for ischaemic stroke in Central African patients with HIV/AIDS (Longo-Mbenza *et al*. 2011). A Nigerian-based study by Owalobi *et al*. (2012) showed the increased frequency of HIV as a risk factor for stroke among young adults. A study conducted in black African patients in the KZN province of South Africa (the province with the highest estimated incidence of HIV seropositivity in Sub-Saharan Africa); found that the chances of having a stroke were greater in HIV positive as opposed to HIV negative individuals (Patel *et al*. 2005). Outside Africa, in a US-based study, Chow *et al*. (2012) documented that the increased risk of ischaemic stroke in their HIV cohort was pronounced in the younger age groups, 18-49 years.

CONCLUSION

HIV is omnipresent. It affects the human body in a number of ways, with the burden of disease attributed to HIV resulting in a global epidemic. The nervous system bears the brunt of infection with HIV in a variety of ways. Although controversial, the association between HIV infection and stroke cannot be dismissed. There are numerous mechanisms by which HIV may promote the occurrence of stroke in individuals infected with HIV. Younger adults appear to be particularly vulnerable. For all these reasons, the problem of HIV-related young stroke deserves further investigation. The double burden of HIV and stroke contributes significantly to morbidity and mortality and adversely affects South Africa's economic potential.

CHAPTER 2 - REVIEW OF HAEMOSTASIS, VWF, ADAMTS13 AND THEIR ASSOCIATION WITH HIV AND STROKE

INTRODUCTION

Stroke occurring in the setting of HIV infection in young people has major public health implications. It is linked to a high morbidity with a long duration of symptoms, a large burden of care for health personnel and the family members of patients affected by the stroke, and has a crippling effect on health resources. I was especially interested in the concept of HIV as a pro-thrombotic state and the role of haemostatic and coagulation abnormalities in the pathogenesis of HIV-associated stroke. A literature review led me to the identification of VWF and ADAMTS13, two very important factors involved in haemostasis, and how they may be related to stroke and HIV.

To understand these two dynamic proteins fully, I shall provide a summary of haemostasis to contextualise their role in maintaining vascular homeostasis of the body. In addition, I shall discuss some of the coagulation abnormalities reported in HIV and stroke respectively, including the link between certain coagulation abnormalities seen in both HIV and stroke. I shall summarise the key factors regarding VWF and ADAMTS13, their synthesis, biological factors, regulation of activity and roles in disease. I shall also review the literature regarding the relationships between these factors and stroke causation, with special reference to HIV infection.

2.1 Haemostasis

Haemostasis is the physiological process whereby haemorrhage at the site of vascular injury is arrested while still maintaining normal blood flow elsewhere in the circulation (Gale 2011). It is a dynamic process that is dependent on the interaction between the blood vessel wall, circulating platelets and blood coagulation factors (Mehta *et al*. 2009; Hoffbrand *et al*. 2011). Haemostasis involves two processes viz. primary haemostasis and secondary haemostasis that are interdependent, occur simultaneously and collaborate to bring about successful haemostasis. The fibrinolysis pathway is also vital for normal haemostasis. Pathological bleeding or pathological thrombus formation can occur if any aspect of haemostasis is disrupted (Gale 2011). An intact vessel wall has multiple mechanisms by which it prevents haemostasis under normal circumstances. Endothelial cells produce: (1) prostacyclin, which causes vasodilatation and inhibits

platelet aggregation; (2) thrombomodulin, which results in activated protein C inhibiting coagulation, and (3) tissue plasminogen activator, which promotes fibrinolysis (Mehta *et al*. 2009).

2.1.1 Primary haemostasis

This refers to the initial platelet aggregation and platelet plug formation that occurs at the site of vascular injury following platelet activation (Gale 2011). Platelets thus play a key role in primary haemostasis. Platelet membranes have multiple receptors for collagen, adenosine diphosphate, vessel wall VWF and fibrinogen, and their cytoplasm contains actin, myosin, glycogen, and many secretory organelles, the most important being the alpha (α) granules, dense granules and lysosomes (Barrett *et al*. 2010; Nurden 2011). α granules contain adhesive proteins e.g. VWF, clotting factors and their inhibitors, fibrinolytic factors and their inhibitors, proteases e.g. ADAMTS13 and anti-proteases, growth and mitogenic factors, chemokines and cytokines, antimicrobial proteins and membrane glycoproteins (Nurden 2011).

In normal blood vessel conditions, platelets do not adhere to surfaces or aggregate with each other, but when vascular injury does occur, the endothelium is disrupted and platelets are exposed to the sub-endothelial matrix, with resultant adhesion and activation of platelets (Gale 2011). This process is dependent on the multiple receptors, especially certain glycoprotein (GP) receptors, found on the surface of platelets, and their corresponding ligands (see table 2.1 and figure 2.1).

Platelet receptor	Corresponding ligand
GPIb/GPV/GPIX (forming the GPIb-IX-V complex)	VWF
GPVI	Collagen
$\alpha_{IIb}\beta_3$ (GP IIb/IIIa complex)	Fibrinogen, VWF, fibronectin, vitronectin
$\alpha_2\beta_1$ (GP Ia/IIa complex)	Collagen
$\alpha v\beta_3$	Vitronectin, thrombospondin, VWF, fibronectin, fibrinogen
PAR1, PAR4	Thrombin

Table 2.1 Platelet receptors and their corresponding ligands (Adapted from: Baker *et al*. 2011)
Abbreviations: glycoprotein (GP), von Willebrand factor (VWF), protease-activated receptor (PAR).

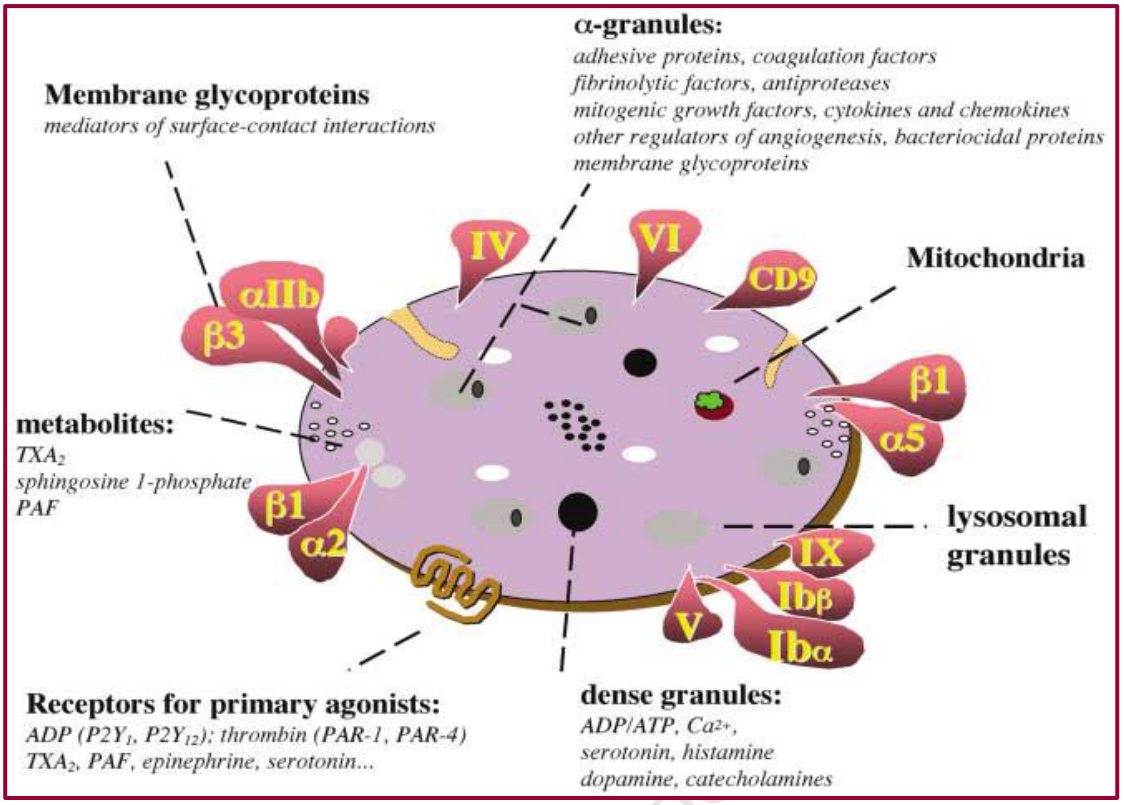


Figure 2.1 Structure of a platelet with the surface receptors involved in haemostasis (From: Anitua *et al*. 2004)
Intracellular organelles whose contents are secreted upon platelet activation and active metabolites synthesised during platelet activation are also indicated. Abbreviations: thromboxane A2 (TXA_2), adenosine diphosphate (ADP), adenosine triphosphate (ATP), protease-activated receptor (PAR), platelet-activating factor (PAF), calcium (Ca^{2+}). $P2Y_1$ and $P2Y_{12}$ are purinergic G protein-coupled receptors that are stimulated by ADP.

Platelet plug formation is a complex sequential process and has been reviewed by Clemetson *et al*. (2012) and Baker *et al*. (2011). Here is a short summary (refer to figure 2.2):

The flowing platelets are slowed down first through the platelet receptor GPIb-IX-V complex and VWF. The VWF binds to the exposed collagen of the sub-endothelial matrix. The shear stress forces of the flowing blood stretch VWF, thus exposing binding sites on the VWF for the platelet GPIb receptor. Thus platelets are tethered down and adhere to the damaged endothelium.

Next, the adherent platelet becomes activated on the vessel wall, releasing the contents of its granules. These secretory products, in turn, further activate the platelets through binding to the platelet receptors and further facilitate platelet binding to the sub-endothelial matrix.

Platelet-platelet interactions, mainly via $\alpha_{IIb}\beta_3$ receptors on these particles, allow for platelet aggregation. VWF plays a key role in this process as well. VWF binds to platelet $\alpha_{IIb}\beta_3$ receptors,

as does fibrinogen. Activated platelets become amoeboid in shape and tight junctions form where two platelets come into contact.

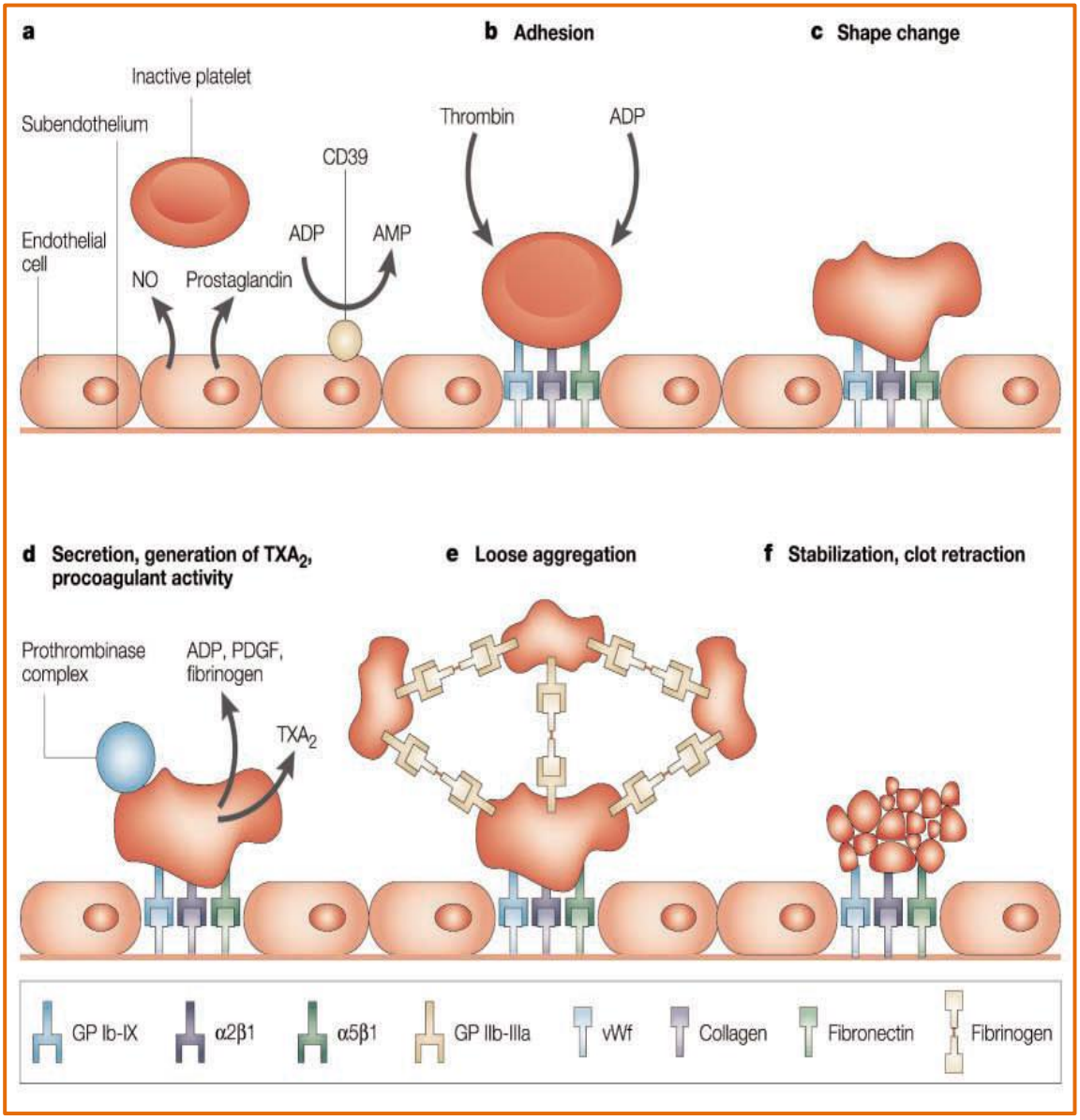


Figure 2.2 Platelets in thrombus formation (From: Bhatt *et al*. 2003)
a. Endothelial cells release nitric oxide (NO), prostacyclin and express cluster of differentiation 39 (CD39) on their cell surface. These inhibit platelet activation. CD39 converts adenosine diphosphate (ADP), a potent inducer of platelet activation, into adenosine monophosphate (AMP). **b and c.** Platelets adhere to the exposed sub-endothelium at sites of vascular injury. This takes place through interactions between collagen, von Willebrand factor (VWF) and fibronectin and their receptors on the platelets, integrin 21, glycoprotein (GP) Ib-IX and integrin 51, respectively. Thrombin and ADP cause platelets to change into an active conformation. **d.** Activated platelets secrete ADP, platelet-derived growth factor (PDGF), and fibrinogen from platelet storage granules, and thromboxane A2 (TXA_2), produced by immediate biosynthesis. ADP and TXA_2 cause circulating platelets to change shape and become activated. **e.** Fibrinogen binds to the glycoprotein IIb/IIIa receptors on the surface of activated platelets and fibrinogen bridges between the platelets are formed, resulting in platelet aggregation. A fibrin mesh (not shown) is formed and leads to the formation of a platelet thrombus. **f.** Clot retraction results in the formation of a stable thrombus.

2.1.2 Secondary haemostasis

Once the coagulation cascade has begun, the process of secondary haemostasis is under way. The end result is a meshwork of insoluble fibrin that is incorporated into and around the platelet plug (Gale 2011). Blood coagulation is usually initiated when the vascular endothelium is damaged or activated, resulting in the exposure of sub-endothelial tissue factor to the flowing blood. The two pathways of the coagulation cascade are the intrinsic and extrinsic pathways. A series of enzymatic reactions, mediated by a host of coagulation factors, results in the generation of thrombin, and the conversion of fibrinogen to fibrin (Ruttman 2006). Fibrinolysis – the process of clot dissolution – occurs at about the same time. There is a dynamic balance between haemostasis and clot formation on the one hand, and fibrinolysis, on the other. Figure 2.3 outlines the process of secondary haemostasis.

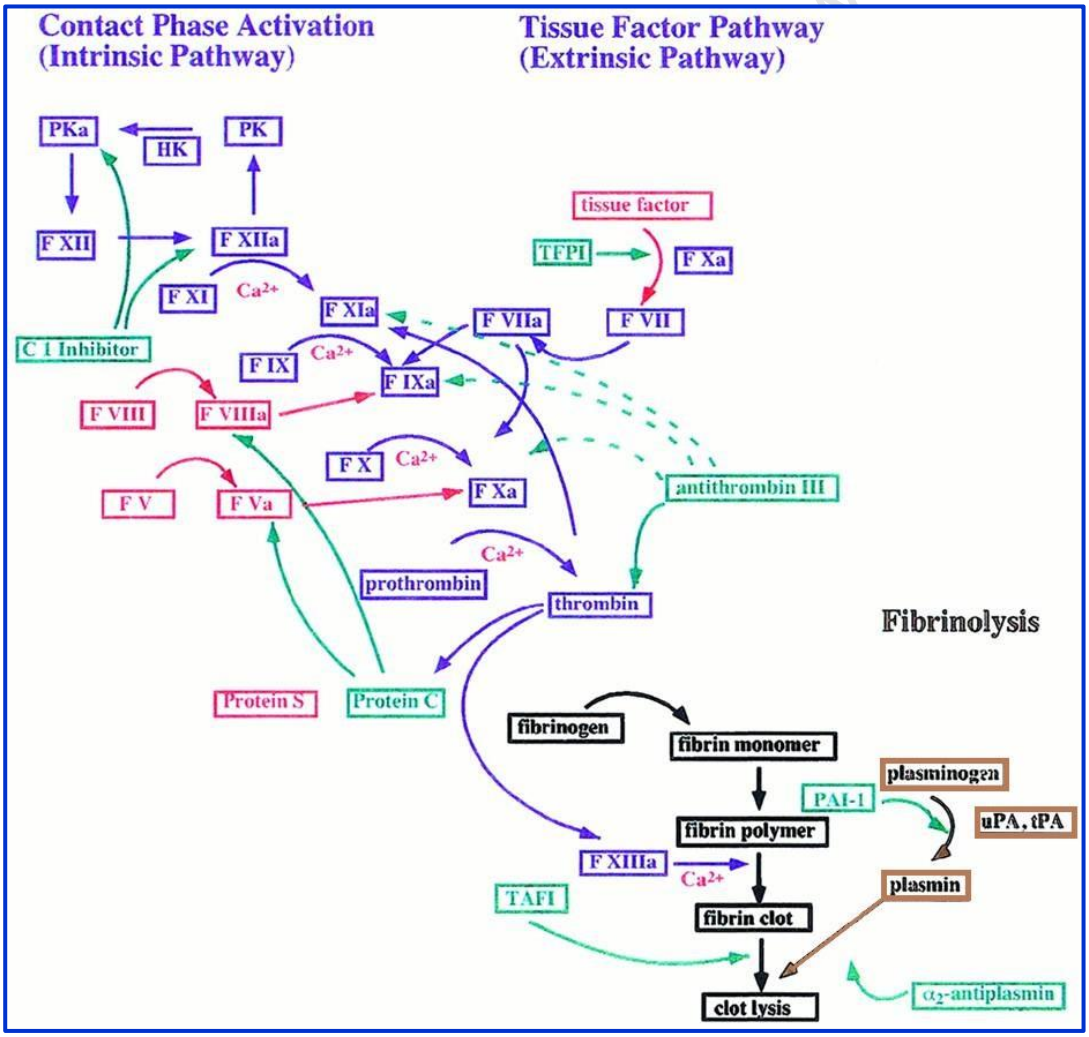


Figure 2.3 Coagulation cascade (From: Tapper *et al*. 2000)
Coagulation factors = blue and "a" indicates activation. Cofactors = red. Coagulation inhibitors = green. Proteins involved in fibrinolysis = brown. Arrows indicate proteolytic conversion. Solid and dotted lines indicate strong and weak interactions respectively. Abbreviations: factor (F), high molecular weight kininogen (HK), plasminogen activator inhibitor-1(PAI-1), plasma kallikrein (PK), thrombin activatable fibrinolysis inhibitor (TAFI), tissue factor pathway inhibitor (TFPI), tissue plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), calcium (Ca^{2+}).

2.2 Coagulation abnormalities in HIV

I have already discussed HIV as a pro-thrombotic state (see section 1.3.3). Defects have been described at multiple levels of the coagulation pathway, including protein C and protein S, antiphospholipid antibodies, antithrombin deficiency and von Willebrand factor. These defects have been noted to correlate with the degree of immunosuppression measured by cluster of differentiation 4 (CD4) cell counts (Saif *et al*. 2001). However, several of these defects have been reported to be epiphenomena rather than causal (Becker *et al*. 2011; Mochan *et al*. 2005).

Heparin cofactor II is a specific thrombin inhibitor and deficiency thereof as well as disorders of plasmin (increased levels of plasminogen activator inhibitor) have been mentioned in the literature in individuals infected with HIV, predisposing them to thrombosis. Even HIV-related nephropathy gives rise to coagulation disturbances in persons infected with HIV (Saif *et al*. 2001). Further complicating the issue, many medical conditions that complicate advanced HIV also predispose to thrombosis e.g. malignancy, sepsis, meningitis etc. The aetiological mix is likely to vary depending on the site of thrombosis e.g. deep vein thrombosis (DVT) versus arterial ischaemia.

2.3 Coagulation abnormalities and stroke

In the absence of hypertension, diabetes, hyperlipidaemia, valvular heart disease or smoking as risk factors in a patient presenting with stroke, a hypercoagulable state should be suspected as a cause (Moster 2003). The factor V Leiden mutation is the most common inherited coagulopathy associated with stroke. Prothrombin has been associated with venous thrombosis (G20210A mutation), but its association with arterial stroke is controversial. Protein C and protein S have been implicated (Soare *et al*. 2010). Hyperhomocysteinaemia and antiphospholipid antibodies have been moderately associated with stroke (Moster 2003).

2.4 Coagulation abnormalities in HIV and their link with stroke

Untangling the cause of stroke in an HIV-infected person can be challenging. However, in the absence of other potential causes, coagulation defects should be considered. Antiphospholipid syndrome, HIV-associated malignancies and infections have been discussed earlier (see section 1.3.3). Other lesser known abnormalities will be described in this section.

Protein C and protein S are part of the body's natural anticoagulant system (Bereczky *et al*. 2010). Deficiencies in these proteins are known to lead to an increased risk of venous thromboembolism, with a lesser association with arterial disease (Bereczky *et al*. 2010; Soare *et al*. 2010). Protein C and protein S deficiency have both been associated with arterial ischaemic stroke, with protein S deficiency linked to a greater propensity for stroke (Soare *et al*. 2010). Protein S deficiency has long been recognised in patients with HIV (Stahl *et al*. 1993; Mochan *et al*. 2005). Various studies found a 27-76% and 0-14% protein S and protein C deficiency respectively, in patients infected with HIV (Erbe *et al*. 2003). Qureshi *et al*. (1997) initially postulated that the increased risk of ischaemic stroke seen in young persons with HIV infection was due to the increased susceptibility of HIV-infected patients to meningitis and protein S deficiency. However, Mochan *et al*. (2005) found no relationship between protein S deficiency in HIV-infected patients and the occurrence of stroke. They attributed the presence of protein S deficiency in HIV positive patients with stroke to be an epiphenomenon of the HIV infection rather than a cause for ischaemic stroke (Mochan *et al*. 2005).

Antithrombin is a glycoprotein whose primary role in the coagulation cascade is the inhibition of activated factor IIa (thrombin) and Xa, and to a lesser extent factors IXa, XIa and XIIa (Maclean *et al*. 2007). The association between antithrombin deficiency and stroke is rare, with the evidence linking the two, weak (Soare *et al*. 2010). Saif *et al*. (2001) have identified reports of antithrombin deficiency in HIV-infected individuals who experienced thrombotic events, but a link with HIV-infected individuals with stroke and the presence of antithrombin deficiency is lacking.

2.5 von Willebrand factor (VWF)

2.5.1 Overview of VWF

VWF is a key protein in the coagulation cascade. In 1926, a Finnish physician, Erik von Willebrand, first described a new type of inherited bleeding disorder that was distinct from haemophilia (Von Willebrand 1926). This disorder was eventually named von Willebrand disease (VWD). The plasma protein implicated in the disease process was only identified 30 years later and was subsequently named von Willebrand factor (De Meyer *et al*. 2012a).

VWF, a multimeric glycoprotein, is present in the plasma and is produced by the endothelium, providing a link with endothelial dysfunction that has been described in HIV (Subbarao *et al*. 2011). It mediates the binding of platelets at sites of vascular injury and is important for platelet adhesion. It also serves as a protective carrier molecule for factor VIII (De Meyer *et al*. 2012a).

VWD is the most common inherited bleeding disorder characterised mainly by mucosal bleeding, but in more severe forms there may be joint, muscle and central nervous system involvement (Schneppenheim *et al*. 2011). Recently, much interest has been focused on VWF as a target for stroke therapy. With the more recent clarification of the structural composition of its cleaving enzyme, ADAMTS13 (see later, section 2.6), the relationship between VWF and ADAMTS13 has become a prominent topic in the current literature.

2.5.2 Synthesis and structure of VWF

VWF is a large multimeric glycoprotein with a long polypeptide chain and a molecular mass of approximately 270 kilodalton (kDa). The gene is located at the tip of the short arm of chromosome 12 (locus 12p13.3) and biosynthesis occurs in endothelial cells and megakaryocytes (Hassan *et al*. 2012; Mendolicchio *et al*. 2005; Peyvandi *et al*. 2011; McGrath *et al*. 2010). Details of its synthesis and secretion are reviewed in McGrath *et al*. (2010), Hassan *et al*. (2012), Peyvandi *et al*. (2011), Schneppenheim *et al*. (2011), Luo *et al*. (2012), Mendolicchio *et al*. (2005) and Sadler (2009). A pre-pro-polypetide is sequentially cleaved to yield pro-VWF monomers that dimerise in the endoplasmic reticulum. Post translational glycosylation occurs in the Golgi complex and ultimately VWF multimers are formed (McGrath *et al*. 2010; Hassan *et al*. 2012; Peyvandi *et al*. 2011; Schneppenheim *et al*. 2011; Luo *et al*. 2012; Mendolicchio *et al*. 2005; Sadler 2009).

The VWF monomer precursor is composed of four types of domains i.e. A, B, C and D, which are constructed as identical repeats in the following order: D1, D2, D´, D3, A1, A2, A3, D4, B1, B2, B3, C1, C2 and CK (Luo *et al*. 2012; Franchini *et al*. 2006) (see figure 2.4).

2.5.3 Storage and secretion of VWF

VWF multimers are stored in its ultra large VWF (ULVWF) form in Weibel-Palade bodies in endothelial cells and in α-granules in platelets (Ruggeri 2007; Hassan *et al*. 2012; Mendolicchio *et al*. 2005; McGrath *et al*. 2010; Crawley *et al*. 2011).

Both constitutive and regulated pathways for VWF secretion from endothelial cells exist. The regulated pathway is influenced by the presence of secretagogues. Secretagogues include histamine, thrombin, collagen, ADP, β-adrenergic agonists, calcium ionophore A23187, phorbol myristate acetate, desmopressin (used in the treatment of VWD), and other important mediators of thrombosis and inflammation (Johnsen *et al* 2008; Ruggeri 2007; Hassan *et al*. 2012; McGrath *et al*. 2010; Sadler 1998). The VWF stored in endothelial cells is secreted into the circulating blood

via apical/luminal secretion or into the sub-endothelial mtrix via basolateral secretion (Reininger 2008).

VWF secretion from megakaryocyte-derived platelets only occurs via the regulated pathway (Ruggeri 2007). The circulating VWF in plasma is predominantly of endothelial cell origin as platelets secrete VWF only in response to activation via a stimulus of the regulated pathway (Bowie *et al*. 1986).

VWF is secreted as large multimers (Sadler 2009). Both the mature VWF multimers and the VWF propeptide are secreted together in 1:1 stoichiometric amounts, with the propeptide dissociating following secretion. VWF multimers, however, have a half-life of approximately 12 hours (Peyvandi *et al*. 2011). Cleavage takes place at the A2 domain of the protein by ADAMTS13 (Hassan *et al*. 2012). The large ULVWF multimers are the most haemostatically active. Therefore cleavage by ADAMTS13 gives rise to smaller multimers that are best suited to carry out the functions of VWF (Crawley *et al*. 2011).

2.5.4 Function of VWF

VWF has a multitude of biological functions. The multidomain structure of VWF provides the binding sites for various interactions. This is shown in figure 2.4.

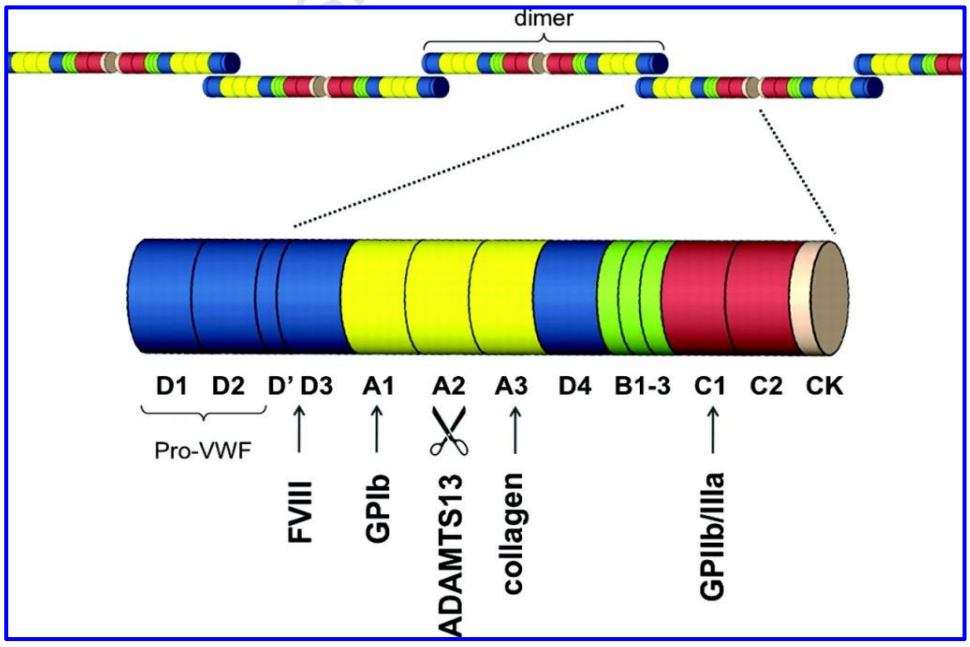


Figure 2.4 Schematic depiction of VWF and its domains indicating the binding sites of major binding partners, and the cleavage site for ADAMTS13 (From: De Meyer *et al*. 2012a) Abbreviations: factor (F), A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), glycoprotein (GP).

a. Binding to platelet GPIb and platelet integrin $\alpha_{IIb}\beta_3$ (see section 2.1.1)

Under normal physiological conditions, soluble VWF and platelets co-exist in the circulation, without any undue interaction. However, when a vessel is injured, VWF binds to the exposed collagen of the sub-endothelial matrix, mainly through its A3 domain (Szántó *et al*. 2012; Hassan *et al*. 2012). VWF has two conformations: a globular form and an elongated form. With high shear stress e.g. at sites of vessel injury or stenosis and atherosclerosis, the circulating globular form unfolds into an elongated form, exposing its A1 domain for platelet binding (Denis *et al*. 2012) (see figure 2.5).

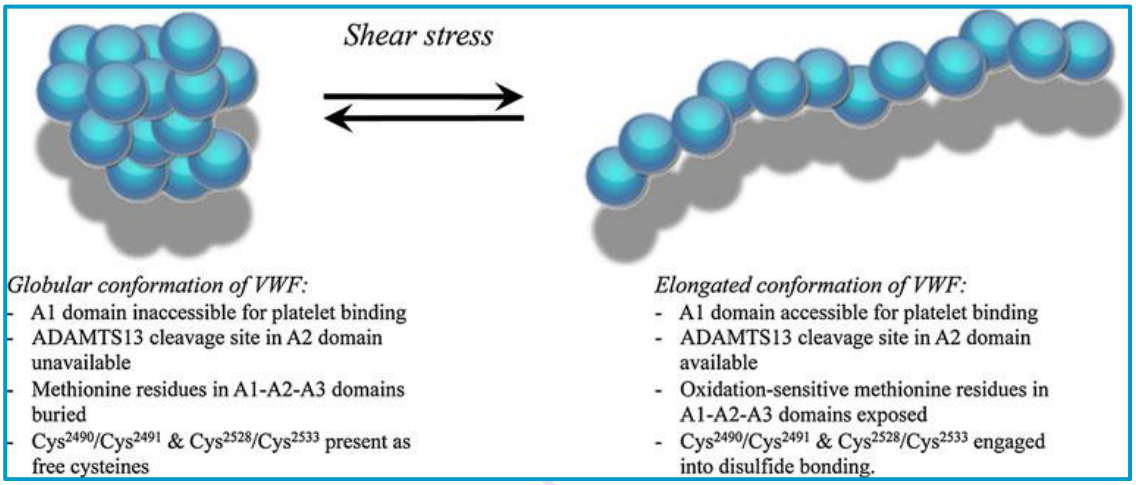


Figure 2.5 Events associated with shear stress-induced conformational changes in VWF (From: Denis *et al*. 2012) Abbreviations: von Willebrand factor (VWF), A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), cysteine (Cys).

Initial binding to collagen at sites of vascular injury slows the VWF down, and also allows for this conformational change to take place (Szántó *et al*. 2012). With the binding sites now exposed, VWF binds to the platelets via the platelet GPIb receptor, more specifically, the GPIb-IX-V complex, with strong affinity resulting in initial platelet tethering, platelet-vessel wall adhesion and initiates the process of platelet activation. The next step, stable platelet adhesion, is facilitated by the binding of VWF to the $\alpha_{IIb}\beta_3$ integrin receptor on platelets that have been activated by thrombin (Hassan *et al*. 2012; Luo *et al*. 2012; Peyvandi *et al*. 2011). This has been described previously (see section 2.1.1). Figure 2.6 indicates the elements involved in VWF-mediated platelet adhesion and aggregation.

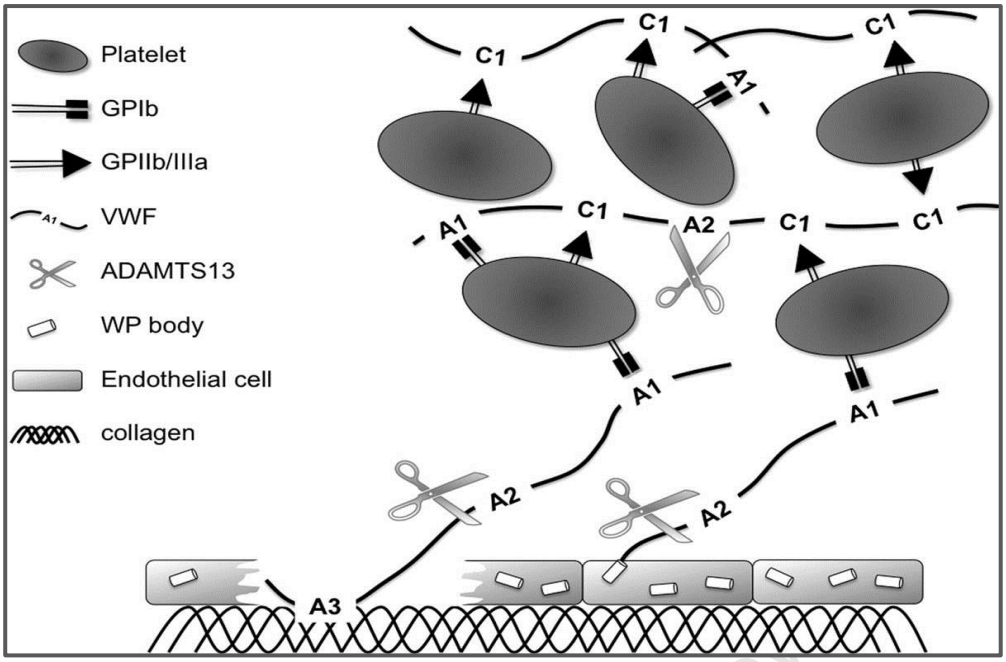


Figure 2.6 Schematic representation of VWF-mediated platelet adhesion and aggregation (From: De Meyer *et al*. 2012a) Abbreviations: glycoprotein (GP), von Willebrand factor (VWF), A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), Weibel-Palade (WP).

b. Stabilisation of blood coagulation factor VIII and binding to collagens, sulfatides and heparin

VWF binds to factor VIII thus stabilising the latter and protecting it from degradation by protein C (Peyvandi *et al*. 2011; Luo *et al*. 2012). By binding to sulfatides, it mediates red blood cell agglutination and also binds to heparin and collagen (Hassan *et al*. 2012).

c. Other biological functions

Multiple other non-haemostatic functions of VWF have been identified. These functions include the regulation of angiogenesis and mitogenic effects on smooth muscle cell proliferation (Luo *et al*. 2012).

2.5.5 Regulation of VWF plasma levels

VWF expresses ABO antigens (the antigens found on the surface of red blood cells). ABO blood grouping is an important genetic determinant of plasma VWF levels: plasma VWF levels have been shown to be lowest in blood group O patients and highest in blood group AB patients (Vischer 2006; Gill *et al*. 1987). Polymorphisms, such as the C/G polymorphism at the -1793 position of the VWF promoter and a mutation in the coding sequence at position 789 (THR789Ala)

have been associated with elevated VWF levels and a higher associated risk of coronary heart disease. Non-genetic factors that can elevate plasma VWF levels over long periods include advancing age, impaired endothelial nitric oxide production, inflammation, diabetes, insulin resistance (metabolic syndrome) and increased reactive oxygen species production (associated with obesity). Increased arterial rigidity associated with ageing may increase endothelial VWF secretion (Vischer 2006).

Rapid, short term increases in VWF secretion can occur through various secretion agonists such as histamine, collagen, epinephrine, as well as processes such as inflammation, endothelial damage, exercise and pregnancy (Hassan *et al*. 2012; McGrath *et al*. 2010; Sadler 1998; Petri *et al*. 2010; Boneu *et al*. 1975; Gonzales *et al*. 2009; Huq *et al*. 2012). Nitric oxide, reactive oxygen species and drugs such as statins may inhibit VWF release from endothelial cells and decrease plasma VWF levels (Vischer 2006; van Schie *et al*. 2010).

2.5.6 VWF and its disease associations

a. Von Willebrand disease and acquired von Willebrand syndrome

VWD is a bleeding disorder caused by inherited defects in the concentration, structure or function of VWF i.e. quantitative or qualitative defects in VWF. Patients with VWD are classified into 3 main categories: type 1, 2 and 3. Type 1 is associated with a partial quantitative deficiency, with type 3 indicating a virtually complete deficiency of VWF (Sadler *et al*. 2006; Schneppenheim 2011).

The acquired von Willebrand syndrome is a rare bleeding disorder that can be caused by lymphoproliferative and myeloproliferative disorders, immune-mediated diseases, cardiovascular disorders, malignancies, hypothyroidism and altered shear stress. These give rise to structural or functional defects of VWF (Tiede *et al*. 2011; Luo *et al*. 2012).

b. VWF and its association with liver disease and tumours

VWF may play a role in the pathophysiology of certain liver diseases. Higher VWF levels have been correlated with poorer outcome in hepatic cirrhosis. VWF may also influence the pathophysiology of other liver diseases such as alcoholic hepatitis. Abnormal VWF levels and activity have been described in certain tumours and metastases as well (Luo *et al*. 2012).

c. VWF and its association with cardiovascular disease: coronary artery disease (CAD) and peripheral arterial disease (PAD)

A number of studies have investigated the predictive value of VWF for coronary artery disease in healthy subjects (Folsom *et al*. 1997; Rumley *et al*. 1999; Meade *et al*. 1994; Thögerson *et al*. 1998; Whincup *et al*. 2002; Morange *et al*. 2004; Danesh *et al*. 2004). In general, VWF is thought to be a poor prognostic marker for CAD in the healthy population. However, it may have a better predictive value for CAD in high-risk patients with diabetes and/or pre-existing atherosclerosis (Vischer 2006). In acute coronary syndromes (especially acute myocardial infarction), VWF has been found to be elevated and its presence has been noted in fresh coronary thrombi (Goto *et al*. 1997; Goto *et al*. 1999; Li *et al*. 2000; Yamashita *et al*. 2006; Hoshiba *et al*. 2006; Spiel *et al*. 2008).

The data concerning the association between VWF and PAD are limited. Smith *et al*. (2000) found that median levels of fibrinogen and VWF were higher in older patients who developed PAD than those who did not. Woodburn *et al*. (1996) showed that high pre-operative VWF levels were associated with poorer outcome of graft surgery. Cassar *et al*. (2005) reported higher levels of VWF in patients with intermittent claudication and critical limb ischaemia, compared with controls. They concluded that coagulation activation and endothelial stimulation were significantly increased in patients with PAD (Cassar *et al*. 2005).

d. VWF, endothelial dysfunction and atherosclerosis

Damage to, or activation of, endothelial cells results in increased VWF release (Boneu *et al*. 1975). Plasma VWF has been identified as a marker of endothelial dysfunction (Blann 1993). Endothelial dysfunction impairs the ability of endothelial cells to function appropriately in the coagulation process, influencing pathological thrombus formation and atherosclerosis (Boneu 1975). Endothelial dysfunction has been shown to be an early step in the development of atherosclerosis, increasing the risk for cardiovascular disease substantially (Sitia *et al*. 2010). Horvath *et al*. (2004) and Lip *et al*. (1997) both advocate VWF measurement as a useful marker for detecting endothelial dysfunction and a surrogate measure for atherosclerosis. Serial measurements may be used as a marker of disease progression (Horvath *et al*. 2004; Lip *et al*. 1997). Horvath *et al*. (2004) showed significant differences in the levels of VWF in acute coronary syndromes and acute strokes compared with patients who had chronic vascular diseases. However, VWF levels in both acute and chronic vascular disease were higher compared with healthy controls (Horvath *et al*. 2004). Therefore measurements of VWF may become increasingly clinically relevant.

2.5.7 VWF and stroke

In comparison to coronary artery disease, less is known about the relationship between VWF and ischaemic stroke. Available data are conflicting. This section attempts to best describe the differing results and the context in which they were found. It is important to bear in mind that these studies have mainly been carried out in older participants, often with upper limits in the range of 75 years old. Also, I could not find any studies that examined this relationship in the context of patients with HIV infection. Thus the role of VWF in HIV-related young stroke patients is a novel study.

a. Increased levels of VWF in case-control studies

Some case-control studies have shown increased levels of VWF in patients with ischaemic stroke. Studies conducted in the early 1980s initially provided evidence for an association between elevated VWF levels and ischaemic cerebrovascular events (Lip *et al*. 1997). Bongers *et al*. (2006), in their study of 124 first-ever ischaemic stroke patients versus 125 age-and sex-matched controls, showed that VWF antigen (VWF:Ag) and activity levels were significantly higher in cases than in controls. When the VWF levels of all participants were analysed, the relative risk (RR) of stroke was significantly increased (RR = 3.21) in those with the highest quartile range of VWF:Ag levels. These data reflected the analysis of VWF in the acute phase (between 7-14 days) following stroke presentation. VWF:Ag levels were not increased in the patients three months post stroke (Bongers *et al*. 2006).

Lip *et al*. (2002) also found significantly raised plasma levels of VWF at 48 hours and 1 week respectively, following an acute stroke. The levels were compared with age-matched healthy controls. However, they raised the question as to whether this elevation in VWF could not be attributed to an acute phase response post-stroke (Lip *et al*. 2002). Similarly, Catto *et al.* (1997) reported elevated levels of VWF initially and 3 months following stroke compared with a healthy population, and found that elevated VWF levels were associated with an increased risk of death after six months follow-up. Stott *et al*. (2001) also showed raised VWF levels in patients in the ischaemic stroke group in their study. They also suggested the association may be due to an acute phase response (Stott *et al*. 2001).

Other case-control studies on VWF and ischaemic stroke also report increased levels of VWF in stroke (Licata *et al*. 2009; Bath *et al*.1998; Qizilbash *et al*. 1997).

VWF propeptide and mature VWF are secreted equimolarly from endothelial cells, therefore VWF propeptide levels reflect the rate of mature VWF secretion (Wagner *et al*. 1987). Van Schie *et al*. (2010) showed that increased VWF propeptide and mature VWF were both individually significantly associated with the occurrence of ischaemic stroke.

Hanson *et al*. (2011) reviewed this relationship in the context of the varying aetiologic subtypes of ischaemic stroke. There are few data in the literature looking at this. Using the TOAST classification system, patients with stroke attributable to large vessel disease and cardio-embolism displayed the highest levels of VWF compared with stroke caused by small vessel disease in the acute phase of the stroke. VWF levels at 3 months were highest in those patients whose stroke was associated with cardio-embolism and cryptogenic causes (Hanson *et al*. 2011).

b. VWF as a predictor of stroke

In case-control studies, we can never be sure whether the high VWF levels reported post-stroke are a cause or a consequence of the event. It is thus necessary to review this association in prospective studies.

The Atherosclerosis Risk in Communities (ARIC) study evaluated the role of a variety of markers of haemostatic function, including VWF, with the risk of ischaemic stroke. The investigators concluded that participants (191 stroke cases and 14 522 non-stroke controls) who had VWF levels in the upper quartile range of VWF, had a 1.7-fold greater risk of developing ischaemic stroke than those with VWF values in the lowest quartile range (Folsom *et al*. 1999). However, other cardiovascular risk factors may have confounded these results.

In the Rotterdam study, a large population-based cohort study with 6250 participants, the risk of all stroke (ischaemic and haemorrhagic) and the risk of ischaemic stroke alone, increased with increasing VWF levels. The association was independent of cardiovascular risk factors (Wieberdink *et al*. 2010).

Tzoulaki *et al*. (2007) showed that elevated plasma VWF levels were associated with future cardiovascular disease even after adjusting for cardiovascular risk factors, sub-clinical atherosclerosis and a baseline history of cardiovascular disease. Baseline VWF levels and many of

the other haemostatic and rheological markers measured in their cohort were higher in people who developed myocardial infarction and stroke, compared with those who remained free of cardiovascular disease. However, they did not advocate the use of VWF as a clinical marker for assessing cardiovascular risk but suggested instead that there might be a place for the measurement of multiple markers in clinical practice to aid with cardiovascular risk assessment (Tzoulaki *et al*. 2007).

Conway *et al*. (2003) found that plasma VWF levels were a significant predictor of stroke and vascular events (stroke, myocardial infarction or vascular death in atrial fibrillation) in patients with atrial fibrillation. The risk of stroke was greatest with the highest levels of VWF. However, after adjusting for traditional risk factors, the relationship between VWF and stroke was no longer significant (Conway *et al*. 2003).

c. Studies with a lack of association between VWF and stroke

Other studies, however, did not show an association between VWF and stroke. In a large study of only men (n = 2208) and a median follow-up of 13 years, Smith *et al*. (2005) examined the contribution of various haemostatic markers to the risk of coronary artery disease and ischaemic stroke. VWF level was measured in nearly 2000 of these participants but no association between VWF and ischaemic stroke risk was noted (Smith *et al*. 2005). Johansson *et al*. (2002) also showed no association between VWF and the development of first-ever ischaemic stroke in their prospective study. Similarly Yip *et al*. (2007) and Cherian *et al*. (2003) failed to demonstrate an association in their case-control studies.

d. VWF levels in ischaemic versus haemorrhagic stroke

Few studies have examined VWF levels in relation to haemorrhagic stroke. Liu *et al*. (1993) reported higher VWF levels in patients with thrombotic strokes compared to patients with haemorrhagic strokes. Others showed an increase in VWF levels in both ischaemic and haemorrhagic stroke in the acute phase (Bath *et al*. 1998). No significant association between VWF and haemorrhagic stroke was found in a prospective study by Johansson *et al*. (2002).

e. Murine VWF stroke studies

Murine (mouse) studies on the role of VWF in ischaemic stroke have provided much needed clues to understanding this relationship in humans. A murine study showed that VWF deficiency protects mice from ischaemic stroke, without causing intracerebral haemorrhage. In this study, they demonstrated that 24 hours after reperfusion, VWF-deficient mice had significantly smaller infarctions, approximately reduced to 60% of the infarct volumes of the wild-type controls, and fewer neurologic deficits when compared with the wild-type control mice (Kleinschnitz *et al*. 2009).

Other murine studies investigated the role of platelet receptors for VWF and their role in stroke development. Stoll *et al*. (2010) recently established that the GPIbα platelet receptor (of the GPIb-IX-V complex) and its interaction with VWF played a central role in initiating thrombus formation in the ischaemic brain. Inhibition of VWF binding to GPIbα by blockade of the receptor binding site with before- and after-transient middle cerebral artery occlusion, has been shown to protect mice from ischaemic brain infarction. The resulting brain infarct volume was significantly reduced compared with control mice (Kleinschnitz *et al*. 2007).

Animal model studies have shown that VWF deficiency plays a protective role in arterial thrombosis, with VWF-platelet interactions responsible for a protective effect on atherogenesis. Human studies have been inconclusive in this regard, but VWF is still considered significant in the human body's interplay between haemostasis and atherosclerosis (van Galen *et al*. 2012).

f. VWF, metabolic syndrome and stroke

The metabolic syndrome refers to a cluster of risk factors that include dyslipidaemia, hypertension, insulin resistance and abdominal obesity as the core components of the syndrome. These increase the risk of atherosclerotic vascular disease (Towfighi *et al*. 2008; Kassi *et al*. 2011). The role of chronic pro-inflammatory and pro-thrombotic states in the syndrome have recently been added (Eckel *et al*. 2005; Kassi *et al*. 2011). Elevation of fibrinogen and plasminogen activator inhibtor 1 has been implicated as the cause of the pro-thrombotic risk (Eckel *et al*. 2005).

However, the role of VWF in the metabolic syndrome has not been well researched. Endothelial dysfunction and the metabolic syndrome have been linked (Fornoni *et al*. 2005; Tziamolos *et al*. 2010). The metabolic syndrome and stroke have been linked (Towfighi *et al*. 2008; Boden-Albala

et al. 2008). VWF has been linked to endothelial dysfunction and to stroke, therefore VWF measured in the metabolic syndrome may be predictive of stroke.

g. VWF gene polymorphisms and stroke

A number of polymorphisms have been reported on the gene encoding VWF, but their relationships to ischaemic stroke have not been conclusively proven (Stankovic *et al*. 2010; Smith *et al*. 2010; van Schie *et al*. 2012; Dai *et al*. 2001).

h. New VWF based stroke therapeutic interventions

Therapeutic interventions in stroke are now also investigating VWF antagonism as a target in stroke therapy. Compounds such as 82D6A3, 6B4, h6B4, AJvW2, AJW200, GPG-290, ARC1779, ALX-0081 and ALX-0681 are being investigated as possible inhibitors of VWF-mediated platelet adhesion (De Meyer *et al*. 2012a). Stoll *et al*. (2008) have highlighted the VWF-GPIb interaction as an important target for future stroke prevention and treatment.

2.5.8 VWF and HIV

a. VWF, HIV-related endothelial dysfunction, metabolic syndrome, platelet reactivity and opportunistic infections

We have already established that VWF is a marker of endothelial dysfunction (Horvath *et al*. 2004). Raised VWF correlates with platelet adhesiveness and VWF may enhance a thrombotic tendency by promoting platelet adhesion and aggregation, especially in stenotic vessels, where shear stress is increased (Qizlbash *et al*. 1997). There is evidence that HIV infection promotes chronic arterial inflammation and injury. The latter, in turn, gives rise to endothelial dysfunction, atherosclerosis and thrombosis (Fourie *et al*. 2011). It is conceivable that in this chronic inflammatory state, VWF levels may parallel that of the degree of endothelial dysfunction, thus further increasing the propensity for thrombosis.

The possible associations between VWF, metabolic syndrome and stroke discussed earlier are especially relevant in the context of HIV-related stroke (see section 2.5.7). In a retrospective case-control study by Ances *et al*. (2009), a higher percentage of HIV-associated cryptogenic stroke cases met the criteria for metabolic syndrome, when compared to HIV positive controls (36% versus 11%). The HIV positive participants in this study were classified into ART regimens, either

protease inhibitor (PI) or non-PI based. More patients with HIV-associated cryptogenic stroke tended to be on a PI-based regimen compared with non-stroke contols (Ances *et al*. 2009).

Platelets and HIV infection are linked. Platelets are able to internalise HIV particles, platelet-bound HIV-1 may infect permissive cells and chronic thrombocytopaenia is one of the haematological complications of HIV infection. Increased platelet reactivity has been documented in patients on HAART and those that are HAART-naïve (Gresele *et al*. 2012). Satchell *et al*. (2010) detected significant differences in platelet reactivity in HIV-infected patients compared with HIV negative controls. Abacavir (an antiretroviral drug) containing regimens and increased platelet activity have also been linked (Satchell *et al*. 2011; Francisci *et al*. 2011). Since activated platelets release VWF, altered platelet function in the setting of HIV may be a reason for the raised VWF levels in HIV-infected individuals. Further studies are required to confirm this.

Cytomegalovirus (CMV) is an opportunistic infection frequently seen in individuals infected with HIV (Steininger *et al*. 2006). There is evidence that cells infected with CMV show increased secretion of soluble VWF and increased cell-surface expression of VWF (Rahbar *et al*. 2005).

b. VWF and ART

The relationships between HIV infection, HAART and endothelial adhesion markers e.g. vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), P-selection, E-selectin, plasminogen activator inhibitor-1, thrombomodulin and VWF, have received much attention over the past decade. De Gaetano Donati *et al*. (2004) provide an excellent review in this field. Previous studies have documented elevated levels of VWF and other endothelial activation markers in ART-naïve patients (Lafeuillade *et al*. 1992; Seigneur *et al*. 1997). The possible mechanisms that lead to this increase are multifactorial and may be related to endothelial dysfunction itself, chronic inflammatory states, the influence of opportunistic diseases, pro-thrombotic states and other factors that contribute to this aberrant bodily homeostasis caused by infection with HIV. The latter were discussed in chapter 1.

In contrast, other studies have shown a decrease in VWF levels following ART initiation (Wolf *et al*. 2002; Aukrust *et al*. 2000). However, van Vonderen *et al*. (2009) have noted that not all markers of endothelial function improve following ART initiation and some even becoming further elevated. This elevation may be attributable to the varying metabolic effects of the different ARV

drug classes and individual drugs within each class or to direct damage of endothelial cells by HAART (de Gaetano Donati *et al*. 2004; van Vonderon *et al*. 2009). However, data are limited. Wolf *et* al. (2002) documented that 5 months following ART initiation, the levels of VWF were lower in plasma compared to pre-ART levels but did not completely reach normal levels. This held true for groups receiving either the PI or NNRTI-based regimens (Wolf *et al*. 2002). Therefore HAART was not fully able to counteract the influence of HIV on endothelial dysfunction. Thus, our protein of interest, VWF, could continue to exert its pro-thrombotic/pro-atherosclerotic/pro-inflammatory effects.

VWF levels are also influenced by viral load, CD4 count and overall immune status, with higher viral load and lower CD4 count correlating with elevated VWF levels (Lafeuillade *et al*. 1992; Seigneur *et al*. 1997).

2.6 A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13)

2.6.1 Overview of ADAM, ADAMTS and ADAMTS13 proteins

A Disintegrin and Metalloproteinase (ADAM) proteins are a group of transmembrane proteins that display a host of functions which range from regulation of the nervous system to signalling in the tumour micro-environment. Their functional ability is aided by their structure which is made up of several domains. These include an amino (N)-terminal propeptide domain, a metalloprotease domain, a disintegrin domain, a cysteine-rich region with an epidermal growth factor (EGF)-like sequence and a cytoplasmic carboxy (C)-terminal tail involved in intracellular signalling (see figure 2.7) (Yang *et al*. 2006; Murphy 2008; Benarroch 2012). A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS) proteins lack EGF-like repeats and a transmembrane domain and are therefore secreted, in contrast to ADAM proteins that are membrane-bound. They also possess one or more thrombospondin type 1 (TSP) and variable additional C-terminal domains compared with ADAM proteins (Levy *et al*. 2005; Cal *et al*. 2002).

Furlan *et al*. (1996) and Tsai (1996) were the first investigators to describe the VWF-cleaving protease and its activity in human plasma in 1996. Following in-depth structural and functional analysis and identification as an ADAMTS protein, ADAMTS13 received its status as a novel member of the ADAMTS family in 2001. ADAMTS13 is the 13th and one of the largest members

of the ADAMTS family. It differs from other members of the ADAMTS family by the presence of 2 C-terminal CUB domains (Levy *et al*. 2005). Its ability to perform a multitude of functions arises from the presence of its many different domains (Bornstein 2001; McLane *et al.*1998; Levy *et al*. 2005). Figure 2.7 provides a schematic depiction of ADAM, ADAMTS metalloproteases and ADAMTS13.

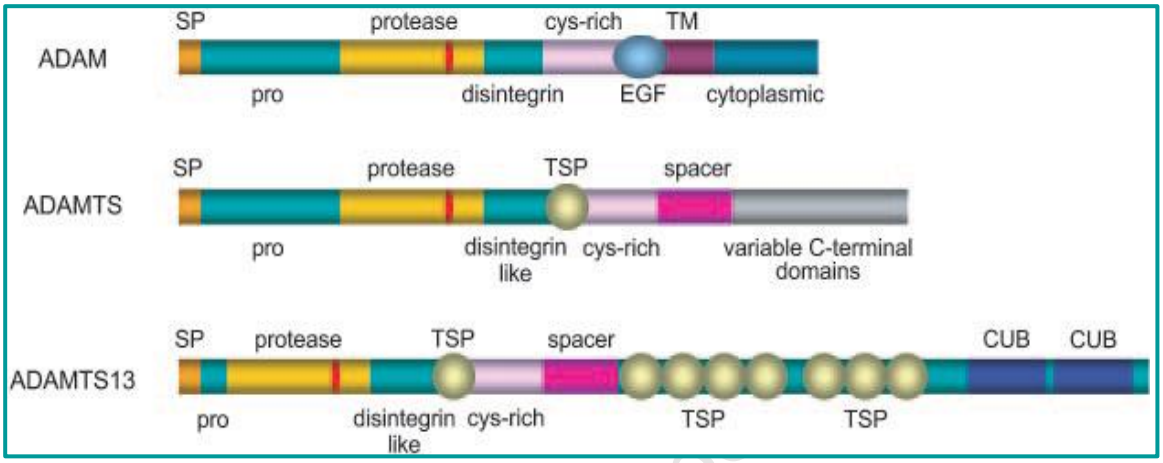


Figure 2.7 Schematic depiction of ADAM, ADAMTS and ADAMTS13 (From: Levy *et al*. 2005)
A Disintegrin and Metalloproteinase (ADAM), A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS) and A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13) are shown with the following structural components: signal peptide (SP), propeptide (pro), protease, metalloprotease (location of zinc-binding motif in red), disintegrin, disintegrin-like (dis-like) domain, cysteine-rich (cys-rich) domain, thrombospondin type-1 (TSP) motif, epidermal growth factor (EGF)-like repeat, transmembrane (TM) domain, CUB domain.

2.6.2 Synthesis and structure of ADAMTS13

The gene that encodes for ADAMTS13 is located on chromosome 9q34 (Levy *et al*. 2001). It encodes a protein composed of 1427 amino acids with a calculated molecular mass of 145kDa (Levy *et al*. 2005). Synthesis takes place predominantly in the liver, with synthesis and release also identified in endothelial cells (Levy *et al*. 2001; Zheng *et al*. 2001; Uemura *et al*. 2005; Turner *et al*. 2006). Suzuki *et al*. (2004) also showed that ADAMTS13 messenger RNA was expressed in platelets. A lesser degree of expression was identified in placenta and skeletal muscle (Zheng *et al*. 2001).

The 1427 amino acid-containing protein is synthesised as a precursor polypeptide made up of a signal peptide, a short 41 amino acid propeptide and a series of domains (Lämmle *et al*. 2005; Majerus *et al*. 2003). The mature form of ADAMTS13 begins with the metalloprotease domain which confers its catalytic ability (Lämmle *et al*. 2005). Refer to figure 2.7 for an overview of ADAMTS13 domain structure.

2.6.3 Storage and release of ADAMTS13

ADAMTS13 is a unique protease in that it normally circulates with VWF in an active form. However, it is only under conditions of high shear stress that ADAMTS13 is activated to carry out proteolysis (Tsai 2012). ADAMTS13 is also stored in platelet α granules and is released upon platelet activation (Nurden 2011).

2.6.4 Function of ADAMTS13

ADAMTS13 cleaves VWF. It was previously known as the VWF-cleaving protease. Haemostatically active ULVWF multimers are cleaved into smaller multimers that are less haemostatically active. Thus ADAMTS13 controls VWF size such that its activity is suitably matched for the haemostatic problem (Crawley *et al*. 2011). However, this is an intricate process facilitated by the integrated action of the various ADAMTS13 domains. I shall not discuss the details of these processes. For a complete review of the process, see Crawley *et al*. (2011), Xiang *et al*. (2011), Tsai (1996), and Denis *et al*. (2012).

VWF is exposed to elevated shear stress in areas of vascular injury, in stenosed blood vessels, when blood passes through the microvasculature and when it becomes tethered on exposed sub-endothelial collagen following vascular injury. It is this high shear stress that unfolds the globular VWF into an elongated, active string-like form (refer back to figure 2.5). Following unfolding, numerous other binding sites (exosites) are exposed and the interaction between the enzyme and substrate continues. Cleavage of VWF takes place at the Tyr1605-Met1606 bond located within the central VWF A2 domain (Crawley *et al*. 2011; Denis *et al*. 2012; Xiang *et al*. 2011).

2.6.5 Regulation of ADAMTS13 activity

The main regulator of ADAMTS13 activity is flow shear stress, with elevated rheologic shear forces causing conformational change of its substrate, VWF (Shelat *et al*. 2005; Crawley *et al*. 2011). Other factors that influence the interaction between VWF and ADAMTS13 include: certain structural elements of VWF, heparin sulphate, platelets or GPIbα, sodium chloride, inflammatory cytokines, chloride ions, haemoglobin, thrombin, factor Xa and plasmin (Shelat *et al*. 2005).

2.6.6 ADAMTS13 and its disease associations

a. Thrombotic Thombocytopaenic Purpura (TTP)

TTP was first described in a 16 year old girl by Dr Eli Moschcowitz in 1924 (Moschcowitz 1924). TTP is a type of thrombotic microangiopathy that manifests with ischaemic neurologic, renal and other organ dysfunction. It is commonly recognised in clinical practice by a pentad of clinical

features: miroangiopathic haemolytic anaemia with schistocytes in the peripheral blood smear, thrombocytopaenia, neurologic signs and symptoms, fever and renal impairment (Moake 2002; Lämmle *et al*. 2005). Deficiency of ADAMTS13 gives rise to TTP and occurs secondary to a congenital deficiency of ADAMTS13 or to deficiency brought about by autoimmune inhibitors (acquired TTP) targeted at ADAMTS13 (Tsai 2010). The platelet thrombi in TTP are thus rich in VWF as a result of impaired VWF cleavage by ADAMTS13 and insufficient processing of the ULVWF multimers (Moake 2002; Pos *et al*. 2011).

b. Congenital, acquired (idiopathic and secondary) and HIV-related TTP

Numerous mutations in the gene that encodes for ADAMTS13 have been identified and are implicated in the congenital forms of TTP (Lotta *et al*. 2010; Kokame *et al*. 2004; Levy *et al*. 2001). Acquired TTP is TTP that is not attributed to any form of genetic aberration. Idiopathic TTP and secondary TTP fall into this category. In idiopathic acquired TTP, auto-antibodies hinder ADAMTS13 activity by inhibiting its enzymatic function or by clearing it from the circulating blood (Pos *et al*. 2011). TTP that occurs in the presence of a predisposing factor is labelled secondary TTP. Autoimmune haemolysis, disseminated intravascular coagulation (DIC), cancer, eclampsia, drug toxicity (e.g. treatment with calcineurin inhibitors), haematopoietic stem cell transplantation, HIV infection or malignant hypertension may predispose patients to secondary TTP (Sadler 2008).

HIV is associated with an increased incidence of TTP (Blazes *et al*. 2004). It is the most common virus precipitating TTP (Opie 2012). Its presentation has been associated with advanced HIV infection (Novitzky *et al*. 2005). There is, however, heterogeneity with regard to ADAMTS13 activity and inhibitors in patients with HIV-related TTP (Gunther *et al*. 2007). Acquired inhibitors (auto-antibodies) have been detected in HIV-related TTP, but not in all studies (Gunther *et al*. 2007; Meiring *et al*. 2012; Miller *et al*. 2005). Gunther *et al*. (2007) proposes either a "consumptive deficiency" mediated by direct endothelial cell injury caused by HIV itself, or damage due to inflammatory cytokines seen in infection with HIV as an alternative mechanism. These mechanisms lead to an excessive release of VWF, with resultant inability of ADAMTS13 to cope with the increased cleaving burden (Gunther *et al*. 2007).

c. Other causes of ADAMTS13 deficiency and increased amounts of soluble ULVWF multimers

The presence of ULVWF multimers with or without severely reduced levels of ADAMTS13 has been reported in patients with sepsis, DIC, liver diseases, infection with plasmodium falciparum,

transplantation and immunosuppression with cyclosporin and sickle cell disease (Tsai 2010; Turner *et al*. 2012). However, these high ULVWF multimer levels and ADAMTS13 deficiency states do not necessarily always cause severe thrombosis and their significance in the clinical setting is uncertain (Tsai 2010).

d. ADAMTS13 and its association with coronary artery disease (CAD) and atherosclerosis

There is an association between ADAMTS13 and CAD. Kaikita *et al*. (2006) and Matsukawa *et al*. (2007) have reported reduced ADAMTS13 activity in acute myocardial infarction. They also noted that an early reduction in ADAMTS13 levels following acute myocardial infarction predicted future thrombotic events (Kaikita *et al*. 2006; Matsukawa *et al*. 2007). The imbalance between VWF and ADAMTS13 activity confers a state of prolonged thrombogenicity on patients with unstable angina (Fuchigami *et al*. 2008).

In a recent study by De Meyer *et al*. (2012b), ADAMTS13 was shown to play an important role in ischaemia/reperfusion injury in myocardial infarction. Following ischaemia/reperfusion injury to the myocardium, ADAMTS13-deficient mice developed larger myocardial infarcts than their ADAMTS13-replete wild-type counterparts (De Meyer *et al*. 2012b).

Schettert *et al*. (2010) reported on associations between ADAMTS13 genotypes and cardiovascular disease. Polymorphisms in the ADAMTS13 gene (especially the ADAMTS13 A900V variant) was associated with decreased ADAMTS13 activity and an increased risk of death in patients with CAD (Schettert *et al*. 2010).

Mouse models of ADAMTS13 and atherosclerosis have shown that ADAMTS13 can reduce excessive vascular inflammation and plaque formation during early atherosclerosis, and that more extensive atherosclerotic plaque formation occurs in ADAMTS13-deficient mice fed a high-fat diet (Gandhi *et al*. 2012; Jin *et al*. 2012). These findings provide a link between ADAMTS13 and atherosclerosis and, therefore, stroke aetiology.

2.6.7 ADAMTS13 and stroke

a. Current data

There are limited data but these do suggest an association between ADAMTS13 and stroke. The field, however, is expanding. Bongers *et al*. (2006) were the first to determine the levels of ADAMTS13 in patients with ischaemic stroke. They observed a trend towards lower ADAMTS13

activity levels in ischaemic stroke patients. Also, the ischaemic stroke risk was lowest in patients with the highest measured ADAMTS13 activity levels (Bongers *et al*. 2006).

In an experimental model, ADAMTS13-deficient mice displayed an increased susceptibility to focal cerebral ischaemia. The infusion of a high dose of recombinant ADAMTS13 into a wild-type mouse immediately before reperfusion reduced the final infarct volume (Zhao *et al*. 2009). Fujioka *et al*. (2010) also demonstrated that ADAMTS13 deficiency exacerbates ischaemic brain injury in their murine model. These studies highlight a potential therapeutic role of ADAMTS13 as a neuro-protector in stroke treatment (Nieswandt *et al*. 2010).

Fujioka *et al*. (2012) have recently reported the effect of ADAMTS13 on inflammation after brain ischaemia. Following ischaemia-reperfusion, mice deficient in ADAMTS13 had larger brain infarcts and a greater increase in high-mobility group box 1 (HMGB1), a marker of post-stroke inflammation, compared with wild-type mice. ADAMTS13 may therefore regulate inflammation following brain ischaemia and therefore protect the nervous system from ischaemia-reperfusion injury (Fujioka *et al*. 2012).

Enhanced formation of reactive oxygen species and peroxynitrite can occur in the clinical setting of stroke, coronary artery disease and chronic inflammatory disease, amongst other conditions. Peroxynitrite hinders the proteolytic cleaving action of ADAMTS13, thus possibly contributing to a pro-thrombotic state (Lancelloti *et al*. 2010). This may represent another mechanistic link between ADAMTS13 and stroke.

b. VWF and ADAMTS13 joint influence on stroke risk

Previous studies have mainly investigated VWF and stroke association in isolation. Few have looked at the joint influence on VWF and ADAMTS13 on stroke risk. As mentioned previously, Bongers *et al*. (2006) were the first to investigate the effect of ADAMTS13 levels on ischaemic stroke risk. They also confirmed the negative association of VWF and ADAMTS13 levels as reported in other studies (Bongers *et al*. 2006).

Andersson *et al*. (2012) characterised the joint risk in a gender-specific study of young women aged 18-49 years. They showed that high VWF and low ADAMTS13 plasma levels were both associated with an increased risk of ischaemic stroke and myocardial infarction. This risk was further increased with the use of oral contraceptives. The joint risk conferred by having both high VWF and low ADAMTS13 levels was higher than that of the risk of stroke attributable to the

individual abnormal VWF and ADAMTS13 levels (Andersson *et al*. 2012). A similar finding of greater cardiovascular disease risk in individuals with the combination of lowest ADAMTS13 and highest VWF levels was observed in a study that included patients with stroke, coronary artery disease and peripheral arterial disease (Bongers *et al*. 2009). The study of Andersson *et al*. (2012) differed, however, from many studies by its exclusive female population and younger age group. The latter were studied to minimise the influence of atherosclerotic risk (Andersson *et al*. 2012).

c. ADAMTS13 polymorphisms and stroke risk

A number of mutations in the ADAMTS13 gene have been identified (Shelat *et al*. 2005). The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) investigated the association between various single nucleotide polymorphisms of the ADAMTS13 gene and ischaemic stroke in a group of patients from western Sweden. The rs4962153 single nucleotide polymorphism was significantly associated with an increased risk of ischaemic stroke and the subtype of cryptogenic strokes (Hanson *et al*. 2009).

2.6.8 ADAMTS13 and HIV

We have already described the link between HIV and TTP. Early initiation of HAART and plasma exchange therapy for HIV-infected patients with TTP has been proposed (Hart *et al*. 2011). ADAMTS13 deficiency and diminished activity have also been reported in other HIV-associated thrombotic microangiopathies (Malak *et al*. 2008). This adds to the evidence linking ADAMTS13 and HIV infection. No studies exist examining this relationship directly in HIV-infected individuals with stroke. Our study will therefore attempt to fill the gap in this knowledge.

CONCLUSION

Vascular haemostasis is a complex dynamic process. Infection with HIV can compromise the haemostatic process. Certain coagulation abnormalities may cause stroke and some of these abnormalities are often recognised in HIV-infected individuals.

VWF is an intricate protein with multiple roles. Deficiencies or aberrant function of this molecule may give rise to life threatening conditions such as von Willebrand disease. Increased levels of VWF have been associated with stroke. However, the data on this subject are limited and mechanisms for the association are uncertain. VWF levels may predict stroke, but further investigation is required before we can advocate its routine testing in clinical settings. HIV, with its many effects on vasculature and the chronic inflammatory environment that it creates, provides a

link for the association between raised levels of VWF and HIV infection. A link between HIV-related stroke and VWF is therefore plausible and will be investigated in the younger adults in this study.

ADAMTS13 is a protease whose biological structure and function has generated much recent research interest. It is clinically important in a host of illnesses, and has revolutionised the understanding of TTP since its discovery. The role that it plays in VWF cleavage is pivotal to its involvement in the pathogenesis of stroke. ADAMTS13 functioning is altered by HIV infection and stroke occurs more frequently in young, HIV positive individuals. Thus, we can propose a mechanistic role for ADAMTS13 in the pathogenesis of HIV-related stroke.

CHAPTER 3 – AIMS AND HYPOTHESES

INTRODUCTION

In the previous two chapters, we reviewed the literature on HIV and stroke and identified important gaps in our current knowledge. This short chapter is intended to provide the reader with our aims and hypotheses which we hoped would address those gaps in our knowledge.

3.1 Aims

Our main aim was to investigate the role of VWF and ADAMTS13 in the pathogenesis of HIV-related young stroke. In order to minimise the influence of confounding variables in our study, we documented the presence of other potential risk factors for stroke.

3.2 Hypotheses

The evidence indicates that HIV infection is associated with abnormalities in the coagulation system and that these result in a pro-thrombotic state. HIV infection also appears to be associated with an increased occurrence of stroke and a younger age of presentation with cerebrovascular events. I therefore proposed the following hypotheses:

3.2.1 Hypothesis 1

HIV positive young stroke participants will have a pro-thrombotic coagulation profile characterised by higher levels of VWF compared with HIV negative young stroke participants. This hypothesis will be tested by measuring the quantity of VWF in the plasma of these two groups, using the VWF antigen (VWF:Ag) assay.

A secondary hypothesis (1a) related to this, is that HIV positive young stroke participants whose stroke is "idiopathic" (i.e. cannot be attributed to any other identifiable cause) will have a pro-thrombotic coagulation profile characterised by higher levels of VWF compared with HIV negative young stroke participants whose stroke is also "idiopathic."

3.2.2 Hypothesis 2

HIV positive young stroke participants will have a pro-thrombotic coagulation profile characterised by higher levels of VWF compared with HIV positive young control participants who have not had a stroke. This will also be determined using the VWF:Ag assay.

3.2.3 Hypothesis 3

HIV positive young stroke participants will have a pro-thrombotic coagulation profile characterised by higher levels of VWF activity compared with HIV negative young stroke participants. This will be measured using the VWF collagen binding (VWF:CB) assay.

A secondary hypothesis (3a) predicts that HIV positive young stroke participants whose stroke is "idiopathic" will have a pro-thrombotic coagulation profile characterised by higher levels of VWF activity compared with HIV negative young "idiopathic" strokes.

3.2.4 Hypothesis 4

HIV positive young stroke participants will have a pro-thrombotic coagulation profile characterised by higher levels of VWF activity (VWF:CB assay) compared with HIV positive young non-stroke control participants.

3.2.5 Hypothesis 5

HIV positive young stroke participants will have a pro-thrombotic coagulation profile characterised by lower levels of ADAMTS13 compared with HIV negative young stroke participants. This will be measured using the ADAMTS13 antigen (ADAMTS13:Ag) assay.

A secondary hypothesis (5a) is that HIV positive young stroke participants with "idiopathic" strokes will have a pro-thrombotic coagulation profile characterised by lower levels of ADAMTS13 compared with HIV negative young "idiopathic" strokes.

3.2.6 Hypothesis 6

HIV positive young stroke participants will have a pro-thrombotic coagulation profile characterised by lower levels of ADAMTS13 compared with HIV positive young non-stroke control participants. This will be determined using the ADAMTS13:Ag assay.

3.2.7 Hypothesis 7

Levels of VWF (7a) and VWF activity (7b) will be negatively correlated with CD4 counts in HIV positive participants with and without strokes i.e. with advancing immunosuppression, VWF levels will increase.

ADAMTS13 levels (7c) will be positively correlated with CD4 counts in HIV positive participants with and without strokes i.e. with advancing immunosuppression, ADAMTS13 levels will decrease.

The results of the hypotheses will be presented in chapter 6.

CHAPTER 4 – METHODS

INTRODUCTION

In this chapter, I shall describe the study design, participant groups, inclusion and exclusion criteria, participant recruitment and laboratory methods used to analyse the samples. I shall also discuss the question of sample size and the statistical tests used to analyse the data. Finally, summary flow diagrams of the methods will be presented. The study was approved by the University of Cape Town (UCT)/Groote Schuur Hospital (GSH) Human Research Ethics Committee (HREC) (HREC REF 178/2010).

4.1 Study design and research setting

The study was an observational case-control study. It formed part of a larger on-going study of HIV-related young stroke. It was based in Cape Town, South Africa, in the study hospitals of the University of Cape Town, as well as the community health centres in the greater metropolitan area of the city.

4.2 Participant group allocation

The participants in this study were divided into 3 groups. All of the participants who had suffered a stroke were categorised as case groups and those participants without stroke were categorised into control groups. The groups were enrolled according to the inclusion and exclusion criteria, as summarised in table 4.1. They were all ≥ 18 years of age and ≤ 45 years of age.

Inclusion criteria	Exclusion criteria
Applicable to all participants • Participants had to be aged ≥ 18 or ≤ 45 years • Participants had to have signed informed consent **Applicable to case participants only** • Participants had to have had a recent ischaemic stroke i.e. only participants referred within 7 days from stroke onset were eligible • If HIV positive, a confirmed diagnosis of HIV infection at the time of enrolment and no history of HAART use • If HIV negative, a confirmed diagnosis that participant was not HIV positive **Applicable to control participants only** • Participants had to have had no history of current or prior stroke • Participants had to have a confirmed diagnosis of HIV infection at the time of enrolment • No history of HAART use at the time of enrolment	**Applicable to all participants** • Age < 18 or > 45 years • Patient refusal to participate • Inability to obtain consent • Subarachnoid, subdural or epidural haemorrhage • Pregnancy **Applicable to case participants only** • If stroke was a haemorrhagic stroke • If meningitis was the cause of the stroke

Table 4.1 Inclusion and exclusion criteria Abbreviations: Human Immunodeficiency Virus (HIV), highly active antiretroviral therapy (HAART).

4.2.1 Cases

a. HIV negative young stroke patients

All of the participants in this group were HIV negative and had had a confirmed stroke within 7 days of the time of enrolment into the study.

b. HIV positive young stroke patients (HAART-naïve)

All of these participants were HIV positive, had had a confirmed stroke, and had not yet started treatment with HAART when enrolled in the study.

4.2.2 Control group

a. HIV positive young non-stroke patients (HAART-naïve)

All of the participants in this group were HIV positive, had not suffered a stroke and had not yet started treatment with HAART at the time of enrolment in the study.

4.3 Sample size and power calculation

VWF has been studied in the setting of stroke (see chapter 2, section 2.5.7). Participant numbers were small in these studies. There has been little research on ADAMTS13 in the setting of stroke (see chapter 2, section 2.6.7). Furthermore, to our knowledge, these factors have not been studied directly in the setting of HIV, let alone in the setting of HIV-related stroke. This therefore is a pilot study and, to our knowledge, the first of its kind.

Our study numbers were limited to 100 participants due to a number of factors. These included short study duration, financial limitations and lack of available human resources. A total of 91 suitable stroke participants (20 HIV positive and 71 HIV negative) and 40 suitable HIV positive non-stroke controls were enrolled during our study period. We therefore best matched according to age and sex, 40 HIV negative stroke participants to the two HIV positive groups (stroke and non-stroke) to complete our predefined number of 100 participants (See figures 4.4 and 4.5). Table 4.2 summarises the participant group allocation.

	HIV positive young stroke (HAART-naïve)	**HIV negative young stroke**	**HIV positive young non-stroke controls (HAART-naïve)**	**Total participants**
No. of participants	20	40	40	100

Table 4.2 Participant groups Abbreviations: highly active antiretroviral therapy (HAART), number (No.).

Power calculations were therefore performed using the fixed sample sizes which we had for each group. With $\alpha = 0.05$ and between-group comparisons fixed at a 1:2 ratio (20:40 participants), table 4.3 presents the power that could have been attained if the effect sizes measured in our study mirrored any of those values in the table.

Fixed factors	Power	Effect size
Between group comparisons: Always 20:40 participant ratio (n = 60) α = 0.05	50 %	0.55
	60 %	0.62
	70 %	0.70
	75 %	0.74
	80 %	0.79
	85 %	0.84
	90 %	0.91
	95 %	1

Table 4.3 Power and corresponding effect sizes based on our sample size Abbreviation: sample size (n).

The table shows that our study is capable of detecting a 0.79 effect size with 80% power, given the total sample size used (n = 60) for each between-group comparisons.

It is important to reiterate that this study is a pilot study. A larger study would not have been feasible or appropriate at the time. However, as a result of this study, we shall have the data that can be used to calculate sample sizes and power calculations for future larger studies (see chapter 8, section 8.3).

4.4 Recruitment of participants

My study was part of a larger study, in which a total of 240 participants were enrolled between May 2010 and August 2012. All the files were reviewed and participant selection for this study was narrowed down. Patients on HAART were included in the larger study but excluded in my study which looked specifically at HAART-naïve participants. Overall a total of 20 HIV positive young stroke HAART-naïve participants were considered eligible. Please refer to the flow chart in figure 4.4 for details of the recruitment process, attrition and exclusions. The second flow chart (figure 4.5) describes the selection of participants in my study for this project.

4.4.1 Cases

All participants were recruited from tertiary and secondary-level public hospitals located in the Western Cape. The four hospitals were: Groote Schuur Hospital, New Somerset Hospital (NSH), Victoria Hospital (VHW), and GF Jooste Hospital (GFJ). The treating physicians at these hospitals were aware of the study through advertising and referred potential participants to us. If a patient

met the inclusion criteria, the patient's primary physician would refer the patient to the study. These patients were then assessed initially by one of the study doctors, either myself or Dr Alan Stanley, a senior registrar in neurology and a colleague involved in the greater study. The purpose of this assessment was to ascertain participants' suitability for the study (see inclusion and exclusion criteria, table 4.1). Only participants referred to us within seven days of the onset of their stroke were eligible for the study. To standardise the timing of blood sampling, blood samples for the study were all obtained on day 6 or 7 post-stroke onset.

Participants enrolled during their hospital admission for treatment of their stroke, received the usual standard of care. They did not receive remuneration for participating in the study. However, some patients who had been discharged within 7 days of their stroke onset and who were subsequently enrolled in the study as outpatients were reimbursed for their transport costs.

4.4.2 Controls

The majority of the control participants were recruited from the Gugulethu Community Health Centre (CHC), a primary health care facility located in the township of Gugulethu, Cape Town. The CHC runs a wellness clinic for HIV positive patients who are relatively healthy, apart from infection with HIV. Patients attend this clinic prior to ART initiation. Consent was granted by the Gugulethu CHC facility manager to allow participant recruitment in this way. The nursing staff of the clinic identified patients who they felt met the inclusion and exclusion criteria for control participants. Potential participants were informed about the study and were asked if they would be interested in participating. Those who were agreeable were contacted telephonically. They were then invited to attend the GSH research clinic where they were enrolled. If I was on site at the CHC, I would inform patients of the study directly. Control participant recruitment at the CHC spanned a short period, from June – August 2012.

One control participant was initially recruited as a case as her presentation mimicked that of a hemiparetic stroke. Only after further investigation, was stroke ruled out. This patient was, however, HIV positive and had consented to study participation. She was therefore included in the control group. All control participants were reimbursed R150 for transport costs and lunch.

4.5 Ethical considerations and consent

All study procedures for the main/larger HIV-related young stroke project were approved by the Human Research Ethics Committee of the University of Cape Town and Groote Schuur Hospital

(HREC REF:178/2010). An amendment to include the specific laboratory analyses of this study was approved on 12th March 2012. The participant information leaflet and consent forms (in English and isiXhosa) were approved by the Ethics Committee and are available on request. The study adhered to the principles laid down in the Helsinki Declaration of 2008 (World Medical Association 2008) and South African Medical Research Council guidelines for research on human subjects (South African Medical Research Council 2002).

Potential participants were provided with a full explanation of the study and the procedures involved. It was emphasised that the study would not in any way compromise their normal standard of care. In fact, participants in the study underwent a more thorough investigation of their stroke than they would otherwise have received in the course of a "routine" hospital admission. Participants were informed that they would be free to withdraw from the study at any stage without necessarily having to give a reason. Nor would this affect their current treatment or jeopardise any future treatments they might need. In the event of the participant being unable to provide informed consent, a close family member/guardian/treatment partner was interviewed, informed of the study and invited to agree on the patient's behalf. The process of informed consent was in the language of choice of the participant/relative. A copy of the signed consent was given to the patient. Study data were kept in locked files and identified by a research code number. Confidentiality of the data was protected at all times.

4.6 Assessment of participants

After consent was obtained, all participants underwent a thorough assessment that included a demographic questionnaire, history and full physical examination, and blood tests. Patients were assessed in the language of their choice (isiXhosa, English or Afrikaans). Data were collected on standardised forms for patients and controls, respectively. A full-time research nurse assisted with the assessments and translations where necessary.

4.6.1 Cases

a. History taking and examination

The history included an ascertainment of the participant's baseline clinical characteristics, with the emphasis on identifying potential cerebrovascular risk factors. In HIV positive participants, a more in-depth history was obtained in relation to the HIV diagnosis and possible treatments. A general systemic examination was performed with special emphasis placed on the neurological examination.

b. Stroke assessment tools and classification

All cases were assessed according to a set of three standardised stroke assessment tools: the National Institutes of Health Stroke Scale (NIHSS), the modified Rankin Scale and the Barthel Index (Brott *et al*. 1989; van Swieten *et al*. 1988; Mahoney *et al*. 1965). The NIHSS is a 15-item clinical stroke assessment tool. It is used to evaluate and document neurological status in acute stroke, and assists with predicting lesion size and assessing stroke severity. I completed an online tutorial and test to become certified in using this scoring system. This reduced the bias in the assessment of participants, as both I and the other doctor enrolling patients completed the online assessment. The modified Rankin Scale is a valid and clinically relevant functional assessment tool that describes the range of global disability of a stroke sufferer and is used to assess recovery from stroke (Banks *et al*. 2007). The Barthel Index is a disability scale used to measure outcome following a stroke. It does so by measuring the patient's performance in 10 activities of daily living (Sulter *et al*. 1999).

c. Investigations

A number of investigations were carried out on case participants as they required extensive work-up for the cause of their stroke. As these patients were young, the investigative work-up was guided by the GSH stroke unit protocol/guidelines devised for the work-up of a young stroke (Bryer 2008/2009). All the participating hospitals implemented the same guidelines under the supervision of the attendant physicians. A Young Stroke Investigation Tick Sheet was devised as reminder to the attendant physicians of the necessary investigations that needed to be performed. Abnormal findings were discussed with the patient and treated according to normal standards of hospital care.

Blood investigations: These included: a full blood count (FBC), the international normalised ratio (INR), partial thromboplastin time (PTT), erythrocyte sedimentation rate (ESR), creatinine, electrolytes, urea, total cholesterol and lipid profile, fasting glucose, C-reactive protein (CRP), rapid plasma reagin (RPR) test/Venereal Disease Research Laboratory (VDRL) test, fluorescent treponemal antibody absorption (FTA) test, antinuclear antibody (ANA) test, anti-double stranded DNA (Anti-DS DNA) test, anti-neutrophil cytoplasmic antibody (ANCA) test, anticardiolipin and lupus anticoagulant. If a recent CD4 count for HIV positive young stroke participants was available in previous patient records (within 15 days before or after stroke onset), this measurement was then not repeated by the attending doctor.

Lumbar puncture: A lumbar puncture for the collection of cerebrospinal fluid (CSF) was part of the routine work-up for a young patient with stroke. CSF protein, glucose and cell count were measured. Cryptococcal Latex Agglutination Test (CLAT) work-up and RPR/FTA tests were done, and the CSF sample was cultured to detect bacterial or fungal infection. Some specimens were sent for TB culture if TB was suspected, and viral polymerase chain reaction (PCR) for varicella zoster virus (VZV) or herpes simplex virus (HSV) were performed in patients where these were clinically suspected. An additional 5ml of CSF was collected for tests that were part of the larger HIV-related young stroke project. The latter were not required for my study.

Imaging: The presence of stroke in patients was confirmed with neuro-imaging. Computed tomography (CT) scan of the brain was routinely performed, with further imaging such as MRI of the brain performed where necessary. A chest x-ray, CT angiogram of the head and neck vessels, and cardiac echocardiogram study were performed. Carotid duplex doppler studies for estimation of the carotid intima-media thickness (CIMT) were also part of the larger HIV-stroke study.

4.6.2 Controls

Demographic data were collected, as for the cases. They also underwent a full clinical and neurological examination. Stroke scores and classification systems did not apply here. Controls were HAART-naïve and attended their local HIV Wellness clinic. They were considered to be in relatively good general health, apart from infection with HIV. Investigations in this group were limited to a FBC, ESR, INR, PTT, RPR and CD4 count. On occasions, old patient records were accessed to obtain a recent CD4 count measurement. The only imaging performed in the controls was that of the carotid duplex doppler testing with CIMT measurement. The latter was required for analysis in the larger HIV-related young stroke project. A specially trained ultra-sonographer was employed to perform these studies. Any controls with abnormalities that required further investigation or treatment were referred to the appropriate health care facility.

4.6.3 Laboratory analysis of study specimens

Blood samples from all participants were used to determine the levels and activity of VWF and ADAMTS13. A total of 30ml of blood was drawn from each participant, and preserved in a citrate tube to prevent clotting. The samples were stored on ice immediately after collection. They were centrifuged at 4000 revolutions per minute (rpm) for 10 minutes before being aliquotted into cryoprecipitate tubes in volumes of around 500 microlitres (µl) each. These were stored at -80 degrees Celsius (ºC) and batched for further analyses.

4.7 Specific specimen laboratory analysis

The specific assays required for the analyses of VWF and ADAMTS13 for my study were performed in the laboratory of Professor Muriel Meiring, Associate Professor and Specialist Scientist of the Department of Haematology and Cell Biology of the University of the Free State, South Africa, and Head of the Haematology Division of the Bloemfontein National Health Laboratory Service (NHLS) laboratory. Her laboratory has a special interest in VWD, TTP, and more specifically, HIV-associated TTP, and is internationally accredited.

The specimens were transported on dry ice by air freight from Cape Town to Bloemfontein and reached the laboratory on the same day. They were immediately transferred to the -80ºC freezer at the Bloemfontein NHLS laboratory and stored before being analysed in 3 batches over a period of 6 months. The specific tests are described next.

4.7.1 VWF antigen (VWF:Ag) assay

This enzyme-linked immunosorbent assay (ELISA) measures the actual amount of VWF in plasma.

An ELISA plate was coated at 4ºC overnight with a rabbit anti-human VWF antibody [DAKO, South Africa, 1:6000 dilution in phosphate buffered saline (PBS) solution: 5.84 grams per litre (g/L) NaCl, 4.76 g/L Na_2HPO_4 and 2.64 g/L $NaH_2PO_4.2H_2O$, pH 7.2]. The VWF in the plasma being tested was captured by this antibody and measured. Following antibody coating, 100µl of 1:50 dilution of plasma (study participants') was then added in duplicate to the wells and incubated for 2 hours at 37ºC. The plasma was diluted in PBS/0.1% Tween-20 (Merck, South Africa). After 4 washing steps with PBS/0.1% Tween-20, a rabbit anti-VWF antibody conjugated to peroxidase (DAKO, South Africa, 1:8000 dilution in PBS/0.1% Tween-20), was added and incubated for one hour at room temperature. This conjugated antibody completes the "sandwich" technique of ELISA tests by binding to the remaining free antigenic determinants of VWF. The bound enzyme peroxidase is revealed by its activity in a predetermined time on the substrate ortho-phenylenediamine (OPD) in the presence of hydrogen peroxide (10ml of 0.2 molar (M) Na_2HPO_4, 10 ml of 0.1 M Citric Acid, 200µl of 50 milligram per litre (mg/L) OPD, and 8µl of 30% H_2O_2). The intensity of the colour produced is directly related to the VWF concentration present in the plasma. After 3 minutes the reaction was stopped by adding 4 M H_2SO_4 [sulphuric acid; 30 microlitre per well (µl/well)]. The absorbance was then measured at 490 nanometre (nm) minus 630nm with a plate reader (Bio Tek SYNERGY HT, Analytical & Diagnostic Products, South

Africa). A standard curve of calibrated human plasma (WHO 6th FVIII/VWF standard) was used as the standard against which the participant plasmas were measured. The accompanying Gen5 software was used to analyse the data. Figure 4.1 illustrates the ELISA process for the VWF:Ag assay.

"Results were expressed as percentages. The normal range for VWF:Ag levels that probably represents that of the general population in South Africa is from 50 to 150%. This correlates with reference ranges of other laboratories in literature. Each laboratory needs to use its own normal range with which to compare patient samples (Meiring *et al*. 2012)."

This test is mostly used in the diagnosis of patients with suspected VWD. It is now frequently used for research studies as interest in VWF has increased significantly over the past few years.

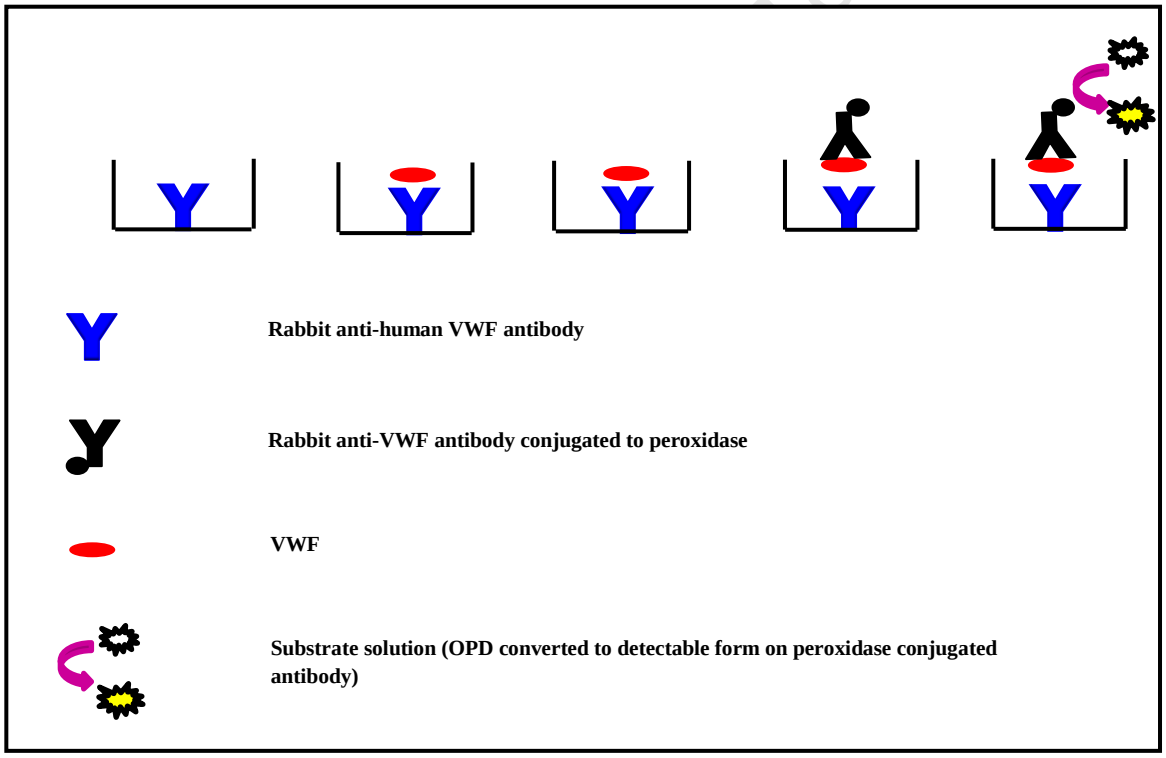


Figure 4.1 ELISA process for the VWF antigen assay Abbreviations: von Willebrand factor (VWF), ortho-phenylenediamine (OPD).

4.7.2 VWF collagen binding (VWF:CB) assay

The VWF:CB assay is based on the ability of the larger multimers of VWF to preferentially bind to collagen. It is an ELISA-based assay and is done in conjunction with the VWF:Ag test. An ELISA plate was coated with 2.5 microgram per millilitre (µg/ml) Collagen type III in PBS. The participants' plasma was added in the same concentration as with the VWF:Ag assay. The rabbit

anti-VWF antibody conjugated to peroxidase was added in a 1:3000 dilution in PBS. The same standard was used to measure the collagen binding activity. Figure 4.2 illustrates the test process.

The values were also expressed as percentages. Again, the normal range for VWF:CB activity levels in the laboratory is from 50 to 150%.

This test is frequently used as a screening test in patients with VWD. It assists with diagnosis of the subtype of VWD, especially that of types 2A and 2B VWD. It is used extensively in research related to VWF and VWD.

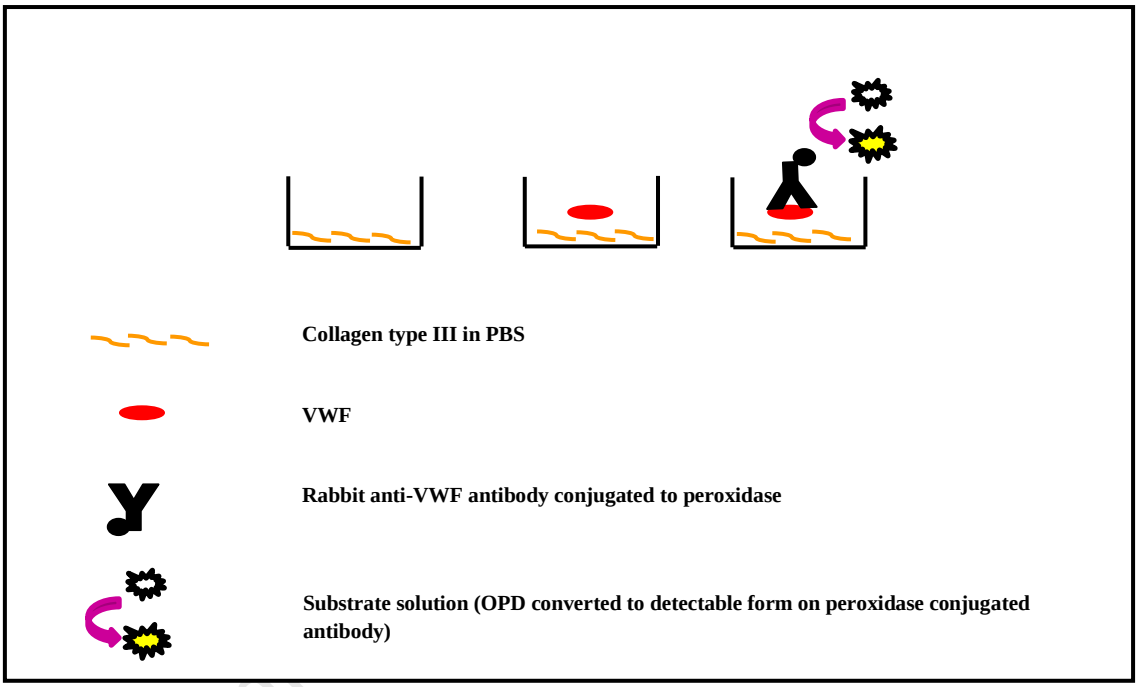


Figure 4.2 ELISA method for the VWF collagen binding assay Abbreviations: von Willebrand factor (VWF), phosphate buffered saline (PBS), ortho-phenylenediamine (OPD).

4.7.3 ADAMTS13 antigen (ADAMTS13:Ag) assay

This ELISA-based assay measures the level of the VWF-cleaving protease, ADAMTS13, in human plasma.

An ELISA plate was coated overnight with a mouse monoclonal antibody against ADAMTS13 (R&D Systems, 1:1000 dilution PBS, 100µl/well). The following morning, the plate was washed with PBS/0.1% Tween-20 and the plasma samples were added in duplicate (100 µl/well) in a 1:10 dilution in PBS/2% bovine serum albumin (BSA), and incubated for 2 hours at 37ºC. This was followed by another wash step. Subsequently, a rabbit polyclonal immunoglobulin G (IgG)

antibody against ADAMTS13 (Santa Crux Biotechnology, CA, USA) was added (1:100 dilution) and incubated for 1 hour at room temperature. A polyclonal goat anti-rabbit antibody conjugated with horseradish peroxidase (HRP) was then added after another wash step and incubated for 1 hour at room temperature. This antibody was added in a 1:2000 dilution. OPD (50mg/L) was used as the substrate for HRP (the same concentration as with the VWF levels). Once again, as described in the VWF antigen assay, a standard curve of calibrated human plasma (WHO 6th FVIII/VWF standard) was used as the standard against which the participants' plasma samples were measured. Figure 4.3 depicts the ELISA process of this assay.

Results are expressed as percentages. The ADAMTS13 assays lack international standardised ranges. The normal range is once again dependent on a number of factors and individual laboratories tend to devise their own normal range based on their testing. The normal range of our laboratory was between 50 – 150%.

The ADAMTS13 antigen assay is used predominantly in the clinical setting for investigating patients with suspected TTP. As with VWF, the recent interest in ADAMTS13 has increased the use of this test in research settings.

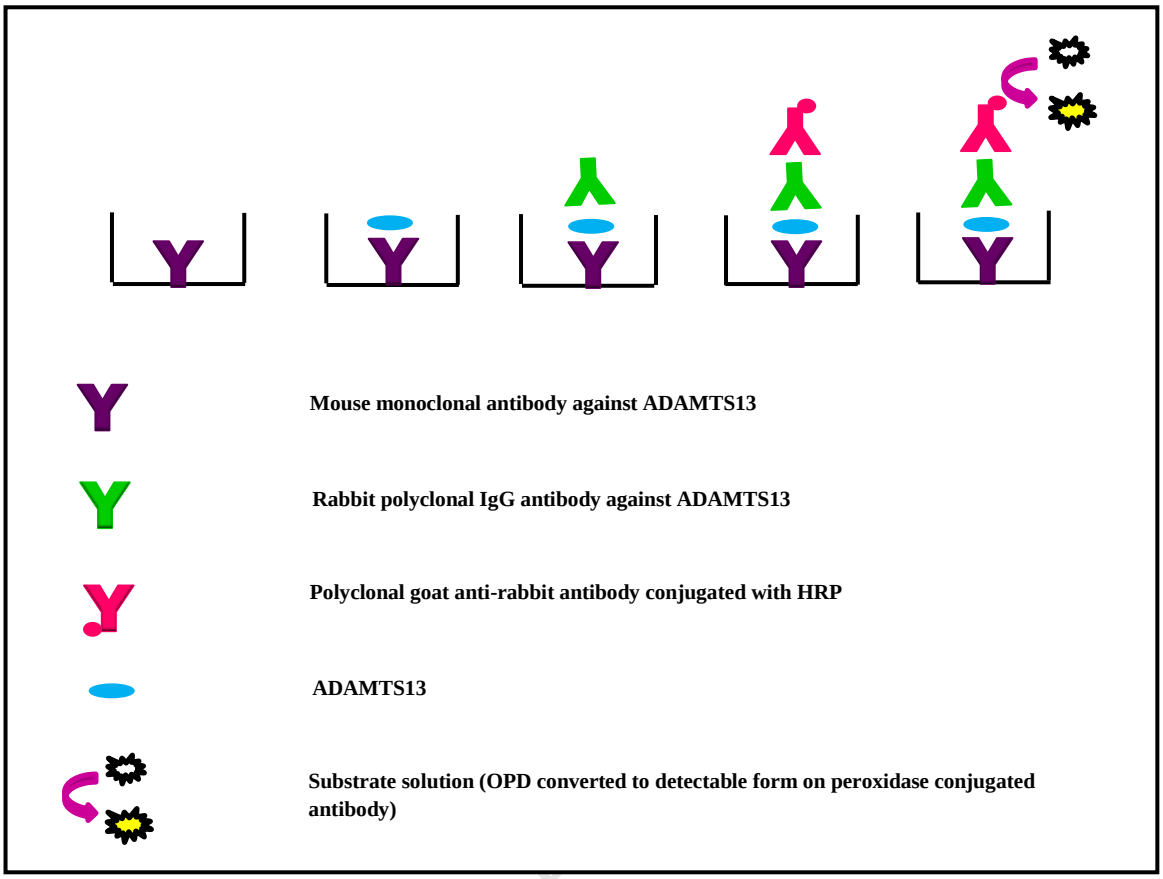


Figure 4.3 ELISA procedure of the ADAMTS13 antigen assay Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), immunoglobulin G (IgG), horseradish peroxidase (HRP), ortho-phenylenediamine (OPD).

4.8 Data management and analysis

All data collected from participants in the larger HIV-related young stroke study were entered in a Microsoft ACCESS programme. This programme enables the user to create a database. For my own study, a modified database was created using the Microsoft EXCEL programme. The database was prepared for analysis and any outstanding information obtained. A final thorough, and methodical data check procedure was undertaken prior to analysis to ensure that all data were entered correctly.

Dr Maia Lesosky, a medical biostatistician based at the University of Cape Town, Department of Medicine, was consulted on several occasions for assistance with data analysis. She was consulted in the early phase of this study to assist with power calculations. At the end of my participant recruitment phase, she was once again consulted for assistance with data analysis. The following computer software packages were used for statistical analysis: Statistical Package for the Social

Sciences (SPSS) version 21 and Statistica version 11. The level of statistical significance was set at $\alpha = 0.05$.

4.9 Summary of methods: Flow diagrams of the study process

The flow diagrams in figures 4.4 and 4.5 summarise the participant recruitment process, the attrition rate and the main tests performed.

Figure 4.4 Flow diagram of study process

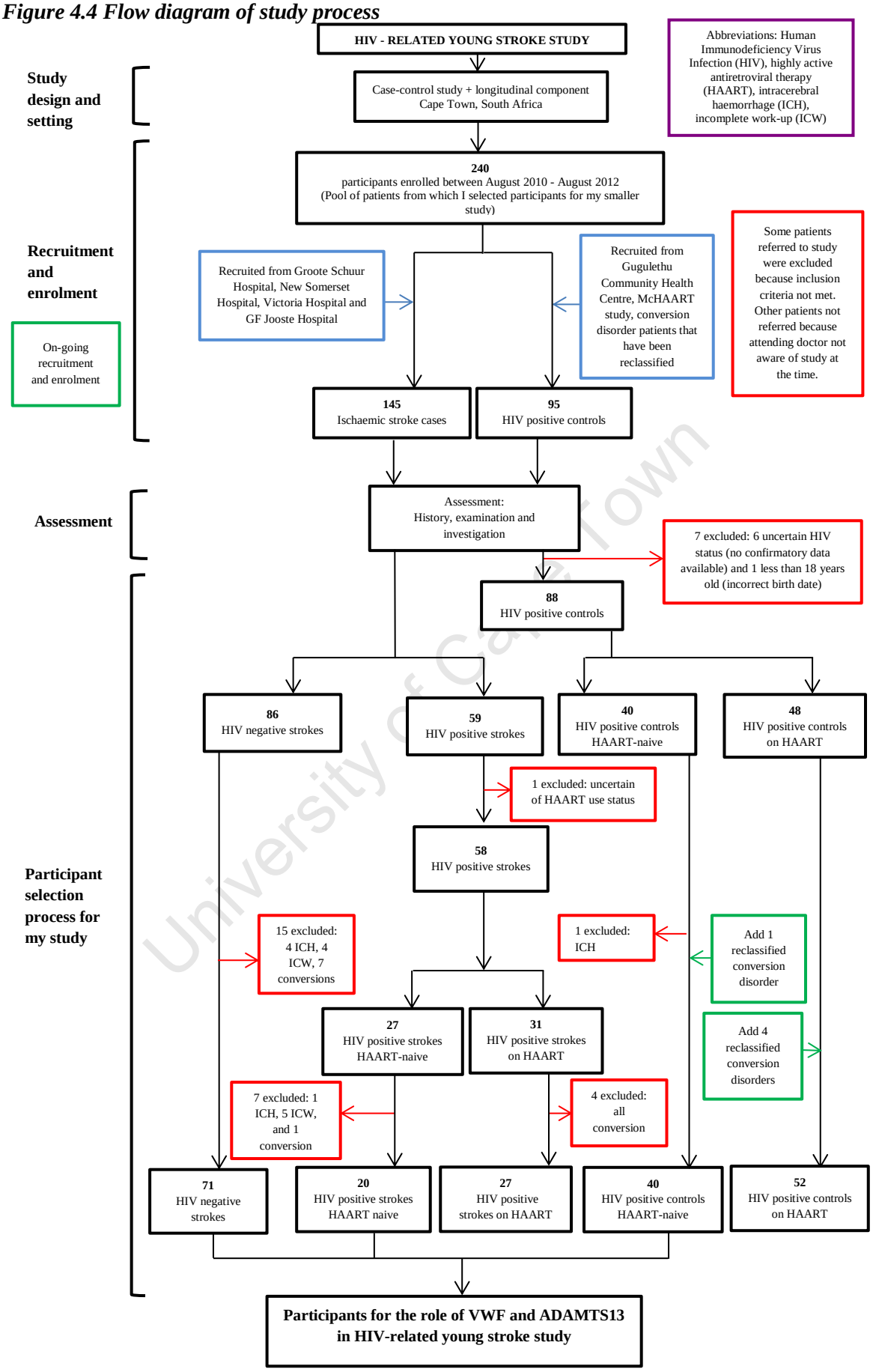
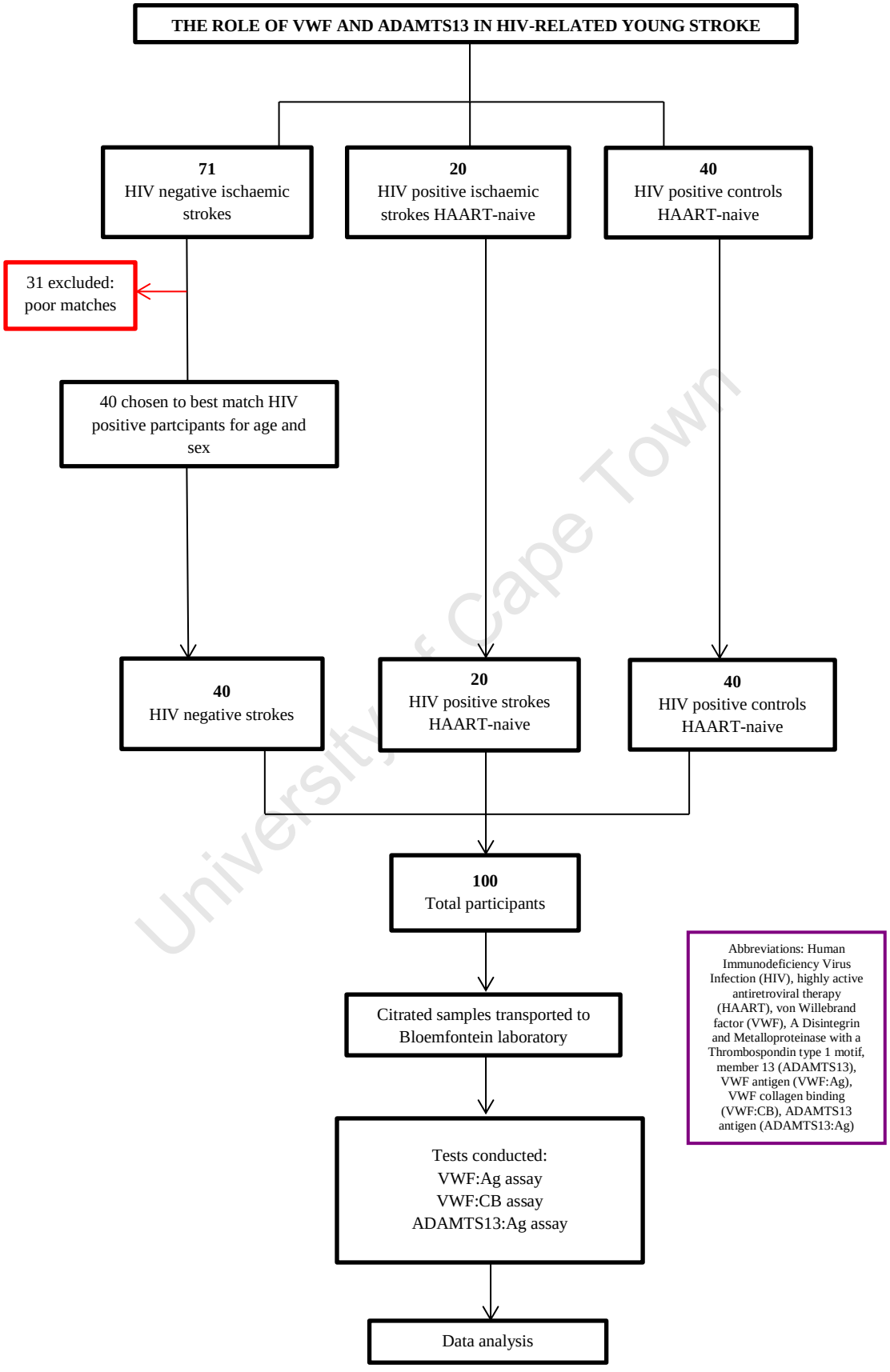


Figure 4.5 Flow diagram of my study



CHAPTER 5 - RESULTS I: PARTICIPANT CHARACTERISTICS

INTRODUCTION

In this chapter, I shall describe the characteristics of the study participants. The basic demographic data, information regarding the stroke episode in those participants who suffered a stroke, details of co-morbidities and associated relevant medical history will be reported. In addition, the CD4 count of HIV positive participants and the physical characteristics of the participants will be presented. Using this information, I was able to determine how comparable the 3 participant groups were and to ascertain the presence of potential confounding factors or variables.

5.1 Analysis of data

The distribution of numerical variables was determined initially and thus the appropriate measures of central tendency and summary statistical analyses were applied depending on whether the data displayed symmetry or not i.e. were normally or non-normally distributed. Visual inspections of histograms, coupled with the interpretation of the Shapiro-Wilk test for normality, were used to assess whether data were normally distributed or not. We chose to use the findings of the Shapiro-Wilk test over that of the Kolmogorov-Smirnov test of normality, as the former is more reliable in the setting of small sample sizes. Numeric data that were not normally distributed underwent log transformation and square root transformation in an attempt to normalise them and allow for the use of parametric statistical tests. However, none of the demographic variables displayed a normal distribution post transformation and thus non-parametric measures of central tendency such as the median (*Mdn*) and interquartile range (*IQR*) were used. The *IQR* presented in this chapter ranges from the 25^{th} to 75^{th} quartile values. In addition, the Mann-Whitney U test was the non-parametric test used for the analysis of the non-symmetric numeric data to obtain inferential statistical data.

Categorical variables were analysed as percentage distributions and Chi-Squared analysis was used to evaluate all categorical data for the presence of any statistically significant associations between these variables and the 3 participant groups.

Results that are statistically significant or tending towards significance will be presented with their associated inferential statistic values accompanying them in the text and in the tables that follow. The following abbreviations will apply: p = p value, U = U statistic, r = effect size, χ^2 = chi-squared statistic, df = degrees of freedom, r_s = Spearman's rho, n = sample size and vs. = versus.

5.2 Participant characteristics

5.2.1 Demographic characteristics (see tables 5.2, 5.3 and 5.4)

A total of 100 participants were included in this study. The breakdown into the 3 main groups is indicated in table 5.1.

	HIV positive young stroke (HAART-naïve)	HIV negative young stroke	HIV positive young non-stroke controls (HAART-naïve)	Total participants
No. of participants	20	40	40	100

Table 5.1 Participant groups Abbreviations: highly active antiretroviral therapy (HAART), number (No.).

The following demographic data were collected for all participants: age, sex, "race," access to basic amenities, educational status and employment. Information regarding participants' access to water and electricity, their type of dwelling, education level attained, and employment status helped us to characterise their socio-economic environment. Each demographic variable will be discussed next and the results are summarised in tables 5.2, 5.3 and 5.4. In all the sections that follow, the HAART-naïve HIV positive young stroke participants, HIV negative young stroke patients and the HIV positive HAART-naïve young non-stroke controls, will be referred to as HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str) and HIV positive young controls respectively (HIV pos con), for easier readability. There was no need to differentiate between ARV use as all our participants were HAART-naïve.

a. Age, sex and "race" (see table 5.2)

Age: Our definition of "young stroke" for this study was patients aged between 18 and 45 years. The participants' ages ranged from age 19 to 45 years. Age data were not normally distributed. Median age for the entire sample was 35 years (*IQR* 30 – 38 years). The Mann-Whitney U test showed no significant differences between the 3 groups.

Sex: The final sample comprised a total of 55 females and 45 males. All the subgroups displayed a predominance of female over male participants. Using Chi-Squared analysis, no statistically significant differences were noted in the between-group sex distribution.

"Race": Due to participant recruitment only being carried out at public health facilities and the specific areas in Cape Town from which we recruited, our participants were predominantly classified "Black", with some "Coloured" participants. The past system of race classification/inequality and its current resonating effects, still have an influence on the demography of patients attending public health facilities in this country. Chi-Squared analyses showed that statistically significant differences existed when comparing the HIV positive young control group to both the HIV positive young stroke group [χ^2 (1, n = 60) = 5.66, p = 0.017] and the HIV negative young stroke group [χ^2 (1, n = 80) = 17.58, $p < 0.0001$] respectively. This was expected as the HIV positive young control group consisted only of individuals classified "Black" African. The HIV positive young stroke group and the HIV negative young stroke group did not differ significantly with respect to "race."

	HIV pos str (n = 20)	**HIV neg str (n = 40)**	**HIV pos con (n = 40)**	**All participants (n = 100)**	**HIV pos str vs. HIV neg str**	**HIV pos str vs. HIV pos con**	**HIV neg str vs. HIV pos con**
***Mdn* age (yrs)** ***IQR* (yrs)**	35 29.5 - 36.8	36.5 30.5 - 41	33.5 29.3 - 38	35 30 - 38	p = 0.16	p = 0.86	p = 0.21
Sex	60% female 40% male	52.5% female 47.5% male	55% female 45% male	55% female 45% male	p = 0.78	p = 0.93	p > 0.99
Race	80% Black 20% Coloured	60% Black 40% Coloured	100% Black	80% Black 20% Coloured	p = 0.21	χ^2 = 5.66 df = 1 p = 0.017	χ^2 = 17.58 df = 1 $p < 0.0001$

Table 5.2 Age, sex and "race" distribution Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), median *(Mdn)*, interquartile range *(IQR)*, years (yrs), p value (*p*), chi-squared statistic (χ^2), degrees of freedom (*df*).

b. Domestic environment (see table 5.3)

Home: Participants had to choose 1 of 5 types of dwelling to describe where they lived. The choices were a brick house, a flat, a backyard dwelling, a shack and or other option. Only one participant fell into the latter category as he was homeless. Most participants resided in brick houses (55%) and shacks (32%). The majority of HIV positive young stroke participants resided in shacks (45%). This is in stark contrast to the HIV negative young stroke participants, 75% of whom occupied brick houses. The HIV positive young controls displayed an almost even distribution of residency in brick houses (45%) and shacks (47.5%). Of the study participants who were HIV positive (n = 60), shacks (46.6%) and brick houses (41.6%) dominated as types of dwellings. Chi-Squared analysis showed that these between-group differences with regards to type of home lived in were statistically significant. Thus, HIV positive participants had poorer types of dwellings compared with HIV negative participants: HIV positive young stroke vs. HIV negative

young stroke group [χ^2 (4, n = 60) = 14.28, p = 0.006] and HIV negative young stroke vs. HIV positive young controls [χ^2 (4, n = 80) = 14.32, p = 0.006].

Electricity: Participants indicated whether they had access to electricity or not at home. In both the HIV negative young stroke and HIV positive young control groups, 95% of the participants had access to electricity. However, only 75% of participants in the HIV positive young stroke group had access to electricity. There was thus a trend towards significance when comparing the HIV positive young stroke group to the HIV negative young stroke group [χ^2 (1, n = 60) = 3.42, p = 0.065] and when comparing the HIV positive young stroke group with the HIV positive young control group [χ^2 (1, n = 60) = 3.42, p = 0.065]. Access to electricity was therefore less in the HIV positive young stroke group.

Water: Participants had to identify which of these three water sources they had access to at home: an internal water source (a tap inside their home), external water source (a tap near the outside of their home that belonged to the participant) or a communal tap (a tap where multiple members of the community could fetch water from). There were statistically significant differences when comparing all 3 groups. The HIV negative young stroke group had the greatest access to internal water when compared with the HIV positive young stroke group [χ^2 (2, n = 60) = 12.75, p = 0.002], as well as when compared with the HIV positive young control group [χ^2 (2, n = 80) = 13.79, p = 0.001]. The HIV positive young control group had better access to internal and external water sources when compared with the HIV positive young stroke group [χ^2 (2, n = 60) = 6.80, p = 0.033].

Toilet: Participants had to identify one of three options: an inside toilet (a toilet inside their home), an external toilet (a toilet outside their home that belonged to the participant) or a communal toilet (a toilet that was accessible to all members of the community). More than 50% of the participants had access to an internal toileting option, with the majority who did have access belonging to the HIV negative young stroke group. As with water accessibility, there were statistically significant between-group differences. The results showed that toilets inside the home were mainly accessible to the HIV negative young stroke group. HIV negative young stroke group vs. HIV positive young stroke group [χ^2 (2, n = 60) = 6.02, p = 0.049] and HIV negative young stroke group vs. HIV positive young control group [χ^2 (2, n = 80) = 15.04, p = 0.001].

In summary, participants in the HIV negative young stroke group had better access to basic amenities than participants in the HIV positive young stroke group and HIV positive young control group.

	HIV pos str (n = 20)	HIV neg str (n = 40)	HIV pos con (n = 40)	All participants (n = 100)	HIV pos str vs. HIV neg str	HIV pos str vs. HIV pos con	HIV neg str vs. HIV pos con
Home							
brick house	7 (35%)	30 (75%)	18 (45%)	55 (55%)			
flat	4 (20%)	3 (7.5%)	2 (5%)	9 (9%)	$\chi^2 = 14.28$		$\chi^2 = 14.32$
backyard	NA	2 (5%)	1 (2.5%)	3 (3%)	$df = 4$	$p = 0.28$	$df = 4$
shack	9 (45%)	4 (10%)	19 (47.5%)	32 (32%)	$p = 0.006$		$p = 0.006$
other	NA	1 (2.5%)	NA	1 (1%)			
Electricity							
yes	15 (75%)	38 (95%)	38 (95%)	91 (91%)	$\chi^2 = 3.42$	$\chi^2 = 3.42$	
no	5 (25%)	2 (5%)	2 (5%)	9 (9%)	$df = 1$	$df = 1$	$p = 1$
					$p = 0.065$	$p = 0.065$	
Water							
internal	11 (55%)	33 (82.5%)	17 (42.5%)	61 (61%)	$\chi^2 = 12.75$	$\chi^2 = 6.80$	$\chi^2 = 13.79$
external	NA	4 (10%)	11 (27.5%)	15 (15%)	$df = 2$	$df = 2$	$df = 2$
communal	9 (45%)	3 (7.5%)	12 (30%)	24 (24%)	$p = 0.002$	$p = 0.033$	$p = 0.001$
Toilet							
internal	11 (55%)	31 (77.5%)	14 (35%)	56 (56%)	$\chi^2 = 6.02$	$\chi^2 = 7.22$	$\chi^2 = 15.04$
external	1 (5%)	4 (10%)	15 (37.5%)	20 (20%)	$df = 2$	$df = 2$	$df = 2$
communal	8 (40%)	5 (12.5%)	11 (27.5%)	24 (24%)	$p = 0.049$	$p = 0.027$	$p = 0.001$

Table 5.3 Domestic environment A frequency count is given, with percentages in parentheses. Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), not applicable (NA), p value (*p*), chi-squared statistic (χ^2), degrees of freedom (*df*).

c. Education and employment (see table 5.4)

Data regarding schooling and employment status were also used as indicators of participants' education level and their capacity/ability to be economically active.

Highest level of education attained: Participants were asked to indicate the highest level of education they had attained at the time of enrolment into the study. The options ranged from no schooling to university level education. 78% of all the participants completed high school i.e. 12 years of schooling. Between-group comparisons showed no statistically significant differences.

Employment status: Participants were asked to comment on whether they were employed at the time of enrolment into the study. No differences were detected when performing between-group comparisons. Despite more than 75% of the total group completing high school, just under two-thirds of participants were employed (63%).

	HIV pos str (n = 20)	HIV neg str (n = 40)	HIV pos con (n = 40)	All participants (n = 100)	HIV pos str vs. HIV neg str	HIV pos str vs. HIV pos con	HIV neg str vs. HIV pos con
Education							
prim school	3 (15%)	4 (10%)	3 (7.5%)	10 (10%)			
high school	16 (80%)	29 (72.5%)	33 (82.5%)	78 (78%)			
tech college	NA	3 (7.5%)	2 (5%)	5 (5%)	$p = 0.64$	$p = 0.31$	$p = 0.25$
college	NA	NA	2 (5%)	2 (2%)			
university	1 (5%)	3 (7.5%)	NA	4 (4%)			
no school	NA	1 (2.5%)	NA	1 (1%)			
Employment							
yes	13 (65%)	22 (55%)	28 (70%)	63 (63%)	$p = 0.64$	$p = 0.92$	$p = 0.25$
no	7 (35%)	18 (45%)	12 (30%)	37 (37%)			

Table 5.4 Education and employment status A frequency count is given, with percentages in parentheses. Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), not applicable (NA), p value (*p*), primary (prim), technical (tech).

5.2.2 Stroke characteristics (see table 5.5)

This section only applies to those 60 participants who had had a stroke. The characteristics of these participants' strokes are discussed next and summarised in table 5.5.

Oxfordshire Community Stroke Project (OCSP) classification: The OCSP classification is used to classify strokes into 4 categories based on the blood vessel territory of the cerebral circulation that is involved. It aids in clinical localisation and is also useful for prognostic purposes. A full description was provided in chapter 1 (see section 1.2.3).

Our stroke participants displayed a predominance of strokes classified into the LACI and PACI categories, 40% and 31.7% respectively. These are the stroke categories normally associated with a better prognosis. Close to half (47.5%) of all the HIV negative young stroke participants had strokes classified as LACI, whereas only a quarter (25%) of the HIV positive young stroke participants were categorised in the LACI group. Both groups had a similar distribution of PACIs, 30% and 35%, for the HIV negative and HIV positive young stroke groups, respectively. Of the HIV negative participant group, 15% fell into the TACI and 7.5% into the POCI categories,

respectively. The remaining distribution amongst the HIV positive young stroke group was equal, with both TACI and POCIs representing 20% of the remaining participants each. We could infer from the OCSP classification that the majority of the HIV negative young stroke group participants had a good prognosis following their stroke. Chi-Squared analysis showed no statistically significant between-group differences when the pooled combination of the various OSCP classifications in each group were analysed.

Stroke cause: In our study, the cause of a stroke was determined after investigative work-up according to standard guidelines as described in chapter 4 (section 4.6), and all cases were reviewed by experienced neurologists.

In our study, stroke aetiology was varied. Chi-Squared analysis showed a statistically significant difference between the HIV positive young stroke group and HIV negative young stroke group [χ^2 (9, n = 60) = 19.46, p = 0.022]. In the HIV negative young stroke group, almost half (42.5%) of the participants' were classified into the "idiopathic" group. This means that despite undergoing a comprehensive diagnostic work-up, the cause of the stroke was still unidentified. 8 participants (20%) and 7 participants (17.5%) in the HIV negative young stroke group had strokes that were attributable to a cardio-embolic source and dissection, respectively. Hypertensive small vessel disease (7.5%), Takayasu's arteritis (2.5%), neurosyphilis (2.5%), varicella vasculopathy (2.5%), an occluded internal carotid artery (2.5%), and large vessel atherosclerosis (2.5%) accounted for the remainder of the causes in the HIV negative young stroke group. In the HIV positive young stroke group, similar to the HIV negative young stroke group, close to one half (45%) of participants had no identified cause for their stroke. Therefore they are considered to be afflicted with a HIV-associated "idiopathic" stroke, and the entity of HIV-associated vasculopathy furthermore accounted for stroke causation in three of the participants classified into the HIV-associated "idiopathic" stroke group. In 30% of HIV positive stroke participants, a varicella vasculopathy was identified as the cause of their stroke. This was confirmed by the presence of varicella zoster virus in the CSF. The remaining causes included cardio-embolic stroke (10%), neurosyphilis (10%) and aortic aneurysm (5%) for the HIV positive young stroke group.

Using Chi-Squared analysis, dissection [χ^2 (1, n = 7) = 3.96, p = 0.047] and varicella vasculopathy [χ^2 (1, n = 7) = 9.78, p = 0.002] were the two causes that were found to be statistically significantly different between the 2 groups, with dissection only in the HIV negative young stroke group, and varicella vasculopathy being higher in the HIV positive young stroke group (this is not shown in table 5.5).

Of the sixty stroke participants, no cause could be found in 43.3% of the cases. The second most common cause of stroke was cardio-embolism (16.67%). As discussed in chapter 1, the aetiology of stroke in both HIV positive and HIV negative groups differs, but it is evident in our study group that "idiopathic" stroke still occupies a large piece of the stroke causation puzzle in young stroke patients.

Stroke complications: Out of the total 60 stroke participants, three patients had had a complication that had arisen secondary to their stroke. Of these three, two patients (one from either stroke group) died as a result of the extensive neurological impact of their stroke, and one HIV negative stroke participant suffered a malignant middle cerebral artery infarct with cerebral oedema and a depressed level of consciousness.

	HIV pos str (n = 20)	**HIV neg str (n = 40)**	**All stroke participants (n = 60)**	**HIV pos str vs. HIV neg str**
OCSP classification				
LACI	5 (25%)	19 (47.5%)	24 (40%)	
PACI	7 (35%)	12 (30%)	19 (31.7%)	$p = 0.28$
TACI	4 (20%)	6 (15%)	10 (16.7%)	
POCI	4 (20%)	3 (7.5%)	7 (11.7%)	
Stroke cause				
idiopathic	9 (45%)	17 (42.5%)	26 (43.3%)	
cardio-embolic	2 (10%)	8 (20%)	10 (16.7%)	
varicella vasculopathy	6 (30%)	1 (2.5%)	7 (11.67%)	
neurosyphillis	2 (10%)	1 (2.5%)	3 (5%)	$\chi^2 = 19.46$
dissection	0	7 (17.5%)	7 (11.67%)	$df = 9$
hypertensive small vessel disease	0	3 (7.5%)	3 (5%)	$p = 0.022$
Takayasu's arteritis	0	1 (2.5%)	1 (1.67%)	
aortic aneurysm	1 (5%)	0	1 (1.67%)	
occluded internal carotid artery	0	1 (2.5%)	1 (1.67%)	
large vessel atherosclerosis	0	1 (2.5%)	1 (1.67%)	
Stroke complications				
no complications	19 (95%)	38 (95%)	57 (95%)	$p = 0.69$
complication	0	1 (2.5%)	1 (1.67%)	
death	1 (5%)	1 (2.5%)	2 (3.33 %)	

Table 5.5 Stroke characteristics A frequency count is given, with percentages in parentheses. Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), sample size (n), versus (vs), Oxfordshire Community Stroke Project (OCSP), lacunar infarct (LACI), partial anterior circulation infarct (PACI), total anterior circulation infarct (TACI), posterior circulation infarct (POCI), p value (*p*), chi-squared statistic (χ^2), degrees of freedom (*df*).

5.2.3 Co-morbidities and relevant medical history (see tables 5.6, 5.7, 5.8 and 5.9)

a. Chronic diseases

The presence of certain co-morbidities, chronic diseases, known stroke risk factors, and a relevant past medical and family history were ascertained in all participants. Many of the chronic conditions known to influence stroke risk and aetiology have already been described in the literature review of this thesis (see chapter 1, section 1.2). I will briefly describe the distribution of these conditions in our participants. The statistical analysis of these variables is presented in table 5.6.

Hypertension (HPT): The diagnosis of HPT is according to the criteria presented in the South African hypertension guideline 2011 (Seedat *et al*. 2011). Stroke participants were classified as having HPT if they had a diagnosis of HPT prior to their stroke episode or if they were diagnosed during their hospital admission for stroke management. Control participants were classified as suffering from hypertension if they were previously known to be hypertensive at the time of enrolment into the study. Blood pressure measurements for control participants were only performed on the day of enrolment and therefore any elevated blood pressure readings were not considered an indicator of HPT as they may have been spuriously elevated. These patients were, however, advised to report to their nearest primary health care facility for repeat blood pressure measurements.

Only 20% of all participants had diagnosed HPT at the time of enrolment into the study. Chi-Squared analysis showed a significant between-group difference when comparing the HIV negative young stroke group to HIV positive young control group [χ^2 (1, n = 80) = 4.78, p = 0.029]. More participants in the HIV negative young stroke group had HPT, than those in the HIV positive young control group.

Diabetes: The diagnosis of diabetes is based on the criteria for diagnosis of diabetes found in the 2012 Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) revised guideline for the management of type 2 diabetes (Amod *et al*. 2012). Similar to the classification of HPT, stroke participants were classified as having diabetes if they already had a diagnosis of diabetes prior to their stroke episode or if they were diagnosed during their hospital admission. Control participants were classified as suffering from diabetes if they were previously known to be diabetic at the time of enrolment into the study. A fasting blood glucose test performed on control participants on their enrolment day showed none of their results exceeded the normal values.

Only 7% of all participants had been diagnosed with diabetes at the time of enrolment into the study. All of these belonged to the HIV negative young stroke group. This brought about a significant difference when comparing the between-group diabetes prevalence in the HIV negative young stroke group to the HIV positive young control group [χ^2 (1, n = 80) = 5.64, p = 0.018].

Peripheral vascular disease (PVD): Participants were asked whether they had a known diagnosis of PVD and questioned about signs and symptoms associated with PVD if not diagnosed with the condition. None of the participants was known to have peripheral vascular disease at the time of enrolment.

Elevated cholesterol: This was diagnosed according to the South African dyslipidaemia guideline consensus statement (Klug *et al*. 2012). Only one participant was identified as having elevated cholesterol at enrolment. This participant belonged to the HIV negative young stroke group and as expected there were no statistically significant between-group differences.

Cardiovascular disease (CVD): Only 2 out of 20 HIV positive young stroke patients had CVD (10%) and 6 out of 40 HIV negative young stroke patients had CVD (15%). The types of cardiovascular disease varied, and included coronary artery disease, cardiomyopathy, valvular heart disease, rheumatic heart disease, aortic aneurysm and even cardiac injury secondary to a previous praecordial stab. None of the HIV positive control participants had CVD. HIV negative young stroke participants therefore had significantly more diagnoses of CVD than the HIV positive young control group [χ^2 (1, n = 80) = 4.51, p = 0.034].

	HIV pos str (n = 20)	**HIV neg str (n = 40)**	**HIV pos con (n = 40)**	**All participants (n = 100)**	**HIV pos str vs. HIV neg str**	**HIV pos str vs. HIV pos con**	**HIV neg str vs. HIV pos con**
HPT	3 (15%)	13 (32.5%)	4 (10%)	20 (20%)	p = 0.26	p = 0.89	χ^2 = 4.78 df =1 p = 0.029
Diabetes	0	7 (17.5%)	0	7 (7%)	p = 0.12	NA	χ^2 = 5.64 df =1 p = 0.018
Elevated cholesterol	0	1 (2.5%)	0	1 (1%)	p > 0.99	NA	p > 0.99
CVD	2 (10%)	6 (15%)	0	8 (8%)	p = 0.89	p = 0.20	χ^2 = 4.51 df = 1 p = 0.034

Table 5.6 Co-morbidities and relevant medical history A frequency count is given, with percentages in parentheses. Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), not applicable (NA), hypertension (HPT), cardiovascular disease (CVD), p value (p), chi-squared statistic (χ^2), degrees of freedom (df).

b. Other co-morbid diseases

Apart from chronic illness, the presence of other co-morbid diseases that could have influenced our results, were collected. These diseases are discussed below.

Current TB: The manifestations of infection with TB can influence stroke risk and the coagulation profile as described in chapter 1 (see section 1.3.3). Five participants were known to be infected with TB at the time of study enrolment. They were all confirmed TB positive on sputum microscopy. Two participants each were divided amongst the HIV negative young stroke group and HIV positive young control group respectively. The remaining TB sufferer was in the HIV positive young stroke group. There were no statistically significant differences between the groups with regards to current TB infection.

Coagulopathy: Only one participant in the HIV positive young stroke group reported the presence of a previous coagulation problem viz. a DVT. None of the participants had any history of a coagulation disorder. Thus the groups were similar.

Cancer: One of the participants in the HIV negative young stroke group suffered from oesophageal cancer.

c. Previous stroke history

A history of a previous stroke was documented in all participants and its prevalence is discussed below. Table 5.7 summarises this information.

Previous stroke: 4 participants had experienced a previous stroke prior to enrolment into this study. They all belonged to the HIV negative young stroke group and there were no statistically significant between-group differences.

	HIV pos str (n = 20)	HIV neg str (n = 40)	HIV pos con (n = 40)	All participants (n = 100)	HIV pos str vs. HIV neg str	HIV pos str vs. HIV pos con	HIV neg str vs. HIV pos con
Previous stroke	0	4 (10%)	0	4 (4%)	$p = 0.36$	NA	$p = 0.12$

Table 5.7 Previous stroke history A frequency count is given, with percentages in parentheses. Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), not applicable (NA), p value (*p*).

d. Smoking, alcohol and substance use history

Cigarette smoking and excessive alcohol intake are both known risk factors for stroke. A history of illegal substance use was also obtained. However, these data should be interpreted with some caution as participants might have withheld or denied such information for fear of legal action. Our findings are described next and the data are summarised in table 5.8.

Smoking: A total of 44% of all study participants were current smokers at the time of enrolment. HIV negative young stroke participants displayed the highest percentage of current smokers (52.5%). This was followed by a 45% prevalence of smoking among HIV positive controls and the lowest prevalence of 25% amongst the HIV positive young stroke participants. Chi-Squared analysis showed no statistically significant between-group differences.

Alcohol use: Current alcohol use peaked in HIV positive controls at a prevalence of 57.5%, with 42.5% and 35% prevalence in the HIV negative young stroke and HIV positive young stroke groups respectively. Similar to current smoking, close to 50% of study participants were using alcohol at the time of enrolment. Statistical analysis revealed no statistically significant between-group differences.

Substance use: Use of illegal substances, as reported by study participants, was minimal. A total of 5 participants used illegal substances, 2 of whom were in the HIV negative young stroke group and 3 in the HIV positive young control group. Substances used were limited to marijuana and cocaine. Once again, no statistically significant differences were identified between the groups.

	HIV pos str (n = 20)	**HIV neg str (n = 40)**	**HIV pos con (n = 40)**	**All participants (n = 100)**	**HIV pos str vs. HIV neg str**	**HIV pos str vs. HIV pos con**	**HIV neg str vs. HIV pos con**
Smoking	5 (25%)	21 (52.5%)	18 (45%)	44 (44%)	$p = 0.080$	$p = 0.22$	$p = 0.65$
Alcohol use	7 (35%)	17 (42.5%)	23 (57.5%)	47 (47%)	$p = 0.78$	$p = 0.17$	$p = 0.26$
Substance abuse	0	2 (5%)	3 (7.5%)	5 (5%)	$p = 0.80$	$p = 0.53$	$p > 0.99$

Table 5.8 Smoking, alcohol and substance use history A frequency count is given, with percentages in parentheses. Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), p value (*p*).

e. Family history

Data with regards to a family history of stroke and coronary artery disease (CAD) were also collected from participants. This is summarised in table 5.9.

Family history of stroke: 14% of the total study participants reported a family history of stroke. Chi-Squared analysis revealed no statistically significant between-group differences.

Family history of CAD: Only 6 % of all participants reported any family history of CAD, with no statistically significant between-group differences with regards to a family history of CAD.

	HIV pos str (n = 20)	HIV neg str (n = 40)	HIV pos con (n = 40)	All participants (n = 100)	HIV pos str vs. HIV neg str	HIV pos str vs. HIV pos con	HIV neg str vs. HIV pos con
Family history of stroke	4 (20%)	3 (7.5%)	7 (17.5%)	14 (14%)	$p = 0.32$	$p = 0.91$	$p = 0.31$
Family history of CAD	1 (5%)	4 (10%)	1 (2.5%)	6 (6%)	$p = 0.87$	$p = 0.80$	$p = 0.36$

Table 5.9 Family history of stroke and CAD A frequency count is given, with percentages in parentheses. Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), coronary artery disease (CAD), p value (*p*).

5.2.4 Laboratory investigations (see table 5.10)

CD4 count: This was ascertained for all HIV positive participants. All except one participant had a recent CD4 count measured from or on the day of their stroke occurrence/or enrolment into the study. The 1 exception belonged to the HIV positive young control group. The remaining HIV positive control participants had a CD4 count measured on the day of enrolment into the study (day 0). The one participant in whom no count was obtained did, however, have a recorded CD4 count 296 days before enrolment into the study. We opted to exclude this participant from any analysis involving CD4 counts as the result was outdated. All the HIV positive stroke participants had had a CD4 count measured within a period from day 0 to day 15 following their stroke onset. The majority of the CD4 counts in this group were measured within the first seven days following the stroke. Only 2 participants had CD4 counts measured on day 8 and day 15 following the stroke episode, respectively.

The CD4 count distribution for all the HIV positive participants included in this analysis (n = 59) was non-normal. Nor was the distribution normal when the CD4 counts for the HIV positive young

stroke group and HIV positive young control group were plotted separately. The median CD4 count for the 59 HIV positive participants included in this analysis was 326 cells per cubic millimetre (cells/mm^3). The Mann-Whitney U test showed a tendency towards a statistically significant difference between the two HIV positive young participant groups (U = 271.5, p = 0.06, r = 0.25). The median CD4 count for the HIV positive young stroke group was 234 cells/mm^3, with the median CD4 count of the HIV positive young control group (n = 39) being 383 cells/mm^3. The *IQR* for both groups were also different. This shows that the HIV positive young stroke group tended to have lower CD4 counts than the HIV positive young participants who had not suffered a stroke.

	HIV pos str (n = 20)	**HIV pos con [n = 39 (40-1)]**	**All HIV positive participants [n = 59 (60-1)]**	**HIV pos str vs. HIV pos con**
Median (cells/mm^3) ***IQR* (cells/mm^3)**	234 100.8 - 381.3	383 205 - 520	326 160 - 451	U = 271.5 p = 0.058 r = 0.25

Table 5.10 CD4 count Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), interquartile range (*IQR*), cells per cubic millimetre (cells/mm^3), p value (p), U statistic (U), effect size (r).

5.2.5 Physical characteristics of participants (see tables 5.11 and 5.12)

Weight: Despite our protocol requiring all participants to be weighed on the day of enrolment, a total of 8 participants had missing weight measurements. The weights for the 92 participants who were weighed did not show a symmetrical distribution, as did the weight measurements of the 3 groups individually. The median weight of all was 69 kilograms (kg) (*IQR* 61 – 85kg). The weights of the HIV positive young stroke group were significantly different from those of the HIV negative young stroke group [Mann-Whitney U test (U = 160, p = 0.014, r = 0.34)]. The HIV positive young stroke participants (*Mdn* = 61kg, *IQR* 51.6 – 72kg) weighed significantly less than their counterparts in the HIV negative young stroke group (*Mdn* = 76.5kg, *IQR* 64 - 86.3kg).

	HIV pos str (n = 15)	**HIV neg str (n = 38)**	**HIV pos con (n = 39)**	**All participants (n = 92)**	**HIV pos str vs. HIV neg str**	**HIV pos str vs. HIV pos con**	**HIV neg str vs. HIV pos con**
Median (kg) ***IQR* (kg)**	61 51.6 - 72	76.5 64 - 86.3	65 60.5 - 89	69 61 - 85	U = 160 p = 0.014 r = 0.34	p = 0.14	p = 0.19

Table 5.11 Weight Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), interquartile range (*IQR*), kilograms (kg), p value (p), U statistic (U), effect size (r).

Body mass index (BMI): We calculated the BMI of our participants in order to obtain a better overall estimate of participants' body habitus. Participants were assigned to one of four groups: underweight (BMI < 18.5), normal weight (BMI = 18.5 - 24.9), overweight (BMI = 25 – 29.9) or obese (BMI ≥ 30). We were unable to calculate the BMI for eight of our participants due to lack of either a weight or height measurement. From table 5.12 we can see that close to half of the HIV positive participants had normal BMI, 46.67% and 43.59% in the HIV positive young stroke and HIV positive young control group respectively. The numbers of normal weight and obese participants were similar in the HIV negative young stroke group (14 and 15 respectively). In both the HIV negative young stroke group and the HIV positive young control group, more than 50% of the participants whose BMI was measured were categorised into the overweight and obese group. Due to the varying BMI levels, data were not symmetrically distributed, with the median for the total group just reaching the overweight range (*Mdn* = 25.4, *IQR* 21.4 - 30.5). The Mann-Whitney U test comparing the median BMI of the HIV positive young stroke group with that of the HIV negative young stroke group, tended towards significance (U = 179, p = 0.036, r = 0.29). Using Chi-Squared analysis, no statistically significant differences were found when the distribution of the different BMI categories in all three participant groups was compared to one another.

	HIV pos str (n = 15)	**HIV neg str (n = 38)**	**HIV pos con (n = 39)**	**All participants (n = 92)**	**HIV pos str vs. HIV neg str**	**HIV pos str vs. HIV pos con**	**HIV neg str vs. HIV pos con**
Mdn* BMI** ***IQR	22.3 18.5 - 28	26.8 22.3 - 31.9	25.2 21.5 - 30	24.5 21.4 - 30.5	U = 179 p = 0.036 r = 0.29	p = 0.099	p = 0.45
BMI categories							
Underweight	3 (20%)	1 (2.63%)	1 (2.56%)	5 (5%)			
Normal weight	7 (46.67%)	14 (36.84%)	17 (43.59%)	38 (38%)			
Overweight	3 (20%)	8 (21.05%)	10 (25.64%)	21 (21%)	p = 0.086	p = 0.13	p = 0.88
Obese	2 (13.33%)	15 (39.47%)	11 (28.21%)	28 (28%)			
Missing values	5 (25%)	2 (5%)	1 (2.5%)	8 (8%)			

Table 5.12 BMI A frequency count is given, with percentages in parentheses. Abbreviations: HIV positive young stroke (HIV pos str), HIV negative young stroke (HIV neg str), HIV positive young controls (HIV pos con), sample size (n), versus (vs.), body mass index (BMI), median (*Mdn*), interquartile range (*IQR*), p value (*p*), U statistic (*U*), effect size (*r*).

5.2.6 Participant characteristics for the "idiopathic" stroke groups

A total of 26 participants were classified "idiopathic" strokes. The breakdown is indicated in table 5.13.

	HIV positive young "idiopathic" stroke	HIV negative young "idiopathic" stroke	Total "idiopathic" stroke participants
No. of participants	9	17	26

Table 5.13 "Idiopathic" stroke participant groups Abbreviation: number (No.)

All the statistical analyses that have been carried out on the three main participant groups described in sections 5.2.1 – 5.2.5 have been carried out on this subset of participants. The median age for the total "idiopathic" stroke group is 36 years (*IQR* 32 – 42 years). The participant characteristics were found to be very similar to their counterpart groups which include all stroke causes, i.e. the HIV positive young stroke group and the HIV negative young stroke group. None of the subject characteristics was found to be significantly different when performing between-group comparisons for the "idiopathic" stroke groups.

5.3 Summary of participant characteristics

All three of the participant groups were comparable with regards to age and sex.

Obvious discrepancies existed in relation to home circumstances and access to basic amenities with HIV positive individuals bearing the brunt of a poor socio-economic status.

The presence of any co-morbid diseases did not prove to be statistically significantly different when conducting between-group comparisons amongst the two stroke groups and amongst that of the two HIV positive groups. There were, however, statistically significant co-morbid disease differences between the HIV negative young stroke group and the HIV positive young control group, but they did not affect our study as we did not conduct any comparisons between these two groups for our hypotheses.

There were no significant differences when comparing current alcohol use, smoking and substance use amongst the three groups.

There were no significant differences when comparing a family history of stroke or CAD between the three groups.

HIV negative participants were nutritionally better off than HIV positive participants. Weight and BMI measurement showed that HIV negative participants weighed significantly more and had higher BMI values than their HIV positive counterparts.

Stroke causation varied amongst the two stroke groups, but stroke classification was not significantly different between the two groups. Complications secondary to stroke were also rare in our study participants.

The between-group comparison of the CD4 count between the two HIV positive groups tended towards significance, with HIV positive stroke participants having lower CD4 counts.

CONCLUSION

The results of all biological data needed to be interpreted in the context of the participants' background, their social environment and their medical history. We will therefore discuss the participant characteristics and how they relate to our study in chapter 7.

CHAPTER 6 – RESULTS II: TESTING OF HYPOTHESES

INTRODUCTION

In this chapter I shall first describe the distribution of the test results of VWF and ADAMTS13 for the entire participant population (n = 100). I shall then present the results of the tests for the individual hypotheses comparing the three participant groups: HIV positive young stroke (n = 20), HIV negative young stroke (n = 40) and HIV positive young controls (n = 40).

6.1 Analysis of data

This was carried out as described in chapter 4 (see section 4.8) and chapter 5 (see section 5.1). Based on an inspection of the frequency distribution histograms of the data and the Shapiro-Wilks tests for normality, neither VWF nor ADAMTS13 data were normally distributed. Log transformation of the VWF antigen (VWF:Ag) assay results resulted in a normal distribution of these data, but neither log transformation nor square root transformation of the VWF collagen binding (VWF:CB) and ADAMTS13 antigen (ADAMTS13:Ag) data resulted in normalisation of these data. We therefore used non-parametric tests to analyse all the data relating to the hypotheses. Results that were statistically significant or tending towards significance will be presented with their associated inferential statistic values in the text and in the tables that follow. All interquartile ranges (*IQR*) are presented from the 25^{th} to 75^{th} quartile.

None of the variables that were considered to be possible confounders in our study was significantly different when conducting between-group analyses (see chapter 5). It was therefore not necessary to control for the influence of these confounding variables when testing our hypotheses.

6.2 Overall results (see table 6.1)

This section compares all the results of the 100 participants for each test performed. All results are presented as a percentage, with 50 - 150% being the normal range (see chapter 4, section 4.7) All descriptive statistics for the individual tests are summarised in table 6.1. Figure 6.1 depicts the medians of the three tests graphically.

6.2.1 VWF:Ag assay

This quantitative assay measures the actual amount of VWF in the participant's plasma. A frequency plot of VWF showed an asymmetric distribution, with the graph skewed to the left. The median was 134.5% (*IQR* 103.3 – 178%) and falls within the normal range. However, when categorised according to the frequency distribution around the normal range, two fifths of all participants had values that were outside the normal range. The majority of these abnormal results (n = 40) were above normal values (>150%).

6.2.2 VWF:CB assay

Larger VWF multimers preferentially bind to collagen. The VWF:CB assay is thus a functional assay that quantifies the ability of the large multimers of VWF to bind to collagen (Meiring *et al*. 2007). Results were asymmetrically distributed and skewed to the left. The median was 84.5% (*IQR* 52.3 - 134.5%). Despite a median and *IQR* within the normal range, once again, just over two fifths of the total group of participants had abnormal values. Of the latter, approximately half were above the upper limit of normal of 150% and half were below the lower limit of normal of 50%.

6.2.3 ADAMTS13:Ag assay

This test measures the level of ADAMTS13 in human plasma. The results were distributed asymmetrically and markedly skewed to the left. The median was 0% (*IQR* 0 - 41.5%) for all participants. This was due to a "floor effect." Only 7 participants had values that were within the normal range. 78 participants had values that were below the lower limit of 50%.

	VWF:Ag assay (n = 100)	**VWF:CB assay (n = 100)**	**ADAMTS13:Ag assay (n = 100)**
Median (%)	134.5	84.5	0
***IQR* (%) (25^{th} – 75^{th} quartile)**	103.3 - 178	52.3 - 134.5	0 - 41.5
Range (%) (minimum – maximum)	48 - 427	0 - 294	0 - 1075
% values within normal range (50% - 150 %)	60%	59%	7%
% values > 150%	39%	19%	15%
% values < 50%	1%	22%	78%

Table 6.1 Overall test results Abbreviations: von Willebrand factor (VWF), A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), VWF antigen (VWF:Ag), VWF collagen binding (VWF:CB), ADAMTS13 antigen (ADAMTS13:Ag), sample size (n), interquartile range (*IQR*).

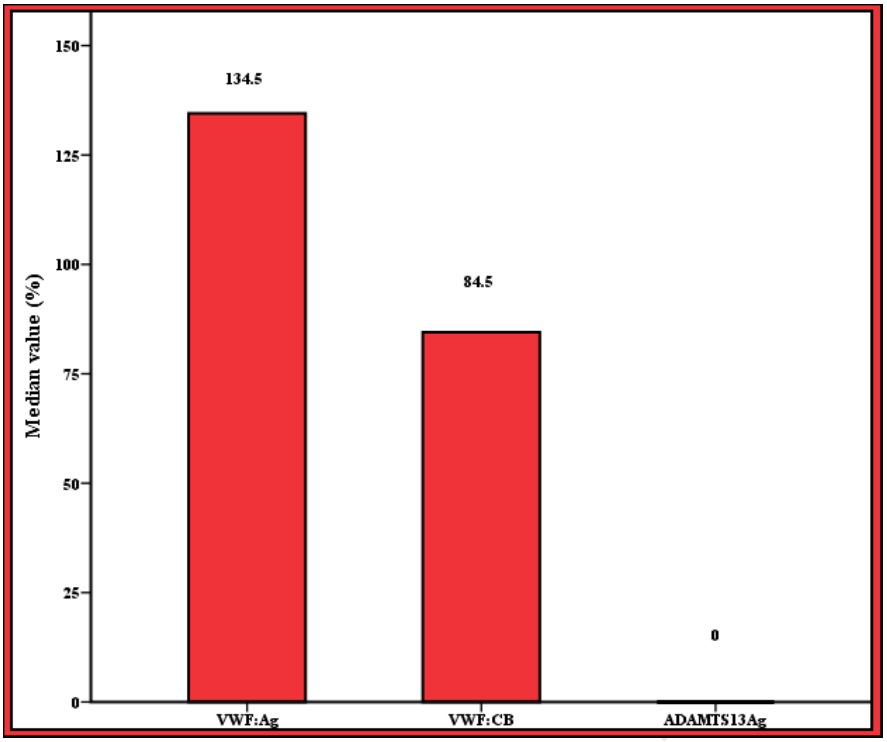


Figure 6.1 A comparison of the median values of the three assays for all 100 participants
Abbreviations: von Willebrand factor (VWF), A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), VWF antigen (VWF:Ag), VWF collagen binding (VWF:CB), ADAMTS13 antigen (ADAMTS13:Ag).

6.3 Results of hypotheses

Box and whisker plots will be used to illustrate the results graphically. Figure 6.2 is a key to interpreting the box and whisker plot.

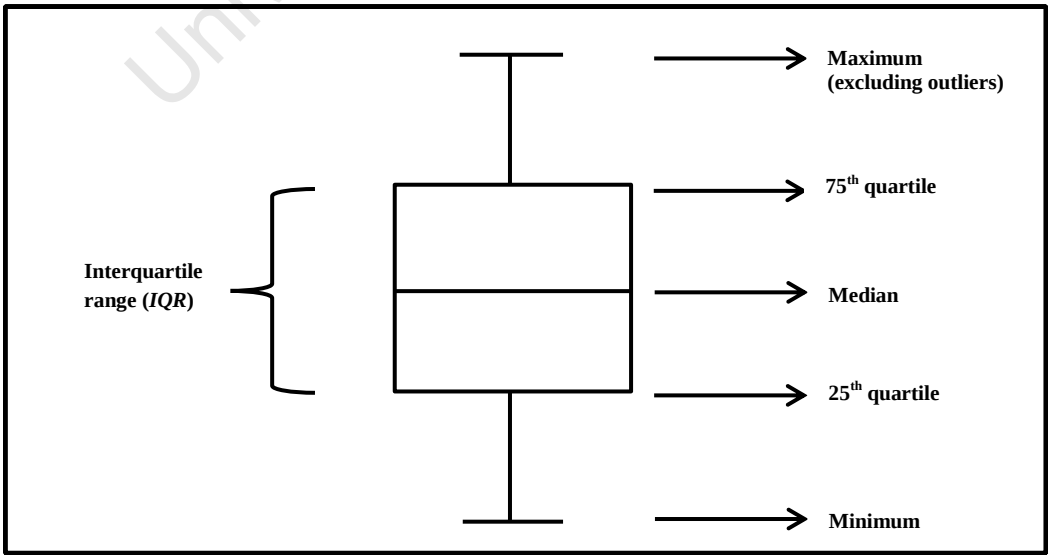


Figure 6.2 Key to interpreting the box and whisker plot

6.3.1 Hypothesis 1

Hypothesis 1: HIV positive young stroke participants would have a pro-thrombotic coagulation profile characterised by higher levels of VWF compared with HIV negative young stroke participants. This hypothesis was tested by measuring the quantity of VWF in the plasma of these two groups using the VWF:Ag assay.

Participant numbers in each group:

	Participant group	Number of participants
Young strokes	HIV positive	n = 20
	HIV negative	n = 40
	All young strokes	n = 60

Table 6.2 Number of participants in each group for hypothesis 1

Distribution of results for all young strokes (n = 60):

- Not normally distributed.
- Range: 70 - 374%
- Median = 138.5%
- *IQR* (25^{th} - 75^{th} quartile): 111.3 – 182%

Distribution of results for individual young stroke groups:

The VWF levels of the individual young stroke groups were also not normally distributed, with a bimodal distribution in the HIV positive young stroke group (n = 20) and skewing to the left in the HIV negative young stroke group (n = 40). The median VWF level in the HIV positive young stroke group was elevated above the upper limit of the normal range, whereas the median of the HIV negative young stroke group remained within the upper limits of the normal range.

Hypothesis testing: The two groups were significantly different [Mann-Whitney U test (U = 263, p = 0.032, r = 0.28)]. Outlier values were identified in our study sample. However, even after performing the analysis with those values excluded, our findings were very similar and we therefore chose to include the outliers in our final analysis. Table 6.3 summarises the results and figure 6.3 depicts them in a box and whisker plot.

VWF:Ag assay	HIV positive young stroke (n = 20)	HIV negative young stroke (n = 40)	All stroke participants (n = 60)	HIV positive young stroke vs. HIV negative young stroke
Median (%)	173.5	135	138.5	$U = 263$ $p = 0.032$ $r = 0.28$
IQR (%) **(25^{th} – 75^{th} quartile)**	118.3 - 215.5	107.5 - 168	111.3 - 182	
Range (%) (minimum– maximum)	83 - 374	70 - 327	70 - 374	

Table 6.3 VWF:Ag assay results for all stroke participants Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*), U statistic (*U*), effect size (*r*).

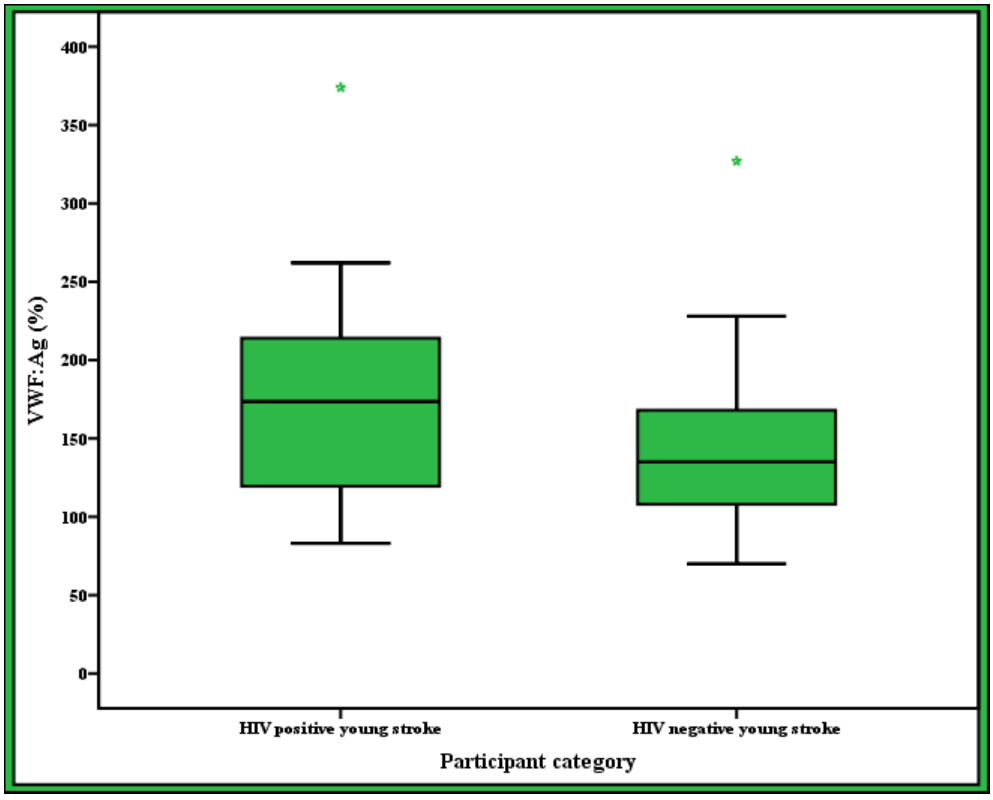


Figure 6.3 Comparison of VWF:Ag assay levels between the two stroke groups * Indicates outlier values. Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag).

Hypothesis 1 is confirmed: HIV positive young stroke participants have higher levels of VWF compared with HIV negative young stroke participants.

Hypothesis 1a: HIV positive young stroke participants whose stroke was "idiopathic" (i.e. could not be attributed to any other identifiable cause) would have a pro-thrombotic coagulation profile characterised by higher levels of VWF compared with HIV negative young stroke participants whose stroke was also "idiopathic."

Participant numbers in each group:

	Participant group	Number of participants	Percentage of total young strokes
Young "idiopathic" strokes	HIV positive	n = 9	45%
	HIV negative	n = 17	42.5%
	All young "idiopathic" strokes	n = 26	43.3%

Table 6.4 Number of participants in each group for hypothesis 1a

Distribution of results for all young "idiopathic" strokes (n = 26):

- Non-normal, skewed distributed.
- Range: 70 - 327%
- Median = 142%
- *IQR* (25^{th} - 75^{th} quartile): 112.5 – 198%

Distribution of results for individual young "idiopathic" stroke groups:

The VWF levels for the individual young "idiopathic" stroke groups were non-normally distributed. The median for the HIV positive young "idiopathic" stroke group is above the upper limit of normal at 175% (*IQR* 122.5 – 203%), whereas that of the HIV negative young "idiopathic" stroke group falls within the normal range at 139% (*IQR* 103.5 - 187.5%). These values are similar to that seen in hypothesis 1 for all young stroke participants.

Hypothesis testing: There was no statistically significant difference between the two groups when a Mann-Whitney U test was performed. The presence of outliers did not change our findings and were therefore included in the final analysis. Table 6.5 summarises the results and figure 6.4 depicts them in a box and whisker plot.

VWF:Ag assay	HIV positive young "idiopathic" strokes (n = 9)	HIV negative young "idiopathic" strokes (n = 17)	All "idiopathic" stroke participants (n = 26)	HIV positive young "idiopathic" strokes vs. HIV negative young "idiopathic" strokes
Median (%)	175	139	142	$p = 0.35$
IQR (%) **(25^{th} – 75^{th} quartile)**	122.5 - 203	103.5 - 187.5	112.5 - 198	
Range (%) (minimum – maximum)	102 - 234	70 - 327	70 - 327	

Table 6.5 VWF:Ag assay results for all "idiopathic" stroke participants Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*).

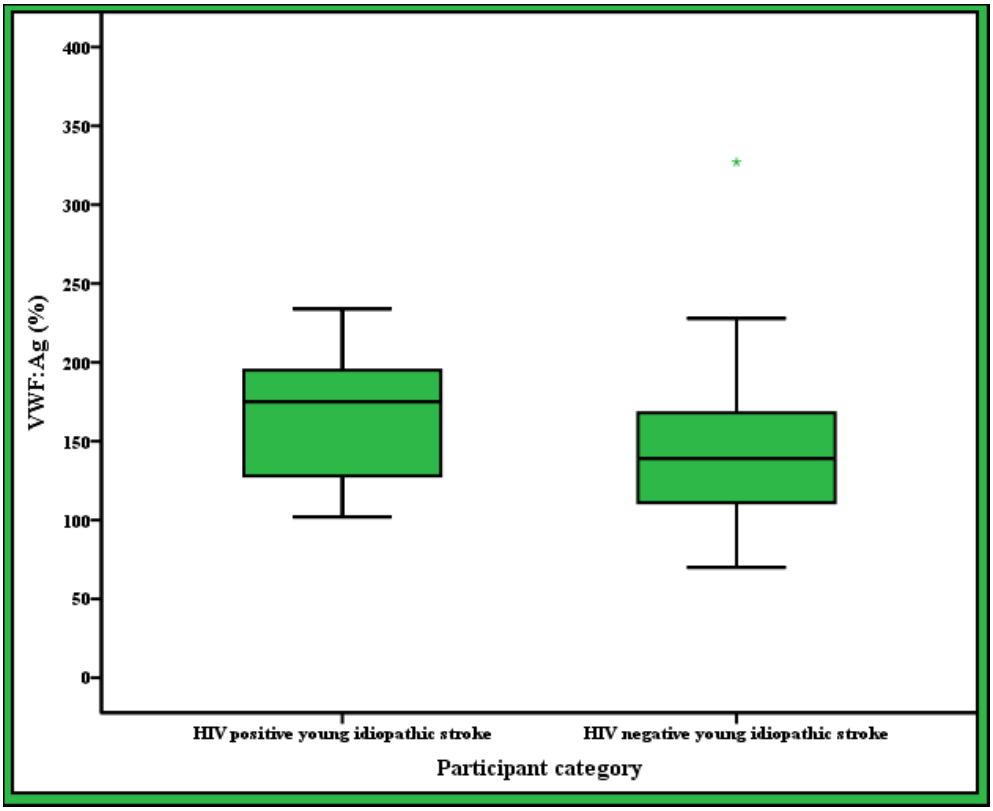


Figure 6.4 Comparison of VWF:Ag assay levels between the two "idiopathic" stroke groups * Indicates outlier values. Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag).

Hypothesis 1a is not confirmed: In the "idiopathic" stroke groups, the VWF:Ag levels of the HIV positive and HIV negative young stroke groups were not significantly different.

6.3.2 Hypothesis 2

Hypothesis 2: HIV positive young stroke participants would have a pro-thrombotic coagulation profile characterised by higher levels of VWF compared with HIV positive young non-stroke control participants. This was determined using the VWF:Ag assay.

Participant numbers in each group:

	Participant group	Number of participants
Young HIV positive	Strokes	n = 20
	Non - stroke	n = 40
	All young HIV positive	n = 60

Table 6.6 Number of participants in each group for hypothesis 2

Distribution of results for all young HIV positive (n = 60)

- Not normally distributed, skewing to the left
- Range: 48 – 427%
- Median = 134.5%
- *IQR* (25^{th} - 75^{th} quartile): 102 – 193%

Distribution of results for individual HIV positive groups:

Distribution of the VWF levels in the HIV positive young non–stroke control group was asymmetrical and remained within the upper limit of the normal range. The bimodal distribution in the HIV positive young stroke group was mentioned earlier under hypothesis 1 (see section 6.3.1).

Hypothesis testing: The two groups were not significantly different, but tended close to statistical significance [Mann-Whitney U test (U = 280.5, p = 0.061, r = 0.24)]. Calculations with and without outlier values did not change the results. We therefore included the outliers in our final analysis. Table 6.7 summarises the results and figure 6.5 depicts them in a box and whisker plot.

VWF:Ag assay	HIV positive young stroke (n = 20)	HIV positive young controls (n = 40)	All HIV positive participants (n = 60)	HIV positive young stroke vs. HIV positive young controls
Median (%)	173.5	129	134.5	*U* = 280.5 *p* = 0.061 *r* = 0.24
IQR (%) **(25^{th} – 75^{th} quartile)**	118.3 - 215.5	94.8 - 175	102 - 193	
Range (%) (minimum – maximum)	83 - 374	48 - 427	48 - 427	

Table 6.7 VWF:Ag assay results for all HIV positive participants Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*), U statistic (*U*), effect size (*r*).

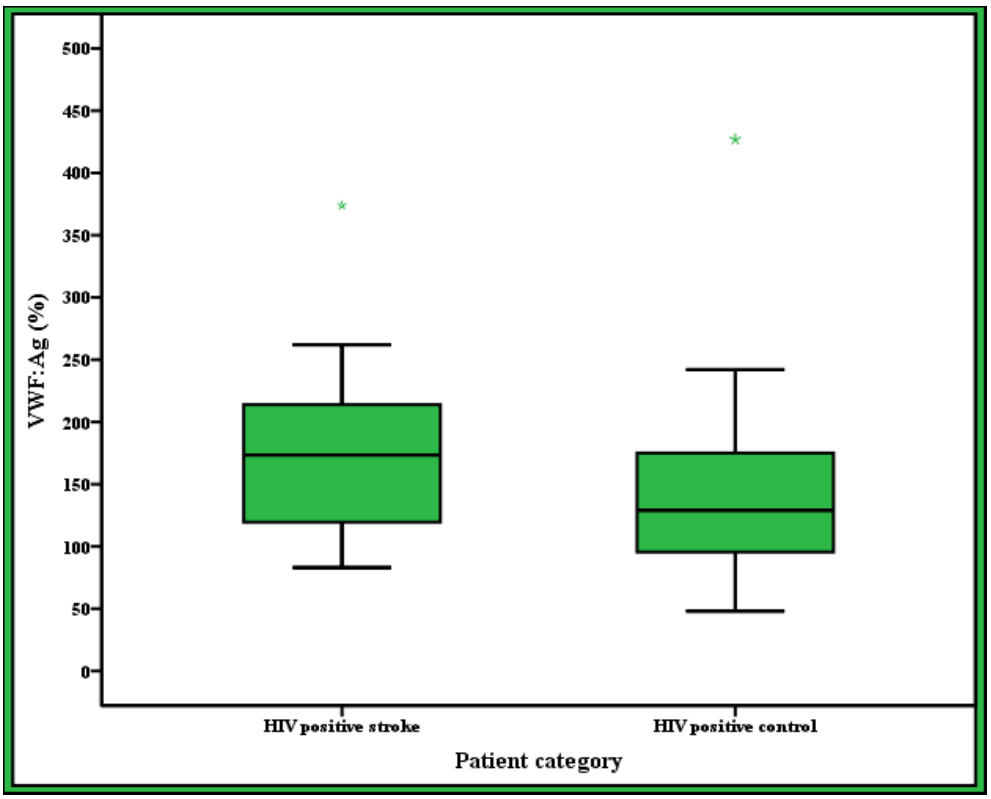


Figure 6.5 Comparison of VWF:Ag assay levels between the two HIV positive groups * Indicates outlier values. Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag).

Hypothesis 2 is not confirmed: HIV positive young stroke participants do not have higher levels of VWF compared with HIV positive young non-stroke control participants. However, the difference came close to being significant.

6.3.3 Hypothesis 3

Hypothesis 3: HIV positive young stroke participants would have a pro-thrombotic coagulation profile characterised by higher levels of VWF activity compared with HIV negative young stroke participants. This was measured using the VWF:CB assay.

Participant numbers in each group:

	Participant group	Number of participants
Young Strokes	HIV positive	n = 20
	HIV negative	n = 40
	All young strokes	n = 60

Table 6.8 Number of participants in each group for hypothesis 3

Distribution of results for all young strokes (n = 60):

- Not normally distributed.
- Range: 0 - 294%
- Median = 68.5%
- *IQR* (25^{th} - 75^{th} quartile): 42.8 – 112%

Distribution of results for all young strokes (n = 60):

For the individual stroke groups, the data were asymmetrically distributed, with pronounced skewing to the left. The medians for both individual stroke groups remained within the normal range. Both groups had 25^{th} percentiles values that were extremely close; 42% in the HIV positive young stroke group, and 42.8% in the HIV negative young stroke group. These lower than normal 25^{th} percentile values were in keeping with the overall low value for the total stroke participant group. Their individual 75^{th} percentile values did, however, differ markedly; 166% in the HIV positive young stroke group, and 108.8% in the HIV negative young stroke group. We therefore see a slight deviation above normal limits in those individuals infected with HIV and suffering from stroke simultaneously.

Hypothesis testing: Despite these differences, the two groups were not statistically significantly different. This finding held true when the analysis was conducted with and without outlier values. Table 6.9 summarises the results and figure 6.6 depicts them in a box and whisker plot.

VWF:CB assay	HIV positive young stroke (n = 20)	HIV negative young stroke (n = 40)	All stroke participants (n = 60)	HIV positive young stroke vs. HIV negative young stroke
Median (%)	81	67.5	68.5	$p = 0.37$
IQR (%) **(25^{th} – 75^{th} quartile)**	42 - 166	42.8 - 108.8	42.8 - 112	
Range (%) (minimum – maximum)	0 - 294	0 - 277	0 - 294	

Table 6.9 VWF:CB assay results for all stroke participants Abbreviations: von Willebrand factor (VWF), VWF collagen binding (VWF:CB), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*).

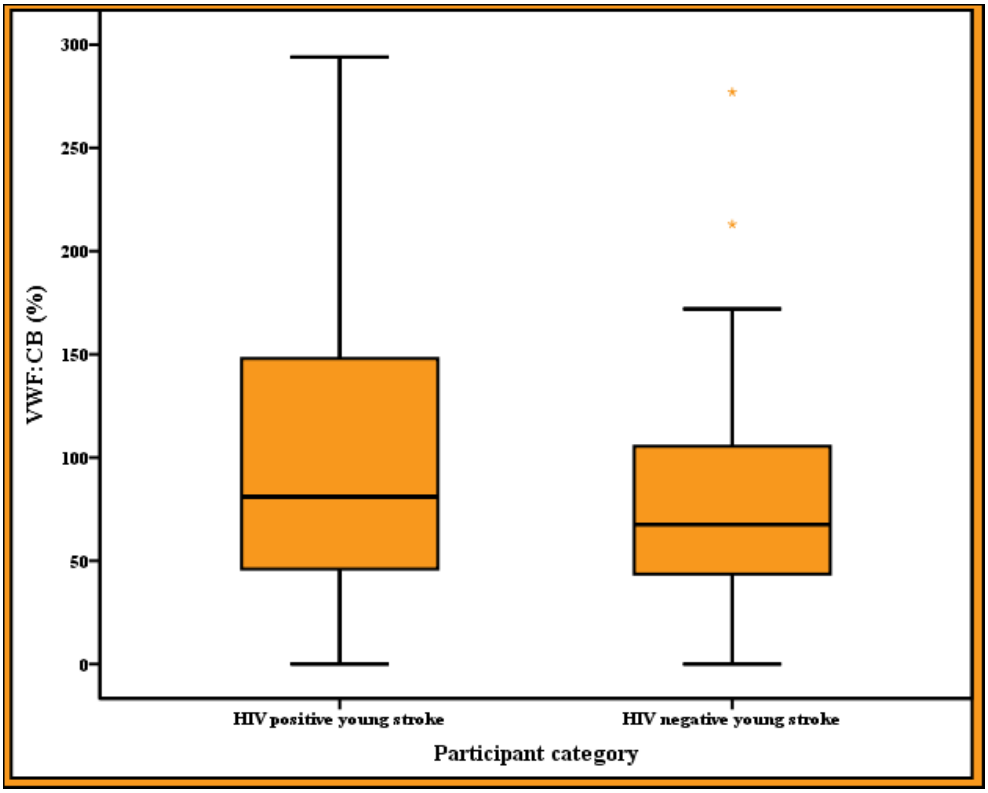


Figure 6.6 Comparison of VWF:CB assay levels between the two stroke groups * Indicates outlier values. Abbreviations: von Willebrand factor (VWF), VWF collagen binding (VWF:CB).

Hypothesis 3 is not confirmed: HIV positive young stroke participants do not have higher levels of VWF activity compared with HIV negative young stroke participants. On these criteria, therefore, HIV positive young stroke participants did not have a pro-thrombotic coagulation profile compared with HIV negative young stroke participants.

Hypothesis 3a: HIV positive young stroke participants whose stroke was "idiopathic" would have a pro-thrombotic coagulation profile characterised by higher levels of VWF activity compared with HIV negative young stroke participants whose stroke was "idiopathic."

Participant numbers in each group:

	Participant group	Number of participants
Young "idiopathic" strokes	HIV positive	n = 9
	HIV negative	n = 17
	All young idiopathic strokes	n = 26

Table 6.10 Number of participants in each group for hypothesis 3a

Distribution of results for all "idiopathic" young strokes (n = 26):

- Non-normal, skewed distributed.
- Range: 0 - 294%
- Median = 78%
- *IQR* (25 - 75th quartile): 54 – 100%

Distribution of results for individual young "idiopathic" stroke groups:

The distributions of the individual young "idiopathic" stroke groups were also non-normal. The median for the individual HIV positive and HIV negative young "idiopathic" stroke groups were both within the normal limits at 85% (*IQR* 58.5 - 157.5%) and 68% (*IQR* 53.5 - 93.5%), respectively. These values are similar to that seen in hypothesis 3 for all stroke participants.

Hypothesis testing: There was no statistically significant difference between the two groups upon completion of a Mann-Whitney U test. Table 6.11 summarises the results and figure 6.7 depicts them in a box and whisker plot.

VWF:CB assay	HIV positive young "idiopathic" strokes (n = 9)	HIV negative young 'idiopathic" strokes (n = 16)	All "idiopathic" stroke participants (n = 26)	HIV positive young "idiopathic" strokes vs. HIV negative young "idiopathic" strokes
Median (%)	85	68	78	p = 0.26
IQR (%) **(25^{th} – 75^{th} quartile)**	58.5 - 157.5	53.5 - 93.5	54 - 100	
Range (%) (minimum – maximum)	13 - 294	0 - 150	0 - 294	

Table 6.11 VWF:CB assay results for all "idiopathic" stroke participants Abbreviations: von Willebrand factor (VWF), VWF collagen binding (VWF:CB), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*).

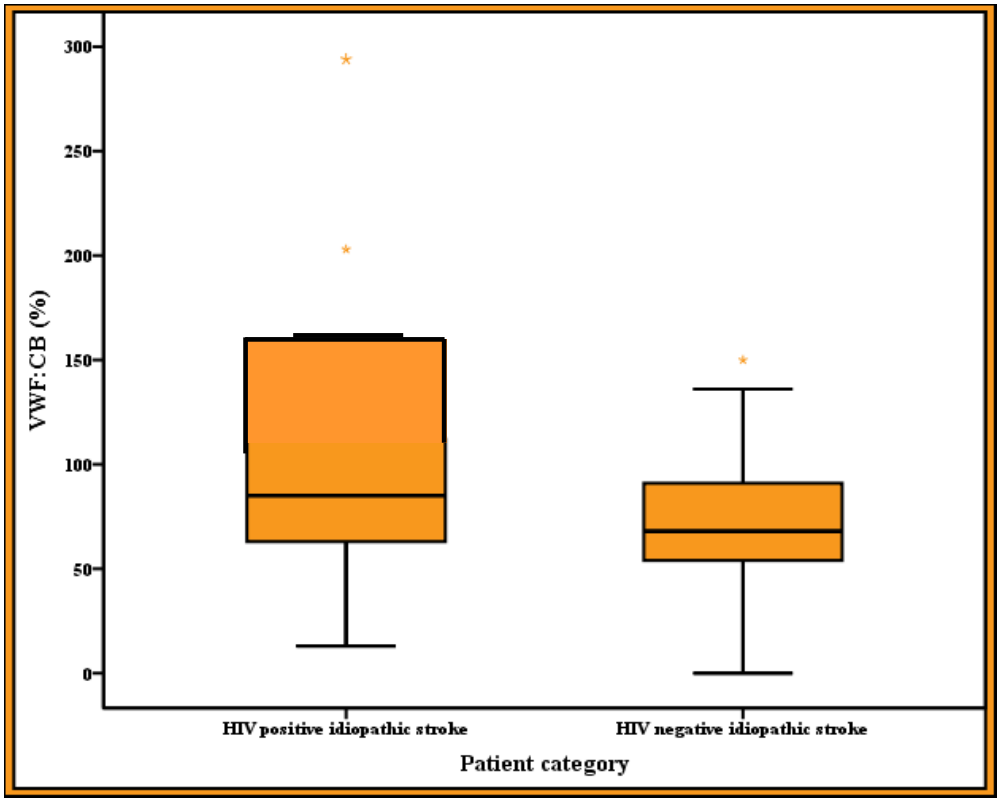


Figure 6.7 Comparison of VWF:CB assay levels between the two "idiopathic" stroke groups
* Indicates outlier values. Abbreviations: von Willebrand factor (VWF), VWF collagen binding (VWF:CB).

Hypothesis 3a is not confirmed: In the "idiopathic" stroke groups, the VWF activity (VFW:CB assay levels) of the HIV positive and HIV negative young stroke groups were not significantly different.

6.3.4 Hypothesis 4

Hypothesis 4: HIV positive young stroke participants would have a pro-thrombotic coagulation profile characterised by higher levels of VWF activity compared with HIV positive young non-stroke control participants. This was measured using the VWF:CB assay.

Participant numbers in each group:

	Participant group	Number of participants
Young HIV positive	Strokes	n = 20
	Non - stroke	n = 40
	All young HIV positive	n = 60

Table 6.12 Number of participants in each group for hypothesis 4

Distribution of results for all young HIV positive (n = 60):

- Not normally distributed, skewing to the left
- Range: 0 – 294%
- Median = 102.5%
- *IQR* (25^{th} - 75^{th} quartile): 60 – 154.3%

Distribution of results for individual HIV positive groups:

The VWF:CB assay results in the HIV positive young non-stroke control group were normally distributed. However, the VWF:CB assay results in the HIV positive young stroke group were non-normally distributed. Both groups had median values within the normal range. The *IQR*s of the two groups did not differ much, with the 25^{th} percentile value of the HIV positive young stroke group only slighter below the normal limit at 42%.

Hypothesis testing: The two groups were not significantly different. Table 6.13 summarises the results and figure 6.8 depicts them in a box and whisker plot.

VWF:CB assay	HIV positive young stroke (n = 20)	HIV positive young controls (n = 40)	All HIV positive participants (n = 60)	HIV positive young stroke vs. HIV positive young controls
Median (%)	81	107	102.5	$p = 0.26$
IQR (%) **(25^{th} – 75^{th} quartile)**	42 - 166	68.5 - 154.3	60.3 - 154.3	
Range (%) (minimum – maximum)	0 - 294	0 - 270	0 - 294	

Table 6.13 VWF:CB assay results for all HIV positive participants Abbreviations: von Willebrand factor (VWF), VWF collagen binding (VWF:CB), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*).

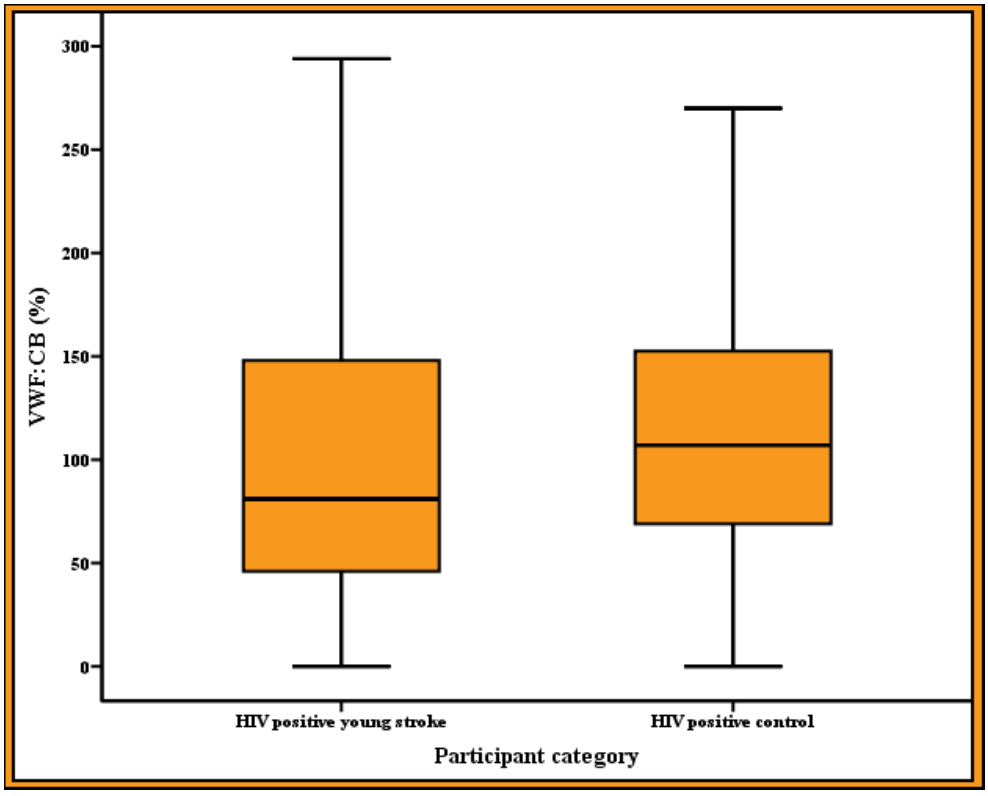


Figure 6.8 Comparison of VWF:CB assay levels between the two HIV positive groups Abbreviations: von Willebrand factor (VWF), VWF collagen binding (VWF:CB).

Hypothesis 4 is not confirmed: HIV positive young stroke participants do not have higher levels of VWF activity compared with HIV positive young non-stroke control participants. On these criteria, therefore, HIV positive young stroke participants did not have a pro-thrombotic coagulation profile compared with HIV positive young non-stroke control participants.

6.3.5 Hypothesis 5

Hypothesis 5: HIV positive young stroke participants would have a pro-thrombotic coagulation profile characterised by lower levels of ADAMTS13 compared with HIV negative young stroke participants. This was measured using the ADAMTS13:Ag assay.

Participant numbers in each group:

	Participant group	Number of participants
Young Strokes	HIV positive	n = 20
	HIV negative	n = 40
	All young strokes	n = 60

Table 6.14 Number of participants in each group for hypothesis 5

Distribution of results for all young strokes (n = 60):

- Not normally distributed.
- Range: 0 - 674%
- Median = 0%
- *IQR* (25^{th} -75^{th} quartile): 0 – 5.5%

A value of 0% does not necessarily mean that the participant has no ADAMTS13 present in their plasma, but it does imply that the amount is very low and is not detectable with the test we used.

Distribution of results for individual young stroke groups:

Both groups were non-normally distributed and skewed to the left. The medians of the two groups were both 0% and *IQR*s were both low. However the *IQR* (0 - 1.5%) of the HIV positive young stroke group indicated that ADAMTS13 levels in those participants were mostly non-detectable. In addition, the maximum ADAMTS13 level detected in the HIV positive young stroke group was measured at 674%. This value is in stark contrast to the maximum value of 123% measured in the HIV negative young stroke group. We can therefore see that none of the HIV negative young stroke participants had an excess amount of ADAMTS13 in their peripheral blood.

Hypothesis testing: Mann-Whitney U analysis revealed no statistically significant difference between the two stroke groups. Table 6.15 summarises the results and figure 6.9 depicts them in a box and whisker plot.

ADAMTS13:Ag assay	HIV positive young stroke (n = 20)	HIV negative young stroke (n = 40)	All stroke participants (n = 60)	HIV positive young stroke vs. HIV negative young stroke
Median (%)	0	0	0	*p* = 0.71
IQR (%) **(25^{th} – 75^{th} quartile)**	0 - 1.5	0 - 6	0 - 5.5	
Range (%) (minimum – maximum)	0 - 674	0 - 123	0 – 674	

Table 6.15 ADAMTS13:Ag assay results for all stroke participants Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), ADAMTS13 antigen (ADAMTS13:Ag), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*).

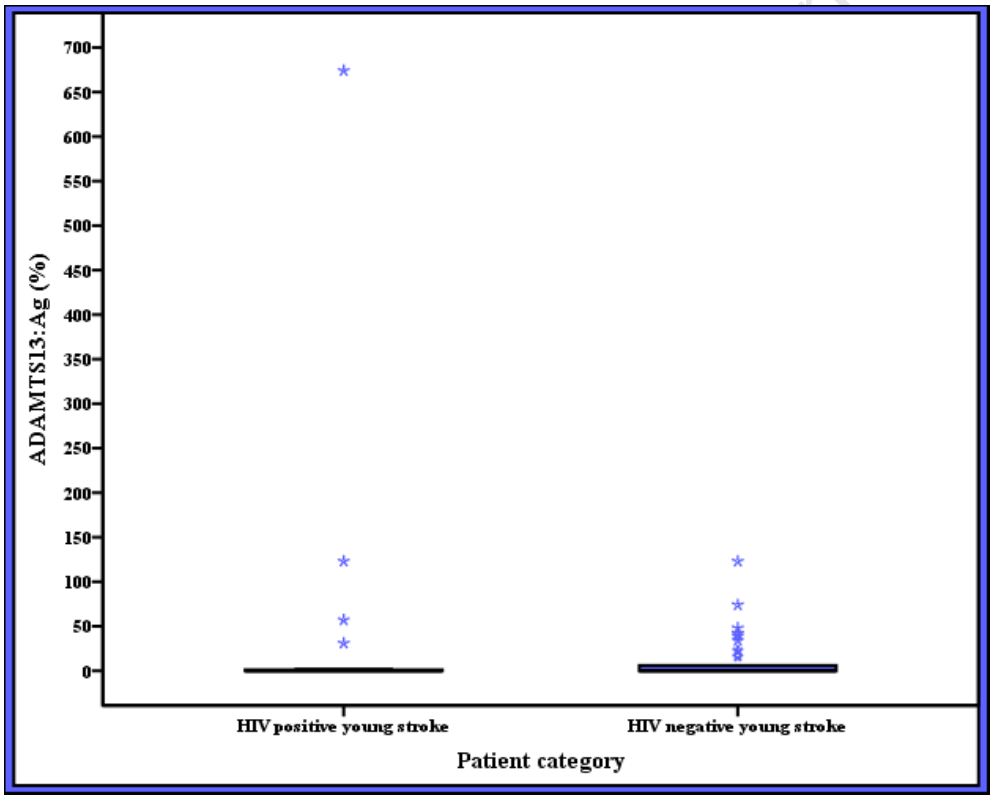


Figure 6.9 Comparison of ADAMTS13:Ag levels between the two stroke groups * Indicates outlier values. Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), ADAMTS13 antigen (ADAMTS13:Ag).

Hypothesis 5 is not confirmed: HIV positive young stroke participants do not have lower levels of ADAMTS13 compared with HIV negative young stroke participants. On these criteria, therefore, HIV positive young stroke participants did not have a pro-thrombotic coagulation profile compared with HIV negative young stroke participants.

Hypothesis 5a: HIV positive young stroke participants whose stroke was "idiopathic" would have a pro-thrombotic coagulation profile characterised by lower levels of ADAMTS13 compared with HIV negative young stroke participants whose stroke was "idiopathic." This was measured using the ADAMTS13:Ag assay.

Participant numbers in each group:

	Participant group	Number of participants
Young "idiopathic" strokes	HIV positive	n = 9
	HIV negative	n = 17
	All young idiopathic strokes	n = 26

Table 6.16 Number of participants in each group for hypothesis 5a

Distribution of results for all "idiopathic" young strokes (n = 26):

- Non-normal, skewed distributed.
- Range: 0 - 123%
- Median = 0%
- *IQR* (25^{th} - 75^{th} quartile): 0 – 3%

Distribution of results for individual young "idiopathic" stroke groups:

The distribution of the ADAMTS13:Ag levels for the individual "idiopathic" stroke groups was also non-normal as in hypothesis 5. The median for the HIV positive young "idiopathic" stroke group and the HIV negative young "idiopathic" stroke group are both equal to 0% with extremely small *IQRs* for both individual groups.

Hypothesis testing: Statistical analysis with a Mann-Whitney U test did not reveal any statistically significant difference between the two groups. Table 6.17 summarises the results and figure 6.10 depicts them in a box and whisker plot.

ADAMTS13:Ag	HIV positive young "idiopathic" strokes (n = 9)	HIV negative young "idiopathic" strokes (n = 17)	All "idiopathic" stroke participants (n = 26)	HIV positive young "idiopathic" strokes vs. HIV negative young "idiopathic" strokes
Median (%)	0	0	0	$p = 0.43$
IQR (%) **(25^{th} – 75^{th} quartile)**	0 - 0	0 - 6	0 - 3	
Range (%) (minimum – maximum)	0 - 123	0 - 48	0 - 123	

Table 6.17 ADAMTS13:Ag assay results for all "idiopathic" stroke participants Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), ADAMTS13 antigen (ADAMTS13:Ag), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*).

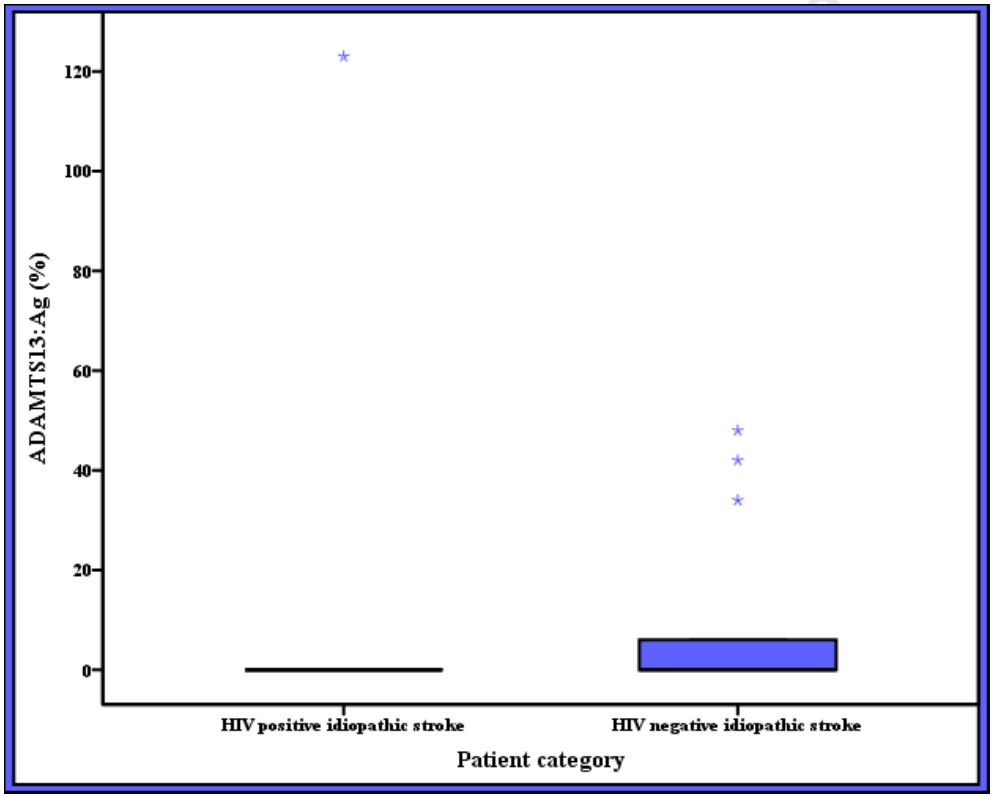


Figure 6.10 Comparison of the ADAMTS13:Ag levels between the two "idiopathic" stroke groups * Indicates outlier values. Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), ADAMTS13 antigen (ADAMTS13:Ag).

Hypothesis 5a is not confirmed: In the "idiopathic" stroke groups, the ADAMTS13:Ag levels of the HIV positive and HIV negative young stroke groups were not significantly different.

6.3.6 Hypothesis 6

Hypothesis 6: HIV positive young stroke participants would have a pro-thrombotic coagulation profile characterised by lower levels of ADAMTS13 compared with HIV positive young non-stroke control participants. This was measured using the ADAMTS13:Ag assay.

Participant numbers in each group:

	Participant group	Number of participants
Young HIV positive	Strokes	n = 20
	Non - stroke	n = 40
	All young HIV positive	n = 60

Table 6.18 Number of participants in each group for hypothesis 6

Distribution of results for all young HIV positive (n = 60):

- Not normally distributed, skewing to the left
- Range: 0 – 1075%
- Median = 0%
- *IQR* (25^{th} - 75^{th} quartile): 0 – 165.8%

Distribution of results for individual HIV positive groups:

The ADAMTS13 distributions of the individual young HIV positive groups were also not normal. The median value of 23.5% for the HIV positive young control group is in stark contrast to that of the median of 0% in the HIV positive young stroke group. The *IQRs* also differed remarkably when comparing the two groups, as did the range of values. One of the participants in the HIV positive young control group had a value of 1075%. This was the highest value of all the participants and was seven times the upper limit of the normal range. The HIV positive young controls appeared to have higher plasma levels of ADAMTS13 compared with their HIV positive counterparts with stroke. Despite this difference, it is important to note that the HIV positive young control group still had a median value that was substantially lower than the lowest limit of the normal range of 50%.

Hypothesis testing: The two groups were significantly different [Mann-Whitney U test (U = 262, p = 0.018, r = 0.31)]. An analysis conducted without the outliers still yielded statistically

significant results that were similar to the results presented here, and thus the decision was made to include the outlier values in our final analysis. Table 6.19 summarises the results and figure 6.11 depicts them in a box and whisker plot.

ADAMTS13:Ag assay	**HIV positive young stroke (n = 20)**	**HIV positive young controls (n = 40)**	**All HIV positive participants (n = 60)**	**HIV positive young stroke vs. HIV positive young controls**
Median (%)	0	23.5	0	$U = 262$ $p = 0.018$ $r = 0.31$
IQR (%) **(25^{th} – 75^{th} quartile)**	0 - 1.5	0 - 240.8	0 - 165.8	
Range (%) (minimum – maximum)	0 - 674	0 - 1075	0 - 1075	

Table 6.19 ADAMTS13:Ag assay results for all HIV positive participants Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), ADAMTS13 antigen (ADAMTS13:Ag), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*), U statistic (*U*), effect size (*r*).

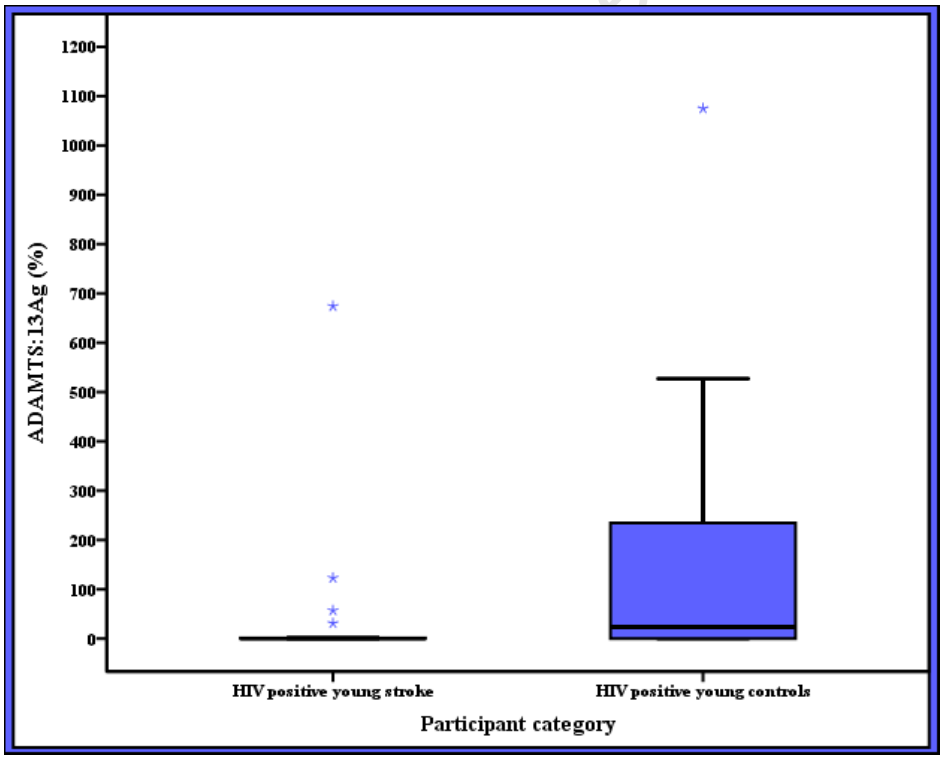


Figure 6.11 Comparison of ADAMTS13:Ag levels between the two HIV positive groups * Indicates outlier values. Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), ADAMTS13 antigen (ADAMTS13:Ag).

Hypothesis 6 is confirmed: HIV positive young stroke participants have lower levels of ADAMTS13 compared with HIV positive young non-stroke control participants.

6.3.7 Hypothesis 7

Hypothesis 7: Levels of VWF (7a) and VWF activity (7b) would be negatively correlated with CD4 counts in all HIV positive participants (with and without strokes) i.e. with advancing immunosuppression (lower CD4 counts), VWF levels would increase. ADAMTS13 levels (7c) would be positively correlated with CD4 counts in all HIV positive participants (with and without strokes) i.e. with advancing immunosuppression (lower CD4 counts), ADAMTS13 levels would decrease.

Participant numbers in each group:

	Participant group	Number of participants whose CD4 count was correlated with VWF
Young HIV positive	Strokes	n = 20
	Non - stroke	n = 39
	All young HIV positive	n = 59

Table 6.20 Number of participants in each group for hypothesis 7

Results of the correlations and hypothesis testing:

VWF levels in HIV positive participants were significantly negatively correlated with CD4 count [Spearman's Rank Order correlation $r_s(59) = 0.36$, $p = 0.006$] (see figure 6.12). This negative correlation remained significant when the analysis was performed in the HIV positive control group on its own. VWF activity was very weakly negatively correlated with CD4 count but did not reach statistical significance (7b). No obvious correlation existed between ADAMTS13 levels and CD4 counts (7c).

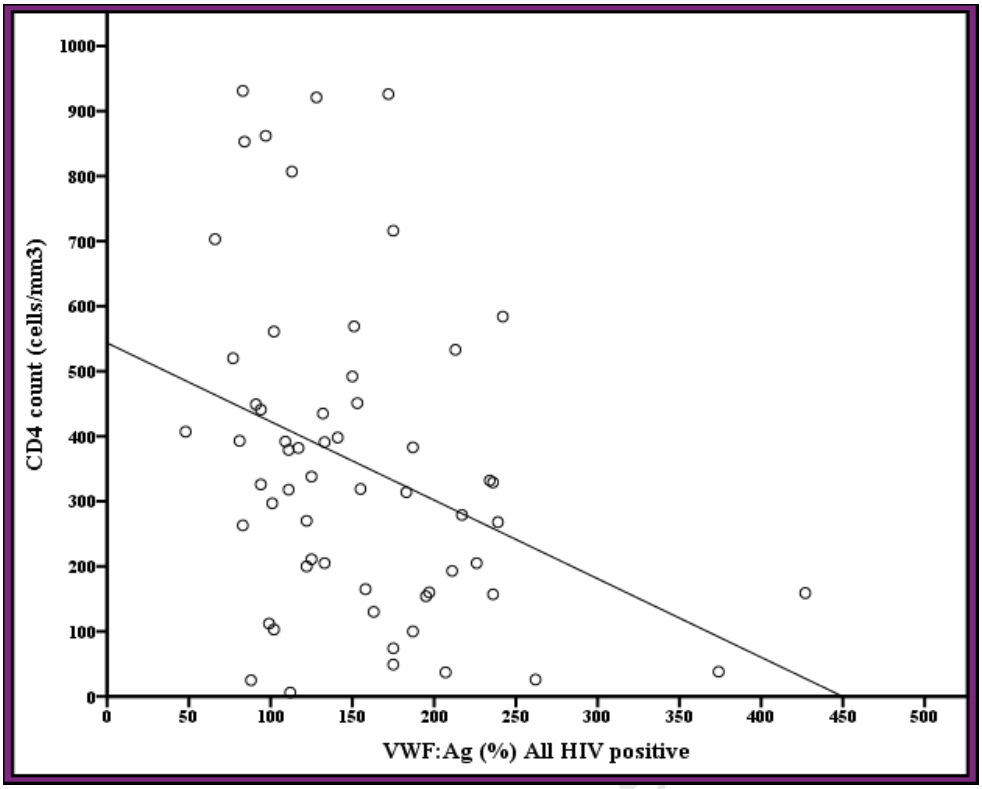


Figure 6.12 VWF levels and CD4 count correlation in all HIV positive participants Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag), cells per cubic millimetre (cells/mm^3).

Hypothesis 7a is confirmed: VWF levels in HIV positive participants are significantly negatively correlated with CD4 count.

6.4 Summary of results of hypotheses

Hypothesis 1, 6 and 7a were the three hypotheses that were found to be statistically significant. The HIV positive young stroke group had significantly higher VWF levels than the HIV negative young stroke group (U = 263, p = 0.032, r = 0.28). The HIV positive young stroke group had significantly lower levels of ADAMTS13 than the HIV positive young non-stroke control group (U = 262, p = 0.018, r = 0.31). VWF levels in HIV positive participants were significantly negatively correlated with CD4 count [Spearman's Rank Order correlation $r_s(59) = 0.36$, p = 0.006].

Hypothesis 2 came close to, but did not quite reach, significance (U = 280.5, p = 0.061, r = 0.24). The HIV positive young stroke group had a higher median level of VWF than the HIV positive young control group.

Hypothesis 3, 4 and 5 were not proven to be statistically significant. In addition, the sub-hypotheses 1a, 3a and 5a that focused on participants with "idiopathic" strokes were also not proven.

CHAPTER 7 – DISCUSSION I: PARTICIPANT CHARACTERISTICS

INTRODUCTION

In this chapter, I shall discuss the participant characteristics relating to the demographic findings, stroke presentation and associated medical findings of the 3 participant groups.

7.1 Discussion of participant characteristics

7.1.1 Demographic characteristics

All three groups were comparable with regards to age and sex. A striking feature in the characterisation of the participants was the socio-economic differences between the HIV positive and HIV negative groups on all measures used (type of home, access to electricity, water and toilet facilities). HIV positive participants had poorer socio-economic circumstances with poorer access to basic amenities. In the small population sample we have studied, HIV/AIDS is clearly linked to poverty and social deprivation. Interestingly, the HIV positive and HIV negative groups were comparable with regards to education and employment status i.e. they had similar total number of years of education and similar rates of employment/unemployment. In the South African context, number of years of education is not synonymous with quality of education. Twelve years of education in a school in a deprived area is not equivalent to schooling in a more affluent area. There were equal levels of employment/unemployment in both groups, but again, the type and quality of the employment was not specifically reported and could have differed markedly.

7.1.2 Stroke characteristics

There were 60 young stroke participants, 20 HIV positive and 40 HIV negative. The majority of stroke participants had strokes localised to lacunar vessels (LACI) or secondary to partial occlusions of the vessels in the anterior cerebral circulation (PACI). Posterior circulation infarcts (POCIs) and total anterior circulation infarcts (TACIs) were in the minority in both HIV positive and HIV negative stroke groups. Overall, the distribution of the different types of strokes was not significantly different in the two stroke groups.

Cardio-embolism and carotid artery dissection were two common causes of stroke in our study and were commoner in HIV negative young strokes. This is consistent with the findings in the literature (Yamamoto 2012, Ferro *et al*. 2010). In keeping with the current literature regarding stroke

causation in younger adults, "idiopathic" causes accounted for a significant proportion (~ 40%) of all strokes (Griffiths *et al*. 2011). Despite a similar ratio of "idiopathic" stroke amongst the two stroke groups, other causes of stroke differed significantly. Unlike the HIV positive young stroke group, the HIV negative young stroke group had aetiologies that are commonly known causes of stroke and more frequently applicable to older stroke sufferers. These include hypertensive small vessel disease and large vessel atherosclerosis. The HIV positive young stroke group were significantly more likely to have varicella vasculopathy as a cause for their stroke. Infection with varicella zoster virus (VZV) is a known occurrence in HIV positive individuals, and its association with stroke causation has been previously described in children as well as in adults (Vafai *et al*. 2001; De La Blanchardiere *et al*. 2000; Stanley *et al*. 2012). Nagel *et al* (2011) described 3 cases of stroke that were histologically confirmed to be secondary to varicella vasculopathy, with one participant having AIDS. Stanley *et al*. (2012) described a case from our hospital (Groote Schuur Hospital) of an aneurysmal vasculopathy-associated stroke in an HIV-infected individual with concomitant VZV. They emphasised the importance of investigating for the presence of VZV in these cases (Stanley *et al*. 2012). Our study confirms this increased frequency of varicella vasculopathy infection associated with stroke.

Within the "idiopathic" causes for the HIV positive young stroke group, there were 3 participants who suffered a stroke attributable to HIV-associated vasculopathy. This represents 15% of our HIV positive young stroke participants and closely matches the 20% of HIV positive strokes attributed to HIV-associated vasculopathy in a study by Tipping *et al*. (2007) conducted at Groote Schuur Hospital. Our findings therefore add to the growing literature on this entity of HIV-related vasculopathy and its link with stroke. In conclusion, although the "idiopathic" groups were sizeable in both HIV positive and HIV negative young strokes, the HIV positive group had a greater diversity of causes, including VZV and HIV-associated vasculopathy.

There were few observable complications associated with stroke in our study. This might relate to the fact that LACI and PACI strokes are generally associated with a good prognosis.

7.1.3 Co-morbidities and relevant medical history

When comparing HIV positive and HIV negative stroke groups, three traditional stroke risk factors viz. hypertension, diabetes and cardiovascular disease, were not significantly different. Nor was current TB infection or a previous coagulopathy different between the two groups. All three participant groups were comparable with respect to cigarette smoking, alcohol use and illegal substance use. We might have expected the latter to be higher in the HIV positive stroke group, given that some substances like cocaine and methamphetamine are associated with increased stroke

risk and increased HIV infection. However, validity of the reports could be questioned, given the perceived legal implications on the part of the patient.

7.1.4 CD4 count

The median CD4 count for all the HIV positive participants was 326 cells/mm^3. The cut-off CD4 count for initiation of HAART in South Africa is < 350 cells/mm^3, yet none of the participants had commenced treatment with ART yet. We had, of course, selected our participants on the basis of their being HAART-naïve, yet a substantial number of the HIV positive participants should have been eligible for HAART given their most recent CD4 count. Reasons for sub-optimal ART initiation in our participants included poor follow-up clinic attendance, lack of routine CD4 count monitoring at local clinics, fear of taking ART due to its side-effects and stigmata associated with its use. These facts reflect poorly on the state of the ARV roll-out plan in South Africa. We referred all eligible participants to their local clinic for the initiation of HAART.

The evidence from the literature suggests that stroke in HIV infection tends to be associated with advanced immunosuppression and therefore lower CD4 counts (Dobbs *et al*. 2009). Our results tended to show that the HIV positive stroke group had lower median CD4 counts compared with HIV positive non-stroke controls (234 vs. 383 cells/mm^3). The difference did not quite reach statistical significance ($p = 0.058$).

7.1.5 Weight and BMI

Median weights and median overall BMIs were lower in the HIV positive groups (stroke and non-stroke) compared with the HIV negative young stroke group. The association between HIV and weight loss/wasting is well known, and BMI measurement is considered a useful prognostic tool in individuals with HIV (Wanke *et al*. 2000; Grinspoon *et al*. 2003; van der Sande *et al*. 2004). The higher obesity levels in the HIV negative young stroke group would also explain the greater prevalence of co-morbid hypertension, diabetes and CVD in this group. There were no significant differences when comparing HIV positive strokes with HIV positive controls. We might have expected more underweight individuals in the HIV positive stroke group, given the association between stroke and advanced disease. This was not the case. There were, however, 8 exclusions from the weight and BMI analyses (5 from the HIV positive young stroke group) because of lack of data and these might have altered the findings.

CONCLUSION

For the most part, the differences between the groups were not entirely unexpected and many of our findings are consistent with those reported previously in the literature. There are three striking aspects with respect to the data on participant characteristics. Firstly, there are socio-economic discrepancies between HIV positive and HIV negative individuals and this reflects the need for social reform in South Africa itself. Secondly, more than 40%, a sizeable proportion of young strokes (both HIV positive and HIV negative) end up without an identified cause ("idiopathic") despite extensive investigation. Finally, there are some stroke causes peculiar to HIV infection, such as varicella vasculopathy and an HIV-associated vasculopathy.

CHAPTER 8 – DISCUSSION II: HYPOTHESES, STUDY LIMITATIONS AND RECOMMENDATIONS

INTRODUCTION

In this chapter, I shall firstly summarise the key findings and then discuss and interpret the results.

8.1 Key findings

Table 8.1 summarises the outcomes of our hypothesis testing.

Hypothesis	Findings
Hypothesis 1 (H1)	HIV positive young stroke participants had significantly higher levels of VWF in their plasma (VWF:Ag) compared with HIV negative young stroke participants. In the subgroup analysis of "idiopathic" strokes, there was no significant difference in the VWF levels between the two groups (H1a).
Hypothesis 2 (H2)	HIV positive young strokes tended to have high levels of VWF compared with HIV positive non-stroke controls. This tendency nearly reached statistical significance.
Hypothesis 3 (H3)	There was no significant difference in the activity of VWF as measured using the VWF:CB assay between the young HIV positive and HIV negative stroke groups. In the subgroup analysis of "idiopathic" strokes, there was no significant difference in the VWF activity between the two groups (H3a).
Hypothesis 4 (H4)	There was no difference between the HIV positive young stroke group and the HIV positive young non-stroke controls with regards to VWF activity as measured by the VWF:CB assay.
Hypothesis 5 (H5)	ADAMTS13 levels were low in both HIV positive and HIV negative young stroke groups, but not significantly different when comparing the two groups. In the subgroup analysis of "idiopathic" strokes, there was also no significant difference in the ADAMTS13 levels between the two groups (H5a).
Hypothesis 6 (H6)	The ADAMTS13 levels were significantly lower in the HIV positive young stroke group compared with the HIV positive non-stroke controls.
Hypothesis 7 (H7)	CD4 count was negatively correlated with VWF levels in HIV positive participants (HIV positive young strokes and HIV positive non-stroke controls combined) (H7a). However, CD4 count was not correlated with VWF activity (H7b) or ADAMTS13 levels (H7c) in HIV positive participants.

Table 8.1 Key findings Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag), VWF collagen binding (VWF:CB), A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), ADAMTS13 antigen (ADAMTS13:Ag).

8.2 Discussion of results

8.2.1 VWF:Ag assay results

The HIV positive young participants who had suffered a stroke had higher VWF levels in their plasma than the HIV negative young stroke participants (H1). This could mean that HIV positive individuals who have had a stroke have a hyper-coagulable/increased pro-thrombotic state compared with HIV negative young strokes. At the same time, HIV positive young stroke participants tended to have higher VWF levels compared with HIV positive non-stroke controls (H2). While the second finding did not quite reach statistical significance, the two results taken together suggest that stroke in the setting of HIV infection is associated with a hyper-coagulable state. These findings were established using the VWF:Ag assay, but were not confirmed using the VWF:CB assay (H3 and H4), which will be discussed later in section 8.2.2.

The two important questions that have been raised by the findings of H1 and H2 are:

1. What were the putative basal levels of VWF in the HIV positive and HIV negative participants with stroke, before the stroke occurred?
2. Are they causal i.e. did the pro-thrombotic state precede and lead to stroke, or were the findings consequential upon the stroke. That is, did they result from the stroke itself e.g. as an acute phase reactant?

Question 1

Obviously, we did not have basal VWF levels for the stroke groups. Ideally, question 1 could only have been directly addressed had our study been a huge longitudinal cohort study. A baseline VWF level would then have been measured in non-stroke HIV positive and HIV negative participants and a repeat measurement would have followed in any participants who presented with a stroke. However, this would have required large participant numbers, with long-term follow-up. Ultimately, it would have been a very expensive study to execute. The lack of baseline VWF levels is therefore a limitation. However, the most practical alternative is to have an HIV positive non-stroke control group in our study and to use an already defined population norm for a non-HIV non-stroke control group from a previous South African study (Meiring *et al*. 2012). The VWF levels in the HIV positive non-stroke control group acts as a surrogate baseline level for the HIV positive young stroke group, and the VWF levels in the HIV negative non-stroke group represents the putative baseline values of the HIV negative young stroke group. Ideally, of course, we would have liked the baseline norms of the HIV negative young stroke group to have been derived from the exact same population we studied. However, the values obtained from Prof Meiring's study, using the same assays and same laboratory, was the best overall substitute available.

Figure 8.1 represents a model to help understand question 1. In this graph, the findings of the VWF:Ag assays of all three participant groups, including the normal range of 50 - 150% based on the population norms of a HIV negative non-stroke population, are plotted.

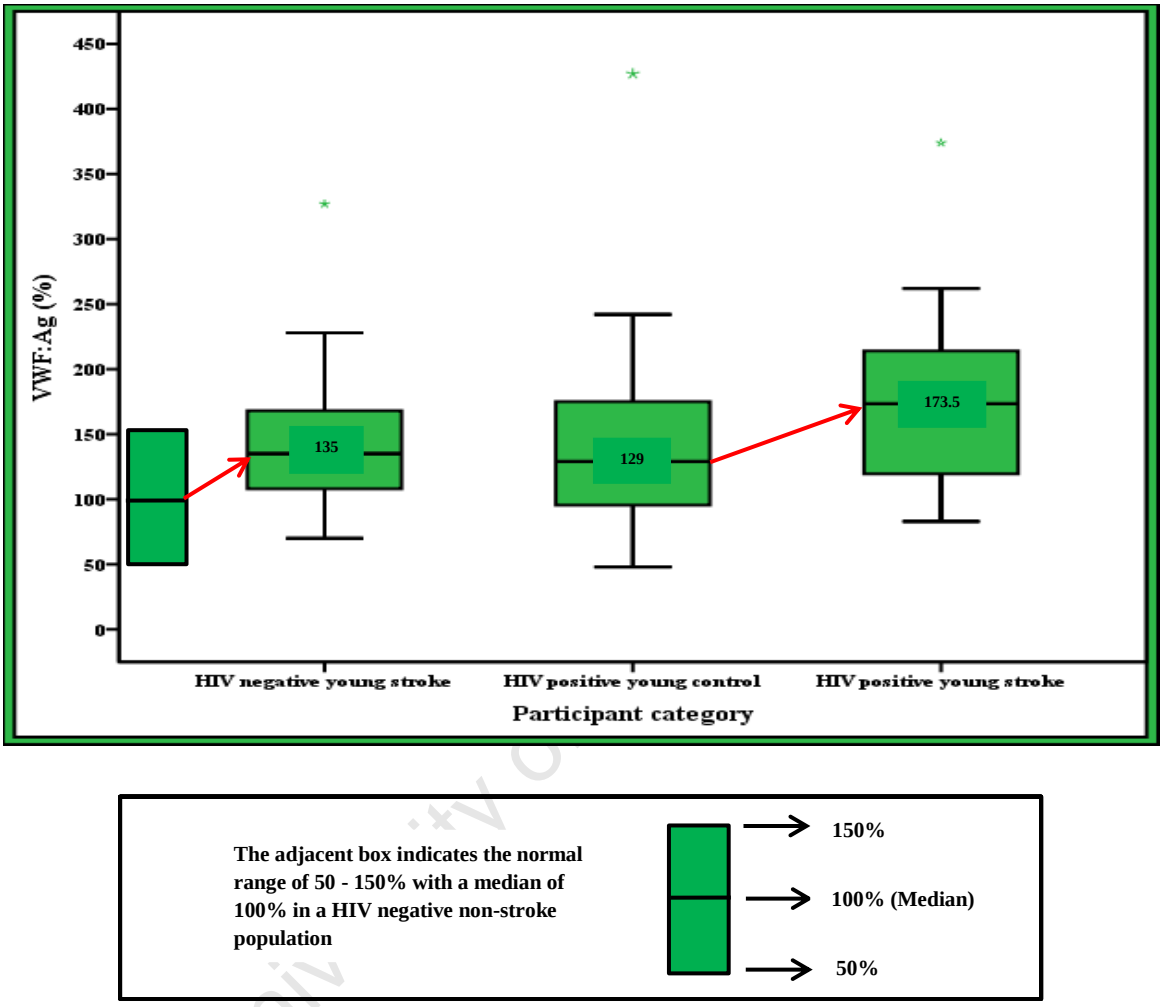


Figure 8.1 Comparison of the median VWF levels of all participant groups measured using the VWF:Ag assay Median values are indicated at the median line, * indicates outlier values and → shows the median change between groups. Abbreviations: von Willebrand factor (VWF), VWF antigen (VWF:Ag).

From this model, we can see that HIV positive non-stroke controls had a median value of 129%, which, although greater than the normal median of 100% in a HIV negative, non-stroke population, was still within the normal range.

This model, however, suggests that stroke is related to increased VWF levels in the following way:

There were no major changes in VWF:Ag levels when a HIV negative young individual developed a stroke i.e. median of 100% increased to 135%. This reflects a small rise within the normal range only. However, the level of VWF in the HIV positive young stroke group was definitely greater

than that in HIV negative young strokes and statistically significantly different. Also, the median VWF levels in the HIV positive young stroke group were greater than that in the HIV positive non-stroke control group, and the difference between these two groups tended close to statistical significance.

Therefore stroke in HIV infection is associated with higher VWF levels and a pro-thrombotic state.

Question 2

Following on from question 1, we should like to know whether this pro-thrombotic state in HIV infection arose prior to the stroke or as a consequence of the stroke i.e. did it precede the stroke and was it causative, or was it secondary e.g. following an acute phase reaction?

Figure 8.2 represents a model that attempts to answer this question. This model follows a hypothetical scenario whereby the VWF levels (based on our study findings and the previously accepted norms) are depicted in the progression from a HIV negative non-stroke state to a HIV-infected stroke state.

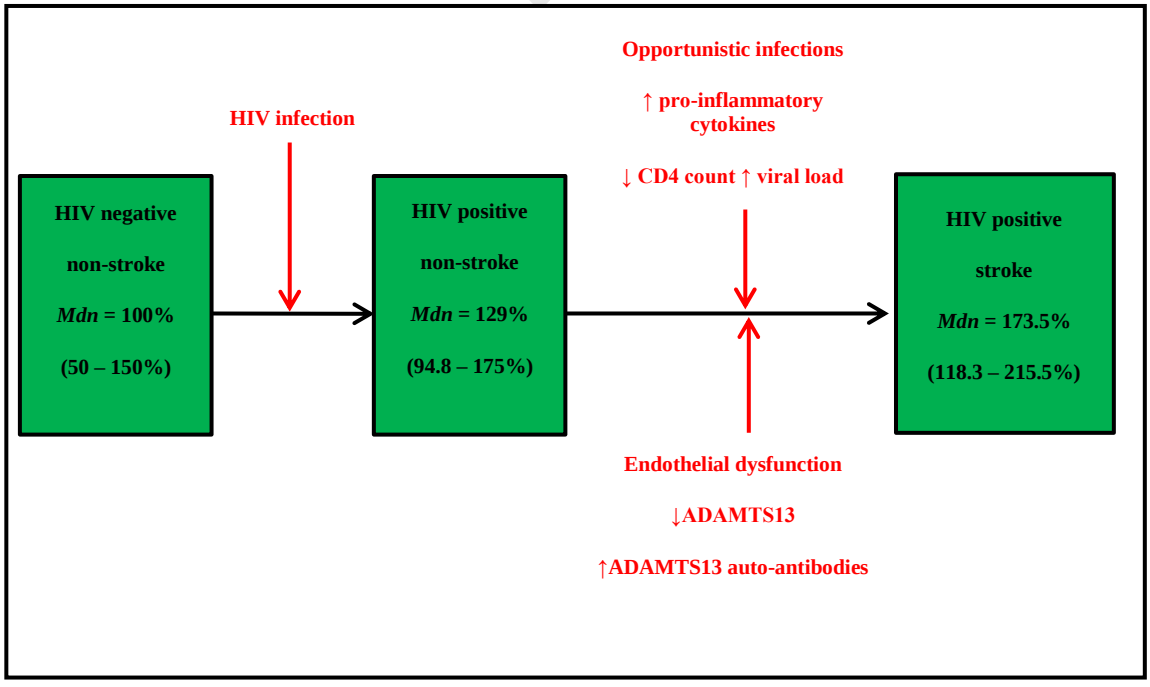


Figure 8.2 Progression of VWF levels from a HIV negative non-stroke state to a HIV-infected stroke state Interquartile range is given in parentheses. Abbreviations: von Willebrand factor (VWF), A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), median (*Mdn*).

In addition, figure 8.3 aids the arguments that follow. It is a diagram that tracks VWF levels in HIV negative participants from a non-stroke to a stroke state.

Figure 8.3 Progression of VWF levels from an HIV negative non-stroke state to a HIV negative stroke state Interquartile range is given in parentheses. Abbreviations: von Willebrand factor (VWF), median (*Mdn*).

Findings favouring a causative relationship for this pro-thrombotic state are as follows:

The pro-thrombotic state in stroke with HIV infection is not a consequence of the stroke, because HIV negative individuals who develop a stroke had only a slightly raised VWF median level than those without stroke, but still within the normal range (see figure 8.3). When an HIV negative non-stroke individual becomes infected with HIV, there is a slight increase from a median VWF level of 100% to 129%. This slight increase, however, remains within the normal range (see figure 8.2). A significant subsequent elevation from a median of 129 to 173% occurs in HIV-infected individuals with stroke (see figure 8.2).

Infection with HIV therefore causes a slight rise in baseline VWF, still within the normal range. This rise in VWF may bring about a pro-thrombotic state that either results in a stroke or facilitates its advent with or without other causative factors. Aiding this causative theory are data indicating that raised VWF levels are associated with an increased risk for pro-thrombotic states (Sadler 2005; Denis *et al*. 2012). There are, however, fewer data available on this association between VWF levels and thrombosis in HIV-infected individuals (Lafeuillade *et al*. 1992; Seigneur *et al*. 1997). The majority of the literature is centred on the influence of ART on VWF levels (Wolf *et al*. 2002; Aukrust *et al*. 2000; de Gaetano Donati *et al*. 2004; van Vonderon *et al*. 2009). Our findings now go one step further, reporting on HIV positive young individuals with stroke. They suggest that these individuals have a greater potential for a preceding hyper-coagulable state than their stroke counterparts who are not infected with HIV. Furthermore, from our demographic data, there were no obvious systemic infections or co-morbidities to account for the rise in VWF levels (see

chapter 5, section 5.2.3). It is therefore reasonable to surmise that our findings are a function primarily of HIV infection.

Also supporting a causal role for the pro-thrombotic state in stroke in HIV-infected individuals are the following:

HIV infection is associated with a state of chronic immune activation (Appay *et al*. 2008). Therefore, elevated levels of "acute phase reactants," such as ESR and CRP, have been noted in HIV-infected individuals (Nixon *et al*. 2010; Ndakotsu *et al*. 2009). VWF, an acute phase reactant itself, may therefore also be raised in the setting of chronic immune activation in HIV. However, it may be that only when the VWF levels reach a certain threshold/critical level, do they result in a pro-thrombotic state that may lead to complications which can include stroke. HIV positive stroke patients therefore represent a subgroup of HIV positive patients whose VWF levels have reached a critical pro-thrombotic level.

Figure 8.4 is a model illustrating the notion of the threshold level in stroke causation in HIV positive individuals.

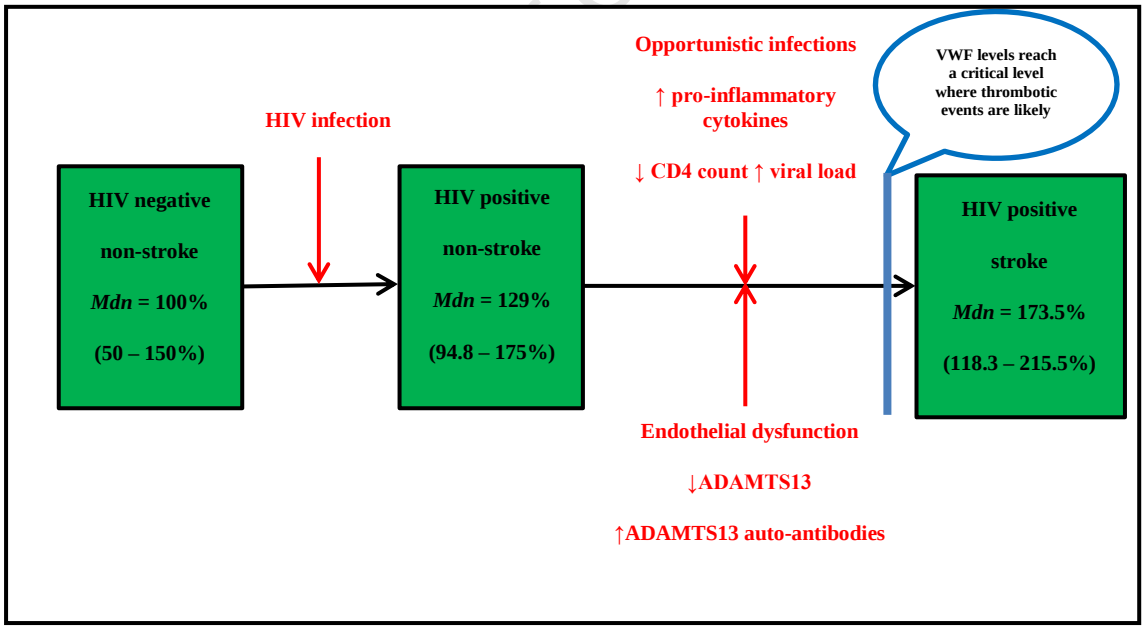


Figure 8.4 VWF threshold/critical level model in HIV positive stroke Interquartile range is given in parentheses. Abbreviations: von Willebrand factor (VWF), A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), median (*Mdn*).

However, a counter-argument to all of the previous theories can be made. This proposes that VWF elevation is secondary to an acute phase response following stroke and thus a consequence of stroke in HIV-infected individuals. The argument is as follows:

The elevation in VWF in both stroke groups from an initial lower baseline (100 to 135% in the HIV negative young stroke group, and 129 to 173.5% in the HIV positive stroke group), may be due to the effects of the acute phase response that follows many acute illnesses. VWF has long been recognised as an acute phase reactant (Pottinger *et al*. 1989). This proposed consequential rise in VWF following a stroke may be secondary to an acute phase or inflammatory response initiated by the evolution of a stroke. The VWF median of 129% in the HIV positive non-stroke control group is within normal limits. Serial measurements following the initial baseline measurements would have allowed us to track any VWF progression, if present, in the HIV positive non-stroke control group. This would have allowed us to determine if it followed a similar course as other acute phase reactants in HIV infection. We did not have the resources to do this and this is a limitation of our study (see section 8.3). Therefore, the elevation in VWF levels measured post-stroke in our study, may also have occurred secondary to the acute inflammatory reaction that follows a stroke (acute phase response).

One possible way of resolving the question as to whether the findings are consequential upon the stroke, is to perform a kinetic study of VWF in the acute post-stroke phase. For example, it could be measured at 1, 3, 5 and 7 days post-stroke. Rapid rises and falls would favour the acute phase (consequential) explanation and a study of this kind should be considered in future research (see section 8.3).

Despite these arguments, we also found additional data within our larger study that did not support the notion of an acute phase response being responsible for the elevated VWF levels:

The elevated levels of acute phase reactants, such as ESR and CRP, as a result of the chronic immune activation conferred by HIV infection, have been mentioned earlier. CRP was not routinely tested in all our study participants. However, the ESR was measured in the majority of the participants. We performed a post-hoc analysis using the ESR measurements. Only ESR measurements performed within 7 days of stroke onset in the stroke participants and on day 0 of enrolment into the study for the HIV positive non-stroke control groups were used for the post-hoc analysis. Therefore, only 25 and 14 ESR measurements for the HIV negative young stroke group and the HIV positive young stroke group, respectively, could be used. ESR was measured in all HIV positive non-stroke control participants on day 0 (n = 40). The findings for the three participant groups are shown in table 8.2.

ESR	HIV positive young stroke (n = 14)	HIV negative young stroke (n = 25)	HIV positive young controls (n = 40)	HIV positive young stroke vs. HIV negative young stroke	HIV positive young stroke vs. HIV positive young controls
Median (mm/hr)	82	16	67	$U = 48$ $p < 0.0001$ $r = 0.60$	$p = 0.4$
***IQR* (mm/hr)**	59 – 98	11 - 39	36 -107		

Table 8.2 ESR comparisons between the participant groups Abbreviations: erythrocyte sedimentation rate (ESR), millimetres per hour (mm/hr), sample size (n), versus (vs.), interquartile range (*IQR*), p value (*p*), U statistic (*U*), effect size (*r*).

The table illustrates the ESR levels in all three participant groups. They were significantly elevated in the HIV positive young stroke group when compared with the HIV negative young stroke group [Mann-Whitney U test ($U = 48$, $p < 0.0001$, $r = 0.60$)]. The *IQR*s indicate that most of the ESR values in the HIV positive stroke group were much higher than the values in the HIV negative stroke group. Both HIV positive groups (stroke and non-stroke) had elevated ESRs. There was no significant difference when performing a between-group comparison of the HIV positive groups. Since ESR is elevated in the HIV positive individuals in our study, and the HIV negative stroke participants had significantly lower ESR values compared with the HIV positive stroke group, it does not appear that the acute phase response following a stroke could have caused the elevation in ESR in the HIV positive stroke group.

Furthermore, post-hoc analysis correlating ESR with VWF showed that the ESR levels were not significantly correlated with VWF in either stroke groups. However, VWF was significantly positively correlated with ESR in all HIV positive participants [Spearman's Rank Order correlation $r_s(54) = 0.57$, $p < 0.0001$] and especially in the HIV positive non-stroke control group [Spearman's Rank Order correlation $r_s(40) = 0.58$, $p < 0.0001$] i.e. VWF levels increased as ESR levels increased (see figures 8.5 and 8.6). Therefore, VWF is positively associated with ESR in HIV positive participants. Thus, VWF behaves in a similar manner to ESR i.e. it is elevated in HIV infection due to the state of chronic immune activation and is independent of an acute stroke event. Elevated VWF levels observed in HIV positive participants following a stroke may well therefore have preceded the stroke and are therefore not necessarily post-stroke related.

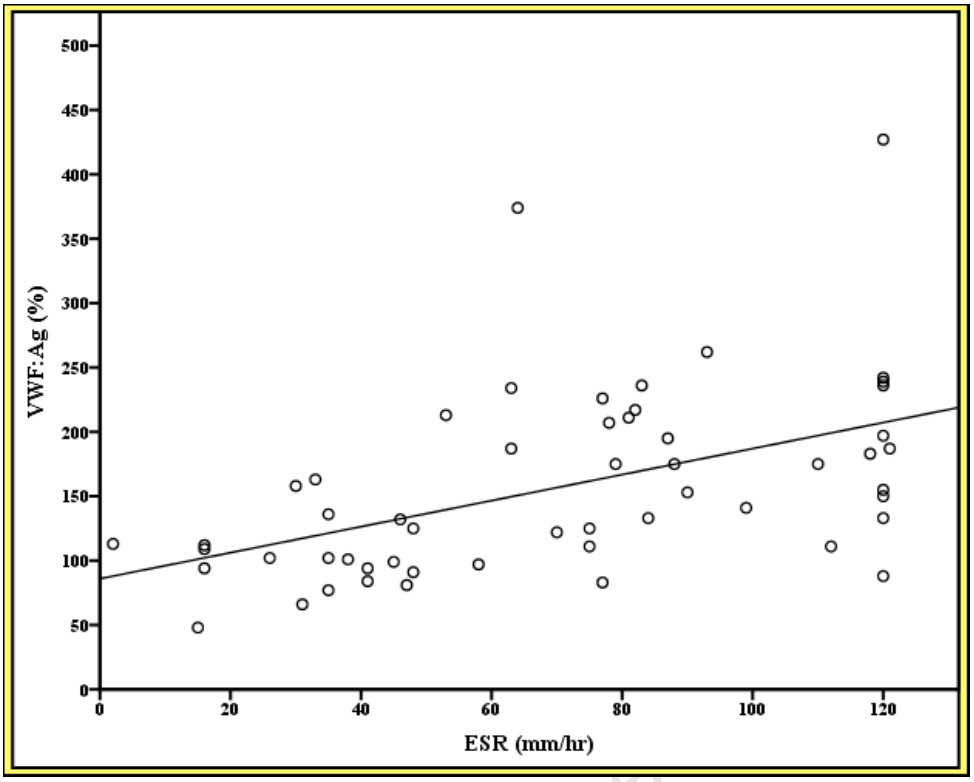


Figure 8.5 ESR and VWF correlation in all HIV positive participants Abbreviations: erythrocyte sedimentation rate (ESR), millimetres per hour (mm/hr), von Willebrand factor (VWF), VWF antigen (VWF:Ag).

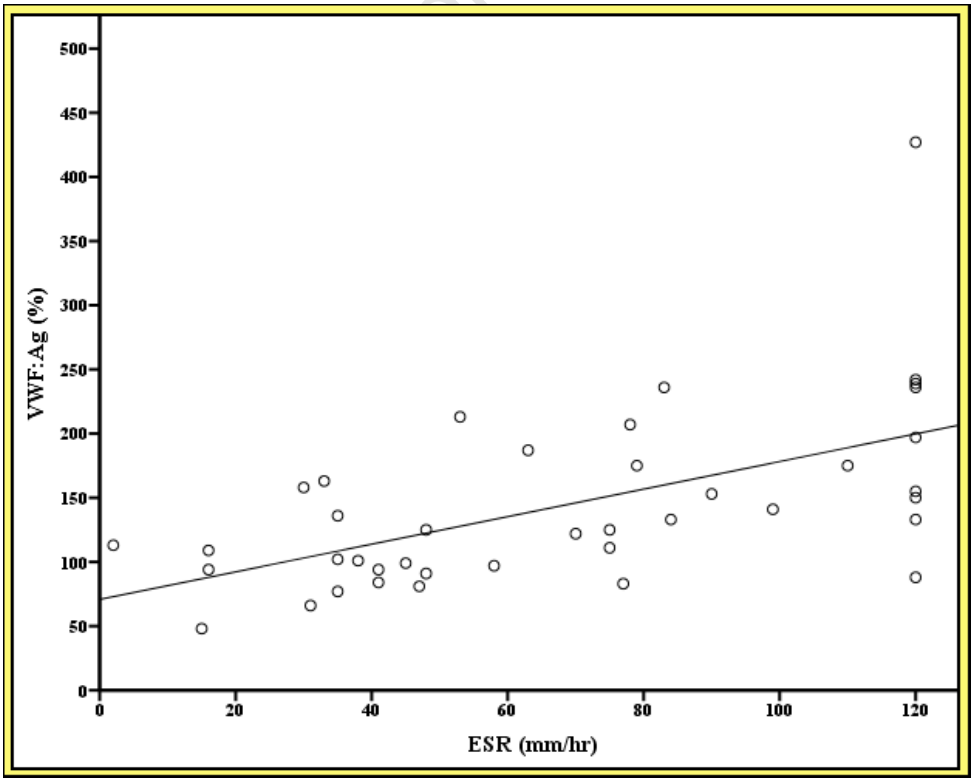


Figure 8.6 ESR and VWF correlation in HIV positive non-stroke control group Abbreviations: erythrocyte sedimentation rate (ESR), millimetres per hour (mm/hr), von Willebrand factor (VWF), VWF antigen (VWF:Ag).

Using data on stroke classification i.e. big (TACI and PACI) versus small stroke (LACI), we were able to investigate question 2 further. If the rise in VWF was part of an acute phase response to the stroke, then one might have expected larger strokes such as TACIs and PACIs to show a greater rise in VWF compared with small lacunar strokes as the inflammatory response could be expected to be larger in a bigger stroke. I did another post-hoc analysis comparing post-stroke VWF levels in big strokes (PACIs and TACIs) versus small strokes (LACIs) in all stroke participants (HIV positive and HIV negative). This showed no statistically significant differences in the VWF levels when comparing big with small strokes. These facts add to the argument favouring a causative relationship i.e. the pro-thrombotic profile preceded the stroke event.

It may, however, be that this pro-thrombotic state seen in HIV infection both precedes stroke onset and also partly arises as a consequence of the stroke i.e. the two explanations may not be mutually exclusive. We need a larger study to answer this question. An ideal study would have been a large longitudinal prospective cohort study, in which baseline VWF and ADAMTS13 levels would be available for all participants. This, however, would have been impossibly large, expensive and require a long follow-up time. Practically, however, a 6 months post-stroke VWF measurement, allowing for the acute effects of stroke to subside, will have provided data that might have better reflected pre-stroke baseline levels (see section 8.3).

Question 3

Most of our data suggests a causative role for the pro-thrombotic state in HIV-associated stroke. The next question that arises is whether this rise in VWF levels preceding a stroke is a slow progressive one that follows infection with HIV or whether it results from a specific insult or insults at particular point(s) in time?

We suggest the following model shown in figure 8.7. It proposes that this rise is multifactorial with the combination of a slow rise associated with advancing, untreated HIV infection and punctuated by more acute elevations in VWF related to superimposed insults or infections.

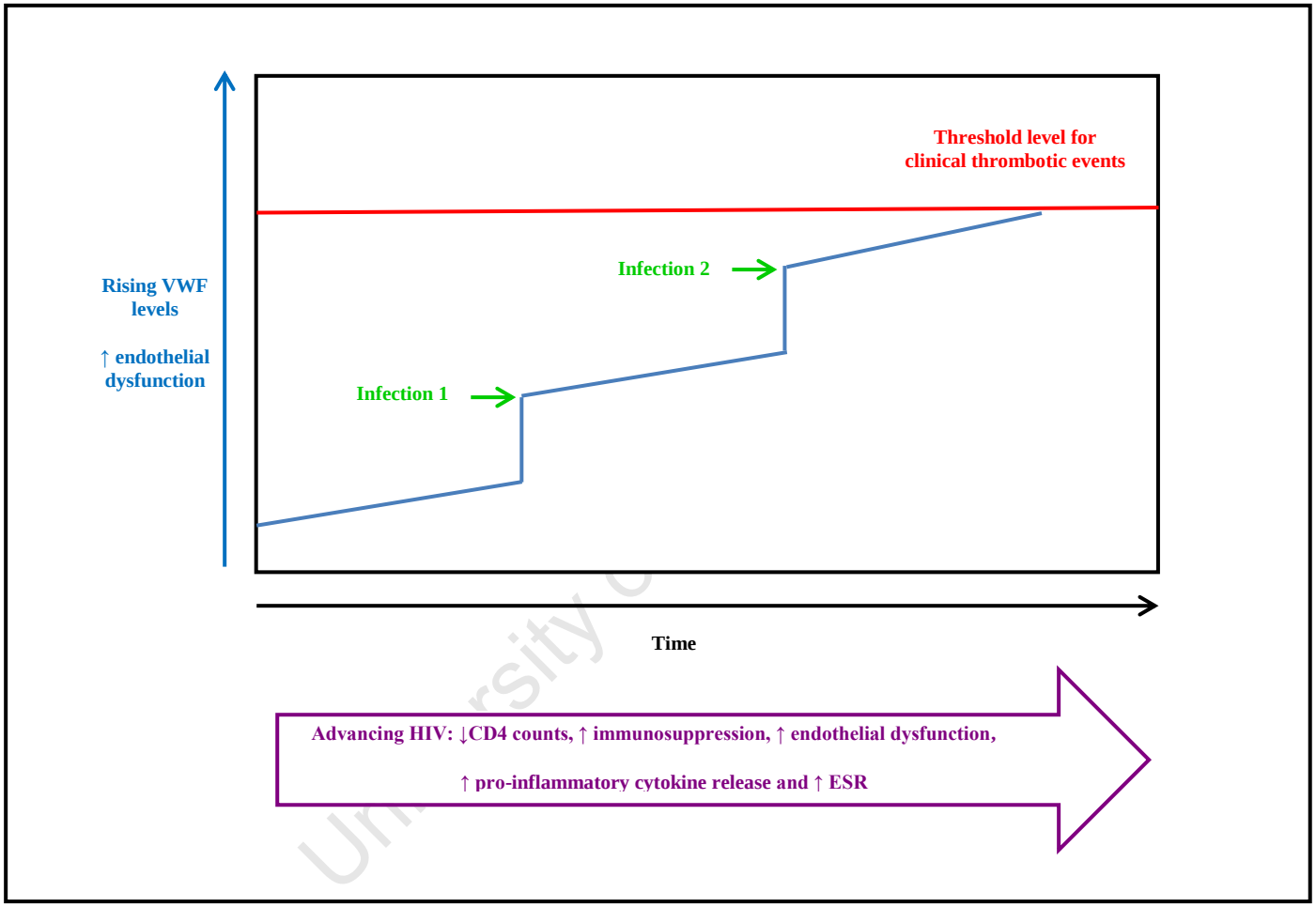


Figure 8.7 Model for the rise of VWF levels in HIV-infected individuals Abbreviations: von Willebrand factor (VWF), erythrocyte sedimentation rate (ESR).

This model shows the slow background rise in VWF, with advancing HIV and its concomitant declining CD4 count/worsening endothelial dysfunction, punctuated by acute insults. The latter may include superimposed opportunistic infections, haemostatic defects and HIV-related TTP which accelerates the rise in VWF.

Data from our study favour the notion that the cause for this pro-thrombotic state is partly due to a slow steady rise in VWF following infection with HIV:

CD4 count is often used as a proxy marker of immunosuppression and the decline in CD4 count is an indicator of advancing immunosuppression. We have shown that the CD4 counts of the HIV positive HAART-naïve participants in our study were negatively correlated with VWF levels (H7a). We can therefore surmise that as CD4 counts fall, VWF levels increase. Raised VWF levels are therefore a function of advancing immunosuppression. This has been noted previously by Lafeuillade *et al*. (1992) and Seigneur *et al*. (1997) who commented on the effects that higher viral loads and lower CD4 counts had on elevation of the VWF levels. Therefore, advancing immunosuppression represented by the usual slowly progressive decline in CD4 count in HIV infection (unless the CD4 count is rapidly lowered by an acute insult), may be the reason for the slow build-up of a pro-thrombotic state that precedes the stroke.

Two other factors that support the notion of a slow progressive rise in VWF following infection with HIV are endothelial dysfunction and pro-inflammatory cytokine release:

In HIV-infected individuals, endothelial dysfunction is a vascular disease process with a complex and as yet not fully understood aetiology (Subbarao *et al*. 2011; Monsuez *et al*. 2009). It is an insult that is multifactorial in aetiology and may worsen over time. VWF is a marker of endothelial dysfunction and raised levels in the HIV positive young stroke group may reflect the presence and evolution of this complex disease process. The HIV-induced inflammatory cascade which is a function of the chronic state of immune activation conferred by HIV infection [specifically CRP, tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6)] and adhesion markers (ICAMs and VCAMs) have been linked to the pathogenesis of HIV-related endothelial dysfunction (Subbarao *et al*. 2011).

Certain disease processes that are more likely to occur in the setting of HIV infection may prove to be the insults that tip the scales in favour of thrombosis and stroke presentation. While the difference between the CD4 counts only tended toward significance when comparing the two HIV positive participant groups, the median CD4 counts were lower in the HIV positive young stroke group compared with the HIV positive young controls (234 vs. 383 cells/mm^3). The more

advanced immunosuppression, reflected by the lower CD4 counts in the HIV positive young stroke group, coupled with the following additional insults, may account for the rise in VWF levels that led to a pro-thrombotic state:

One of the very few predictable facts about HIV is that opportunistic infections become more prevalent as the CD4 count declines. It is a sign of increasing immunodeficiency (Kovacs *et al*. 2000). A more abrupt increase in VWF may thus occur following an opportunistic infection(s) and the pro-inflammatory milieu they create. Defects in the haemostatic system e.g. protein C and protein S deficiency, that may lead to pro-thrombotic states in HIV-infected individuals, are known to correlate with the degree of immunosuppression measured by CD4 cell counts (Saif *et al*. 2001). Elevated VWF levels may be a function of these aberrant haemostatic processes. The effect of HIV-related TTP and decreased ADAMTS13 levels may also play a role, but will be discussed in section 8.2.3.

Once again, our model must be investigated in a large longitudinal cohort study in order to verify our theories.

8.2.2 VWF:CB assay results

The intention of H3 and H4 were not to quantify the VWF levels as previously done in H1 and H2, but rather to provide an assessment of the functional activity of the VWF. The VWF:CB assay is therefore a surrogate test for the presence of ULVWF multimers. This, in turn, should reflect the thrombotic activity occurring in an individual as the ULVWF multimers are known to be functionally more haemostatically active and therefore pro-coagulant.

The results for H3 and H4 did not mirror that which were found in H1 and H2. We had expected to see a similar pattern of results with the VWF:CB assay. However, both sets of results were not statistically significant with no between-group differences and with median values all within the normal range.

The findings for VWF:CB tests of all three participant groups are summarised in the form of a box and whisker plot shown in figure 8.8. The median values for all three groups were within the normal range.

One interpretation of these findings of similar VWF collagen binding activities is that levels of the larger VWF multimers were normal in all three participant groups. None of the groups therefore had a tendency for pro-coagulant activity conferred by excessive amounts of the larger VWF

multimers. However, this does not exclude the possibility that other types of VWF molecules contribute to the elevated VWF levels found in the HIV positive young stroke group, as seen in H1. VWF molecules in the blood include an array of molecules of varying sizes. It is known that the ULVWF multimers are the most pro-thrombotic (see chapter 2, section 2.5.3), but all the other smaller molecules of VWF themselves are also conducive to clotting in various degrees. Therefore, had we measured all the forms of VWF for our participants, we might have identified a different form of VWF that could be associated with an even higher tendency for thrombosis in individuals with HIV.

The results of our VWF:CB assay were verified using the same test with a different type of collagen from that described in the methods section. Both sets of results were similar. However, we also need to consider whether our results might have arisen due to some other technical difficulty when performing the test. This appears to be highly unlikely though, considering that the tests were conducted in a laboratory which routinely ran them and they were performed by personnel familiar with the methods. Could there be a substance in HIV-infected plasma, such as an auto-antibody perhaps, that is interfering with the collagen binding? To answer this, one would have to perform the assay using various dilutions of the plasma (see recommendations, section 8.3).

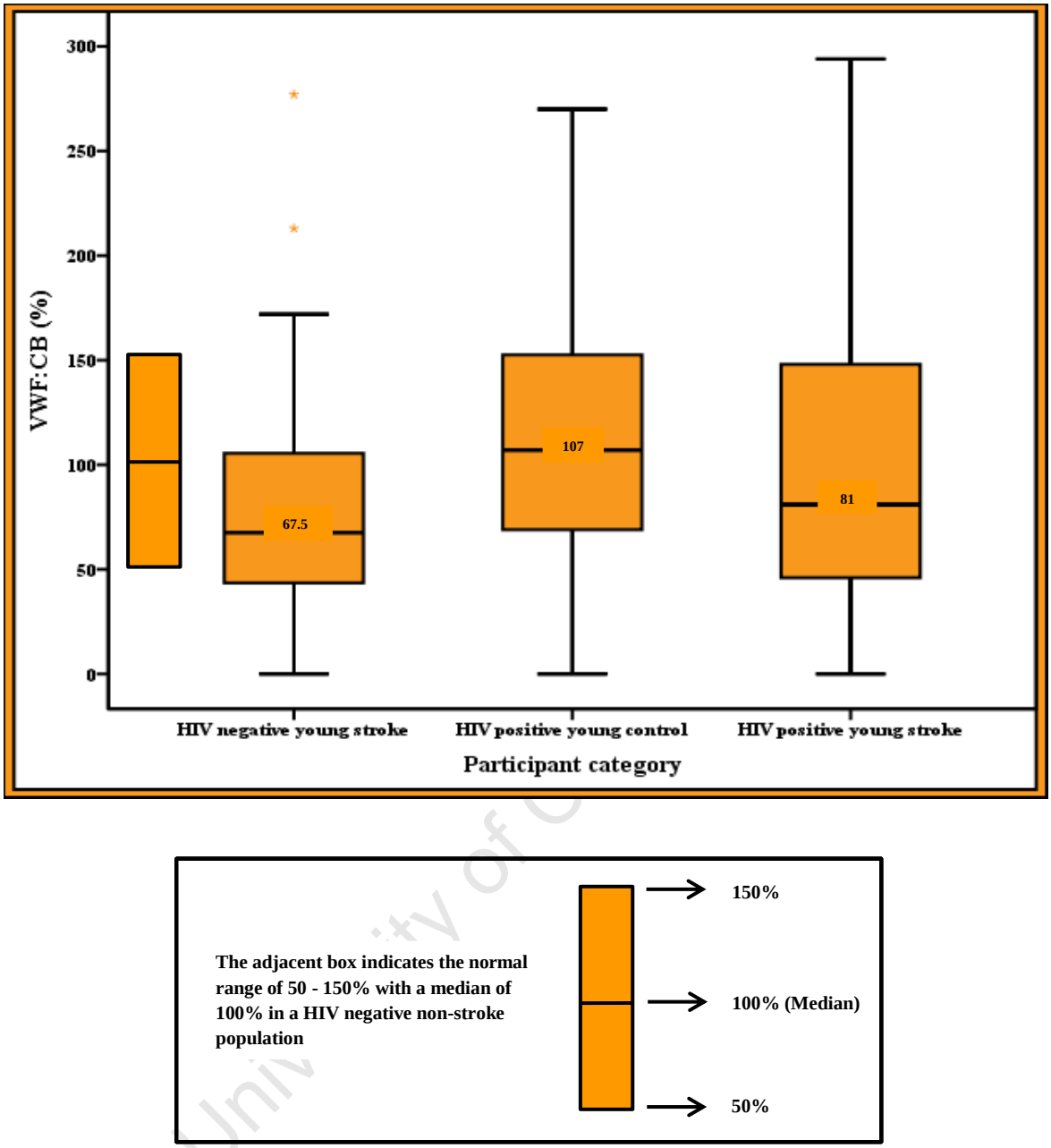


Figure 8.8 Median VWF:CB assay values for all participant groups Median values are indicated at the median line, * indicates outlier values. Abbreviations: von Willebrand factor (VWF), VWF collagen binding (VWF:CB).

8.2.3 ADAMTS13:Ag assay results

The result of testing H5 was not statistically significant. We had initially stated that HIV positive young stroke sufferers would have lower levels of ADAMTS13 compared with HIV negative young stroke sufferers. Interestingly, what we detected was that both stroke groups had very low levels of ADAMTS13 with median values of 0% for both stroke groups. As mentioned previously, a value of 0% does not mean that there is a complete absence of ADAMTS13 in an individual's blood, but rather that levels are so low that they are undetectable and immeasurable by the assay

used. This implies that the occurrence of a stroke in HIV positive and HIV negative individuals is associated with a decrease in ADAMTS13 levels.

In contrast, H6 was statistically significant. The HIV positive non-stroke control group already had considerably lower ADAMTS13 levels (23.5%) than the HIV negative non-stroke controls (100%). However, the 0% measurement in the HIV positive young stroke group proved to be significantly lower than the levels of HIV positive non-stroke control group (23.5%).

The following important questions have been raised by the findings of H5 and H6:

4. What were the basal levels of ADAMTS13 in the HIV negative and HIV positive stroke participants, before the stroke occurred?
5. Why are the ADAMTS13 levels that have been measured in our stroke study participants at such low levels and are these findings causal i.e. did the low levels precede and lead to stroke, or were the findings consequential upon the stroke i.e. did they result from the stroke itself?
6. What causes the low ADAMTS13 levels in HIV positive non-stroke individuals and why or how did they drop so much lower in HIV positive patients with stroke?

Question 4

We have already discussed the difficulties surrounding the lack of basal values for all three participant groups in our study in the VFW:Ag section of the discussion (see section 8.2.1). The same discussion applies here. Also, we used the same HIV negative non-stroke population for ADAMTS13 norms as we did for the VWF.

Figure 8.9 is a suggested model to help understand question 4. In this graph, the findings of the ADAMTS13:Ag assays of all three participant groups, including the normal range of 50 - 150% based on the population norms of an HIV negative, non-stroke population, are plotted.

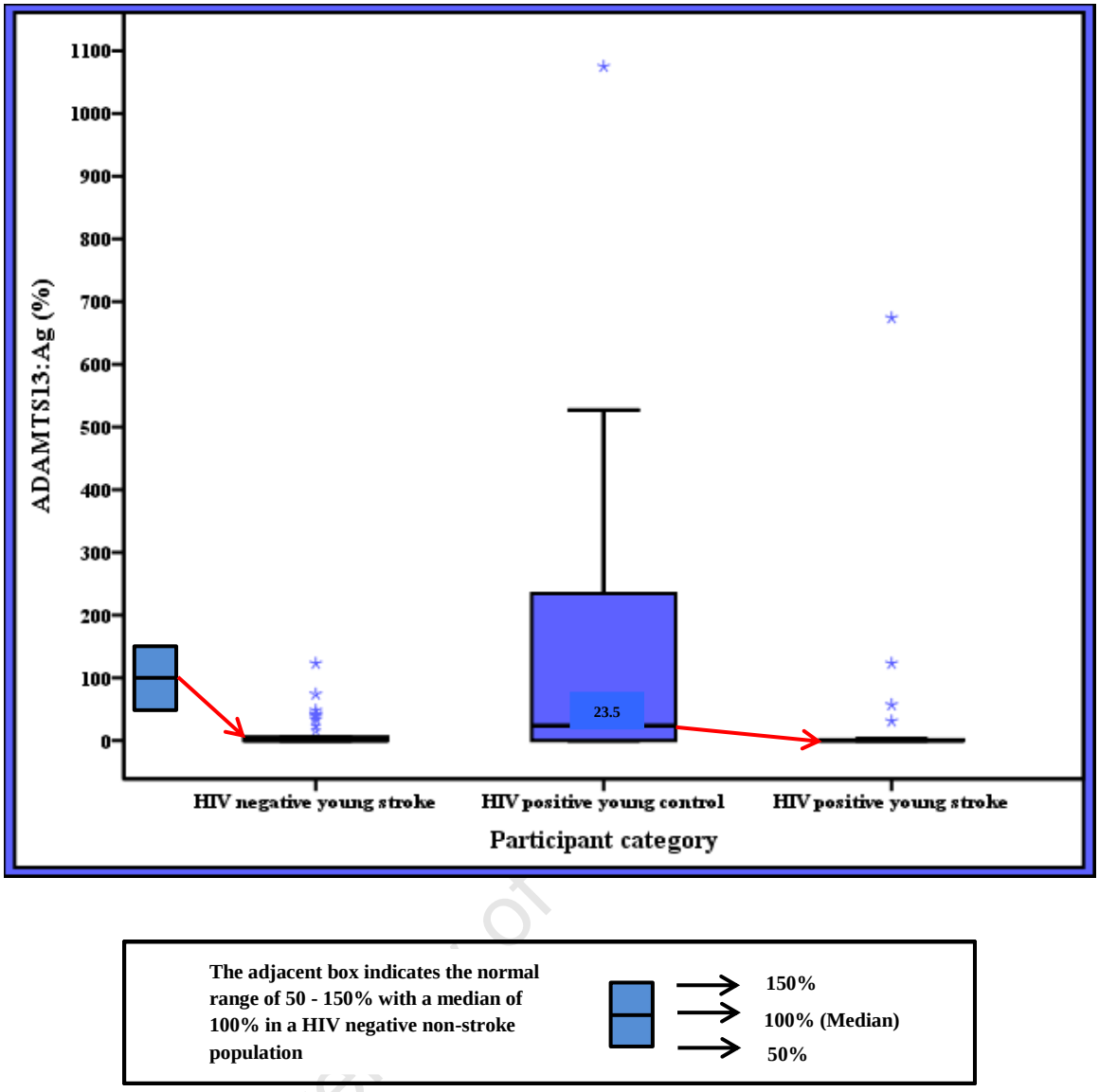


Figure 8.9 Comparison of the median ADAMTS13 levels of all participant groups measured using the ADAMTS13:Ag assay Median values are indicated at the median line, * indicates outlier values and → shows the median change between groups. Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), ADAMTS13 antigen (ADAMTS13:Ag).

From this model, we can see that the baseline median ADAMTS13 level in the HIV positive non-stroke group (23.5%) was lower than the baseline in a HIV negative non-stroke control population (100%). This model suggests the presence of an even lower than normal baseline ADAMTS13 level in HIV-infected non-stroke individuals compared with the baseline of an HIV negative non-stroke population. The potential reasons for this will be discussed in question 6.

Question 5

Our findings of a median ADAMTS13 value measured at 0% in both stroke groups, warrants further investigation. These data represent a "floor effect" i.e. the inability of the ELISA assay to detect very low levels of ADAMTS13 in the plasma.

Another factor to consider is the effect that aberrant liver function may have on ADAMTS13 levels, since it is known to be synthesised in the liver (see chapter 2, section 2.6.2). We did not perform routine liver function tests, but the INR was measured in 93 study participants. We reviewed these values post-hoc. INR is used in clinical practice as a marker of synthetic liver function, and after excluding INR values that were done after 7 days from stroke onset/enrolment and in patients on warfarin, the 80 remaining participants had INR values that were all within the normal range. We can therefore assume that the majority of our participants had normal synthetic liver function. Therefore, we can surmise that abnormal liver functioning was not the cause for the low ADAMTS13 levels in our study participants.

To determine whether low levels of ADAMTS13 in the stroke groups were causative or consequential, the timelines in figure 8.10 and 8.11 have been devised. Figure 8.10 follows the hypothetical scenario whereby ADAMTS13 levels (based on our study findings and the previously accepted norms) are depicted in a progression from a HIV negative non-stroke to a HIV-infected stroke state.

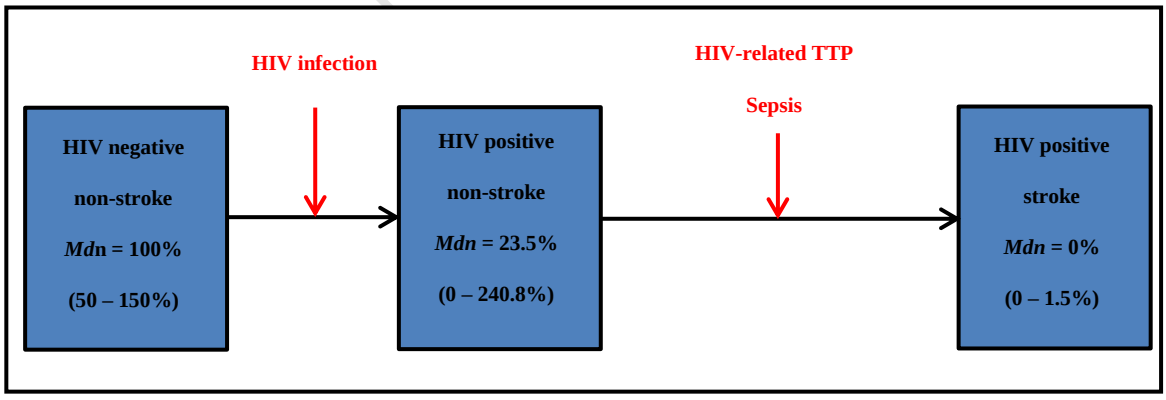


Figure 8.10 Progression of ADAMTS13 levels from a HIV negative non-stroke state to a HIV-infected stroke state Interquartile range is given in parentheses. Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), thrombotic thrombocytopaenic purpura (TTP), median (*Mdn*).

Figure 8.11 indicates the progressing ADAMTS13 levels in HIV negative participants from a non-stroke to a stroke state.

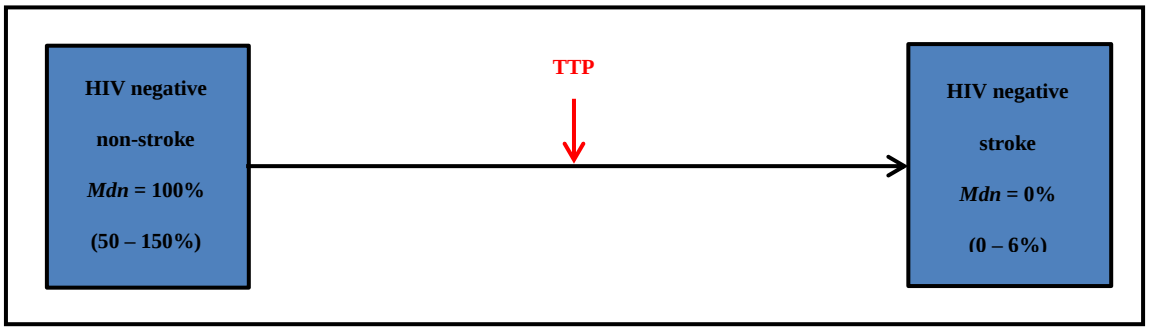


Figure 8.11 Progression of ADAMTS13 levels from a HIV negative non-stroke state to a HIV negative stroke state Interquartile range is given in parentheses. Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), thrombotic thrombocytopaenic purpura (TTP), median (*Mdn*).

Low ADAMTS13 levels result in diminished cleavage of the pro-coagulant ULVWF multimers and may thus bring about a pro-thrombotic state. Plausible reasons favouring a causative relationship between this pro-thrombotic state and stroke causation are as follows:

Prior to the stroke episode, the baseline levels of both non-stroke groups (HIV negative non-stroke and HIV positive non-stroke controls) might have been higher than when measured post-stroke. It may be that these stroke participants had been affected by some form of sub-clinical TTP, which had not been clinically identified earlier. This may account for the low ADAMTS13 levels and increased potential for thrombotic episodes.

Other factors such as sepsis, DIC, liver disease, infection with plasmodium falciparum, transplantation and immunosuppression with cyclosporine and sickle cell disease are also known to cause low ADAMTS13 levels (Tsai 2010; Turner *et al*. 2012). These factors, however, were not known to be present in our study groups (see chapter 5).

Zhao *et al*. (2009) and Fujioka *et al*. (2010) have explored the role of ADAMTS13 in murine models. Deficiency of ADAMTS13 in mice appears to increase the susceptibility of these animals to cerebral ischaemia and can exacerbate ischaemic brain injury, with infusion of ADAMTS13 having a protective role to play in infarct size reduction. With the findings of low levels in both stroke groups, it may be that we are seeing a similar pattern of low levels of ADAMTS13 having caused an increased susceptibility of our human participants to cerebral ischaemia as seen in murine models. Stroke therefore occurs in a select group of patients whose ADAMTS13 levels fall below a critical level.

A post-hoc analysis comparing big versus small strokes described previously in the VWF:Ag discussion (see section 8.2.1, question 2), was also performed in relation to ADAMTS13 levels. It, too, showed no statistically significant differences between stroke sizes and ADAMTS13 levels. One might have expected larger strokes to show a greater drop in ADAMTS13 levels compared with smaller strokes due to the larger inflammatory response that might accompany a big stroke. These findings therefore tend to negate the explanation of a consequential inflammatory response and support a causative relationship for a pro-thrombotic state conferred by low ADAMTS13 levels.

However, a strong counter-argument favouring the notion of low ADAMTS13 levels being a consequence of an acute phase response following stroke is as follows:
The levels of ADAMTS13 fall to low/undetectable levels in *both* HIV positive and HIV negative stroke groups. These findings support the idea that it is a reaction to the stroke.

A kinetics study of ADAMTS13 in the acute post-stroke phase, like that of VWF described previously (see section 8.2.1, question 2), is a possible way of resolving the question of causality versus consequence and should be considered in future research (see section 8.3).

Bongers *et al*. (2006) conducted the first study to measure ADAMTS13 in patients with ischaemic stroke. Using a different technique from the one used in our study, they found that levels of ADAMTS13 were slightly lower in patients with ischaemic stroke compared to controls. In addition, participants with the lowest levels of ADAMTS13 were at greater risk for stroke compared with participants who had the highest ADAMTS13 measurements (Bongers *et al*. 2006). They did not manage to determine if the reduced levels of ADAMTS13 was causative or consequential from their study.

Lambers *et al*. (2013) reported that reduced ADAMTS13 activity posed a risk for the development of arterial ischaemic stroke in children. ADAMTS13 was measured 6-12 months following the stroke. ADAMTS13 activity was significantly lower in patients compared with controls. There were significantly more patients with ADAMTS13 levels below the 10th percentile in the stroke group compared with the control group (Lambers *et al*. 2013). It is important to note that this study was not carried out in the acute phase.

Our study cannot answer the question of causation versus consequence with certainty, and the influence of HIV infection raises the question of many other potential pathogenic mechanisms being involved.

Question 6

What causes the low ADAMTS13 levels in HIV positive non-stroke individuals and why or how did they drop so much lower in HIV positive patients with stroke? We have to consider that this is a function of infection with HIV itself. The virus itself may drive this initial drop by a variety of mechanisms.

The findings of hypothesis 6 could be related to the concept of HIV-associated TTP. HIV-associated TTP is a well-known entity (Blazes *et al*. 2004; Opie 2012; Novitzky *et al*. 2005; Gunther *et al*. 2007). Our findings of diminished ADAMTS13 levels in the HIV positive participants may be explained by this phenomenon, even in a sub-clinical form. Meiring *et al*. (2012) proposed a mechanism by which HIV-related TTP arises. She described a dynamic mechanism by which HIV infection itself results in increased cytokine release, micronutrient deficiency and the production of auto-antibodies to ADAMTS13. This, in turn, results in a cascade of events that interplay to create an environment of increased VWF synthesis, decreased ADAMTS13 synthesis and release by endothelium, and the production of inhibitory antibodies to ADAMTS13, with the subsequent haematological events that give rise to HIV-associated TTP (Meiring *et al*. 2012). Figure 8.12 is a summary of this proposed mechanism.

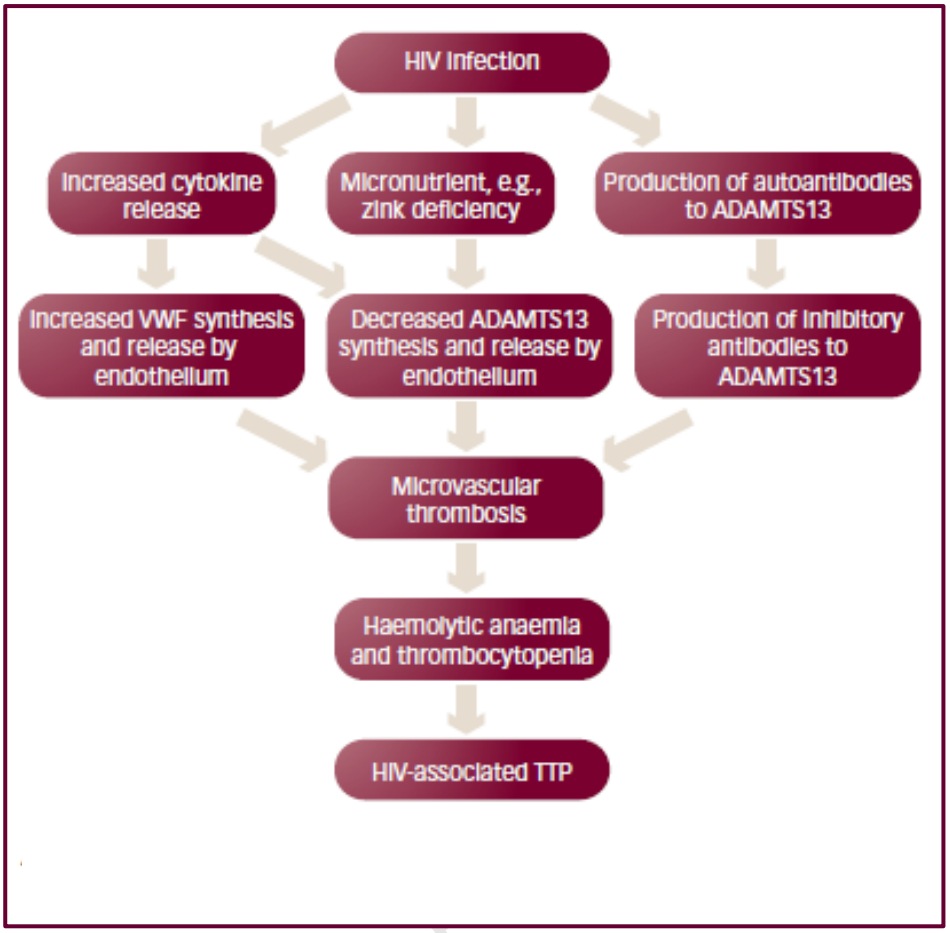


Figure 8.12 Proposed mechanism for the initial onset of HIV-associated TTP (From: Meiring *et al*. 2012) Abbreviations: A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13), thrombotic thrombocytopaenic purpura (TTP).

A possible reason for our findings in the HIV positive participants could therefore relate to the presence of an as yet undiagnosed HIV-related TTP in these individuals. This hypothesis would have been supported by the finding of auto-antibodies to ADAMTS13. This is definitely a factor to consider for future studies as the concept of HIV-related TTP gains ground (see section 8.3).

Furthermore, CD4 count did not correlate positively with ADAMTS13 i.e. ADAMTS13 levels did not decrease with decreasing CD4 counts (H7c). But, an obvious lower median CD4 count in the HIV positive stroke group, with the difference between the two HIV positive groups tending towards significance, suggests that lower ADAMTS13 levels are related to advancing immunosuppression in HIV. This may therefore account for the low pre-stroke ADAMTS13 levels.

8.2.4 The joint influence of VWF and ADAMTS13 on stroke causation/risk

In the HIV positive young stroke group, the VWF median was reported above the upper limit of normal, with the ADAMTS13 level below the lower limit of normal. There are data to support the idea that a combination of high VWF levels and low ADAMTS13 level confers a greater stroke risk than either finding in isolation in a single individual (Andersson *et al*. 2012). This combined abnormal finding is not present in the HIV negative young stroke group. However, it is present in the HIV positive young stroke group, and may be a mechanism underlying stroke causation. This combined finding should be investigated further in future studies (see section 8.3).

8.2.5 Hypotheses related to "idiopathic" strokes

The sub-hypotheses, H1a, H2a and H3a aimed to investigate whether VWF and ADAMTS13 played a role in stroke causation in those individuals in our study with "idiopathic" stroke. Close to half of the participants in either stroke group were classified as "idiopathic." We might have expected the associations in our main hypotheses to apply especially to the "idiopathic" stroke group, where no obvious cause could be identified, but this was not the case. None of the hypotheses was confirmed, suggesting that stroke causation is multifactorial in these groups.

8.3 Study limitations and recommendations

Our study had a number of limitations. In this section, I shall discuss these and how we attempted to best deal with them. In addition, recommendations for future studies will also be discussed.

Firstly, the number of participants in our study was small. The reasons for this was that this was a pilot study and participant numbers for the HIV positive young stroke group were self-limiting i.e. there were only 20 eligible HIV positive strokes enrolled during our study enrolment period (see chapter 4, figures 4.5 and 4.6).

Secondly, some selection bias is present. We only included participants who were HAART-naïve in our study. However, this allowed us to interpret our findings in the context of HIV infection itself, without having to account for the confounding factor of ARVs with their known increased cardiovascular risk/stroke risk and metabolic side effects. This is a major strength of the study as it is probably not possible to perform such a study in an industrialised country where ART availability is more widespread. Future studies should include an HIV positive stroke group on HAART to assess the influence of HAART on stroke causation.

In addition, the stroke participants were recruited from tertiary and secondary public hospitals located in the Western Cape, South Africa. This means that only cases that presented to these facilities were eligible for inclusion in the study. This could have resulted in people who had an HIV-related stroke, but who did not seek medical attention, being excluded from the study. Stroke, however, is a condition that in most instances results in the patient affected or his/her relatives seeking medical attention. Therefore, stroke patients generally end up in hospitals. Also, the fact that we recruited participants from more than one facility, each serving the people of a varied and vast geographic area, means that we were able to obtain participants from a large population that was representative of the Western Cape region.

Another possible but practically irremediable source of selection bias may have occurred if patients with large strokes died within a few hours post-stroke. Our study would not have included these participants and one might therefore argue that recruitment was biased against large severe strokes. These patients might have had massive thromboses with very high VWF and very low ADAMTS13 levels.

The fact that control participants were recruited from primary (public) health care facilities might have resulted in control participants who were in a better state of general baseline health compared with the stroke cases at the time of enrolment into the study. That is, there may have been a selection bias in the controls.

Our study was limited to public (state) health care facilities and this may be considered a limitation. However, HIV infection generally affects people of lower socio-economic status in South Africa who mostly use the public (state) health care facilities.

Time and financial constraints also limited the study. Despite the element of selection bias, our groups were nonetheless comparable. Cases and controls were well matched with regards to age and sex. Age inclusion criteria limitations were part of the study so as to avoid the confounding effects of age-associated cerebrovascular and neurodegenerative diseases.

A third limitation was that we did not have baseline VWF and ADAMTS13 levels for the stroke groups. Our study lacked HIV negative non-stroke population norms that were specific to our study population. We used the HIV positive non-stroke control group from our study and the HIV negative non-stroke controls from another South African study by Meiring *et al*. (2012), as proxy indicators of putative pre-stroke values. A six month post-stroke VWF and ADAMTS13 levels for

the groups would have also provided potential surrogate baseline levels for the stroke groups. Therefore, future studies should include a HIV negative non-stroke control group and 6 months post-stroke measurements.

Ideally, a large longitudinal cohort-study, with a long follow-up time, will address the limitations regarding small study population numbers, selection bias, population norms and baseline levels. In addition, it will allow for paired tests and repeated measures to be conducted. This type of study will, however, be very expensive. The idea of a 6 month post-stroke follow-up of the factors of interest is a more practical solution. Our pilot study has been useful in bringing these issues to light.

Given the fact that high VWF levels and low ADAMTS13 levels in combination are associated with a greater stroke risk than either finding alone (see section 8.2.4), a composite score of these two factors could be derived. Future stroke studies could then determine if any statistically significant differences exist with regard to this composite score between participant groups.

The measurement of cytokines and inhibitory ADAMTS13 auto-antibodies should be considered for future studies, as they may provide more insight into the association between HIV-related TTP and stroke. Since ADAMTS13 is synthesised by the liver, other tests of synthetic liver function should perhaps also be performed.

A kinetic study of VWF and ADAMTS13 in the acute phase post-stroke (see section 8.2.1, question 2 and section 8.2.3, question 5), might be able to resolve the issue of causality versus consequence.

Another recommendation is to perform the assays using various dilutions of plasma (see section 8.2.2). A substance might be present in HIV-infected plasma that might have interfered with collagen binding. Serial dilutions should be able to minimise the interfering effect of this substance and provide more accurate measurements.

ABO blood group typing of participants should also be considered in future studies. There is evidence that individuals with blood group O have lower VWF levels and that non-blood group O (A, B and AB) individuals have an increased incidence of arterial and venous thrombotic disease, compared with their group O counterparts (Goodeve 2010; Jenkins *et al*. 2006).

Finally, to better answer the question of causality or consequence, other acute phase reactants, such as CRP, should also be routinely measured and correlated with VWF and ADAMTS13 levels.

8.4 Summary of discussion

VWF and ADAMTS13 appear to be associated with HIV-related young stroke. Our findings indicate that:

- Stroke in HIV infection is associated with a pro-thrombotic state.
- This pro-thrombotic state, resulting from raised VWF and lowered ADAMTS13 levels, is probably causal rather than consequential upon the stroke in HIV infection.
- The chronic state of immune activation associated with HIV may increase VWF and decrease ADAMTS13 levels as CD4 counts fall with advancing, untreated HIV infection.
- HIV-related TTP is a potential driving force behind this pro-thrombotic state in HIV infection.
- Stroke occurs in those individuals whose VWF and ADAMTS13 concentrations reach a critical pro-thrombotic level.

Our proposed models based on our study findings indicate that raised VWF and low ADAMTS13 levels contribute to a pro-thrombotic state that can cause stroke in young HIV-infected individuals. However, we cannot entirely exclude the notion that our findings are due to an acute phase response post-stroke. It may be that high VWF and low ADAMTS13 are both causal and partly consequential upon HIV-related stroke.

CONCLUSION

The present period is an ideal time to conduct research in the field of HIV-related stroke. The global HIV/AIDS epidemic has taken firm root in sub-Saharan African which bears the brunt of the epidemic. South Africa, in particular, has the largest number of people living with HIV in the world (UNAIDS 2011).

The list of diseases complicating HIV infection is long and continues to expand. Stroke in HIV positive patients is a recent addition to this list. It affects young people especially and thus adds significant years of disability and unemployment to an already struggling population. The causes of HIV-associated stroke in the young are probably many and varied. HIV-associated vasculopathy is an ill-understood entity which accounts for at least some of the cases.

This study aimed to further our knowledge about the aetiology of HIV-associated stroke by investigating factors that have recently begun to be studied in the fields of stroke and HIV infection, respectively. However, to our knowledge, none of these two factors, viz. VWF and ADAMTS13, has been studied together in a group of young patients afflicted by both HIV and stroke. Furthermore, ADAMTS13 is a relatively new area of research, having only recently been identified and characterised.

Our results suggest that elevated VWF and low ADAMTS13 levels contribute to a pro-thrombotic state in HIV-infected individuals. The precise mechanisms by which this happens are not entirely clear. The question of whether our findings are causative or a consequence of stroke has not yet been resolved, but our findings tend to favour a causative explanation. However, larger studies will be required to verify the results of this initial study.

Despite our study limitations, the results are interesting and provide new insights into HIV-related stroke in young adults. Studies of VWF and ADAMTS13, especially in the context of HIV infection, are seldom performed in South Africa. We have added in a small way to both HIV and haematological research in the country.

While there is a growing international body of literature on VWF and ADAMTS13, these do not necessarily apply in the context of South Africa, with its specific circumstances and divergent demography. But our study was prompted by the international literature and it can also add to the global pool of knowledge in the field.

We hope that our own study and the recommendations we made for future studies will encourage further research on this important clinical problem in South Africa, in the rest of the continent and internationally.

REFERENCES

Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, *et al*. Classification of subtype of acute ischaemic stroke. Definitions for use in a multicentre clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993 Jan; 24(1): 35-41.

Addo J, Ayerbe L, Mohan KM, Crichton S, Sheldenkar A, Chen R *et al*. Socioeconomic status and stroke: an updated review. Stroke. 2012 Apr; 43(4): 1186-91. Epub 2012 Feb 23. doi: 10.1161/STROKEAHA.111.639732. PubMed PMID: 22363052.

Allen CL. Bayraktutan U. Risk factors for ischaemic stroke. Int J Stroke. 2008 May; 3(2): 105-16. doi: 10.1111/j.1747-4949.2008.00187.x. PubMed PMID: 18706004.

Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Hennerici MG. New approach to stroke subtyping: the A-S-C-O (phenotypic) classification of stroke. Cerebrovasc Dis. 2009; 27(5): 502-8. Epub 2009 Apr 3. doi: 10.1159/000210433. PubMed PMID: 19342826.

Amod A, Ascott-Evans BH, Berg GI, Blom DJ, Brown SL, Carrihill MM *et al*. The 2012 SEMDSA Guideline for the Management of Type 2 Diabetes (Revised). JEMDSA. 2012; 17(2)(Suppl 1): S1-S95.

Ances BM, Bhatt A, Vaida F, Rosario D, Alexander T, Marqui-Beck J, *et al*. Role of metabolic syndrome components in human immunodeficiency virus-associated stroke. J Neurovirol. 2009 May; 15(3): 249-56. doi: 10.1080/13550280902962443. PubMed PMID: 19562611.

Andersson HM, Siegerink B, Luken BM, Crawley JT, Algra A, Lane DA, *et al*. High VWF, low ADAMTS13, and oral contraceptives increase the risk of ischemic stroke and myocardial infraction in young women. Blood. 2012 Feb 9; 119(6): 1555-60. Epub 2011 Nov 22. doi: 10.1182/blood-2011-09-380618. PubMed PMID: 22110247.

Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004 Jan; 91(1): 4-15.

Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. J Pathol. 2008 Jan; 214(2): 231-41.

Asherson RA, Cervera R. Antiphospholipid antibodies and infections. Ann Rheum Dis. 2003 May; 62(5): 388-93.

Aukrust P, Bjørnsen S, Lunden B, Otterdal K, Ng EC, Ameln W, *et al*. Persistently elevated levels of von Willebrand factor antigen in HIV infection. Downregulation during highly active antiretroviral therapy. Thromb Haemost. 2000 Aug; 84(2): 183-7.

Baker DC, Brassard J. Review of continuing education course on hemostasis. Toxicol Pathol. 2011 Jan; 39(1): 281-8. Epub 2010 Dec 3. doi: 10.1177/0192623310389476. PubMed PMID: 21131603.

Balci K, Utku U, Asil T, Celik Y. Ischaemic stroke in young adults: risk factors, subtypes, and prognosis. Neurologist. 2011 Jan; 17(1): 16-20. doi: 10.1097/NRL.0b013e3181f954a7. PubMed PMID: 21192186.

Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. Lancet. 1991 Jun 22; 337 (8756): 1521-6.

Banks JL, Marotta CA. Outcomes validity and reliability of the modified Rankin scale: implications for stroke clinical trials: a literature review and synthesis. Stroke. 2007 Mar; 38(3): 1091-6. Epub 2007 Feb 1.

Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, *et al*. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 1983 May 20; 220(4599): 868-71.

Barrett KE, Barman SM, Boitano S, Brooks HL. Ganong's Review of Medical Physiology. 23rd ed. Singapore: McGraw-Hill; 2010.

Basavanagowdappa H, Babu MS, Karuturi S. HIV infection and thromboembolism. J Assoc Physicians India. 2011 Jun; 59: 380-382.

Bath PM, Blann A, Smith N, Butterworth RJ. Von Willebrand factor, P-selectin and fibrinogen levels in patients with acute ischaemic stroke and haemorraghic stroke, and their relationship with stroke sub-type and functional outcome. Platelets. 1998; 9(3-4): 155-9.

Becker AC, Jacobson B, Singh S, Sliwa K, Stewart S, Libhaber E, *et al*. The thrombotic profile of treatment-naive HIV-positive Black South Africans with acute coronary syndromes. Clin Appl Thromb Hemost. 2011 Jun; 17(3): 264-72. Epub 2010 May 11. doi: 10.1177/1076029609358883. PubMed PMID: 20460356.

Benarroch EE. ADAM proteins, their ligands, and clinical implications. Neurology. 2012 Mar 20; 78(12): 914-20. doi: 10.1212/WNL.0b013e31824c4728. PubMed PMID: 22431739.

Benjamin LA, Bryer A, Emsley HC, Khoo S, Solomon T, Connor MD. HIV infection and stroke: current perspectives and future directions. Lancet Neurol. 2012 Oct; 11(10): 878-90. doi: 10.1016/S1474-4422(12)70205-3. PubMed PMID: 22995692; PubMed Central PMCID: PMC3460367.

Bereczky Z, Kovács KB, Musbek L. Protein C and protein S deficiencies: similarities and differences between two brothers playing in the same game. Clin Chem Lab Med. 2010 Dec; 48 Suppl 1: S53-66. Epub 2010 Nov 5. doi: 10.1515/CCLM.2010.369. PubMed PMID: 21054189.

Berger JR, Harris JO, Gregorios J, Norenberg M. Cerebrovascular disease in AIDS: a case control study. AIDS. 1990 Mar; 4(3): 239-44.

Bhatt DL, Topol EJ. Scientific and therapeutic advances in antiplatelet therapy. Nat Rev Drug Discov. 2003 Jan; 2(1): 15-28.

Blann A. von Willebrand factor and the endothelium in vascular disease. Br J Biomed Sci. 1993 Jun; 50(2): 125-34.

Blazes DL, Decker CF. Thrombotic thrombocytopaenic purpura in HIV-infected patients. Infect Dis Clin Pract. 2004; 12(2): 99-106.

Blum A, Vaispapir V, Keinan-Boker L, Soboh S, Yehuda H, Tamir S. Endothelial dysfunction and procoagulant activity in acute ischemic stroke. J Vasc Interv Neurol. 2012 Jun; 5(1): 33-9.

Boden-Albala B, Sacco RL, Lee HS, Grahame-Clarke C, Rundek T, Elkind MV, *et al*. Metabolic syndrome and ischaemic stroke risk: Northern Manhattan Study. Stroke. 2008 Jan; 39(1): 30-5. Epub 2007 Dec 6.

Boissé L, Gill MJ, Power C. HIV infection of the Central Nervous System: clinical features and neuropathogenesis. Neurol Clin. 2008 Aug; 26(3): 799-819. doi: 10.1016/j.ncl.2008.04.002. PubMed PMID: 18657727.

Boneu B, Abbal M, Plante J, Bierme R. Letter: Factor-VIII complex and endothelial damage. Lancet. 1975 Jun 28; 1 (7922): 1430.

Bongers TN, de Maat MP, van Goor ML, Bhagwanbali V, van Vliet HH, Gómez Garcia EB, *et al*. High von Willebrand factor levels increase the risk of first ischemic stroke: influence of ADAMTS13, inflammation, and genetic variability. Stroke. 2006 Nov; 37(11): 2672-7. Epub 2006 Sep 21.

Bongers TN, de Bruijne EL, Dippel DW, de Jong AJ, Deckers JW, Poldermans D, *et al*. Lower levels of ADAMTS13 are associated with cardiovascular disease in young patients. Atherosclerosis. 2009 Nov; 207(1): 250-4. Epub 2009 Apr 17. doi: 10.1016/j.atherosclerosis.2009.04.013. PubMed PMID: 19439298.

Bonnet F, Chêne G. Evolving epidemiology of malignancies in HIV. Curr Opin Oncol. 2008 Sep; 20(5): 534-40. doi: 10.1097/CCO.0b013e32830a5080. PubMed PMID: 19106656.

Bornstein P. Thrombospondins as matricellular modulators of cell function. J Clin Invest. 2001 Apr; 107(8): 929-34.

Bowie EJ, Solberg LA Jr, Fass DN, Johnson CM, Knutson GJ, Stewart ML, *et al*. Transplantation of normal bone marrow into a pig with severe von Willebrand's disease. J Clin Invest. 1986 Jul; 78(1): 26-30.

Bradshaw D, Groenewald P, Laubscher R, Nannan N, Nojilana B, Norman R, *et al*. Initial burden of disease estimates for South Africa, 2000. S Afr Med J. 2003 Sep; 93(9): 682-8.

Brainin M, Teuschl Y, Kalra L. Acute treatment and long-term management of stroke in developing countries. Lancet Neurol. 2007 Jun; 6(6): 553-61.

Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, *et al*. Measurements of acute cerebral infarction: a clinical examination scale. Stroke. 1989 Jul; 20(7): 864-70.

Brown TT, Glesby MJ. Management of the metabolic effects of HIV and HIV drugs. Nat Rev Endocrinol. 2011 Sep 20; 8(1): 11-21. doi: 10.1038/nrendo.2011.151. PubMed PMID: 21931374; PubMed Central PMCID: 3371609.

Bryer A, Connor M, Haug P, Cheyip B, Staub H, Tipping B *et al*. South African guideline for management of ischaemic stroke and transient ischaemic attack 2010: a guideline from the South African Stroke Society (SASS) and the SASS Writing Committee. S Afr Med J. 2010 Nov 10; 100(11 Pt 2): 747-78.

Bryer A. Groote Schuur Hospital and University of Cape Town Stroke Unit: Stroke protocol and pathway 2008/2009. Copies can be obtained from: Groote Schuur Hospital and University of Cape Town Stroke Unit.

Burn MJ. Stroke and HIV infection: a review of the literature. Current Medical Literature: Stroke Review. 2006; 10(1): 8-14.

Cal S, Obaya AJ, Llamazares M, Garabaya C, Quesada V, López-Otín C. Cloning, expression analysis, and structural characterization of seven novel human ADAMTSs, a family of metalloproteinases with disintegrin and thrombospondin-1 domains. Gene. 2002 Jan 23; 283(1-2): 49-62.

Cassar K, Bachoo P, Ford I, Greaves M, Brittenden J. Markers of coagulation activation, endothelial stimulation and inflammation in patients with peripheral arterial disease. Eur J Vasc Endovasc Surg. 2005 Feb; 29(2): 171-6.

Catto AJ, Carter AM, Barrett JH, Bamford J, Rice PJ, Grant PJ. von Willebrand factor and factor VIII: C in acute cerebrovascular disease. Relationship to stroke subtype and mortality. Thromb Haemost. 1997 Jun; 77(6): 1104-8.

Centres for Disease Control and Prevention. Pneumocystis pneumonia --- Los Angeles. MMWR Morb Mortal Wkly Rep. 1981 Jun 5; 30(21): 250-2.

Cerrato P, Grasso M, Imperiale D, Priano L, Baima C, Giraudo M *et al*. Stroke in young patients: etiopathogenesis and risk factors in different age classes. Cerebrovasc Dis. 2004; 18(2): 154-9. Epub 2004 Jul 13.

Chahine LM, Khoriaty RN, Tomford WJ, Hussain MS. The changing face of neurosyphilis. Int J Stroke. 2011 Apr; 6(2): 136-43. Epub 2011 Jan 10. doi: 10.1111/j.1747-4949.2010.00568.x. PubMed PMID: 21371276.

Chatzikonstantinou A, Wolf ME, Hennerici MG. Ischemic stroke in young adults: classification and risk factors. J Neurol. 2012 Apr; 259(4): 653-9. Epub 2011 Sep 17. doi: 10.1007/s00415-011-6234-3. PubMed PMID: 21927959.

Cherian P, Hankey GJ, Eikelboom JW, Thom J, Baker RI, McQuillan A, *et al*. Endothelial and platelet activation in acute ischemic stroke and its etiological subtypes. Stroke. 2003 Sep; 34(9): 2132-7. Epub 2003 Aug 7.

Chow FC, Regan S, Feske S, Meigs JB, Grinspoon SK, Triant VA. Comparison of ischemic stroke incidence in HIV-infected and non-HIV-infected patients in a US health care system. J Acquir Immune Defic Syndr. 2012 Aug 1; 60(4): 351-8. doi: 10.1097/QAI.0b013e31825c7f24. PubMed PMID: 22580566.

Clark CR. Neurological disease. In: Kumar P, Clark M, editors. Kumar & Clark's Clinical Medicine. 7th ed. Saunders Elsevier; 2009. p. 1095-1183.

Clemetson KJ. Platelets and primary haemostasis. Thromb Res. 2012 Mar; 129(3): 220-4. Epub 2011 Dec 16. doi: 10.1016/j.thromres.2011.11.036. PubMed PMID: 22178577.

Connor MD, Bryer A. Stroke in South Africa. In: Steyn K, Fourie J, Temple N, editors. Chronic Diseases of Lifestyle in South Africa: 1995 – 2005. Technical Report. Cape Town: South African Medical Research Council; 2006. p. 195-203.

Connor MD, Thorogood M, Casserly B, Dobson C, Warlow CP, SASPI Project Team. Prevalence of stroke survivors in rural South Africa: results from the Southern Africa Stroke Prevention Initiative (SASPI) Agincourt field site. Stroke. 2004 Mar; 35(3): 627-32. Epub 2004 Feb 12.

Connor MD, Lammie GA, Bell JE, Warlow CP, Simmonds P, Brettle RD. Cerebral infarction in adult AIDS patients: observations from the Edinburgh HIV Autopsy Cohort. Stroke. 2000 Sep; 31(9): 2117-26.

Connor MD. Treatment of HIV associated cerebral vasculopathy. J Neurol Neurosurg Psychiatry. 2009 Aug; 80(8): 831. doi: 10.1136/jnnp.2008.169490. PubMed PMID: 19608782.

Conway DS, Pearce LA, Chin BS, Hart RG, Lip GY. Prognostic value of plasma von Willebrand factor and soluble P-selectin as indices of endothelial damage and platelet activation in 994 patients with nonvalvular atrial fibrillation. Circulation. 2003 Jul 1; 107(25): 3141-5. Epub 2003 Jun 9.

Cordonnier C, Leys D. Stroke: the bare essentials. Pract Neurol. 2008 Aug; 8(4): 263-72. doi: 10.1136/jnnp.2008.149401. PubMed PMID: 18644915.

Corral I, Quereda C, Moreno A, Pérez-Elías MJ, Dronda F, Casado JL, *et al*. Cerebrovasc Dis. 2009; 27(6): 559-63. Epub 2009 Apr 24. doi: 10.1159/000214219. PubMed PMID: 19390181.

Cotter PE, Belham M, Martin PJ. Towards understanding the cause of stroke in young adults utilising a new stroke classification system (A-S-C-O). Cerebrovasc Dis. 2012; 33(2): 123-7. Epub 2011 Dec 14. doi: 10.1159/000334183. PubMed PMID: 22179549.

Crawley JT, de Groot R, Xiang Y, Luken BM, Lane DA. Unraveling the scissile bond: how ADAMTS13 recognizes and cleaves von Willebrand factor. Blood. 2011 Sep 22; 118(12): 3212-21. Epub 2011 Jun 29. doi: 10.1182/blood-2011-02-306597. PubMed PMID: 21715306; PubMed Central PMCID: PMC3179391.

Cruse B, Cysique LA, Markus R, Brew BJ. Cerebrovascular disease in HIV-infected individuals in the era of highly active antiretroviral therapy. J Neurovirol. 2012 Aug; 18(4): 264-76. Epub 2012 Apr 14. doi: 10.1007/s13365-012-0092-3. PubMed PMID: 22528476.

Culebras A. International newsletter. Neurology. 2006 Dec 26; 67(12): 2099-2100.

d'Arminio A, Sabin CA, Phillips AN, Reiss P, Weber R, Kirk O, *et al*. Cardio- and cerebrovascular events in HIV-infected persons. AIDS. 2004 Sep 3; 18(13): 1811-7.

D'Ascenzo F, Cerrato E, Biondi-Zoccai G, Moretti C, Omede P, Sciuto F, *et al*. Acute coronary syndromes in human immunodeficiency virus patients: a meta-analysis investigating adverse event rates and the role of antiretroviral therapy. Eur Heart J. 2012 Apr; 33(7): 875-80. Epub 2011 Dec 20. doi: 10.1093/eurheartj/ehr456. PubMed PMID: 22187508.

Dai K, Gao W, Ruan C. The Sma I polymorphism in the von Willebrand factor gene associated with acute ischemic stroke. Thromb Res. 2001 Dec 15; 104(6): 389-95.

Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, *et al*. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004 Apr 1; 350(14): 1387-97.

De Cock KM, Jaffe HW, Curran JW. The evolving epidemiology of HIV/AIDS. AIDS. 2012 Jun 19; 26(10): 1205-13. doi: 10.1097/QAD.0b013e328354622a. Pubmed PMID: 22706007.

de Gaetano Donati K, Rabagliati R, Iacoviello L, Cauda R. HIV infection, HAART, and endothelial adhesion molecules: current perspectives. Lancet Infect Dis. 2004 Apr; 4(4): 213-22.

De La Blanchardiere A, Rozenberg F, Caumes E, Picard O, Lionnet F, Livartowski J, *et al*. Neurological complications of varicella-zoster virus infection in adults with human immunodeficiency virus infection. Scand J Infect Dis. 2000; 32(2): 263-9.

De Meyer SF, Stoll G, Wagner DD, Kleinschnitz C. von Willebrand factor: an emerging target in stroke therapy. Stroke. 2012a Feb; 43(2): 599-606. Epub 2011 Dec 15. doi: 10.1161/STROKEAHA.111.628867. PubMed PMID: 22180250.

De Meyer SF, Savchenko AS, Haas MS, Schatzberg D, Carroll MC, Schiviz A, *et al*. Protective anti-inflammatory effect of ADAMTS13 on myocardial ischemia/reperfusion injury in mice. Blood. 2012b Dec 20; 120(26): 5217-23. Epub 2012 Aug 22. doi: 10.1182/blood-2012-06-439935. PubMed PMID: 22915644.

Denis CV, Lenting PJ. von Willebrand factor: at the crossroads of bleeding and thrombosis. Int J Hematol. 2012 Apr; 95(4): 353-61. Epub 2012 Apr 5. doi: 10.1007/s12185-012-1041-x. PubMed PMID: 22477538.

Dharmasaroja PA, Muengtaweepongsa S, Lechawanich C, Pattaraarchachai J. Causes of ischaemic stroke in young adults in Thailand: a pilot study. J Cerebrovasc Dis. 2011 May-Jun; 20(3): 247-50. Epub 2010 Jun 26. doi: 10.1016/j.jstrokecerebrovasdis.2010.01.004. PubMed PMID: 20580256.

Dobbs MR, Berger JR. Stroke in HIV infection and AIDS. Expert Rev Cardiovasc Ther. 2009 Oct; 7(10): 1263-71. doi: 10.1586/erc.09.72. PubMed PMID: 19814669.

Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005 Apr 16-22; 365(9468): 1415-28.

Elkind MS. Epidemiology and risk factors. Continuum (Minneap Minn). 2011 Dec; 17 (6 2ndary Stroke Prevention): 1213-32. doi: 10.1212/01.CON.0000410031.34477.8d. PubMed PMID: 22810026.

Erbe M, Rickerts V, Bauersachs RM, Lindhoff-Last E. Acquired protein C and protein S deficiency in HIV-infected patients. Clin Appl Thromb Hemost. 2003 Oct; 9(4): 325-31.

Evers S, Nabavi D, Rahmann A, Heese C, Reichelt D, Husstedt IW. Ischaemic cerebrovascular events in HIV infection: a cohort study. Cerebrovasc Dis. 2003; 15(3): 199-205.

Feigin VL, Krishnamurthi R. Stroke in the developing world. Stroke. 2011 Dec; 42(12): 3655-8. Epub 2011 Nov 3. doi: 10.1161/STROKEAHA.110.596858. PubMed PMID: 22052509.

Ferro JM, Massaro AR, Mas JL. Aetiological diagnosis of ischaemic stroke in young adults. Lancet Neurol. 2010 Nov; 9(11): 1085-96. doi: 10.1016/S1474-4422(10)70251-9. PubMed PMID: 20965437.

Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 1997 Aug 19; 96(4): 1102-8.

Folsom AR, Rosamund WD, Shahar E, Cooper LS, Aleksic N, Nieto FJ, *et al*. Prospective study of markers of hemostatic function with risk of ischaemic stroke. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation. 1999 Aug 17; 100(7): 736-42.

Fornoni A, Raij L. Metabolic syndrome and endothelial dysfunction. Curr Hypertens Rep. 2005 Apr; 7(2): 88-95.

Fourie C, Van Rooyen J, Pieters M, Conradie K, Hoekstra T, Schutte A. Is HIV-1 infection associated with endothelial dysfunction in a population of African ancestry in South Africa? Cardiovasc J Afr. 2011 May-Jun; 22(3): 134-40. doi: CVJ-21.049. PubMed PMID: 21713302.

Franchini M, Lippi G. Von Willebrand factor and thrombosis. Ann Hematol. 2006 Jul; 85(7): 415-23. Epub 2006 Mar 28.

Francisci D, Falcinelli E, Belfiori B, Petito E, Fierro T, Mezzasoma AM, *et al*. Possible role of platelet activation in the cardiovascular complications associated with infection: differential effects of abacavir (ABC) versus tenofovir (TDF). J Thromb Haemost. 2011; 9(Suppl 2): 799.

Friis-Møller N, Sabin CA, Weber R, d'Arminio Monforte A, El-Sadr W, Reiss P, *et al*. Combination antiretroviral therapy and the risk of myocardial infarction. N Engl J Med. 2003 Nov 20; 349(21): 1993-2003. Erratum in: N Engl J Med. 2004 Feb 26; 350(9): 955.

Fuchigami S, Kaikita K, Soejima K, Matsukawa M, Honda T, Tsujita K, *et al*. Changes in plasma von Willebrand factor-cleaving protease (ADAMTS13) levels in patients with unstable angina. Thromb Res. 2008; 122(5): 618-23. Epub 2008 Mar 4. doi: 10.1016/j.thromres.2007.12.025. PubMed PMID: 18295305.

Fujioka M, Hayakawa K, Mishima K, Kunizawa A, Irie K, Higuchi S, *et al*. ADAMTS13 gene deletion aggravates ischemic brain damage: a possible neuroprotective role of ADAMTS13 by ameliorating postischemic hypoperfusion. Blood. 2010 Feb 25; 115(8): 1650-3. Epub 2009 Nov 13. doi: 10.1182/blood-2009-06-230110. PubMed PMID: 19965676.

Fujioka M, Nakano T, Hayakawa K, Irie K, Akitake Y, Sakamoto Y, *et al*. ADAMTS13 gene deletion enhances plasma high-mobility group box1 elevation and neuroinflammation in brain ischemia-reperfusion injury. Neurol Sci. 2012 Oct; 33(5): 1107-15. Epub 2012 Jan 3. doi: 10.1007/s10072-011-0913-9. PubMed PMID: 22212812.

Furlan M, Robles R, Lämmle B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood. 1996 May 15; 87(10): 4223-34.

Gale AJ. Continuing education course #2: current understanding of hemostasis. Toxicol Pathol. 2011 Jan; 39(1): 273-80. Epub Nov 30. doi: 10.1177/0192623310389474. PubMed PMID: 21119054.

Gandhi C, Khan MM, Lentz SR, Chauhan AK. ADAMTS13 reduces vascular inflammation and the development of early atherosclerosis in mice. Blood. 2012 Mar 8; 119(10): 2385-91. Epub 2011 Nov 28. doi: 10.1182/blood-2011-09-376202. PubMed PMID: 22123843.

Garg RK. Sinha MK. Tuberculous meningitis in patients infected with human immunodeficiency virus. J Neurol. 2011 Jan; 258(1): 3-13. Epub 2010 Sep 17. doi: 10.1007/s00415-010-5744-8. PubMed PMID: 20848123.

Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood. 1987 Jun; 69(6): 1691-5.

Gonzales JU, Thistlethwaite JR, Thompson BC, Scheuermann BW. Exercise-induced shear stress is associated with changes in plasma von Willebrand factor in older humans. Eur J Appl Physiol. 2009 Jul; 106(5): 779-84. Epub 2009 May 13. doi: 10.1007/s00421-009-1074-x. PubMed PMID: 19437032.

Goodeve AC. The genetic basis of von Willebrand disease. Blood Rev. 2010 May; 24(3): 123-34. Epub 2010 Apr 20. doi: 10.1016/j.blre.2010.03.003. PubMed PMID: 20409624.

Goto S, Sakai H, Ikeda Y, Handa S. Acute myocardial infarction plasma augments platelet thrombus growth in high shear rates. Lancet. 1997 Feb 22; 349(9051): 543-4.

Goto S, Sakai H, Goto M, Ono M, Ikeda Y, Handa S, *et al*. Enhanced shear-induced platelet aggregation in acute myocardial infarction. Circulation. 1999 Feb 9; 99(5): 608-13.

Gresele P, Falcinelli E, Sebastiano M, Baldelli F. Endothelial and platelet function alterations in HIV-infected patients. Thromb Res. 2012 Mar; 129(3): 301-8. Epub 2011 Dec 20. doi: 10.1016/j.thromres.2011.11.022. PubMed PMID: 22192157.

Griffiths D, Sturm J. Epidemiology and etiology of young stroke. Stroke Res Treat. [Online]. 2011 [cited 2012, Feb 23]. doi: 10.4061/2011/209370. PubMed Central PMCID: PMC3140048. Available from: http://www.hindawi.com/journals/srt/2011/209370/

Grinspoon S, Mulligan K, Department of Health and Human Services Working Group on the Prevention and Treatment of Wasting and Weight Loss. Weight Loss and wasting in patients infected with human immunodeficiency virus. Clin Infect Dis. 2003 Apr 1; 36(Suppl 2): S69-78.

Grisold W, Oberndorfer S, Struhal W. Stroke and cancer. Acta Neurol Scand. 2009 Jan; 119(1): 1-16. doi: 10.1111/j.1600-0404.2008.01059.x. PubMed PMID: 18616624.

Gunther K, Garizio D, Nesara P. ADAMTS13 activity and the presence of acquired inhibitors in human immunodeficiency virus-related thrombotic thrombocytopenic purpura. Transfusion. 2007 Sep; 47(9): 1710-6.

Halcox JP, Donald AE, Ellins E, Witte DR, Shipley MJ, Brunner EJ, *et al*. Endothelial function predicts progession of carotid intima-media thickness. Circulation. 2009 Feb 24; 119(7): 1005-12. Epub 2009 Feb 9. doi: 10.1161/CIRCULATIONAHA.108.765701. PubMed PMID: 19204308.

Hanson E, Jood K, Karlsson S, Nilsson S, Blomstrand C, Jern C. Plasma levels of von Willebrand factor in the etiologic subtypes of ischaemic stroke. J Thromb Haemost. 2011 Feb; 9(2): 275-81. doi: 10.1111/j.1538-7836.2010.04134.x. PubMed PMID: 21054779.

Hanson E, Jood K, Nilsson S, Blomstrand C, Jern C. Association between genetic variation at the ADAMTS13 locus and ischemic stroke. J Thromb Haemost. 2009 Dec; 7(12): 2147-8. Epub 2009 Sep 18. doi: 10.1111/j.1538-7836.2009.03617.x. PubMed PMID: 19765208.

Hart D, Sayer R, Miller R, Edwards S, Kelly A, Baglin T, *et al*. Human immunodeficiency virus associated thrombotic thrombocytopenic purpura – favourable outcome with plasma exchange and prompt initiation of highly active antiretroviral therapy. Br J Haematol. 2011 May; 153(4): 515-9. Epub 2011 Mar 10. doi: 10.1111/j.1365-2141.2011.08636.x. PubMed PMID: 21391974.

Hassan MI, Saxena A, Ahmad F. Structure and function of von Willebrand factor. Blood Coagul Fibrinolysis. 2012 Jan; 23(1): 11-22. doi: 10.1097/MBC.0b013e32834cb35d. PubMed PMID: 22089939.

Hassoun A, Al-Khadimi Z, Cervia J. HIV infection and antiphospholipid antibody: literature review and link to the antiphospholipid syndrome. AIDS Patient Care STDS. 2004 Jun; 18(6): 333-40.

Hatano S. Experience from a multicentre stroke register: a preliminary report. Bull World Health Organ. 1976; 54(5): 541-53.

Heikinheimo T, Chimbayo D, Kumwenda JJ, Kampondeni S, Allain TJ. Stroke outcomes in Malawi, a country with high prevalence of HIV: a prospective follow-up study. PLoS One. 2012; 7(3): e33765. Epub 2012 Mar 29. doi:10.1371/journal.pone.0033765. PubMed PMID: 22479439; PubMed Central PMCID: PMC3315584.

HIV: Geography of an Epidemic. [Online]. 2011 [cited 2013, Apr 15]. Available from: http://www.incidence0.org/2011/04/05/hiv-geography-of-an-epidemic/

Hoffbrand AV, Moss PAH. Essential haematology. 6th ed. West Sussex: Wiley-Blackwell; 2011.

Hoffmann M. Stroke in the young in South Africa – an analysis of 320 patients. S Afr Med J. 2000a Dec: 90(12): 1226-37.

Hoffmann M, Berger JR, Nath A, Rayens M. Cerebrovascular disease in young, HIV-infected, black Africans in the KwaZulu Natal province of South Africa. J Neurovirol. 2000b Jun; 6(3): 229-36.

Hogan C, Wilkins E. Neurological complications of HIV. Clin Med. 2011 Dec; 11(6): 571-5.

Horvath B, Hegedus D, Szapary L, Marton Z, Alexy T, Koltai K *et al*. Measurement of von Willebrand factor as the marker of endothelial dysfunction in vascular diseases. Exp Clin Cardiol. 2004 Spring; 9(1): 31-4.

Hoshiba Y, Hatakeyama K, Tanabe T, Asada Y, Goto S. Co-localization of von Willebrand factor with platelet thrombi, tissue factor and platelets with fibrin, and consistent presence of inflammatory cells in coronary thrombi obtained by an aspiration device from patients with acute myocardial infarction. J Thromb Haemost. 2006 Jan; 4(1): 114-20.

Huq FY, Kulkarni A, Agbim EC, Riddell A, Tuddenham E, Kadir RA. Changes in the levels of factor VIII and von Willebrand factor in the puerperium. Haemophilia. 2012 Mar; 18(2): 241-5. Epub 2011 Sep 28. doi: 10.1111/j.1365-2516.2011.02625.x. PubMed PMID: 21951573.

Jenkins PV, O'Donnell JS. ABO blood group determines plasma von Willebrand factor levels: a biologic function after all? Transfusion. 2006 Oct; 46(10): 1836-44.

Jin SY, Tohyama J, Bauer RC, Cao NN, Rader DJ, Zheng XL. Genetic ablation of Adamts13 gene dramatically accelerates the formation of early atherosclerosis in murine model. Arterioscler Thromb Vasc Biol. 2012 Aug; 32(8): 1817-23. Epub 2012 May 31. doi: 10.1161/ATVBAHA.112.247262. PubMed PMID: 22652598.

Johansson L, Jansson JH, Boman K, Nilsson TK, Stegmayr B, Hallmans G. Prospective study on soluble thrombomodulin and von Willebrand factor and the risk of ischemic and hemorrhagic stroke. Thromb Haemost. 2002 Feb; 87(2): 211-7.

Johnsen J, López JA. VWF secretion: what's in a name? Blood. 2008 Aug 15; 112(4): 926-7. doi: 10.1182/blood-2008-04-149245. PubMed PMID: 18684873.

Kaikita K, Soejima K, Matsukawa M, Nakagaki T, Ogawa H. Reduced von Willebrand factor-cleaving protease (ADAMTS13) activity in acute myocardial infarction. J Thromb Haemost. 2006 Nov; 4(11): 2490-3. Epub 2006 Aug 8.

Karp G, Schlaeffer F, Jotkowitz A, Riesenberg K. Syphilis and HIV co-infection. Eur J Intern Med. 2009 Jan; 20(1): 9-13. Epub 2008 Jun 20. doi: 10.1016/j.ejim.2008.04.002. PubMed PMID: 19237085.

Kassi E, Pervanidou P, Kaltsas G, Chrousos G. Metabolic syndrome: definitions and controversies. BMC Med. 2011 May 5; 9: 48. doi: 10.1186/1741-7015-9-48. PubMed PMID: 21542944.

Kengne AP, Anderson CS. The neglected burden of stroke in Sub-Saharan Africa. Int J Stroke. 2006 Nov; 1(4): 180-90. doi: 10.1111/j.1747-4949.2006.00064.x. PubMed PMID: 18706015.

Kidwell CS, Alger JR, Di Salle F, Starkman S, Villablanca P, Bentson *et al*. Diffusion MRI in patients with transient ischemic attacks. Stroke. 1999 Jun; 30(6): 1174-80.

Klein SK, Slim EJ, de Kruif MD, Keller TT, ten Cate H, van Gorp EC, *et al*. Is chronic HIV infection associated with venous thrombotic disease? A systematic review. Neth J Med. 2005 Apr; 63(4): 129-36.

Kleinschnitz C, De Meyer SF, Schwarz T, Austinat M, Vanhoorelbeke K, Nieswandt B, *et al*. Deficiency of von Willebrand factor protects mice from ischemic stroke. Blood. 2009 Apr 9; 113(15): 3600-3. Epub 2009 Jan 30. doi: 10.1182/blood-2008-09-180695. PubMed PMID: 19182208.

Kleinschnitz C, Pozgajoya M, Pham M, Bendszus M, Nieswandt B, Stoll G. Targeting platelets in acute experimental stroke: impact of glycoprotein Ib, VI, and IIb/IIIa blockade on infarct size, functional outcome, and intracranial bleeding. Circulation. 2007 May 1; 115(17): 2323-30. Epub 2007 Apr 16.

Klug E, South African Heart Association (S A Heart), Lipid and Atherosclerosis Society of Southern Africa (LASSA). South African dyslipidaemia guideline consensus statement. S Afr Med J. 2012 Feb 23; 102(3 Pt 2): 178-87.

Knottnerus IL, Ten Cate H, Lodder J, Kessels F, van Oostenbrugge RJ. Endothelial dysfunction in lacunar stroke: a systematic review. Cerebrovasc Dis. 2009; 27(5): 519-26. Epub 2009 Apr 16. doi: 10.1159/000212672. PubMed PMID: 19372654.

Kokame K, Miyata T. Genetic defects leading to hereditary thrombotic thrombocytopenic purpura. Semin Hematol. 2004 Jan; 41(1): 34-40.

Kovacs JA, Masur H. Prophylaxis against opportunistic infections in patients with human immunodeficiency virus infection. N Engl J Med. 2000 May 11; 342(19): 1416-29.

Lafeuillade A, Alessi MC, Poizot-Martin I, Boyer-Neumann C, Zandotti C, Quilichini R, *et al*. Endothelial cell dysfunction in HIV infection. J Acquir Immune Defic Syndr. 1992; 5(2): 127-31.

Lambers M, Goldenberg NA, Kenet G, Kirkham FJ, Manner D, Bernard T, *et al*. Role of reduced ADAMTS13 in arterial ischemic stroke: a pediatric cohort study. Ann Neurol. 2013 Jan; 73(1): 58-64. Epub 2012 Dec 7. doi: 10.1002/ana.23735. PubMed PMID: 23225307.

Lammie GA, Hewlett RH, Schoeman JF, Donald PR. Tuberculous cerebrovascular disease: a review. J Infect. 2009 Sep; 59(3): 156-66. Epub 2009 Jul 25. doi: 10.1016/j.jinf.2009.07.012. PubMed PMID: 19635500.

Lämmle B, Kremer Hovinga JA, Alberio L. Thrombotic thrombocytopenic purpura. J Thromb Haemost. 2005 Aug; 3(8): 1663-75.

Lancellotti S, De Fillipis V, Pozzi N, Peyvandi F, Palla R, Rocca B, *et al*. Formation of methionine sulfoxide by peroxynitrite at position 1606 of von Willebrand factor inhibits its cleavage by ADAMTS-13: A new prothrombotic mechanism in disease associated with oxidative stress. Free Radic Biol Med. 2010 Feb 1; 48(3): 446-56. Epub 2009 Dec 4. doi: 10.1016/j.freeradbiomed.2009.11.020. PubMed PMID: 19969076.

Larrue V, Berhoune N, Massabuau P, Calviere L, Raposo N, Viquer A, *et al*. Etiologic investigation of ischemic stroke in young adults. Neurology. 2011 Jun 7; 76(23): 1938-8. doi: 10.1212/WNL.0b013e31821e5517. PubMed PMID: 21646623.

Levy GG, Motto DG, Ginsburg D. ADAMTS13 turns 3. Blood. 2005 Jul 1; 106(1): 11-7. Epub 2005 Mar 17.

Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, *et al*. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature. 2001 Oct 4; 413(6855): 488-94.

Li M, Goto S, Sakai H, Kim JY, Ichikawa N, Yoshida M, *et al*. Enhanced shear-induced von Willebrand factor binding to platelets in acute myocardial infarction. Thromb Res. 2000 Nov 15; 100(4): 251-61.

Licata G, Tuttolomondo A, Di Raimondo D, Corrao S, Di Sciacca R, Pinto A. Immuno-inflammatory activation in acute cardio-embolic strokes in comparison with other subtypes of ischaemic stroke. Thromb Haemost. 2009 May; 101(5): 929-37.

Lip GY, Blann AD, Farooqi IS, Zarifis J, Sagar G, Beevers DG. Sequential alterations in haemorheology, endothelial dysfunction, platelet activation and thrombogenesis in relation to prognosis following acute stroke: The West Birmingham Stroke Project. Blood Coagul Fibrinolysis. 2002 Jun; 13(4): 339-47.

Lip GY, Blann A. von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? Cardiovasc Res. 1997 May; 34(2): 255-65.

Liu L, Lin Z, Shen Z. Changes of von Willebrand factor and antithrombin III levels in acute stroke: difference between thrombotic and haemorrhagic stroke. Thromb Res. 1993 Nov 15; 72(4): 353-8.

Lo J. Plutzky J. The biology of atherosclerosis: general paradigms and distinct pathogenic mechanisms among HIV-infected patients. J Infect Dis. 2012 Jun; 205 Suppl 3: S368-74. doi: 10.1093/infdis/jis201. PubMed PMID: 22577210.

Longo-Mbenza B, Longokolo Mashi M, Lelo Tshikwela M, Mokondjimobe E, Gombet T, Ellenga-Mbolla B, *et al*. Relationship between Younger Age, Autoimmunity, Cardiometabolic Risk, Oxidative Stress, HAART, and Ischemic Stroke in Africans with HIV/AIDS. ISRN Cardiol. 2011; 2011: 897908. Epub 2011 May 23. doi: 10.5402/2011/897908. PubMed PMID: 22347662; PubMed Central PMCID: PMC3262512.

Lotta LA, Garagiola I, Palla R, Cairo A, Peyvandi F. ADAMTS13 mutations and polymorphisms in congenital thrombotic thrombocytopenic purpura. Hum Mutat. 2010 Jan; 31(1): 11-9. doi: 10.1002/humu.21143. PubMed PMID: 19847791.

Louw S, Jacobson BF, Büller H. Human immunodeficiency virus infection and acute deep vein thrombosis. Clin Appl Thromb Hemost. 2008 Jul; 14(3): 352-5. Epub 2007 Sep 25.

Luo GP, Ni B, Yang X, Wu YZ. von Willebrand factor: more than a regulator of hemostasis and thrombosis. Acta Haematol. 2012; 128(3): 158-69. Epub 2012 Aug 10.

Lynn WA, Lightman S. Syphilis and HIV: a dangerous combination. Lancet Infect Dis. 2004 Jul: 4(7): 456-66.

Maclean PS, Tait RC. Hereditary and acquired antithrombin deficiency: epidemiology, pathogenesis and treatment options. Drugs. 2007; 67(10): 1429-40.

Mahoney FI, Barthel DW. "Functional evaluation: the Barthel Index." Maryland State Med Journal 1965; 14: 56-61. Used with permission.

Majerus EM, Zheng X, Tuley EA, Sadler JE. Cleavage of the ADAMTS13 propeptide is not required for protease activity. J Biol Chem. 2003 Nov 21; 278(47): 46643-8. Epub 2003 Sep 15.

Malak S, Wolf M, Millot GA, Mariotte E, Veyradier A, Meynard JL, *et al*. Human immunodeficiency virus-associated thrombotic microangiopathies: clinical characteristics and outcome according to ADAMTS13 activity. Scand J Immunol. 2008 Sep; 68(3): 337-44. doi: 10.1111/j.1365-3083.2008.02143.x. PubMed PMID: 18782260.

Marini C, Russo T, Felzani G. Incidence of stroke in young adults: a review. Stroke Res Treat [Online]. 2010 Dec 19 [cited 2012 Oct 4]. doi: 10.4061/2011/535672. PubMed Central PMCID: PMC3010685. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3010685/

Marsh JD, Keyrouz SG. Stroke prevention and treatment. J Am Coll Cardiol. 2010 Aug 24; 56(9): 638-91. doi: 10.1016/j.jacc.2009.12.072. PubMed PMID: 20723798.

Martin PJ, Enevoldson TP, Humphrey PRD. Causes of ischaemic stroke in the young. Postgrad Med J. 1997 Jan; 73(855): 8-16.

Matsukawa M, Kaikita K, Soejima K, Fuchigami S, Nakamura Y, Honda T, *et al*. Serial changes in von Willebrand factor-cleaving protease (ADAMTS13) and prognosis after acute myocardial infarction. Am J Cardiol. 2007 Sep 1; 100(5): 758-63. Epub 2007 Jun 12.

Mayosi BM, Flisher AJ, Lalloo UG, Sitas F, Tollman SM, Bradshaw D. The burden of non-communicable diseases in South Africa. Lancet. 2009 Sep 12; 374(9693): 934-947. Epub 2009 Aug 24. doi: 10.1016/S0140-6736(09)61087-4. PubMed PMID: 19709736.

McArthur JC, Brew BJ, Nath A. Neurological complications of HIV infection. Lancet Neurol. 2005 Sep; 4(9): 543-55.

McGrath RT, McRae E, Smith OP, O'Donnell JS. Platelet von Willebrand factor – structure, function and biological importance. Br J Haematol. 2010 Mar; 148(6): 834-43. Epub 2010 Jan 11. doi: 10.1111/j.1365-2141.2009.08052.x. PubMed PMID: 20067560.

McLane MA, Marcinkiewicz C, Vijay-Kumar S, Wierzbicka-Patynowski I, Niewiarowski S. Viper venom disintegrins and related molecules. Proc Soc Exp Biol Med. 1998 Nov; 219(2): 109-19.

Meade TW, Cooper JA, Stirling Y, Howarth DJ, Ruddock V, Miller GJ. Factor VIII, ABO blood group and the incidence of ischaemic heart disease. Br J Haematol. 1994 Nov; 88(3): 601-7.

Mehta AB, Hoffbrand V. Hematology at a Glance. 3rd ed. West Sussex: Wiley-Blackwell; 2009.

Meiring M, Webb M, Goedhals D, Louw V. HIV–associated Thrombotic Thrombocytopenic Purpura – What We Know So Far. European Oncology and Haematology. 2012; 8(2): 89-91.

Meiring M, Badenhorst PN, Kelderman M. Performance and utility of a cost-effective collagen-binding assay for the laboratory diagnosis of Von Willebrand disease. Clin Chem Lab Med. 2007; 45(8): 1068-72.

Mendolicchio GL, Ruggeri ZM. New perspectives on von Willebrand factor functions in hemostasis and thrombosis. Semin Hematol. 2005 Jan; 42(1): 5-14.

Miller RF, Scully M, Cohen H, Roedling S, Starke R, Edwards SG, *et al*. Thrombotic thrombocytopaenic purpura in HIV-infected patients. Int J STD AIDS. 2005 Aug; 16(8): 538-42.

Mizusawa H, Hirano A, Llena JF, Shintaku M. Cerebrovascular lesions in acquired immune deficiency syndrome (AIDS). Acta Neuropathol. 1988; 76(5): 451-7.

Moake JL. Thrombotic microangiopathies. N Engl J Med. 2002 Aug 22; 347(8): 589-600.

Mochan A, Modi M, Modi G. Stroke in black South African HIV-positive patients: a prospective analysis. Stroke. 2003 Jan; 34(1): 10-5.

Mochan A, Modi M, Modi G. Protein S deficiency in HIV associated ischaemic stroke: an epiphenomenon of HIV infection. J Neurol Neurosurg Psychiatry. 2005 Oct; 76(10): 1455-6.

Modi G, Modi M, Mochan A. Is stroke a HIV-related neurologic manifestation? Expert Rev Neurother. 2008 Aug; 8(8): 1247-53. doi: 10.1586/14737175.8.8.1247. PubMed PMID: 18671668.

Modi M, Singla V, Ahluwalia J, Sharma A, Prabhakar S, Khandelwal N, *et al*. HIV infection presenting as cerebral venous sinus thrombosis. Am J Emerg Med. 2012 Mar; 30(3): 517.e1-3. Epub 2011 Mar 15. doi: 10.1016/j.ajem.2011.01.022. PubMed PMID: 21406317.

Monsuez JJ, Charniot JC, Escaut L, Teicher E, Wyplosz B, Couzigou C, *et al*. HIV-associated vascular diseases: structural and functional changes, clinical implications. Int J Cardiol. 2009 Apr 17; 133(3): 293-306. Epub 2009 Jan 7. doi: 10.1016/j.ijcard.2008.11.113. PubMed PMID: 19131130.

Morange PE, Simon C, Alessi MC, Luc G, Arveiler D, Ferrieres J, *et al*. Endothelial cell markers and the risk of coronary heart disease: the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study. Circulation. 2004 Mar 23; 109(11): 1343-8. Epub 2004 Mar 15.

Moschcowitz E. Hyaline thrombosis of the terminal arterioles and capillaries: a hitherto undescribed disease. Proc N Y Pathol Soc. 1924; 24: 21-4.

Moster ML. Coagulopathies and arterial stroke. J Neuroophthalmol. 2003 Mar; 23(1): 63-71.

Muir KW. Stroke. Medicine. 2009; 37(2): 109-114.

Mukherjee D, Patil CG. Epidemiology and the global burden of stroke. World Neurosurg. 2011 Dec; 76 (6 Suppl): S85-90. doi: 10.1016/j.wneu.2011.07.023. PubMed PMID: 22182277.

Murphy G. The ADAMs: signalling scissors in the tumour microenvironment. Nat Rev Cancer. 2008 Dec; 8(12): 929-41. Epub 2008 Nov 13. doi: 10.1038/nrc2459. PubMed PMID: 19005493.

Naess H, Tatlisumak T, Kõrv J. Editorial. Stroke in the young. Stroke Res Treat. [Online]. 2011 [cited 2012, Mar 12]. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3270574/pdf/SRT2011-271979.pdf

Naess H, Waje-Andreassen U. Review of long-term mortality and vascular morbidity amongst young adults with cerebral infarction. Eur J Neurol. 2010 Jan; 17(1): 17-22. Epub 2009 Nov 24. doi: 10.1111/j.1468-1331.2009.02868.x. PubMed PMID: 19968702.

Nagel MA, Mahalingam R, Cohrs RJ, Gilden D. Virus vasculopathy and stroke: an under-recognized cause and treatment target. Infect Disord Drug Targets. 2010 Apr; 10(2): 105-11.

Nagel MA, Traktinskiy I, Azarkh Y, Kleinschmidt-DeMasters B, Hedley-Whyte T, Russman A, *et al*. Varicella zoster virus vasculopathy: analysis of virus-infected arteries. Neurology. 2011 Jul 26; 77(4): 364-70. Epub 2011 Jul 13. doi: 10.1212/WNL.0b013e3182267bfa. PubMed PMID: 21753174.

Napierala Mavedzenge SM, Doyle AM, Ross DA. HIV prevention in young people in sub-Saharan Africa: a systematic review. J Adolesc Health. 2011 Dec; 49(6): 568-86. Epub 2011 Jun 12. doi: 10.1016/j.jadohealth.2011.02.007. PubMed PMID: 22098767.

Ndakotsu MA, Salawu L, Durosinmi MA. Relation between erythrocyte sedimentation rate, clinical and immune status in HIV-infected patients. Niger J Med. 2009 Apr-Jun; 18(2): 208-10.

Nieswandt B, Stoll G. The smaller, the better: VWF in stroke. Blood. 2010 Feb 25; 115(8): 1477-8. doi: 10.1182/blood-2009-12-255000. PubMed PMID: 20185592.

Nixon DE, Landay AL. Biomarkers of immune dysfunction in HIV. Curr Opin HIV AIDS. 2010 Nov; 5(6): 498-503. doi: 10.1097/COH.0b013e32833ed6f4. PubMed PMID: 20978393; PubMed Central PMCID: PMC3032605.

Nogueras C, Sala M, Sasal M, Viñas J, Garcia N, Bella MR, *et al*. Recurrent stroke as a manifestation of primary angiitis of the central nervous system in a patient infected with human immunodeficiency virus. Arch Neurol. 2002 Mar; 59(3): 468-73.

Novitzky N, Thomson J, Abrahams L, du Toit C, McDonald A. Thrombotic thrombocytopenic purpura in patients with retroviral infection is highly responsive to plasma infusion therapy. Br J Haematol. 2005 Feb; 128(3): 373-9.

Nurden AT. Platelets, inflammation and tissue regeneration. Thromb Haemost. 2011 May; 105 Suppl 1: S13-33. Epub 2011 Apr 11. doi: 10.1160/THS10-11-0720. PubMed PMID: 21479340.

O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, *et al*. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. Lancet. 2010 Jul 10; 376 (9735): 112-23. Epub 2010 Jun 17. doi: 10.1016/S0140-6736(10)60834-3. PubMed PMID: 20561675.

Onwuchekwa AC, Onwuchekwa RC, Asekomeh EG. Stroke in young Nigerian adults. J Vasc Nurs. 2009 Dec; 27(4): 98-102. doi: 10.1016/j.jvn.2009.08.001. PubMed PMID: 19914570.

Opie J. Haematological complications of HIV infection. S Afr Med J. 2012 Mar 2; 102(6): 465-8.

Ortiz G, Koch S, Romano JG, Forteza AM, Rabinstein AA. Mechanisms of ischemic stroke in HIV-infected patients. Neurology. 2007 Apr 17; 68(16): 1257-61.

Ovbiagele B, Nath A. Increasing incidence of ischemic stroke in patients with HIV infection. Neurology. 2011 Feb 1; 76(5): 444-50. Epub 2011 Jan 19. doi: 10.1212/WNL.0b013e31820a0cfc. PubMed PMID: 21248273; PubMed Central PMCID: PMC3034413.

Owolabi LF, Ibrahim A. Stroke in young adults: a prospective study from northwestern Nigeria. ISRN Neurol. 2012; 2012: 468706. Epub 2012 Feb 16. doi: 10.5402/2012/468706. PubMed PMID: 2247460; PubMed Central PMCID: PMC3302105.

Panichpisal K, Rozner E, Levine SR. The management of stroke in antiphospholipid syndrome. Curr Rheumatol Rep. 2012 Feb; 14(1): 99-106. doi: 10.1007/s11926-011-0223-5. PubMed PMID: 22109663.

Patel VB, Sacoor Z, Francis P, Bill PL, Bhigjee AI, Connolly C. Ischaemic stroke in young HIV-positive patients in Kwazulu Natal, South Africa. Neurology. 2005 Sep 13; 65(5): 759-61.

Petri B, Broermann A, Li H, Khandoga AG, Zarbock A, Krombach F, *et al*. von Willebrand factor promotes leukocyte extravasation. Blood. 2010 Nov 25; 116(22): 4712-9. Epub 2010 Aug 17. doi: 10.1182/blood-2010-03-276311. PubMed PMID: 20716766.

Peyvandi F, Garagiola I, Baronciani L. Role of von Willebrand factor in the haemostasis. Blood Transfus. 2011 May; 9 Suppl 2: S3-8. doi: 10.2450/2011.002S. PubMed PMID: 21839029; PubMed Central PMCID: PMC3159913.

Pinto AN. AIDS and cerebrovascular disease. Stroke. 1996 Mar; 27(3): 538-43.

Pos W, Luken BM, Sorvillo N, Kremer Hovinga JA, Voorberg J. Humoral immune response to ADAMTS13 in acquired thrombotic thrombocytopenic purpura. J Thromb Haemost. 2011 Jul; 9(7): 1285-91. doi: 10.1111/j.1538-7836.2011.04307.x. PubMed PMID: 21535387.

Pottinger BE, Read RC, Paleolog EM, Higgins PG, Pearson JD. von Willebrand factor is an acute phase reactant in man. Thromb Res. 1989 Feb 15; 53(4): 387-94.

Pratt G, Gascoyne K, Cunningham K, Tunbridge A. Human immunodeficiency virus (HIV) in older people. Age Ageing. 2010 May; 39(3): 289-94. Epub 2010 Feb 26. doi: 10.1093/ageing/afq009. PubMed PMID: 20190230.

Price RW. Neurological complications of HIV infection. Lancet. 1996 Aug 17; 348(9025): 445-52.

Qizilbash N. Duffy S. Prentice CR, Boothby M, Warlow C. Von Willebrand factor and risk of ischemic stroke. Neurology. 1997 Dec. 49(6): 1552-6.

Qureshi AI, Janssen RS, Karon JM, Weissman JP, Akbar MS, Safdar K, *et al*. Human immunodeficiency virus infection and stroke in young patients. Arch Neurol. 1997 Sep; 54(9): 1150-3.

Rahbar A, Söderberg-Nauclér C. Human cytomegalovirus infection of endothelial cells triggers platelet adhesion and aggregation. J Virol. 2005 Feb; 79(4): 2211-20.

Reininger AJ. VWF attributes – impact on thrombus formation. Thromb Res. 2008; 122 Suppl 4: S9-13. doi: 10.1016/S0049-3848(08)70028-8. PubMed PMID: 18929523.

Rickerts V, Brodt H, Staszewski S, Stille W. Incidence of myocardial infarctions in HIV-infected patients between 1983 and 1998: the Frankfurt HIV-cohort study. Eur J Med Res. 2000 Aug 18; 5(8): 329-33.

Roquer J, Segura T, Serena J, Castillo J. Endothelial dysfunction, vascular disease and stroke: the ARTICO study. Cerebrovasc Dis. 2009; 27 Suppl 1: 25-37. Epub 2009 Apr 3. doi: 10.1159/000200439. PubMed PMID: 19342831.

Ross AC, Rizk N, O'Riordan MA, Dogra V, El-Bejjani D, Storer N, *et al*. Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. Clin Infect Dis. 2009 Oct 1; 49 (7): 1119-27. doi: 10.1086/605578. PubMed PMID: 19712036.

Ruggeri ZM. The role of von Willebrand factor in thrombus formation. Thromb Res. 2007; 120 Suppl 1: S5-9. Epub 2007 May 9.

Rumley A, Lowe GD, Sweetnam PM, Yarnell JW, Ford RP. Factor VIII, von Willebrand factor and the risk of major ischaemic heart disease in the Caerphilly Heart Study. Br J Haematol. 1999 Apr; 105(1): 110-6.

Rutten-Jacobs LC, Maaijwee NA, Arntz RM, Van Alebeek ME, Schaapsmeerders P, Schoonderwalt HC *et al*. Risk factors and prognosis of young stroke. The FUTURE study: a prospective cohort study. Study rationale and protocol. BMC Neurol. [Online]. 2011 Sep 20 [cited 2012, Oct 3]. doi: 10.1186/1471-2377-11-109. PubMed PMID: 21933424; PubMed Central PMCID: PMC3185265. Available from: http://www.biomedcentral.com/1471-2377/11/109

Ruttmann T. Coagulation for the clinician. S Afr J Surg. 2006 Feb; 44(1): 22, 24-6, 28-30; passim.

Sadler JE. Von Willebrand factor assembly and secretion. J Thromb Haemost. 2009 Jul; 7 Suppl 1: 24-7. doi: 10.1111/j.1538-7836.2009.03375.x. PubMed PMID: 19630761.

Sadler JE. Biochemistry and genetics of von Willebrand Factor. Annu Rev Biochem. 1998; 67(1): 395-424.

Sadler JE, Budde U, Eikenboom JC, Favaloro EJ, Hill FG, Holmberg L, *et al*. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. J Thromb Haemost. 2006 Oct; 4(10): 2103-14. Epub 2006 Aug 2.

Sadler JE. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. Blood. 2008 Jul 1; 112(1): 11-8. doi: 10.1182/blood-2008-02-078170. PubMed PMID: 18574040.

Sadler JE. von Willebrand factor: two sides of a coin. J Thromb Haemost. 2005 Aug; 3(8): 1702-9.

Saif MW, Greenberg B. HIV and thrombosis: a review. AIDS Patient Care STDS. 2001 Jan; 15(1): 15-24.

Sangle NA, Smock KJ. Antiphospholipid antibody syndrome. Arch Pathol Lab Med. 2011 Sep; 135(9): 1092-6. doi: 10.5858/2010-0325-RSR.1. PubMed PMID: 21877992.

Satchell CS, Cotter AG, O'Connor EF, Peace AJ, Tedesco AF, Clare A, *et al*. Platelet function and HIV: a case-control study. AIDS. 2010 Mar 13; 24(5): 649-57. doi: 10.1097/QAD.0b013e328336098c. PubMed PMID: 20177361.

Satchell CS, O'Halloran JA, Cotter AG, Peace AJ, O'Connor EF, Tedesco AF, *et al*. Increased platelet reactivity in HIV-1-infected patients receiving abacavir-containing antiretroviral therapy. J Infect Dis. 2011 Oct 15; 204(8): 1202-10. doi: 10.1093/infdis/jir509. PubMed PMID: 21917893.

Schettert IT, Pereira AC, Lopes NH, Hueb WA, Krieger JE. Association between ADAMTS13 polymorphisms and risk of cardiovascular events in chronic coronary disease. Thromb Res. 2010 Jan; 125(1): 61-6. Epub 2009 May 8. doi: 10.1016/j.thromres.2009.03.008. PubMed PMID: 19427680.

Schneppenheim R, Budde U. von Willebrand factor: the complex molecular genetics of a multidomain and multifunctional protein. J Thromb Haemost. 2011 Jul; 9 Suppl 1: 209-15. doi: 10.1111/j.1538-7836.2011.04324.x. PubMed PMID: 21781257.

Schneppenheim R. The pathophysiology of von Willebrand disease: therapeutic implications. Thromb Res. 2011; 128 Suppl 1: S3-7. doi: 10.1016/S0049-3848(12)70002-6. PubMed PMID: 22221847.

Seedat YK, Rayner BL, Southern African Hypertension Society. South African hypertension guideline 2011. S Afr Med J. 2011 Dec 14; 102(1 Pt 2): 57-83.

Seigneur M, Constans J, Blann A, Renard M, Pellegrin JL, Amiral J, *et al*. Soluble adhesion molecules and endothelial cell damage in HIV infected patients. Thromb Haemost. 1997 Apr; 77(4): 646-9.

Sen S, Rabinstein AA, Elkind MS, Powers WJ. Recent developments regarding human immunodeficiency virus infection and stroke. Cerebrovasc Dis. 2012; 33(3): 209-18. Epub 2012 Jan 19. doi: 10.1159/000335300. PubMed PMID: 22261608.

Sène D, Piette JC, Cacoub P. Antiphospholipid antibodies, antiphospholipid syndrome and infections. Autoimmun Rev. 2008 Feb; 7(4): 272-7. Epub 2007 Oct 23. doi: 10.1016/j.autrev.2007.10.001. PubMed PMID: 18295729.

Shapshak P, Kangueane P, Fujimura RK, Commins D, Chiappelli F, Singer E, *et al*. Editorial neuroAIDS review. AIDS. 2011 Jan 14; 25(2): 123-41. doi: 10.1097/QAD.0b013e328340fd42. PubMed PMID: 21076277.

Shelat SG, Ai J, Zheng XL. Molecular biology of ADAMTS13 and diagnostic utility of ADAMTS13 proteolytic activity and inhibitor assays. Semin Thromb Hemost. 2005 Dec; 31(6): 659-72.

Shen YM, Frenkel EP. Thrombosis and a hypercoagulable state in HIV-infected patients. Clin Appl Thromb Hemost. 2004 Jul; 10(3): 277-80.

Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Pillay-van-Wyk V, *et al*. South African national HIV prevalence, incidence, behaviour and communication survey 2008: A turning tide among teenagers? [Online]. Cape Town: HSRC Press; 2009 [cited 2012 Nov 12]. Available from: http://www.hsrcpress.ac.za/product.php?productid=2264&freedownload=1

Sierra C, Coca A, Schiffrin EL. Vascular mechanisms in the pathogenesis of stroke. Curr Hypertens Rep. 2011 Jun; 13(3): 200-7. doi: 10.1007/s11906-011-0195-x. PubMed PMID: 21331606.

Sitia S, Tomasoni L, Atzeni F, Ambrosio G, Cordiano C, Catapano A, *et al*. From endothelial dysfunction to atherosclerosis. Autoimmun Rev. 2010 Oct; 9(12): 830-4. Epub 2010 Jul 30. doi: 10.1016/j.autrev.2010.07.016. PubMed PMID: 20678595.

Smith FB, Lee AJ, Hau CM, Rumley A, Lowe GD, Fowkes FG. Plasma fibrinogen, haemostatic factors and prediction of peripheral arterial disease in the Edinburgh Artery Study. Blood Coagul Fibrinolysis. 2000 Jan; 11(1): 43-50.

Smith A, Patterson C, Yarnell J, Rumley A, Ben-Shlomo Y, Lowe G. Which hemostatic markers add to the predictive value of conventional risk factors for coronary heart disease and ischemic stroke? The Caerphilly Study. Circulation. 2005 Nov 15; 112(20): 3080-7.

Smith NL, Chen MH, Dehghan A, Strachan DP, Basu S, Soranzo N, *et al*. Novel associations of multiple genetic loci with plasma levels of factor VII, factor VIII, and von Willebrand factor: The CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium. Circulation. 2010 Mar 30; 121(12): 1382-92. Epub 2010 Mar 15. doi: 10.1161/CIRCULATIONAHA.109.869156. PubMed PMID: 20231535; PubMed Central PMCID: PMC2861278.

Soare AM, Popa C. Deficiencies of proteins C, S and antithrombin and factor V Leiden and the risk of ischemic strokes. J Med Life. 2010 Jul-Sep; 3(3): 235-8.

South African Medical Research Council. Guidelines on ethics for medical research: general principles. 4th ed. South African Medical Research Council. [Online]. 2002 [cited 2012, Mar 6]. Available from: http://www.mrc.ac.za/ethics/ethicsbook1.pdf

Spengos K, Vemmos K. Risk factors, etiology, and outcome of first-ever ischaemic stroke in young adults aged 15 to 45 – the Athens young stroke registry. Eur J Neurol. 2010 Nov; 17(11): 1358-64. doi: 10.1111/j.1468-1331.2010.03065.x. PubMed PMID: 20482604.

Spiel AO, Gilbert JC, Jilma B. von Willebrand factor in cardiovascular disease: focus on acute coronary syndromes. Circulation. 2008 Mar 18; 117(11); 1449-59. doi: 10.1161/CIRCULATIONAHA.107.722827. PubMed PMID: 18347221.

Stahl CP, Wideman CS, Spira TJ, Haff EC, Hixon GJ, Evatt BL. Protein S deficiency in men with long-term human immunodeficiency virus infection. Blood. 1993 Apr 1; 81(7): 1801-7.

Stankovic S, Majkic-Singh N. Genetic aspects of ischemic stroke: coagulation, homocysteine, and lipoprotein metabolism as potential risk factors. Crit Rev Clin Lab Sci. 2010 Mar-Apr; 47(2): 72-123. doi: 10.3109/10408361003791520. PubMed PMID: 20590502.

Stanley A, Candy S, Levin C, Heckmann JM. The complexity of HIV vasculopathy. S Afr Med J. 2012 Mar 2; 102(6): 474-6.

Steininger C, Puchhammer-Stöckl E, Popow-Kraupp T. Cytomegalovirus disease in the era of highly active antiretroviral therapy (HAART). J Clin Virol. 2006 Sep; 37(1): 1-9. Epub 2006 May 3.

Stoll G, Kleinschnitz C, Nieswandt B. The role of glycoprotein Ibalpha and von Willebrand factor interaction in stroke development. Hamostaseologie. 2010 Aug; 30(3): 136-8.

Stoll G, Kleinschnitz C, Nieswandt B. Molecular mechanisms of thrombus formation in ischemic stroke: novel insights and targets for treatment. Blood. 2008 Nov 1; 112(9): 3555-62. Epub 2008 Aug 1. doi: 10.1182/blood-2008-04-144758. PubMed PMID: 18676880.

Stott DJ, Spilg E, Campbell AM, Rumley A, Mansoor MA, Lowe GD. Haemostasis in ischaemic stroke and vascular dementia. Blood Coagul Fibinolysis. 2001 Dec; 12(8): 651-7.

Subbarao V, Lowe D, Aghamohammadzadeh R and Wilkinson RJ. Endothelial dysfunction in HIV. In: Elaheh Aghdassi, editor. HIV infection in the era of highly active antiretroviral treatment and some of its associated complications. [Online]. InTech; 2011 [cited 2012, Mar 19]. p. 17-36. Available from: http://www.intechopen.com/articles/show/title/endothelial-dysfunction-in-hiv

Sulter G, Steen C, De Keyser J. Use of the Barthel index and modified Rankin scale in acute stroke trials. Stroke. 1999 Aug; 30(8): 1538-41.

Suzuki M, Murata M, Matsubara Y, Uchida T, Ishihara H, Shibano T, *et al*. Detection of von Willebrand factor-cleaving protease (ADAMTS-13) in human platelets. Biochem Biophys Res Commun. 2004 Jan 2; 313(1): 212-6.

Szántó T, Joutsi-Korhonen L, Deckmyn H, Lassila R. New insights into von Willebrand disease and platelet function. Semin Thromb Hemost. 2012 Feb; 38(1): 55-63. Epub 2012 Feb 7. doi: 10.1055/s-0031-1300952. PubMed PMID: 22314604.

Tapper H, Herwald H. Modulation of hemostatic mechanisms in bacterial infectious diseases. Blood. 2000 Oct 1; 96(7): 2329-37.

Thögersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, *et al*. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation. 1998 Nov 24; 98(21): 2241-7.

Thurlow J, Gow J, George G. HIV/AIDS, growth and poverty in Kwazulu-Natal and South Africa: an integrated survey, demographic and economy-wide analysis. J Int AIDS Soc. 2009 Sep 16; 12: 18. doi: 10.1186/1758-2652-12-18. PubMed PMID: 19758444; PubMed Central PMCID: PMC2754430.

Tiede A, Rand JH, Budde U, Ganser A, Federici AB. How I treat the acquired von Willebrand syndrome. Blood. 2011 Jun 23; 117(25): 6777-85. Epub 2011 May 3. doi: 10.1182/blood-2010-11-297580. PubMed PMID: 21540459.

Tipping B, de Villiers L, Wainwright H, Candy S, Bryer A. Stroke in patients with human immunodeficiency virus infection. J Neurol Neurosurg Psychiatry. 2007 Dec; 78(12): 1320-4. Epub 2007 Apr 30.

Towfighi A, Ovbiagele B. Metabolic syndrome and stroke. Curr Diab Rep. 2008; 8(1): 37-41.

Tsai HM. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. Blood. 1996 May 15; 87(10): 4235-44.

Tsai HM. Von Willebrand factor, shear stress, and ADAMTS13 in hemostasis and thrombosis. ASAIO J. 2012 Mar-Apr; 58(2): 163-9. doi: 10.1097/MAT.0b013e31824363e7. PubMed PMID: 22370688.

Tsai HM. Pathophysiology of thrombotic thrombocytopaenic purpura. Int J Hematol. 2010 Jan; 91(1): 1-19. doi: 10.1007/s12185-009-0476-1. PubMed PMID: 20058209.

Turner N, Nolasco L, Tao Z, Dong JF, Moake J. Human endothelial cells synthesize and release ADAMTS-13. J Thromb Haemost. 2006 Jun; 4(6): 1396-404.

Turner N, Nolasco L, Moake J. Generation and breakdown of soluble ultralarge von Willebrand factor multimers. Semin Thromb Hemost. 2012 Feb; 38(1): 38-46. Epub 2012 Feb 7. doi: 10.1055/s-0031-1300950. PubMed PMID: 22314602.

Tziomalos K, Athyros VG, Karagiannis A, Mikhailidis DP. Endothelial dysfunction in metabolic syndrome: prevalence, pathogenesis and management. Nutr Metab Cardiovasc Dis. 2010 Feb; 20(2): 140-6. Epub 2009 Oct 14. doi: 10.1016/j.numecd.2009.08.006. PubMed PMID: 19833491.

Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. Relative value of inflammatory, hemostatic, and rheological factors of incident myocardial infarction and stroke: the Edinburgh Artery Study. Circulation. 2007 Apr 24; 115(16): 2119-27. Epub 2007 Apr 2.

Uemura M, Tatsumi K, Matsumoto M, Fujimoto M, Matsuyama T, Ishikawa M, *et al*. Localization of ADAMTS13 to the stellate cells of human liver. Blood. 2005 Aug 1; 106(3): 922-4. Epub 2005 Apr 26.

UNAIDS. UNAIDS world AIDS day report 2011. [Online]. 2011 [cited 2012, Feb 20]. Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/JC2216_WorldAIDSday_report_2011_en.pdf

Vafai A, Berger M. Zoster in patients infected with HIV: a review. Am J Med Sci. 2001 Jun; 321(6): 372-80.

Vance DE, McGuinness T, Musgrove K, Orel NA, Fazeli PL. Successful aging and the epidemiology of HIV. Clin Interv Aging. 2011; 6: 181-92. Epub 2011 Jun 28. doi: 10.2147/CIA.S14726. PubMed PMID: 21822373.

van der Sande MA, Schim van der Loeff MF, Aveika AA, Sabally S, Togun T, Sarge-Njie R, *et al*. Body mass index at time of HIV diagnosis: a strong independent predictor of survival. J Acquir Immune Defic Syndr. 2004 Oct 1; 37(2): 1288-94.

van Galen KP, Tuinenburg A, Smeets EM, Schutgens RE. Von Willebrand factor deficiency and atherosclerosis. Blood Rev. 2012 Sep; 26(5): 189-96. Epub 2012 Jun 19. doi: 10.1016/j.blre.2012.05.002. PubMed PMID: 22721874.

van Schie MC, DE Maat MP, Dippel DW, de Groot PG, Lenting PJ, Leebeek FW, *et al*. von Willebrand factor propeptide and the occurrence of a first ischemic stroke. J Thromb Heamost. 2010 Jun; 8(6): 1424-6. Epub 2010 Mar 19. doi: 10.1111/j.1538-7836.2010.03863.x. PubMed PMID: 20345720.

van Schie MC, Wieberdink RG, Koudstaal PJ, Hofman A, Ikram MA, Witteman JC, *et al*. Genetic determinants of von Willebrand factor plasma levels and the risk of stroke: the Rotterdam Study. J Thromb Haemost. 2012 Apr; 10(4): 550-6. doi: 10.1111/j.1538-7836.2012.04634.x. PubMed PMID: 22257027.

van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke. 1988 May; 19(5): 604-7.

van Vonderen MG, Hassink EA, van Agtmael MA, Stehouwer CD, Danner SA, Reiss P, *et al*. Increase in carotid artery intima-media thickness and arterial stiffness but improvement in several markers of endothelial function after initiation of antiretroviral therapy. J Infect Dis. 2009 Apr 15; 199(8): 1186-94. doi: 10.1086/597475. PubMed PMID: 19275490.

Vischer UM. von Willebrand factor, endothelial dysfunction, and cardiovascular disease. J Thromb Haemost. 2006 Jun; 4(6): 1186-93.

Vita JA. Endothelial function. Circulation. 2011 Dec 20; 124(25): e906-12. doi: 10.1161/CIRCULATIONAHA.111.078824. PubMed PMID: 22184047.

Von Willebrand EA. Hereditary pseudohaemophilia. *Finska Läkaresälskapets Handlingar. Band LXVII. No. 2.* (Transactions of the Finnish Society of Medicine) 1926. Reprinted in: Haemophilia. 1999 May; 5(3): 223-231.

Wagner DD, Fay PJ, Sporn LA, Sinha S, Lawrence SO, Marder VJ. Divergent fates of von Willebrand factor and its propolypeptide (von Willebrand antigen II) after secretion from endothelial cells. Proc Natl Acad Sci USA. 1987 Apr; 84(7): 1955-9.

Wanke CA, Silva M, Knox TA, Forrester J, Speigelman D, Gorbach SL. Weight loss and wasting remain common complications in individuals infected with human immunodeficiency virus in the era of highly active antiretroviral therapy. Clin Infect Dis. 2000 Sep; 31(3): 803-5.

Whincup PH, Danesh J, Walker M, Lennon L, Thomson A, Appleby P, *et al*. von Willebrand factor and coronary heart disease: prospective study and meta-analysis. Eur Heart J. 2002 Nov; 23(22): 1764-70.

Wieberdink RG, van Schie MC, Koudstaal PJ, Hofman A, Witteman JC, de Maat MP, *et al*. High von Willebrand factor levels increase the risk of stroke: the Rotterdam study. Stroke. 2010 Oct; 41(10): 2151-6. Epub 2010 Aug 26. doi: 10.1161/STROKEAHA.110.586289. PubMed PMID: 20798373.

Witz M, Lehmann J, Korzets Z. Acute brachial artery thrombosis as the initial manifestation of human immunodeficiency virus infection. Am J Hematol. 2000 Jun; 64(2): 137-9.

Wolf K, Tsakiris DA, Weber R, Erb P, Battegay M, Swiss HIV Cohort Study. Antiretroviral therapy reduces markers of endothelial and coagulation activation in patients with human immunodeficiency virus type 1. J Infect Dis. 2002 Feb 15; 185(4): 456-62. Epub 2002 Jan 18.

Wood C. Harrington W Jr. AIDS and associated malignancies. Cell Res. 2005 Nov-Dec; 15(11-12): 947-52.

Woodburn KR, Rumley A, Lowe GD, Love JG, Murray GD, Pollock JG. Clinical, biochemical, and rheologic factors affecting the outcome of infrainguinal bypass grafting. J Vasc Surg. 1996 Oct; 24(4): 639-46.

World Medical Association. World Medical Association of Helsinki. Ethical principles for medical research involving human subjects. [Online]. 2008 [cited 2012, Mar 6]. Available from: http://www.wma.net/en/30publications/10policies/b3/index.html.pdf?print-media-type&footer-right=[page]/[toPage]

Xiang Y, de Groot R, Crawley JT, Lane DA. Mechanism of von Willebrand factor scissile bond cleavage by a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13). Proc Natl Acad Sci USA. 2011 Jul 12; 108(28): 11602-7. Epub 2011 Jun 24. doi: 10.1073/pnas.1018559108. PubMed PMID: 21705658.

Yamamoto FI. Ischaemic stroke in young adults: an overview of etiological aspects. Arq Neuropsiquatr. 2012 Jun; 70(6): 462-6.

Yamashita A, Sumi T, Goto S, Hoshiba Y, Nishihira K, Kawamoto R, *et al*. Detection of von Willebrand factor and tissue factor in platelets-fibrin rich coronary thrombi in acute myocardial infarction. Am J Cardiol. 2006 Jan 1; 97(1): 26-8. Epub 2005 Nov 7.

Yang P, Baker KA, Hagg T. The ADAMs family: coordinators of nervous system development, plasticity and repair. Prog Neurobiol. 2006 Jun; 79(2): 73-94. Epub 2006 Jul 7.

Yao H, Bethel-Brown C, Li CZ, Buch SJ. HIV neuropathogenesis: a tight rope walk of innate immunity. J Neuroimmune Pharmacol. 2010 Dec; 5(4): 489-95. Epub 2010 Mar 31. doi: 10.1007/s11481-010-9211-1. PubMed PMID: 20354805; PubMed Central PMCID: PMC3121574.

Yip HK, Lai SL, Lan MY, Chang WN, Liu JS, Kao YF, *et al*. Time course of platelet activation and von Willebrand factor in patients with non-valvular atrial fibrillation after ischemic stroke. Circ J. 2007 Mar; 71(3): 321-6.

Zhao BQ, Chauhan AK, Canault M, Paten IS, Yang JJ, Dockal M, *et al*. von Willebrand factor-cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke. Blood. 2009 Oct 8; 114(15): 3329-34. Epub 2009 Aug 17. doi: 10.1182/blood-2009-03-213264. PubMed PMID: 19687510.

Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic pupura. J Biol Chem. 2001 Nov 2; 276(44): 41059-63. Epub 2001 Sep 13.

Zou W, Berglund L. HIV and highly active antiretroviral therapy: dyslipidemia, metabolic aberrations, and cardiovascular risk. Prev Cardiol. 2007 Spring; 10(2): 96-103; quiz 104-5.