

**THE ROLE OF ATHEROSCLEROSIS IN HIV-RELATED STROKE
IN SOUTH AFRICA**

Dr Mohammad Eitzaz Sadiq

Original published work submitted to the Faculty of Health Sciences,
University of the Witwatersrand, Johannesburg,
in fulfilment of the requirements for the degree of
Doctor of Philosophy (PhD)

Johannesburg, 2025

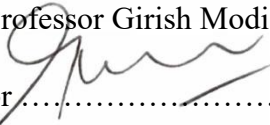
Declaration

Student's contribution to article(s) and agreement of co-author(s)

I, Eitzaz Sadiq, student number 0000464G, declare that this thesis is my own work and that I contributed adequately towards research findings published in the article(s) stated below, which are included in my thesis. It has not been submitted before for any degree or examination at any other University.

Signature of Student..........Date...01/08/2025.....

Name of Primary Supervisor: Professor Girish Modi.....

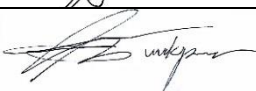
Signature of Primary Supervisor..........Date...16/07/2025.....

Agreement by co-authors:

By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of his thesis. In cases where the student is not the first author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article 1: Stroke as the index presentation of traditional cardiovascular risk factors and Human Immunodeficiency Virus in a South African Population.

Journal of Stroke and Cerebrovascular Diseases. 2024; 33(2):107529

Authors	Name	Signature	Date
1 st Author	E Sadiq		28/01/2025
2 nd Author	A von Klemperer		8/02/2025
3 rd Author	A Woodiwiss		31/01/2025
4 th Author	G Norton		31/01/2025
5 th Author	G Modi		11/02/2025

Comments by primary supervisor: Contribution by E Sadiq: Conceptualisation, study design, data acquisition and analysis, writing draft unassisted, editing and writing the final paper.

Article 2: The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients.

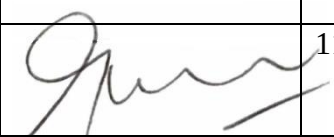
HIV Medicine. 2025;1-10.

Authors	Name	Signature	Date
1 st Author	E Sadiq		20/01/2025
2 nd Author	A Woodiwiss		31/01/2025
3 rd Author	G Tade		07/02/2025
4 th Author	J Nel		06/02/2025
5 th Author	G Norton		31/01/2025
6 th Author	G Modi		11/02/2025

Comments by primary supervisor: Contribution by E Sadiq: conceptualisation, study design, data acquisition and analysis, writing draft unassisted, editing and writing the final paper.

Article 3: Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke patients in South Africa.

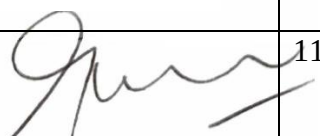
Journal of the Neurological Sciences. 2024;465:123186

Authors	Name	Signature	Date
1 st Author	E Sadiq		28/01/2025
2 nd Author	A Woodiwiss		31/01/2025
3 rd Author	G Tade		07/02/2025
4 th Author	G Norton		31/01/2025
5 th Author	G Modi		11/02/2025

Comments by primary supervisor: Contribution by E Sadiq: conceptualisation, study design, data acquisition and analysis, writing draft unassisted, editing and writing the final paper.

Article 4: Stroke, HIV and the immune reconstitution inflammatory syndrome in the absence of opportunistic infections.

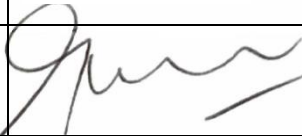
Journal of the Neurological Sciences. 2024;457:122880.

Authors	Name	Signature	Date
1 st Author	E Sadiq		28/01/2025
2 nd Author	S Katzew		6/01/2025
3 rd Author	J Nel		06/02/2025
4 th Author	G Tade		07/02/2025
5 th Author	A Woodiwiss		31/01/2025
6 th Author	G Norton		31/01/2025
7 th Author	G Modi		11/02/2025

Comments by primary supervisor: Contribution by E Sadiq: conceptualisation, study design, data acquisition and analysis, writing draft unassisted, editing and writing the final paper.

Article 5: Cardioembolic stroke in an HIV endemic region: underdiagnosed and severe.

BMJ Neurology Open. 2024;6(2):e000592

Authors	Name	Signature	Date
1 st Author	E Sadiq		28/01/2025
2 nd Author	A Woodiwiss		31/01/2025
3 rd Author	G Norton		31/01/2025
4 th Author	G Modi		11/02/2025

Comments by primary supervisor: Contribution by E Sadiq: conceptualisation, study design, data acquisition and analysis, writing draft unassisted, editing and writing the final paper.



PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Mohammad Eitzaz Sadiq (Student number: 0000464G) am a student
registered for the degree of PhD in the academic year 2025.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____

Date: 01/08/2025

Dedication

To those no longer with us.

My gentle father, Imtiaz Sadiq, my greatest teacher in neurology and in life, and the reason I embarked upon this PhD.

My little son, Samuel Solomon Sadiq, the reason I fought on to complete this PhD.

My inspirational supervisor, Professor Gavin Norton, who motivated me to believe I was worthy of this PhD.

To those who continue to walk this journey

My loving mother, Ghazala, and my caring, forerunning sister, Ammara, who have lovingly held my hand through every triumph and tragedy.

My sweetheart, Louisa, the love of my life, my selfless rock, who sacrifices so much for all those around her. Her talents are limitless, as is my love for her.

My darling daughters Sophia Ava, Sara Luccia, Sabina Zareen and Sania Maria, for showing me what love and joy truly are: I love you all so much. Puggy pug pug :)

Above all, to God be the glory

Publications arising from this research project

Paper 1: Sadiq E, von Klemperer A, Woodiwiss A, Norton G, Modi G. Stroke as the index presentation of traditional cardiovascular risk factors and human immunodeficiency virus in a South African population. *Journal of Stroke and Cerebrovascular Diseases*. 2024; 33(2):107529.

Paper 2: Sadiq E, Woodiwiss A, Tade G, Nel J, Norton G, Modi G. The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients. *HIV Medicine*. 2025;1-10.

Paper 3: Sadiq E, Woodiwiss A, Tade G, Norton G, Modi G. Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke patients in South Africa. *Journal of the Neurological Sciences*. 2024;465:123186.

Paper 3 Response to Letter to the Editor:

Sadiq E, Woodiwiss A, Tade G, Norton G, Modi G. HIV status and carotid intima-media thickness. *Journal of the Neurological Sciences*. 2024;466.

Paper 4: Sadiq E, Katzew S, Nel J, Tade G, Woodiwiss A, Norton G, Modi G. Stroke, HIV and the Immune Reconstitution Inflammatory Syndrome in the Absence of Opportunistic Infections. *Journal of the Neurological Sciences*. 2024;457:122880.

Paper 5: Sadiq E, Woodiwiss A, Norton G, Modi G. Cardioembolic Stroke in an HIV endemic region: underdiagnosed and severe. *BMJ Neurology Open*. 2024;6(2):e000592.

Other publications to which this work contributed:

1. Kolkenbeck-Ruh A, Woodiwiss AJ, Naran R, **Sadiq E**, Robinson C, Motau TH, Monareng T, et al. Carotid intima-media thickness, but not chronic kidney disease independently associates with noncardiac arterial vascular events in South Africa. *Journal of Hypertension*. 2019;37(4):795-804.
2. Kolkenbeck-Ruh A, Woodiwiss AJ, Monareng T, **Sadiq E**, Mabena P, Robinson C, Motau TH, et al. Complementary impact of carotid intima-media thickness with plaque in associations with noncardiac arterial vascular events. *Angiology*. 2020;71(2):122-30.
3. Motau TH, Norton GR, **Sadiq E**, Manyatsi N, Kolkenbeck-Ruh A, Robinson C, Tade G, et al. marked arterial functional changes in patients with arterial vascular events across the early adult lifespan. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2020 Jun;40(6):1574-86.
4. Motau TH, Norton GR, Mmopi KN, Bello H, Peterson VR, Libhaber C, **Sadiq E**, et al. Relations of aortic stiffness with arterial damage beyond brachial pressure are both dependent and independent of central arterial pulsatile load. *Journal of Hypertension*. 2021;39(4):718-28.
5. Mthembu N, Peterson VR, Norton GR, **Sadiq E**, Kolkenbeck-Ruh A, Naran R, Yusuf SM, et al. Proximal aortic stiffness modifies the relationship between heart rate and backward wave and hence central arterial pulse pressure. *Frontiers in Cardiovascular Medicine*. 2022;9:971141.
6. Peterson V, Els D, Fernandes DDS, Kinsey J-L, Dos Santos C, **Sadiq E**, Naran R, et al. Marked increases in proximal aortic characteristic impedance and hence forward wave pressures beyond brachial blood pressure in angiographic proven coronary artery disease. *Journal of Hypertension*. 2023;41(Suppl 3):e181.
7. Peterson VR, Els D, **Sadiq E**, Naran R, Monareng T, Abdool-Carrim T, Cassimjee I et al. Aortic characteristic impedance-induced increases in forward wave but not central arterial pulse pressure beyond brachial blood pressure in coronary artery disease. *J Hypertens*. 2025 Sep 1;43(9):1589-1597

Presentations arising from this research project

Conference Podium Presentations

1. Conventional Risk Factor-Independent Associations Between Indices of Athero- and Arteriosclerosis and Stroke in Human Immunodeficiency Virus Positive Patients in South Africa. Stroke and Hypertension Congress. Johannesburg, South Africa. 18/08/2016. **Awarded Best Oral Presentation.**
2. The Role of Atherosclerosis in HIV-associated Vasculopathy in Young South African Stroke Patients. Neurological Association of South Africa Annual Congress. Bloemfontein, South Africa. 19/04/2024
3. Invited Speaker to the African Stroke Organisation Congress (ASOC) 2024 in Lusaka, Zambia, 04/11/2024. Title of address: The Role of Atherosclerosis in HIV-related Stroke.

Conference Poster Presentations

1. Stroke as the Index Presentation of Traditional Cardiovascular Risk Factors and Human Immunodeficiency Virus. Poster Presentation. Neurological Association of South Africa Annual Congress. Cape Town, South Africa. 05/05/2023.
2. Stroke, HIV and the Immune Reconstitution Inflammatory Syndrome in the Absence of Opportunistic Infections. Poster Presentation. Neurological Association of South Africa Annual Congress. Bloemfontein, South Africa. 19/4/2024. Accepted for presentation at the World Congress of Neurology, Seoul, South Korea (12-15/10/2025)
3. Cardioembolic Stroke in an HIV endemic region: underdiagnosed and severe. African Stroke Organisation Congress (ASOC 2024). Lusaka, Zambia. 05/11/2024. **Awarded Best Poster Presentation.** Accepted for presentation at the World Congress of Neurology, Seoul, South Korea (12-15/10/2025)
4. The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients. Accepted for presentation at the World Congress of Neurology, Seoul, South Korea (12-15/10/2025)
5. Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke patients in South Africa. Accepted for presentation at the World Congress of Neurology, Seoul, South Korea (12-15/10/2025)

Abstract

Stroke in people living with HIV (PLWH) is an incompletely understood entity. The purpose of this cross-sectional research was to study a population of PLWH who have suffered a stroke in a low-and-middle-income country at the epicentre of the world's HIV epidemic. Of particular interest was exploring the role of atherosclerosis, which appears to be the primary mechanism mediating HIV-related stroke in data from high-income countries.

This study was conducted at a quaternary-level hospital in Johannesburg, South Africa, between August 2014 and November 2017. Patients with new-onset stroke were recruited and assessed using investigations including CT angiography and echocardiography. Validated non-invasive biomarkers of subclinical atherosclerosis, namely carotid intima-media thickness and aortic pulse wave velocity, were used to assess the role of atherosclerosis in patients with strokes of different aetiologies.

A total of 346 patients were recruited, of which 87 (25.1%) were PLWH. Of these, 33 (37.9%) were newly diagnosed with HIV on presentation with stroke. In the PLWH with ischaemic stroke ($n=85$, mean age 43.4 ± 12.5 years), vasculopathy was the most common stroke aetiology (28/85, 32.9%), of which only four were due to atherosclerosis (4.7%). There was no evidence of subclinical atherosclerosis in the other 24 PLWH with vasculopathy nor in those with undetermined stroke aetiology. HIV status had no impact on carotid intima-media thickness in multivariate regression models.

Vasculopathy was due to varicella-zoster virus in 8/28 PLWH with vasculopathy (21.4%). This was despite excluding patients with abnormal basic cerebrospinal fluid examination and the unavailability of optimal varicella-zoster diagnostics, suggesting the likely under-diagnosis of this potentially treatable stroke risk factor. Routine testing for varicella-zoster in the cerebrospinal fluid of all PLWH with stroke is thus recommended.

In the first 6 months of starting antiretroviral therapy, 32.1% of patients had a stroke, translating to a 3.5 times greater stroke risk in this period compared to beyond 6 months ($p=0.0002$). This was despite excluding all patients with opportunistic infections, suggesting a novel role of the Immune Reconstitution Inflammatory Syndrome as a stroke risk factor in the absence of such infections. This was also shown to be independent of atherosclerosis.

Cardioembolism accounted for 20% of strokes, compared to 4-9% in previous regional literature. This likely indicates the under-diagnosis of such conventional stroke aetiologies due to resource limitations. Cardiomyopathy was the predominant aetiology of cardioembolic stroke (76.4%), emphasising the need for a cost-effective and accessible biomarker in a region with limited access to echocardiography.

In conclusion, our findings of a low atherosclerotic burden, covert varicella-zoster-related strokes, and the role of immune reconstitution in the absence of opportunistic infections are examples of an alternative view to that in the current literature from high-income countries. This may be more applicable to the management of the vast majority of PLWH globally, who mostly reside in low-and-middle-income countries.

Acknowledgements

I am indebted to my esteemed supervisors.

Professor Girish Modi for his brilliant mind and how he has taught me to search for a unique angle in all research and writing.

Professor Angela Woodiwiss for her meticulous attention to detail, statistical genius and constant support.

The late Professor Gavin Norton for giving me the confidence to believe that my work was indeed worthy. It was an honour and privilege to learn from a true giant in his field.

Funding

I was awarded the Discovery Foundation Academic Fellowship Award (Reference number 035747), which provided financial assistance for the study.

TABLE OF CONTENTS

Declaration.....	ii
Dedication.....	vi
Publications arising from this research project.....	vii
Presentations arising from this research project	ix
Abstract.....	x
Acknowledgements.....	xii
List of Tables	xv
List of Abbreviations	xvi
1. INTRODUCTION	1
2. THE GLOBAL EPIDEMIOLOGY OF STROKE	3
2.1 The Burden of Stroke Disproportionately Affects Countries Such as South Africa...	3
2.2 Given South Africa’s High HIV Prevalence, This Must be Considered as a Separate Risk Factor	3
2.3 A Large Proportion of Stroke Patients Present with Previously Undiagnosed Risk Factors	4
3 STROKE IN PEOPLE LIVING WITH HIV IN SUB-SAHARAN AFRICA.....	5
3.1 Sub-Saharan Africa Needs Its Own Data.....	5
3.2 Stroke Risk In This Population Cannot Be Explained by Traditional Cardiovascular Risk Factors.....	6
3.3 The Role of Opportunistic Infections in HIV-Related Stroke.....	8
4 ATHEROSCLEROSIS IN PEOPLE LIVING WITH HIV IN SUB-SAHARAN AFRICA	9
4.1 Mechanisms of Accelerated Atherosclerosis in People Living with HIV	9
4.2 Atherosclerotic Large Vessel Occlusion is not a Prominent Cause of Stroke in People Living with HIV in sub-Saharan Africa	10
4.3 Exploring Markers of Subclinical Atherosclerosis	11

4.3.1	Carotid intima-media thickness is not affected by HIV status in stroke patients in sub-Saharan Africa.....	11
4.3.2	Pulse wave velocity as a measure of arteriosclerosis in PLWH with stroke	13
5	THE AETIOLOGY OF STROKE IN PLWH	14
5.1	The Challenges in Classifying Stroke Aetiology in PLWH.....	14
5.2	Vasculopathy in People Living With HIV is Predominantly Non-Atherosclerotic ..	19
5.3	Non-Atherosclerotic Vasculopathy: Risk Factors and Mechanisms.....	22
5.4	Stroke Due to Small Vessel Disease is Traditional Cardiovascular Risk Factor-Mediated and May be Unrelated to HIV Status	24
5.5	The Role of Subclinical Opportunistic Infections: Varicella-Zoster Virus.....	25
5.6	HIV-Associated Vasculitis is a Diagnosis of Exclusion	26
5.7	Antiretroviral Therapy and Stroke Risk	27
5.7.1	Antiretroviral therapy is not associated with atherosclerosis in HIV-related stroke in our setting	27
5.7.2	Stroke risk is increased in the first six months following antiretroviral therapy initiation.....	28
5.8	Cardioembolic Stroke is Underdiagnosed in Resource-Poor Settings	29
6.	IMPLICATIONS FOR FUTURE RESEARCH.....	31
6.1	The Role of Statins in Stroke Prevention in PLWH.....	31
6.2	Improved Diagnostics for Subclinical Infections.....	32
6.3	Prospectively Studying Stroke Risk After ART Initiation.....	32
6.4	A Cost-Effective Biomarker for Cardiomyopathy	33
7.	STRENGTHS AND LIMITATIONS.....	33
8.	CONCLUSION	35
	LIST OF REFERENCES	36
	Appendix A: Ethics Clearance Certificates	46
	Appendix B: Similarity Report (Turnitin)	49
	Appendix C: The Published Papers	51

List of Tables

Table 1: The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification. Adapted from Adams et al (1993)	15
Table 2: Excerpts from the proposed diagnostic criteria for the aetiology of stroke in PLWH, adapted from Benjamin et al.	17

List of Abbreviations

AF	Atrial fibrillation
ART	Antiretroviral therapy
AV	Atherosclerotic vasculopathy
CES	Cardioembolic stroke
cIMT	Carotid intima-media thickness
CSF	Cerebrospinal fluid
CT	Computed tomography
CVD	Cardiovascular disease
DM	Diabetes mellitus
ECG	Electrocardiogram
HICs	High-income countries
HIV	Human immunodeficiency virus
HT	Hypertension
IgG	Immunoglobulin G
IRIS	Immune reconstitution inflammatory syndrome
LMIC	Low-and-middle income country
NAV	Non-atherosclerotic vasculopathy
OI	Opportunistic infection
PCR	Polymerase chain reaction
PI	Protease inhibitor
PLWH	People living with human immunodeficiency virus
PWV	Pulse wave velocity
REPRIEVE	Randomized Trial to Prevent Vascular Events in HIV
RF	Risk factor
SSA	Sub-Saharan Africa
SVD	Small vessel disease
TOAST	Trial of ORG 10172 in Acute Stroke Treatment
TRF	Traditional cardiovascular risk factor
VL	Viral load
VZV	Varicella-zoster virus

1. INTRODUCTION

Stroke is the second leading cause of mortality worldwide, behind only ischaemic heart disease.

(1) The incidence in low-and-middle-income countries (LMICs) is rapidly increasing, resulting in 80% of the world's stroke-related deaths. (2) There are almost 40 million people living with HIV (PLWH) globally, of which 70% reside in LMICs. (3) The association between HIV and cardiovascular disease (including stroke) is well described, with HIV infection shown to confer a two-fold increased risk of cardiovascular disease when compared to HIV-negative controls. (4)

The risk factors and mechanisms of stroke in PLWH are not clear. It is also uncertain if these differ between high-income countries (HICs), where most scientific data originates, and LMICs, where most of the world's PLWH population resides. (5) There are many differences between PLWH in HICs and LMICs, including demographics, the prevalence of traditional cardiovascular risk factors (TRFs), and HIV-related factors such as the extent of immunosuppression and antiretroviral therapy (ART) exposure. (5) It may thus be speculative to extrapolate HIC data onto a completely distinct population to whom the described risk factors and mechanisms of stroke may not necessarily apply.

The purpose of this research was to study a population of PLWH who have suffered a stroke in an LMIC at the epicentre of the world's HIV epidemic. We are in a unique position in South Africa to be able to study both treated and untreated HIV infection in an LMIC setting while having access to expertise and diagnostic modalities on par with those in most HICs. The feature of this research, which is truly unique in the HIV-stroke literature, is our exclusion of all PLWH with any overt opportunistic infection (OIs), enabling us to produce a clearer picture of the role of HIV itself.

Our particular interest was exploring the role of atherosclerosis, which appears to be the primary mechanism mediating stroke in PLWH in HICs. (6). We attempted to establish whether this applies to an LMIC PLWH population or if alternate mechanisms are more prevalent. We achieved this using a combination of clinical expertise and diagnostic investigations, including non-invasive biomarkers of subclinical atherosclerosis. The results of our study were unexpected at times. They demonstrated the vast differences in HIV medicine across different regions of the world, emphasising the need for region-specific research such as ours.

This work has thus far contributed to 12 published papers, of which five will constitute this PhD by Publication. All these papers have been published in international peer-reviewed journals accredited by the Department of Higher Education and Training (DHET) in South Africa.

In Paper 1, entitled “*Stroke as the index presentation of traditional cardiovascular risk factors and human immunodeficiency virus in a South African population*”, we started by describing the risk factors for stroke in our population (including PLWH and HIV-negative patients). We analysed how many of these risk factors were only diagnosed at the time of their stroke and the implications thereof. The following two papers reported on the crux of our work, exploring the role of atherosclerosis in stroke in PLWH. Paper 2 (“*The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients*”) provided detailed descriptions of HIV-related vasculopathy and the role of atherosclerosis in this process. We also discussed the role of covert opportunistic infections as an aetiology for stroke, particularly varicella-zoster virus (VZV). Paper 3 (“*Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke patients in South Africa*”) provided compelling evidence for the lack of impact of HIV infection on carotid intima-media thickness (cIMT), a marker of subclinical atherosclerosis. In Paper 4 (“*Stroke, HIV and the immune reconstitution inflammatory syndrome in the absence of opportunistic infections*”), we reported on a novel finding of stroke as a manifestation of the immune reconstitution inflammatory syndrome (IRIS) despite the exclusion of opportunistic infections. The final paper (“*Cardioembolic stroke in an HIV endemic region: underdiagnosed and severe*”) emphasised the need to still consider conventional stroke aetiologies in PLWH (in this case, cardioembolic stroke).

The following chapters will systematically and integratedly discuss each of these papers in the context of the current literature. Relevant methodologies, novel findings and clinical implications will be summarised, and the reader will be referred to the attached papers and supplemental data for further details. This research has been a long and arduous journey. We believe that the results have added meaningfully to local and global knowledge on a topic that is as relevant, uncertain and challenging today as it was when the study commenced.

2. THE GLOBAL EPIDEMIOLOGY OF STROKE

2.1 The Burden of Stroke Disproportionately Affects Countries Such as South Africa

The incidence of cardiovascular disease (CVD) has shown a steady decline in HICs over the past three decades. (7) In contrast, LMICs have seen a rapid increase in CVD burden, likely related to increasing urbanisation and the subsequent epidemiological shift towards non-communicable diseases. (7, 8) Consequently, the burden of disease of stroke now falls disproportionately on LMICs such as South Africa, with 80% of the world's burden of stroke-related deaths and 90% of its disability-adjusted life years. (2, 9) Stroke patients in LMICs also have greater severity of stroke and, as such, have higher 3-month mortality than those in HICs. (10) Despite this disproportionately large burden of disease, LMICs have access to fewer resources to prevent, diagnose and treat stroke. (11, 12)

Large multinational studies affirm that traditional cardiovascular risk factors (TRFs) are responsible for the majority of the population's risk for stroke. (12-15) These TRFs include hypertension (HT), diabetes mellitus (DM), dyslipidaemia, abdominal obesity, unhealthy diet, smoking and sedentary lifestyle. HT is the leading TRF in sub-Saharan Africa (SSA). (16) However, only a quarter of patients in SSA are aware of their HT status. (17) The proportion of individuals aware of the status of their other TRFs is likely to be even lower than this, particularly diseases such as dyslipidaemia which are reliant on laboratory investigations to make the diagnosis.

2.2 Given South Africa's High HIV Prevalence, This Must be Considered as a Separate Risk Factor

It is inappropriate to consider stroke risk factors in South Africa without discussing the contribution of HIV. As of 2024, it was estimated that 8 million South Africans are living with HIV, making it the country with the most PLWH in the world. (17) PLWH have been shown to have a higher incidence of stroke and at younger ages than those who are HIV-negative. (18-21) There is now good evidence to suggest that HIV may be considered an independent risk factor for stroke. (4, 21-24)

In SSA, we are now faced with the colliding epidemics of the world's largest HIV population and a rapidly mounting burden of a non-communicable disease in the form of CVD. (25) Not only are these two epidemics colliding, they are significantly overlapping, with HIV likely to

increase cardiovascular risk further. The inextricable interplay of these two epidemics undoubtedly requires urgent intervention.

2.3 A Large Proportion of Stroke Patients Present with Previously Undiagnosed Risk Factors

There is evidence that the increased burden of stroke in LMICs may, in part, be due to worse control of modifiable TRFs and reduced access to healthcare. (26) The fragile, under-resourced public healthcare system in LMICs is under tremendous pressure to provide a basic life-sustaining service to the population. Screening perceivably ‘normal’ individuals for TRFs to potentially prevent future cardiovascular events is understandably not always a priority.

Identifying shortcomings in TRF screening and diagnosis is the first step in implementing strategies to decrease and control the TRF burden, which has been shown to contribute to major CVD. Thus, in Paper 1 (*“Stroke as the Index Presentation of Traditional Cardiovascular Risk Factors and Human Immunodeficiency Virus in a South African Population”*), we sought to identify what proportion of each TRF, as well as HIV, was first diagnosed only after the occurrence of a dreaded cardiovascular event, in this case, stroke. Our secondary objective was to explore if the missed diagnosis of a risk factor (RF) had any impact on stroke severity.

Each of the RFs had strictly defined criteria for diagnosis as described in the paper, and transient post-stroke physiological changes which may have confounded the diagnosis were taken into account. HPT, DM, dyslipidaemia, atrial fibrillation (AF) and HIV (as a non-traditional RF) were coded as either ‘newly-diagnosed’ or ‘previously-diagnosed’ based on clinical history and available past medical records. Obesity and smoking are difficult to define as “newly diagnosed” and were thus not analysed further.

Of the 346 patients recruited, stroke was the index presentation for at least one RF in 199 patients (57.5%). Furthermore, of the 281 patients known with RFs before their stroke, 162 (57.7%) were newly diagnosed with one or more additional RFs on presentation with stroke. The greatest proportion of newly-diagnosed patients compared to those with a pre-existing diagnosis was in dyslipidaemia (76.0% of dyslipidaemics were newly-diagnosed). Notably, patients newly-diagnosed with dyslipidaemia were significantly younger (median age 53 (IQR 44-63) years vs 62 (IQR 51-71) years, $p=0.02$) and had more severe strokes than those who were previously-diagnosed (median National Institute of Health Stroke Scale of 12 (IQR 8-16) vs 7 (IQR 4-12), $p=0.0007$). Multivariate regression models confirmed that undiagnosed

dyslipidaemia was the main driver of stroke severity (β -Coefficient 3.281 ± 1.350 , $p=0.017$). This provides compelling evidence of the detrimental consequences of a poorly screened RF.

While it is unknown how many of the 199 patients with undiagnosed RFs had ever sought healthcare in the past, the 281 patients with at least one established RF are assumed to have all accessed healthcare. We do not know how many of these RFs were previously screened for (negatively) at the healthcare centre the patient accessed. If they were not screened, this may represent a relatively easily accessible, high-risk population which will potentially benefit greatly from the identification and treatment of all major RFs before they result in stroke and other CVD. While our study was not designed to establish whether HIV infection is a risk factor for stroke, the prevalence of HIV in our stroke population was almost double the expected prevalence in the general population (25.1% vs 13.1%, χ^2 , $p < 0.0001$). (17)

Our work also provided other clues as to which particular populations may need to be targeted for the screening of specific RFs, including higher proportions of undiagnosed DM and dyslipidaemia in younger patients compared to higher proportions of undiagnosed HIV in older patients. There were also sex differences, with males having a significantly higher proportion of undiagnosed HT and HIV when compared to females.

If confirmed in subsequent prospective studies, this may guide policy-makers in revising screening protocols in these particular subgroups of patients. Our data on the high proportion of undiagnosed RFs is surprisingly similar to that from HICs with well-resourced healthcare systems. These include studies from Switzerland (27), Taiwan (28), Austria (29) and France (30), and indicate that this is a global public health challenge. However, to our knowledge, ours is the first study to demonstrate the detrimental effects of poor RF diagnosis on stroke severity.

Overall, our findings point to the need for urgent public health interventions in the screening and diagnosis of RFs to prevent catastrophic cardiovascular outcomes such as stroke.

3 STROKE IN PEOPLE LIVING WITH HIV IN SUB-SAHARAN AFRICA

Having briefly explored the risk factors for stroke in our population in Paper 1, the remainder of this discussion focuses on HIV and its multifaceted impact on stroke.

3.1 Sub-Saharan Africa Needs Its Own Data

Despite being at the epicentre of the HIV epidemic, there is a paucity of data on HIV-related strokes from LMICs, including those in SSA. (21) The differences in HIV between LMICs and

HICs go well beyond just the differences in economies and healthcare resources of the relevant regions. The profile of PLWH also differs markedly. PLWH in HICs are much older, with a subsequently greater burden of TRFs when compared to PLWH in LMICs. (5, 6) They also have higher proportions of cigarette smokers, recreational drug users and men who have sex with men than cohorts from LMICs. (6, 31)

The exposure to ART also differs markedly between HICs and LMICs. This is not only in the proportions of PLWH on treatment (higher in HICs) but also the duration of treatment (the widespread rollout of ART in HICs preceded that in LMICs by over a decade) and the regimens used (including far greater protease inhibitor (PI) usage in HICs). (32, 33) In addition, there are also virological differences, where SSA is predominantly affected by Clade C of the virus, as opposed to Clade B in HICs. (34) Whether this has any significance in stroke pathogenesis is unknown.

The validity of extrapolating evidence from a dissimilar population that harbours less than 20% of the world's HIV population onto a population with significant differences, as described above, has always been a major challenge in HIV research, and this remains the case in HIV-related stroke research.

While the number of studies from LMICs is encouragingly gradually increasing, including invaluable collaborations with academic centres in HICs, most descriptions of stroke in PLWH in LMICs are still plagued by severe resource limitations resulting in large amounts of suboptimal investigations and subsequently underpowered data. (5, 21) This has resulted in the forced over-reliance on data from HICs, despite us being well aware of the marked dissimilarities in PLWH populations between different regions. The importance of region-specific research, particularly from a region with the world's highest prevalence of HIV, is thus paramount.

3.2 Stroke Risk In This Population Cannot Be Explained by Traditional Cardiovascular Risk Factors

Proposed mechanisms of HIV-related stroke in HICs are currently centred on TRFs in an ageing PLWH population. (24, 35) ART itself has also been proposed to contribute to the incidence of stroke in HICs, particularly PIs, and also appears to be TRF-mediated. (24, 36, 37) In the non-stroke literature, most HIC data suggest an increased prevalence of TRFs in PLWH when compared to HIV-negative controls. (38-40) It would thus be logical that PLWH would be at increased risk of stroke based purely on TRFs. However, in SSA, the association

between HIV status and TRF burden does not appear to be as strong as in HICs, as suggested by two large meta-analyses, including only studies from SSA. (31, 41, 42) These showed only weak associations, if any, between HIV status and TRFs. Large South African population-based surveys and data from the subsequent Ndlovu Cohort Study corroborate this. (40, 43, 44) These studies included both ART-naïve and ART-exposed patients.

Previous stroke studies from SSA have reported similar trends regarding TRFs in PLWH. (1, 6) Most have shown a similar TRF profile in PLWH and HIV-negative stroke patients (5, 21, 22, 45), while some have reported a lower prevalence of certain TRFs in PLWH (19, 20, 46). It thus appears doubtful that TRFs play any more significant role in stroke risk in PLWH than in HIV-negative individuals in SSA. (21)

In Paper 2, “*The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients*” we begin with analysing the prevalence of TRFs in PLWH with stroke. We found that, despite being of a relatively young mean age (43.4 ± 12.5 years), the prevalences of TRFs in PLWH with stroke were relatively high at face value (HT 43.5%, smoking 25.9%, obesity 25.8%, dyslipidaemia 24.7% and DM 5.9%) The median number of TRFs in PLWH was however only 1.0 (IQR 0-2).

We then compared the TRF prevalence in PLWH to HIV-negative stroke patients. For this purpose, we age- and sex-matched 109 HIV-negative controls to our 85 PLWH with ischaemic stroke. The controls were recruited simultaneously as part of the same study and underwent the same investigation protocol as the PLWH. We found that PLWH, when compared to the HIV-negative controls, had lower prevalences of HT (43.5% vs 60.6%, $p=0.02$) and DM (5.9% vs 22.9%, $p=0.001$). The difference in the median number of TRFs (1.0 (IQR 0-2) in PLWH vs 2.0 (IQR 1-2) in HIV-negative patients) was also statistically significant ($p=0.02$).

We thus concluded that, while the prevalences of TRFs in PLWH were higher than anticipated given the young mean age, these were significantly lower than in age- and sex-matched HIV-negative stroke patients. However, we must acknowledge that our study design did not include non-stroke controls and thus could not make further inferences about the role of TRFs in stroke patients compared to ‘healthy’ controls. We thus definitely cannot conclude that TRFs are not relevant to stroke in PLWH. However, their role appears to be less prominent than in HIV-negative controls.

This has important implications, as the early identification of the risk of CVD in our PLWH population cannot rely on conventional tools, which are highly reliant on TRF prevalence. This

has been confirmed in previous studies from SSA, showing the compromised validity of risk prediction tools in PLWH (including the American College of Cardiology (ACC)/American Heart Association (AHA) risk prediction tool for atherosclerotic CVD (ASCVD) or the Framingham Risk Score (FRS). (47, 48)

It appears as though cardiovascular risk prevention in PLWH may not be appropriately achieved by solely targeting TRFs. Other factors must play a more significant role and will be discussed below.

3.3 The Role of Opportunistic Infections in HIV-Related Stroke

In SSA, opportunistic infections (OIs) have been shown to contribute to 23-55% of HIV-related stroke. (21) A recent study from Pretoria, South Africa, even reported evidence of OIs in up to 70% of PLWH with stroke. (19) Meningitis appears to be the commonest OI contributing to stroke, diagnosed in almost one-third of PLWH with stroke in SSA. (21) While OIs are more common in ART-naïve patients, they also remain a significant cause of morbidity and mortality in the ART-exposed. There are numerous mechanisms by which OIs may result in stroke, including meningitis, infectious vasculitis, septic emboli or an infection-mediated pro-coagulable state. (6, 18, 49)

In the absence of overt OIs, it is unknown if the aetiology and pathogenesis of stroke in PLWH in SSA is any different to that seen in HIV-negative patients. We thus decided to exclude all patients with evidence of meningitis or other overt OIs from our entire cohort. This is a distinct and unique aspect of our study design, which, to our knowledge, has not been done previously in the global HIV literature. While PLWH are more likely to acquire meningitis than HIV-negative patients due to their compromised immunity, the final mechanism of stroke secondary to meningitis is likely no different in PLWH compared to the HIV-negative. (18) It involves infective vasculitis and endarteritis of small blood vessels and is independent of TRFs. (6)

The inclusion of patients with infection, who are more likely to be severely immunocompromised and have fewer TRFs, may have resulted in falsely low TRF prevalences in PLWH. Our lower TRF prevalence in PLWH, despite the exclusion of meningitis and overt OIs, suggests there may be an alternative underlying mechanism for stroke in PLWH in our population beyond that mediated by TRFs and infections.

Similarly, the perceived role of atherosclerosis in HIV-related stroke may be diluted out in cohorts with high OI burdens and thus potentially under-recognised, which motivated us to

exclude such patients from our cohort. Later in the thesis, we will discuss some remarkably unexpected findings regarding the role of covert OIs such as varicella-zoster virus (VZV).

However, at this point, we will move from our TRF analysis to discussing whether there is any evidence for the presence of accelerated atherosclerosis in PLWH with stroke in SSA, which may be driven by HIV-related factors, as opposed to TRFs.

4 ATHEROSCLEROSIS IN PEOPLE LIVING WITH HIV IN SUB-SAHARAN AFRICA

PLWH are at increased risk of atherosclerotic CVD. A meta-analysis including almost 800 000 PLWH (with 3.5 million person-years follow-up) estimated this risk to be double that of HIV-negative controls. (4) Importantly, most of the 16 cohorts included in the risk ratio calculations were from Europe and North America, with only one from SSA (Tanzania). (23)

4.1 Mechanisms of Accelerated Atherosclerosis in People Living with HIV

There is evidence for accelerated atherosclerosis in PLWH (31, 50), and the mechanisms for this are briefly discussed below.

TRFs are responsible for the overwhelming majority of atherosclerosis, with the evidence for alternate mechanisms not being as robust. As discussed in Section 2.1 above, PLWH in HICs have been reported to have a greater prevalence of TRFs than HIV-negative controls. However, this does not appear to apply to PLWH in SSA. TRF-mediated atherosclerosis is thus unlikely to be a significant role player in HIV-related stroke in SSA.

The role of ART in the atherogenic process has also received much attention. In particular, protease inhibitors (PIs) have been associated with an increased CVD risk, likely related to their metabolic side effects. (36, 51) Due to economic constraints, PI usage in SSA has never been part of first-line treatment; thus, only a small proportion of PLWH have ever been exposed to PIs. (32) The evidence for the atherogenic potential of other antiretroviral agents is not as convincing as it is for PIs.

HIV-related chronic inflammation, immune activation and subsequent endothelial dysfunction have been proposed as key drivers of atherosclerosis. This may occur even in the setting of viral suppression. (18, 52, 53) Of all the proposed mechanisms above, this may be the most significant contributor to atherosclerotic disease in our setting.

However, the focus of our work was not to establish the mechanisms of atherosclerosis in PLWH but to retreat one step and attempt to establish whether or not atherosclerosis is even a major contributor to CVD in PLWH in our setting.

4.2 Atherosclerotic Large Vessel Occlusion is not a Prominent Cause of Stroke in People Living with HIV in sub-Saharan Africa

Previous studies from SSA reporting on stroke aetiology in PLWH have reported very low prevalences of atherosclerotic large vessel occlusion (generally under 10%). (5, 21). This is in striking contrast to data from HICs, where large vessel atherosclerosis is reported to be the most common cause of stroke in PLWH. (6)

Even in the non-HIV literature, atherosclerotic carotid stenosis appears to be a rare cause of stroke in SSA. (54) Suboptimal investigations due to resource constraints are a possible contributor. (5, 21) It has also been proposed that atherosclerosis (particularly large vessel extracranial disease) is a marker of the epidemiological transition, explaining why its prevalence appears low in LMICs which are at earlier stages of the transition. (54)

We detected a very low prevalence of large vessel atherosclerotic stroke in our cohort (only 4 out of 85 PLWH (4.7%)). This was similar to the prevalence in age- and sex-matched HIV-negative controls (3/109 (2.8%), $p=0.47$) and is discussed in the papers titled '*Cardioembolic stroke in an HIV endemic region: underdiagnosed and severe*' and '*The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients*'. Our results were remarkably similar to a study from the same institution over 15 years prior, where only five out of 177 stroke patients (3%) were classified as having large vessel atherosclerotic stroke. HIV status was not reported. (55) Again, this may suggest a different phenotype of patients compared to HICs with a lower atherosclerotic burden. However, the apparent lack of epidemiological transition in almost two decades was a surprising finding.

At face value, this is evidence for atherosclerosis not being a prominent cause of occlusive vascular disease in our setting in both PLWH and HIV-negative patients. However, our findings are insufficient to completely exclude the atherosclerotic process as a role-player. To investigate this further, we tried to establish whether there was any evidence for the presence of subclinical atherosclerosis in our stroke patients. While not necessarily directly causative in the arterial occlusion, the detection of subclinical atherosclerosis in specific patient groups could provide clues that the underlying stroke mechanism in that group may be related to

atherosclerosis. On the contrary, and importantly, the absence of any evidence of subclinical atherosclerosis makes it very unlikely that this is the case.

The following section will discuss the investigations we performed to assess subclinical atherosclerosis.

4.3 Exploring Markers of Subclinical Atherosclerosis

4.3.1 Carotid intima-media thickness is not affected by HIV status in stroke patients in sub-Saharan Africa

Carotid intima-media thickness (cIMT) has been widely investigated and validated as a surrogate marker of atherosclerosis and is regarded as an accurate predictor of future cardiovascular events. (56)

Previous studies on the effect of HIV status on cIMT have yielded inconsistent results. While some have shown evidence of an increased cIMT in PLWH compared to HIV-negative controls (57-61), others have shown no association (40, 43, 62-64). Others have demonstrated only associations in certain groups of patients, including specific age categories (older patients). (65)

A meta-analysis of 26 studies (66) and another large study (57) not part of this prior meta-analysis have suggested that the TRF-independent effects of HIV on cIMT are modest at best (0.02 to 0.06mm higher in PLWH). The meta-analysis reported evidence of publication bias ($p=0.001$), with smaller studies being more likely to report an increased cIMT in PLWH when compared to larger studies, which mostly showed minimal or no effect. (66) There was also significant heterogeneity between studies ($I^2 = 86.5\%$) and evidence of confounding.

Strikingly, most of this data comes from HICs, where the profile of PLWH differs markedly from LMICs, as previously discussed. A large multinational study from four African countries (Burkina Faso, Kenya, Ghana and South Africa) examined cIMT in 8872 patients and reported an inverse association of HIV status with cIMT ($\beta = -8.86$ 95% CI -15.7 to -2.03). (67) No convincing explanation was offered by the authors for this unexpected finding but may again be indicative of a distinct patient profile in SSA in contrast to HICs. Other cohorts from SSA all reported no effect of HIV status on cIMT. (40, 43, 62, 63, 68, 69)

Importantly, all these studies have been done in non-stroke patients. To our knowledge, there is no previously published data on cIMT in PLWH who have suffered a stroke. In our paper titled '*Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke*

patients in South Africa,’ we sought to investigate the relationship between HIV status and cIMT in stroke patients and to establish what the determinants of cIMT are in this population.

Patients were assessed for the presence of TRFs (with clearly defined criteria as described in the paper) and HIV infection and were thoroughly investigated for stroke aetiology as per our study protocol. The methodology is described in the paper as follows:

cIMT was measured by carotid Doppler studies, which were performed by a single operator using high-resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington) employing a linear array 7.5 MHz probe as described previously. (70) Images of at least 1cm length of the far wall of the distal portion of the right common carotid artery from an optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualised simultaneously) at least 1 cm proximal to the flow divider were obtained. cIMT measurements were determined using semi-automated border detection and quality control software. A total of six separate measurements (three on each side) were averaged to a single mean cIMT. All images were saved electronically, allowing for later quality control and re-evaluation if necessary. A cIMT ≥ 0.70 mm was considered to be pathological and suggestive of subclinical atherosclerosis, as per previously published data. (71, 72) Patients whose cIMT data were of insufficient quality were excluded from the study. Due to known differences in cIMT in different racial groups, we chose to perform these analyses only in the commonest racial group in our cohort (i.e. black Africans). (73)

168 patients were included in the analyses, of which 62 (36.9%) were PLWH. The mean cIMT was higher in HIV-negative patients when compared to PLWH (0.79 ± 0.19 mm vs 0.69 ± 0.18 mm, $p=0.0021$). However, after adjusting for age, sex, HT, DM, smoking, total cholesterol, body mass index and stroke aetiology, there was no difference in mean cIMT between the groups (0.76 ± 0.16 mm vs 0.73 ± 0.17 mm, $p=0.29$). Regression models revealed the determinants of cIMT to be age, HT and total cholesterol, while the determinants of increased cIMT (≥ 0.70 mm) were only age and HT. HIV status had no impact on cIMT in these models.

Limitations of the study included the lack of a non-stroke PLWH group to compare cIMT in PLWH with and without stroke. (73)

In conclusion, we found no evidence of additional subclinical atherosclerosis in PLWH with stroke when compared to HIV-negative controls. It remains possible that HIV may confer an increased cardiovascular risk by affecting vessels in a manner which is not detectable on cIMT measurement, i.e. a non-atherosclerotic vasculopathy, which will be discussed in the section on vasculopathy. (21, 74)

Our findings of a low atherosclerotic burden may suggest that the undisputed value of cIMT in cardiovascular risk prediction may be compromised in our setting. The implications of this include the need to develop and test alternative risk prediction tools specific to PLWH in SSA. Traditional CVD risk prediction models have already been shown to be inaccurate in our population. (47, 48) The reasons for this may be partially related to our findings of a stroke population with a low TRF prevalence and a subsequently low atherosclerotic burden.

4.3.2 Pulse wave velocity as a measure of arteriosclerosis in PLWH with stroke

It is presently well accepted that aortic stiffening, indexed by an increase in carotid-femoral pulse wave velocity (PWV), predicts cardiovascular outcomes independent of TRFs. (75) One possible explanation for this relationship is that indices of aortic stiffness may closely reflect the extent of arteriosclerosis. (75) If HIV-associated occlusive arterial disease is indeed related to early atherogenesis, PWV measurement offers an alternative biomarker for cardiovascular risk prediction in PLWH. This measurement is considerably simpler and cheaper to perform than cIMT, or it may complement IMT measurements.

PWV was measured using the following technique: after participants had rested for 15 minutes in the supine position, sequential recordings of the arterial pressure waveform at the carotid and femoral arteries were determined using applanation tonometry. A high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas), interfaced with a computer using SphygmoCor software, version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) was used. Arterial pressure waveform recordings were discarded when the systolic or diastolic variability of consecutive waveforms exceeded 5% or when the amplitude of the pulse wave signal was less than 80 mV. To determine aortic PWV, distances from the suprasternal notch to the carotid sampling site (distance A) and from the suprasternal notch to the femoral artery (distance B) were measured. Pulse wave velocity distance was calculated as distance B minus distance A. Pulse transit time, calculated as the mean time difference between

sites A and B, was determined from the average of 10 consecutive beats. Aortic PWV was calculated as the ratio of the distance in metres to the transit time in seconds. All measurements were done by a single trained operator. A PWV of ≥ 10 m/sec was considered abnormal and suggestive of increased aortic stiffness, as per previously published data. (75, 76)

In our papers titled '*The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients*' and '*Stroke, HIV and the immune reconstitution inflammatory syndrome in the absence of opportunistic infections*,' we used PWV as an adjunct to cIMT in assessing for subclinical atherosclerosis. Our findings are discussed in sections 5.2 - 5.4 and 5.7.2 below.

5 THE AETIOLOGY OF STROKE IN PLWH

5.1 The Challenges in Classifying Stroke Aetiology in PLWH

Describing and defining stroke aetiology for the purposes of research studies is a complex task, particularly in any population of patients in whom there is a greater chance of non-conventional stroke aetiologies. This includes stroke in the young, and stroke in PLWH (77), both of which were common in our cohort. The mean age of our 346 participants was 52.3 ± 15.7 years, with 48.5% of patients fitting the definition of 'young stroke' (age <50 in males and <55 in females). Out of 321 patients, 87 (27.1%) who consented to testing were HIV positive. Thus, we faced a challenge on how best to classify stroke aetiology in our cohort.

In our publications on this work, we decided to use different aetiological classifications for stroke, depending on the data being presented and what was used in previous literature on the particular topic, to enable meaningful comparison.

The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) is the most widely-used stroke aetiological classification. (78) Based on strictly defined criteria, it categorises stroke aetiology into cardioembolic, large vessel atherosclerotic, small vessel disease, stroke of undetermined aetiology, and stroke of other aetiology (See Table 1). The majority of 'classical' stroke patients fall into one of the first three categories. (79)

Table 1: The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification. Adapted from Adams et al. (1993) (78)

Aetiological Category	Criteria
Large-artery atherosclerosis	• $\geq 50\%$ stenosis of a supplying intra- or extracranial artery on Doppler or angiography • Presumably due to atherosclerosis • Exclude cardioembolism
Cardioembolism	• <u>High-risk</u> (including mechanical heart valve, atrial fibrillation, dilated cardiomyopathy, infective endocarditis) • <u>Medium risk</u> (including mitral valve prolapse, patent foramen ovale, atrial flutter)
Small-vessel occlusion (lacunar)	• Traditional lacunar syndrome* • No evidence of cortical dysfunction • Size $< 15\text{mm}$
Stroke of other determined aetiology	• Vasculopathy (non-atherosclerotic) • Hyper-coagulable state • Vessel dissection • Recreational drugs
Stroke of undetermined aetiology	• ≥ 2 causes identified • Negative evaluation • Incomplete evaluation

*The traditional lacunar syndromes include pure motor hemiparesis, pure sensory, sensorimotor, clumsy-hand dysarthria and ataxic hemiparesis.

In Paper 4, ‘*Cardioembolic stroke in an HIV endemic region: underdiagnosed and severe*’, we briefly presented the aetiology of our 85 PLWH and contrasted it to 109 age- and sex-matched HIV-negative controls according to the TOAST classification. We described how the two

groups had similar proportions of cardioembolic (20% vs 30%), large vessel atherosclerotic (5% vs 3%), small vessel disease (17% vs 18%) and strokes of undetermined aetiology (26% vs 36%). However, the proportion of patients with stroke of other aetiology was significantly higher in PLWH (32.9% vs 11.9% in HIV-negative controls, $p < 0.001$). Our proportion of strokes of undetermined aetiology was in keeping with data from most previous international cohorts (including in the non-HIV literature), with incomplete investigations being the commonest reason to be assigned into the category (75%). HIV status was not blinded in our study; thus, PLWH were possibly more extensively investigated than HIV-negative patients, but this difference was not statistically significant.

Our finding of a very high proportion of stroke of other aetiology (almost one-third) was not unexpected for the above reasons regarding the limitations of the TOAST classification in populations such as ours. Similar findings in previous literature were a key motivation for a group of global experts to propose an alternative aetiological classification for stroke in PLWH. This aimed to better define those patients that fall under the ‘undetermined’ and ‘other’ aetiological categories of the TOAST classification (over 50% of PLWH with stroke) and will be discussed below. (77)

In 2016, after the commencement of our study, Benjamin and colleagues published a proposed minimum battery of tests to guide stroke aetiology classification in PLWH. (77) These included radiological investigations (chest x-ray, uncontrasted computed tomography (CT) brain and carotid ultrasound), laboratory investigations (including HIV and syphilis testing, full blood count, coagulation screen, basic cerebrospinal fluid (CSF) analyses) and echocardiography. (77) An ‘optimum’ battery of tests was also proposed, which included angiography, CSF viral studies, transoesophageal echocardiography and Holter electrocardiogram (ECG). Almost all these investigations (as well as many additional ones) were, fortunately, part of our study protocol, except sickle cell disease testing (which has a relatively low prevalence in South Africa compared to other regions on the continent) and CSF varicella-zoster immunoglobulin G (IgG). This test was unavailable at our institution; thus, we only performed VZV polymerase chain reaction (PCR) testing on CSF.

Based on these investigations, Benjamin et al. (2016) proposed an algorithm to guide the classification of stroke aetiology in PLWH (Table 2). (77) These were ranked hierarchically, with better established and treatable aetiologies considered first (such as OIs and cardioembolic stroke), followed by vasculitis, vasculopathy (atherosclerotic and non-atherosclerotic),

coagulopathies and lastly, small vessel disease (SVD). The TOAST category of ‘stroke of undetermined aetiology’ was revised to be either incompletely investigated, multifactorial or cryptogenic stroke (complete defined battery of investigations with no aetiology found). While each of the above case definitions are well described in detail in the paper, including categorising them as either ‘confirmed’ or ‘probable,’ a few aspects of the case definitions may be open to debate. These will be discussed below and pertain particularly to vasculopathy. The definitions for cardioembolic stroke and SVD were almost identical to those in the TOAST classification. The priority given to potentially treatable causes of stroke in the hierarchy was an insightful suggestion from the authors and ought to be implemented in the workup of all stroke patients, not only PLWH.

Table 2: Excerpts from the proposed diagnostic criteria for the aetiology of stroke in PLWH, adapted from Benjamin et al. (2016) (77)

Aetiological Category	Confirmed*	Probable
Varicella-zoster virus (VZV)	Positive CSF VZV PCR or Positive VZV IgG-index	- CSF VZV PCR negative (or unavailable) AND - Zoster rash (trigeminal or cervical) within 12 weeks before stroke
Cardioembolism	Presence of high-risk cardioembolic lesions as per the TOAST criteria	Presence of medium-risk cardioembolic lesions as per the TOAST criteria
Atherosclerotic vasculopathy	- intra/extra-cranial carotid stenosis ($\geq 50\%$) supplying the ischaemic field AND age $>45y$ OR - age $\leq 45y$ AND exposure to TRFs or hepatitis C	- Age $>45y$ or $\leq 45y$ with exposure to TRF or hepatitis C PLUS one of: - Clinical history suggestive of intra/extra-cranial stenosis (i.e. TIA, ischaemic heart disease, peripheral vascular disease)

Aetiological Category	Confirmed*	Probable
		• $\geq 50\%$ stenosis in a non-affected vascular territory • 30-50% stenosis in an extra/intracranial artery
Non-atherosclerotic vasculopathy	• intra/extra-cranial carotid stenosis ($\geq 50\%$) supplying the ischaemic field AND age ≤ 45y AND absence of TRFs	• Age ≤ 45y with no TRFs or hepatitis C PLUS one of: • $\geq 50\%$ stenosis in a non-affected vascular territory • 30-50% stenosis in an extra/intracranial artery
HIV-associated vasculitis	• Histopathology or classic angiographic features of vasculitis AND • Other causes of vasculitis excluded	• CT/MRI confirmation of ischaemic change in >1 vascular territory AND • Other causes of vasculitis excluded
Small vessel disease	• Traditional lacunar syndrome, as per TOAST criteria AND • CT/MRI lesion ≤ 20mm diameter	• Traditional lacunar syndrome, as per TOAST criteria AND • Normal unenhanced CT brain with 24 hours of onset

CSF, cerebrospinal fluid; PLWH, people living with HIV; TIA, transient ischaemic attack; TOAST, Trial of ORG 10172 in Acute Stroke Treatment; TRF, traditional risk factor; VZV, varicella-zoster virus.

*In the absence of histopathological confirmation

Other proposed aetiological categories included infections (tuberculosis, cryptococcus, or syphilis), haematological (antiphospholipid syndrome and thrombotic thrombocytopenic purpura) and undetermined (incompletely investigated, multifactorial or cryptogenic stroke)

We used the basic framework of this classification to report on the aetiology of stroke in Paper 2, *'The Role of Atherosclerosis in HIV-associated Vasculopathy in Young South African Stroke Patients'*, which will be discussed below.

5.2 Vasculopathy in People Living With HIV is Predominantly Non-Atherosclerotic

HIV-associated vasculopathy has been identified as a cause of stroke in PLWH in numerous case series, with a quoted prevalence of 20 to 37%. (5, 80-82)

Vasculopathy is an incompletely understood entity. In the setting of HIV-associated vasculopathy, it is appropriate to use a broad definition, as proposed by Benjamin et al. (2016) (77) Here, it was defined as “an abnormality of the cerebral blood vessels that results directly or indirectly from HIV infection, but excluding vasculitis due to opportunistic infections.”(77) The vessel abnormalities may include stenotic lesions, alternating stenosis and dilatation or even aneurysmal changes, which may result in stroke.

Four phenotypes of HIV-associated vasculopathy have been proposed and defined in the consensus paper: atherosclerotic vasculopathy (AV), non-atherosclerotic vasculopathy (NAV), SVD and HIV-associated vasculitis. (77)

AV occurs due to atherosclerosis-induced stenosis and eventual occlusion of large and medium-sized arteries. In contrast, NAV produces similar stenotic lesions in large and medium-sized arteries with no histopathological evidence of atherosclerosis.

The major challenge in diagnosing AV and NAV is the impracticability of the ante-mortem histopathological examination of intracerebral blood vessels. Thus, the differentiation of AV from NAV has to rely on other indicators. The criteria used by Benjamin et al. (2016) to distinguish AV from NAV were clearly defined; however, the distinction was highly reliant on patient age and the presence of only one TRF. (77) For example, a patient aged >45 years (or age ≤45 years with exposure to a single TRF) with a previous transient ischaemic attack was considered as “clinical history suggestive of atherosclerosis” and diagnosed with “probable AV”. While this is plausible in a population with a high TRF burden, it may be presumptive in the setting of HIV-related stroke in SSA, where the role of TRFs and atherosclerosis is yet to be established. Vitally, the history of a previous cardiovascular event does not necessarily equate to the presence of atherosclerosis. A previous transient ischaemic attack secondary to cardioembolism in a 46-year-old is an example of the significant limitation of the proposed case definition, as such a patient would be considered to have a “clinical history suggestive of atherosclerosis”, even though their stroke mechanism (cardioembolic) was completely independent of atherosclerosis.

Data from a study by Benjamin et al. (2017) conducted in Malawi ascribed 23 out of 64 strokes in PLWH (38%) to HIV-associated vasculopathy. (81) Of these, seven were judged to be atherosclerotic (10.9% of strokes in PLWH). A distinction was made between “confirmed AV” (n=4) and “probable AV” (n=3) based on the aetiological classification above (which was proposed by the same lead author a year before the publication of the above data). (77) The limitations of this classification in defining AV have been discussed above, leaving room for debate, particularly regarding the three patients categorised as “probable AV” and the true prevalence of AV in the study population.

The accurate differentiation between AV and NAV is of great clinical importance, as subsequent treatment and secondary stroke prevention strategies will differ markedly depending on the main driver of the vasculopathy. Current secondary stroke prevention approaches are centred on the management of atherosclerosis (including antiplatelet agents and high-dose statins). (83) However, there is insufficient evidence at present to suggest that atherosclerosis is a major contributor to HIV-related stroke in SSA.

Markers of subclinical atherosclerosis (cIMT and PWV) have never been tested in PLWH with stroke, not to mention in the setting of stroke secondary to HIV-associated vasculopathy. We identified their potential value in discriminating AV from NAV and presented this data in Paper 2 (*‘The Role of Atherosclerosis in HIV-associated Vasculopathy in Young South African Stroke Patients’*). In order to diagnose AV, we required the ultrasound visualisation of atherosclerotic plaque causing a >50% occlusion of the extracranial internal carotid artery or common carotid artery ipsilateral to the stroke. This is in accordance with both the TOAST criteria and those proposed by Benjamin et al. (2016). In the absence of this occlusive plaque, the patient was diagnosed with NAV. Unlike Benjamin, we did not consider TRFs or past history of CVD when differentiating AV from NAV. As our objective was to test the utility of cIMT and PWV to distinguish AV and NAV, we also did not use these measurements in the diagnostic work-up of the patient. We only compared them once a diagnostic category was allocated. Mean cIMT and PWV were compared in AV and NAV and to predetermined, well-recognised cut-off values for subclinical atherosclerosis (cIMT $\geq$ 0.70mm and PWV $\geq$ 10.00m/s), as described earlier.

Overall, we found that 28 out of 85 PLWH had vascular abnormalities on angiography (32.9% of all strokes in PLWH). This excludes patients with SVD (n=14, who all had normal

angiography). Two distinct radiological types of vasculopathy were identified: a large vessel mono-focal occlusion (n=16) and a multifocal vasculitis (n=12).

NAV was the most common cause of mono-focal occlusion (11 patients). Only four PLWH had AV. Compared to those with NAV, those with AV were older (50.0 ± 4.1 years vs 39.2 ± 9.2 years, $p=0.04$) and had more TRFs (median 2.0 (IQR 1.5-2.5) vs 0.0 (IQR 0.0-1.0), $p=0.02$). The mean cIMT in patients with AV was higher than in NAV (1.01 ± 0.07 mm (n=4) vs 0.63 ± 0.04 mm (n=9), $p<0.001$). A comparison of cIMT and PWV to the pre-defined cut-off values revealed all PLWH with AV had measurements above the thresholds, while all except for one of those with NAV were below. HIV-related factors (CD4, viral load (VL), and ART-exposure) were similar between the groups.

We thus demonstrated that HIV-associated vasculopathy in our region appears to be predominantly non-atherosclerotic and showed that cIMT and PWV may be useful adjuncts in distinguishing AV from NAV. Historical data from SSA has also demonstrated a very low prevalence of large vessel atherosclerotic stroke, both in PLWH and HIV-negative populations. (21, 54)

While the extracranial arteries are relatively easily accessible to Doppler examination for atherosclerotic plaque, the assessment of intracranial atherosclerosis remains a challenge. Our data shows that intracranial mono-focal occlusion of a large vessel on angiography is not associated with surrogate markers of subclinical atherosclerosis and is thus unlikely to be atherosclerosis-mediated. This is an important clinical implication which may guide further diagnostic workup.

Although cIMT and PWV are admittedly surrogate measures, they are both well-validated and sensitive markers of subclinical atherosclerosis. (56, 72, 75) We acknowledge that the addition of other radiological biomarkers, such as CT for arterial calcification and even assessing arterial inflammation with positron image tomography (PET) scanning, would have further strengthened our conclusions. (56) However, these modalities were not available to us. We do not feel the absence of these tests was a significant limitation to our study, although including such modalities in future studies would be of great interest.

In conclusion, we suggest caution in diagnosing AV in PLWH with stroke due to mono-focal vessel occlusion purely based on age and the presence of TRFs. Our data has shown that the adjunctive use of markers of subclinical atherosclerosis, particularly cIMT (which is widely available), may be of value and warrants further study. Importantly, although an elevated cIMT

is not diagnostic of AV, the presence of a normal cIMT makes it unlikely that the mono-focal vessel occlusion (whether extra- or intracranial) is a result of atherosclerosis.

5.3 Non-Atherosclerotic Vasculopathy: Risk Factors and Mechanisms

Our findings of mono-focal vessel occlusions without evidence of atherosclerosis (i.e. NAV) of either extra- or intracranial vessels in 11 patients were particularly interesting. This was in the absence of any other vascular abnormalities on angiography and no clear proximal embolic source of these occlusions. Our clinico-radiological diagnosis of NAV correlated well with cIMT and PWV measurements. There have been very few detailed descriptions of similar findings in the literature, and detailed angiographic descriptions or reports on cIMT and PWV are lacking. (5, 21)

While our study was not designed to explore the mechanisms underlying NAV, previous histopathological data suggests that mono-focal stenotic lesions may result from inward vessel wall remodelling secondary to chronic vascular endothelial damage from HIV particles, with subsequent vessel stenosis. (84) Inflammation of the adventitia, as opposed to the intima and media, has been proposed to contribute to outward cerebral vessel remodelling independent of atherosclerosis in PLWH, resulting in aneurysmal and dolichoectatic changes. (84, 85) Gutierrez et al. showed that PLWH were significantly more likely to develop adventitial inflammation than HIV-negative controls after adjusting for demographics and TRFs. In contrast, intima-medial inflammation was similar between the groups. The finding suggests that HIV, particularly with uncontrolled viraemia, may preferentially affect the adventitia, leading to a different phenotype of vascular disease than atherosclerosis seen in the typical ageing population. (85) More protracted immunosuppression and a higher HIV VL have been associated with such outward remodelling. (84)

Mono-focal non-atherosclerotic large vessel occlusion may be considered a result of ‘inward’ remodelling, while multifocal vasculitis (with dolichoectatic or aneurysmal angiographic changes) may suggest ‘outward’ remodelling. In our cohort, we did not identify any apparent difference in patient profile (both HIV- and non-HIV-related) between those with presumed inward and outward vessel wall remodelling. Notably, there was no surrogate evidence of atherosclerosis in either, as evidenced by our normal cIMT and PWV measurements.

The site of vasculopathy, be it intracranial or extracranial, is another aspect of interest. Tipping et al. (2007) from Cape Town described six patients with extracranial non-aneurysmal vasculopathy and six patients with intracranial aneurysmal and non-aneurysmal vasculopathy

(as well as one patient with both intra and extracranial involvement). (80) No clear OI was identified, and the presence or absence of atherosclerosis was not commented on. Post-mortem histology from one of their patients with extracranial vasculopathy showed fibrosis of the adventitia and a lymphoplasmacytic infiltrate, with neutrophilic infiltration of the intima and media. This was in contrast to the histological findings in a patient with intracranial aneurysmal vasculopathy, which showed degenerative ectasia with hyperplasia and concentric fibrosis of the intima. This may suggest different targets depending on the location of vasculopathy, i.e. the intima intracranially and possibly the adventitia extracranially. (86) Of interest is that the vasa vasorum is not present in intracranial vessels, which may partially explain the differences. (87)

In our cohort, we observed with interest how mono-focal occlusion was more prevalent extracranially (10 out of 16), while multifocal vasculitis was more prevalent intracranially (10 out of 12). We did not demonstrate any differences in demographics, TRFs, HIV-related factors or markers of subclinical atherosclerosis between intracranial and extracranial vasculopathy (Table S2 in the supplemental data of Paper 2). This is in contrast to the description by Tipping and colleagues (2007) of extracranial vasculopathy occurring in patients with higher CD4 counts than those with intracranial vasculopathy, implying a possible ‘immunocompetent’ vasculitis extracranially. (80) Aneurysmal changes were also described in their case series. However, although we identified patients with alternating stenosis and dilatation of blood vessels as described earlier (labelled as vasculitis), we found only one patient with a discrete aneurysm (which accompanied vasculitic changes in the other large intracerebral vessels). It is possible that patients with aneurysmal vasculopathy presented with intracerebral or subarachnoid haemorrhage and were thus referred directly to our neurosurgery service. An important difference between our cohort and that from Cape Town remains our attempted exclusion of OIs. For example, the patient described above with intracranial aneurysmal vasculopathy had an active CSF, which would have led to them being excluded from our cohort due to suspicion of OI. (86)

It is plausible that the histological vascular changes described above could affect the measurement and interpretation of our cIMT and PWV examinations. This remains an intrinsic limitation of such tests, as they will always be only surrogate markers of atherosclerosis. A future large study correlating cIMT/PWV with histological vascular changes is unlikely to be practical in the setting of stroke in PLWH, as histology of large intracerebral vessels is not feasible antemortem.

Our study failed to identify any risk factors for NAV, while it was not designed to postulate on possible mechanisms of NAV. Importantly, there is still no convincing evidence from SSA (histological or from our biomarkers) that atherosclerosis is a significant role player in the vasculopathy resulting in stroke in PLWH.

5.4 Stroke Due to Small Vessel Disease is Traditional Cardiovascular Risk Factor-Mediated and May be Unrelated to HIV Status

Small vessel disease (SVD) is a clinico-radiological diagnosis referring to the presence of a traditional lacunar syndrome with a ≤ 20 mm subcortical stroke on CT or MRI. These traditional lacunar syndromes are diagnosed in the absence of any higher cortical dysfunction and may include a pure motor, pure sensory, sensorimotor or spastic ataxic hemiparesis. (88) In clinical practice, such syndromes are inevitably secondary to HT. There is minimal evidence of similar changes seen secondary to HIV in a post-mortem series published in 2000. (89) An association with PI usage has also been described, and SVD may also play a role in the pathogenesis of HIV-associated neurocognitive disorders in the absence of a discrete stroke. (90)

A common clinical challenge remains in a hypertensive PLWH, where it is not possible to confidently conclude whether the SVD is secondary to HT, HIV, or a combination of the two. For this reason, we classified SVD in a separate category and not necessarily as an HIV-related vasculopathy. In our cohort, PLWH with stroke secondary to SVD made up a relatively small proportion (16.5%). These patients' greater age and higher TRF burden suggest that the process of SVD stroke appears to be primarily TRF-driven and possibly even incidental to HIV (with similar patient profiles in PLWH and HIV-negative SVD patients). cIMT was similar in PLWH with SVD compared to HIV-negative with SVD but significantly higher than in PLWH with non-SVD-related stroke (0.78 ± 0.12 (n=11) vs 0.66 ± 0.17 (n=51), $p=0.03$). A normal PWV in SVD was expected, as PWV is a measure of large artery stiffness, not SVD. (75, 76). Angiography was normal in all patients with SVD, although we acknowledge that small vessel vasculitis cannot be excluded with normal angiography.

A small vessel stroke secondary to meningitis may be radiologically indistinguishable from SVD secondary to HT. Our findings of similar patient profiles between PLWH and HIV-negative SVD patients (having excluded meningitis-related strokes) bring into question whether SVD is truly an HIV-associated vasculopathy, once OIs have been excluded.

5.5 The Role of Subclinical Opportunistic Infections: Varicella-Zoster Virus

Vasculitis as a result of OIs has a well-described association with stroke. (91) This is often secondary to meningitis and thus is associated with an abnormal CSF examination. In clinical practice, an infective vasculitis of the brain is considered very unlikely in the setting of normal CSF parameters. Our study design aimed to exclude all patients with meningitis for reasons described earlier. We did this based on stringent CSF criteria (CSF protein $>0.45\text{g/L}$, a serum: CSF glucose ratio $<67\%$, or the presence of any polymorphonuclear cells or a lymphocytic pleocytosis $>5\text{cells/mm}^3$). Such patients were not recruited into the study ($n=20$). PCR testing for viruses on CSF was also performed; however, due to laboratory delays, these results only became available in the following 5-7 days, by which time patients with normal basic CSF parameters had been enrolled into the study and investigations performed.

To our great surprise, we subsequently detected a positive VZV PCR on the CSF of three patients with vasculopathy (all of whom had normal CSF biochemistry and microscopy). A further five PLWH had clinical evidence of recent but inactive cutaneous zoster infection. VZV reactivation has a known association with stroke. (92-98) The cutaneous features of VZV are not always present and may precede stroke by over two years. (92)

In total, VZV was implicated in 26.7% of our cohort of PLWH with presumed vasculopathy despite the exclusion of those with abnormal basic CSF parameters. We elected not to subsequently exclude these patients from the study, as we thought it invaluable data with strong clinical implications. Our patients with VZV-related stroke had evidence of more advanced HIV infection than non-VZV-related stroke (mean CD4 $92.9\pm77.1\text{ cells}/\mu\text{L}$ vs $357.0\pm201.5\text{ cells}/\mu\text{L}$, $p<0.001$), which is in keeping with most OIs. Although five out of eight VZV patients were ART-exposed, only one had viral load suppression. VZV reactivation has previously also been shown to be more common in more advanced immunocompromised patients. (92, 95, 99)

Our significant observations suggest that VZV may be associated with stroke by causing vasculitis in the absence of any CSF response and thus may be overlooked in patients with a normal basic CSF examination. If our study protocol did not include mandatory CSF virology, irrespective of the CSF biochemistry and microscopy, the opportunity to treat this infection with intravenous antiviral agents would have been missed, with the potential for adverse clinical outcomes. We also did not have testing for VZV IgG on CSF available, which has been shown to be more sensitive than PCR, particularly when compared to serum VZV IgG. (92, 99) The true prevalence of VZV-associated strokes is thus likely to be higher than we have

detected, and the index of suspicion needs to remain high to detect this potentially treatable cause of stroke.

The vital clinical implication of our findings is that we recommend testing for VZV in the CSF of all PLWH with stroke, even if the basic CSF examination is within normal parameters and no zoster rash is present. Testing the CSF:serum VZV IgG index is preferable; however, if unavailable, even VZV PCR coupled with a high index of clinical suspicion may suffice.

5.6 HIV-Associated Vasculitis is a Diagnosis of Exclusion

Despite the isolation of HIV particles and antigens in perivascular tissues, the existence of directly HIV-induced vasculitis is uncertain. (100) HIV has not been identified in the vessel wall itself, suggesting a possible immune-mediated mechanism. (18)

Benjamin et al. (2016) suggested that HIV-associated vasculitis be diagnosed in a patient with the classic angiographic features of vasculitis without an identified causative OI. (77) A major challenge with this definition is the uncertainty about the extent of investigations which would constitute an infection being considered excluded. As we have demonstrated with VZV, covert infections may be very difficult to exclude conclusively. Benjamin and colleagues' (2016) proposed definition of "probable HIV-associated vasculitis" is even more non-specific, requiring only the radiological confirmation of ischaemia in more than one vascular territory, having excluded OIs such as syphilis, VZV, tuberculosis and hepatitis B. The minimum suggested investigation is only a non-contrasted CT scan, although angiography is included under optimum investigations. Descriptions of the entity of HIV-associated vasculitis in the literature are very sparse because of the uncertainty in diagnosis.

We cautiously made this diagnosis in six patients with multifocal vasculitis who fulfilled the criteria above (21.4% of vasculopathy patients). All had multifocal vasculitis of the large vessels (intra- or extracranially), with no angiographic evidence of a small vessel vasculitis in any patient. None of our patients had clinical or radiographic features diagnostic of alternate diagnoses such as Moyamoya disease or Reversible Cerebral Vasoconstriction Syndrome, nor any evidence of any systemic autoimmune or inflammatory diseases. When comparing HIV-associated vasculitis to patients with VZV-related vasculitis, patients with VZV had a lower age, CD4 count and mean BMI. This suggests that these are indeed distinct clinical entities. However, there remains a distinct possibility of over-diagnosing HIV-associated vasculitis in the setting of an incomplete or false-negative infection screen. This must be attentively guarded

against, as the patient's management will differ markedly. Our patients diagnosed with HIV-associated vasculitis had evidence of less profound immunocompromise than those with VZV vasculitis, making it less likely that covert OIs were missed. However, we again emphasise that HIV-associated vasculitis remains a diagnosis of exclusion, with a broad differential diagnosis.

5.7 Antiretroviral Therapy and Stroke Risk

5.7.1 Antiretroviral therapy is not associated with atherosclerosis in HIV-related stroke in our setting

The association between ART and increased cardiovascular risk has been a topic of interest over the past two decades. There is good evidence for an increased stroke risk with the use of certain antiretrovirals, particularly PIs including lopinavir-ritonavir and indinavir, as well as a debateable increased risk with the nucleoside reverse transcriptase inhibitor abacavir. (36) This appears to be mediated primarily through the known metabolic side effects of these drugs, including dyslipidaemia. (36, 52) Importantly, most data indicating an elevated cardiovascular risk is from HICs. Due to resource constraints in LMICs, including South Africa, the proportion of PLWH exposed to PIs is significantly lower than in HICs, with PIs infrequently used as first-line agents. (32) The evidence for ART contributing to cardiovascular risk in these regions is thus less robust. (40)

In Paper 2, we report that 40/85 PLWH (47.1%) were on ART at the time of their stroke. Of these patients, 37 were on a regimen containing emtricitabine, tenofovir and efavirenz. The three patients on PI-containing regimens all had no evidence of atherosclerosis-mediated stroke aetiology (two with multifocal vasculitis and one with cardioembolic stroke related to a metallic heart valve, all with normal cIMT and PWV measurements). There was no patient in our cohort with exposure to abacavir. The ART-exposed patients were of similar age and sex distribution and had a similar TRF profile and CD4 counts to the ART-naïve patients. They also had similar proportions of large-vessel atherosclerotic stroke (i.e. AV) (2/40, 5.0% in ART-exposed vs 2/45, 4.4% in ART-naïve, $p=0.92$).

We then studied the markers of subclinical atherosclerosis in ART patients, compared them to the ART-naïve and revealed no difference in cIMT or PWV between the two groups. In Paper 3, '*Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke patients in South Africa*' we also reported on regression analyses, where the use of ART had no impact on cIMT nor increased cIMT ($\geq 0.70\text{mm}$) in PLWH.

This supports previous data from our region suggesting that stroke in PLWH does not appear to be related to ART-induced atherosclerosis, as opposed to in HICs. (19, 21, 101) Our low rates of PI usage may be partly responsible for this, together with the intrinsic differences in our population when compared to those in HICs, as discussed previously.

5.7.2 Stroke risk is increased in the first six months following antiretroviral therapy initiation

A novel mechanism by which ART may increase stroke risk was first reported in a landmark case-control study from Malawi in 2016. (22) They reported that the highest risk of stroke in PLWH was within six months of ART initiation (adjusted OR 15.6, 95% CI 4.21–46.6, $p < 0.001$). Out of 64 PLWH with stroke, 16 (25%) fell into this category. These patients had a median CD4 of 92 cells/mm³, with lower VLs than other categories. Nadir CD4 counts were not reported. It is postulated that these strokes occurred secondary to the Immune Reconstitution Inflammatory Syndrome (IRIS). This is a dysregulated, hyper-inflammatory response that is usually directed at OIs. It almost always occurs within six months of ART initiation and may cause considerable morbidity and mortality. (102) Proposed mechanisms for this relatively novel concept include a dysregulated and destructive excessive immune response to occult OIs, HIV-associated proteins or even self-antigens. (103)

OIs such as VZV and tuberculosis are known to cause stroke, as previously discussed. (91) We were interested in investigating whether this potential phenomenon of IRIS-mediated stroke was still apparent despite the exclusion of OIs. If so, we sought to identify potential risk factors for stroke in these patients and whether there was any evidence of subclinical atherosclerosis (possibly accelerated by an aberrant immune response as is seen in IRIS). Ours is the first study to examine these aspects of IRIS-related stroke.

In Paper 5, '*Stroke, HIV and the immune reconstitution inflammatory syndrome in the absence of opportunistic infections*', we report that 9/28 (32.1%) PLWH with known ART duration had a stroke within the first six months of starting ART (crude incidence rate of 0.73 cases per patient-year). In the period beyond six months, 19 strokes occurred (crude incidence rate of 0.21 cases per patient-year), translating to a 3.5 times greater risk in the first six months ($p = 0.0002$). We emphasise that this was despite the exclusion of OIs. The statistical methods are discussed in detail in the supplementary data section of the paper. This provides striking evidence of the role of an IRIS phenomenon to complement the data from Malawi. Importantly,

we are the first to show that this appears to be independent of OIs, which are usually synonymous with IRIS.

We compared the group of patients with a stroke while on ART for ≤ 6 months to those on ART for >6 months to try to identify risk factors for developing stroke soon after ART initiation but showed no difference in demographics, TRFs, CD4, VL, stroke severity and selected inflammatory markers. There were also no differences in the markers of subclinical atherosclerosis (cIMT and PWV) between the two groups. Comparisons to ART-naïve patients were similarly unremarkable. We thus failed to identify any risk factors, although we did not have data on nadir CD4 counts, which was a noted limitation.

Our high prevalence of possible IRIS-related stroke, despite the exclusion of OIs, supports a role for a non-infectious inflammatory mechanism, which appears to be unrelated to atherosclerosis. Once again, the role of occult OIs cannot be excluded, despite our stringent efforts to exclude them, as was noted with VZV-related vasculopathy described earlier (all of those patients were excluded from the analyses for this paper). IRIS-related stroke remains an area of great interest, which will best be answered with a large prospective study, which we intend to undertake.

5.8 Cardioembolic Stroke is Underdiagnosed in Resource-Poor Settings

As previously noted, a high proportion of stroke in PLWH is of alternative aetiology, including infections and vasculopathy. However, this should not distract one from still considering more conventional stroke aetiologies, including cardioembolic stroke (CES).

In the global non-HIV literature, CES is the likely aetiology in up to 30% of strokes. (104) However, CES has been reported to be a relatively rare cause of stroke in PLWH in SSA, causing only 4-9% of all strokes. (5, 21) Due to severely limited resources, a comprehensive cardiovascular assessment, including echocardiography, is seldom possible in stroke patients in this region. (5) Thus, the true prevalence of CES is likely higher than previously reported. Furthermore, a paucity of data makes it unclear which cardiac pathologies are primarily responsible for CES in PLWH in SSA.

In Paper 5, '*Cardioembolic stroke in an HIV endemic region: underdiagnosed and severe*', we sought to determine the prevalence, aetiology and severity of CES in PLWH in our cohort with the benefit of having access to quaternary-level cardiac diagnostics. We also sought to determine if there were any differences compared to HIV-negative stroke patients.

The paper describes the methodology and results:

“Our cardiac evaluation included a complete cardiovascular clinical examination, a 12-lead (ECG) and transthoracic echocardiography. 24-hour Holter ECG and trans-oesophageal echocardiography were performed where clinically indicated, particularly in patients with large vessel stroke of undetermined aetiology after standard investigations. These were performed and interpreted by a specialist cardiologist, who then assigned the cardiac aetiological category most likely to be responsible for the stroke. Patients with definite cardiac pathology but not meeting the TOAST criteria for CES were categorised as ‘stroke of undetermined aetiology’.” (105)

We found that 20% (17/85) of strokes in PLWH were due to a cardioembolic cause. Of these, 76.4% (n=13) were secondary to cardiomyopathy, with very low prevalences of valvular heart disease and arrhythmia. PLWH with CES suffered from more severe strokes when compared to PLWH with non-CES (mean National Institute of Health Stroke Scale score 14.9 ± 6.7 vs 11.7 ± 5.4 , $p=0.04$) despite similar baseline characteristics. The commonest underlying cardiac pathology differed significantly between PLWH (cardiomyopathy in 76.4%) and age- and sex-matched HIV-negative controls (valvulopathy in 42.5%, $p = 0.03$).

Our prevalence of CES is higher than other PLWH cohorts in SSA and other LMICs (4-9%). (5, 21) However, fewer than 60% of the study participants had an ECG or echocardiography (5) compared to the detailed cardiac assessments performed in our cohort. Our higher prevalence of CES thus likely illustrates the desperate need for improved access to resources to diagnose CES adequately. The clinical implication of a missed cardioembolic source is profound, as these patients are at very high risk of recurrent strokes if not treated with the appropriate anticoagulation. (106)

However, if it is indeed true that the source of CES in our PLWH population is more likely to be cardiomyopathy, this has the potential to guide a targeted search for the most likely sources of CES in a particular population. Our very low arrhythmia prevalence (1 out of 17 PLWH with CES) adds further value to this model. In regions such as SSA, the rational allocation of scarce diagnostic resources is of great importance. Echocardiography, in particular, is an extremely scarce resource. Due to massive economic and healthcare system challenges, it is improbable that widespread access to echocardiography is a realistic solution to diagnosing

CES in populations such as ours. Using alternative cost-effective biomarkers for CES, which are more readily accessible and require less technical expertise, may be a more practical solution. (107, 108) In order to guide the development and testing of these biomarkers, it is important to know which particular cardiac diseases are most prevalent in a particular stroke population, which appears to be cardiomyopathy in our PLWH population.

Our findings of CES being more severe than non-CES in PLWH are alarming. CES stroke unrelated to HIV infection has been shown to cause more severe stroke in extensive HIC studies. (106) However, the majority of these strokes are in older patients with atrial fibrillation and multiple other TRFs. (109) This is a vastly different patient profile to our description, emphasising the novelty of our findings. This increased severity of CES in PLWH, together with the intrinsically higher stroke recurrence risk in untreated CES, points to an urgent need to address the probable underdiagnosis of CES as a cause of stroke in PLWH in SSA.

6. IMPLICATIONS FOR FUTURE RESEARCH

6.1 The Role of Statins in Stroke Prevention in PLWH

We discussed the clinical implications of our significant findings in the discussion above and in each individual paper. However, it is pertinent to emphasise the implications of our main overall conclusion: stroke in PLWH in SSA is largely unrelated to atherosclerosis.

The long-awaited Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) trial was designed to test the efficacy of a statin (pitavastatin) compared to a placebo for the primary prevention of CVD in PLWH. (110) This was based on the premise that PLWH have accelerated atherosclerosis leading to vascular events. The trial was terminated early due to the clear superiority of pitavastatin, resulting in 35% fewer major cardiovascular events over five years. (111)

While this is ground-breaking evidence for PLWH in HICs and likely to motivate a change in clinical management, the data from the LMIC study sites of the REPRIEVE trial was not as robust. Smaller sample sizes at LMIC sites may be partially responsible. Importantly, it should also be noted that the phenotype of patients recruited from SSA into REPRIEVE (median age 50 years) was quite different to the profile of those described in most LMIC stroke studies, including ours, with median ages between 32 to 43 years, and a lower TRF burden. (5, 21) Thus, while atherosclerosis may be relevant in the subgroup of patients from SSA included in REPRIEVE, it remains unclear whether this study population was genuinely representative of

the PLWH stroke population in SSA. Our findings of a very low atherosclerotic burden in PLWH with stroke (both clinical and subclinical) cast further doubt on the applicability of the REPRIEVE conclusions to PLWH in SSA. In a healthcare system with severe resource constraints, at present, there appears to be insufficient evidence to consider a major programme involving the primary prevention of CVD in PLWH using statins.

This may appear contradictory to our recommendations based on Paper 1, where we found a very high prevalence of undiagnosed dyslipidaemia (with subsequently more severe stroke and in younger patients). Firstly, the patients in Paper 1 included both PLWH and HIV-negative and, therefore, had a significantly higher age and TRF burden than our PLWH cohort alone. Furthermore, while statins are the mainstay of therapy for dyslipidaemia, their use in the REPRIEVE trial was based more on the pleiotropic effects of statins on atherosclerosis and the vasculature, as opposed to managing dyslipidaemia. In fact, the exclusion criteria of REPRIEVE excluded patients with cholesterol levels high enough to warrant statin treatment.

Thus, our recommendation for exploring more extensive dyslipidaemia screening programmes in the general population remains, as we have demonstrated how the consequences of poor pre-event RF screening may be catastrophic. Whether or not statins for the primary prevention of stroke in PLWH will be as efficacious in our setting is less certain.

6.2 Improved Diagnostics for Subclinical Infections

A critical issue that arises from our findings on VZV is regarding other subclinical infections, which may be silently contributing to stroke pathogenesis but remain undetected and thus untreated. This may include the effects of HIV itself, despite the apparent peripheral viral load suppression. Future research directions may include next-generation sequencing and other advanced molecular techniques to evaluate the presence of infective pathogens contributing to stroke pathogenesis. While it may be challenging to prove causality in a stroke population such as the one we have described (with a low TRF burden and apparent lack of atherosclerosis), the pursuit of subclinical infections should certainly be considered in future research in the field.

6.3 Prospectively Studying Stroke Risk After ART Initiation

As discussed in Section 5.7.2 above, the concern for an increased stroke risk soon after ART initiation justifies a prospective study, which our group will be undertaking in the near future.

Our objectives include identifying risk predictors for having a stroke soon after ART initiation to guide primary prevention strategies in those who are at greatest risk.

6.4 A Cost-Effective Biomarker for Cardiomyopathy

We found the proportion of stroke due to cardioembolism in our population of PLWH is higher than previously reported in our region. Although this may result from a more thorough cardiac evaluation, our finding that the majority of CES in PLWH is secondary to cardiomyopathy warrants special attention. As discussed in Section 5.8 above, the targeted search for a specific cardiac pathology (in this case, cardiomyopathy) using a cost-effective and accessible biomarker for that condition may diminish the impact of the dire lack of advanced cardiac diagnostics such as echocardiography in most of SSA. Based on our findings, future work into developing and testing such a biomarker in our setting should be encouraged.

7. STRENGTHS AND LIMITATIONS

Our cohort of 85 PLWH with ischaemic stroke is one of the largest from SSA. (5, 21) To our knowledge, only one study from SSA (from Benin), has recruited more PLWH with stroke. (112)

Our study is also one of the most extensively investigated cohorts of PLWH with stroke in an LMIC. We had the benefit of being at a sizeable quaternary hospital in Johannesburg, with access to most investigations which are considered the gold standard in investigating HIV-related stroke (including echocardiography and angiography) as well as being able to study the markers of subclinical atherosclerosis using validated measurement techniques. Patients were comprehensively assessed with access to various specialists (including neurologists, radiologists, cardiologists, vascular surgeons and microbiologists) and laboratory investigations. No other study from SSA on HIV-related stroke has reported our degree of multidisciplinary expertise in the investigation of stroke, ensuring that the aetiology and mechanism of stroke are determined as accurately as possible in each patient.

Examples of novel methodology in our work include excluding patients with OIs, which was particularly striking in our paper on IRIS (a phenomenon generally associated with OIs). It also enabled us to portray a purer comparison between PLWH and HIV-negative controls regarding stroke risk factors, aetiology and the role of atherosclerosis without the confounder of OIs, which are far more likely to affect PLWH. To our knowledge, no previous study on HIV-related stroke has done this.

Our analysis of cIMT and PWV in PLWH who have suffered a stroke is also unique in the global HIV literature. Most previous cIMT and PWV data have been on ‘healthy’ PLWH, i.e. non-stroke patients. Our methods for measuring cIMT and PWV followed validated techniques vigorously and were all performed by a single trained operator, eliminating inter-observer bias.

The limitations of our work are listed in each individual paper, including missing data (which was not imputed) and the small sample sizes of specific subgroups. Overall, the lack of non-stroke control groups was our most significant limitation. This did not permit more robust analyses of stroke risk factors and comparisons of markers of subclinical atherosclerosis in stroke patients when compared to non-stroke controls. Unfortunately, this being an unfunded study, resource constraints did not allow for the recruitment of such control groups.

The selection bias of recruiting stroke patients from a large referral hospital also compromises the applicability of the data to the general PLWH population of the country. However, this limitation also translates into a strength of the study, as one would expect the prevalence of atherosclerosis to be higher in an urban centre such as Johannesburg compared to more rural locations in the country due to the epidemiological transition being more advanced in urban regions. Thus, our finding of a low atherosclerosis burden in our study from the most urbanised city in the country likely suggests an even lower prevalence of atherosclerosis elsewhere in the country.

An important intrinsic caveat in using biomarkers such as cIMT and PWV is that these are merely surrogate markers of atherosclerosis and not necessarily diagnostic of its presence. However, the relatively high negative predictive value is where our study draws its strength, in that the absence of a raised cIMT or PWV makes it very unlikely that subclinical atherosclerosis plays a significant role in stroke pathogenesis. This applies not only to extracranial carotid artery atherosclerosis but also as a marker of atherosclerosis elsewhere in the body, including the intracranial blood vessels (where the direct assessment of atherosclerosis is technically very challenging).

While the recruitment of study participants was completed by the end of 2017, a series of personal and professional challenges unfortunately led to prolonged delays in data analysis and reporting. While it is unlikely that the profile of stroke patients in our setting has changed significantly since then, we acknowledge this as a limitation but do not wish it to stand in the way of disseminating information that may be of value. Many of our findings were comparable to those from a study performed at the same institution between 2000 and 2002. (55) While

that was not an HIV-related study, the similarity in patient profiles in the current study to those in a study completed 15 years prior suggests that these are not likely to have changed significantly between 2017 and the present. A comprehensive literature review was also repeated at the time of writing this thesis. It did not reveal any significant advances in the fundamental understanding of stroke in PLWH in SSA since our initial protocol document was compiled.

8. CONCLUSION

We have demonstrated that most strokes in PLWH in our population appear to be non-atherosclerotic, even after the exclusion of overt OIs. This is in stark contrast to evidence from HICs. (6, 52, 53, 101)

It may seem counter-intuitive to dispute the HIC-data-inspired shift towards stroke in PLWH being considered a chronic disease ‘of lifestyle’ and backtracking to the era of predominantly OI-mediated morbidity in PLWH. However, we know that our PLWH population is very different to that in HICs. While it may be argued that PLWH in SSA are simply lagging in the epidemiological transition, there is a possibility that we will remain an entirely different population of PLWH, with different risk factors and different manifestations of disease.

Our findings of a low atherosclerotic burden, covert VZV-related strokes and the possible role of IRIS in the absence of OIs are examples of this. Concurrently, one still needs to consider more conventional stroke aetiologies, such as cardioembolic stroke, with a particular emphasis on cardiomyopathy.

With this work, we have presented evidence for an alternative view to that in the current literature; one which may be more applicable to the management of the vast majority of PLWH globally, who mostly reside in LMICs such as ours.

LIST OF REFERENCES

1. World Health Organisation. The top 10 causes of death (2019) Available from: <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>. Accessed on 6 May 2021.
2. Collaborators GBDS. Global, regional, and national burden of stroke and its risk factors, 1990-2019: A systematic analysis for the Global Burden of Disease study 2019. *Lancet Neurol.* 2021;20(10):795-820.
3. Kharsany AB, Karim QA. HIV infection and AIDS in sub-Saharan Africa: Current status, challenges and opportunities. *Open AIDS J.* 2016;10:34.
4. Shah AS, Stelzle D, Lee KK, Beck EJ, Alam S, Clifford S et al. Global burden of atherosclerotic cardiovascular disease in people living with HIV: Systematic review and meta-analysis. *Circulation.* 2018;138(11):1100-12.
5. Ransley G, Zimba S, Gadama Y, Saylor D, Benjamin L. Trends and clinical characteristics of HIV and cerebrovascular disease in low- and middle-income countries (LMICS) between 1990 and 2021. *Curr HIV/AIDS Rep.* 2022.
6. Bogorodskaya M, Chow FC, Triant VA. Stroke in HIV. *Can J Cardiol.* 2019;35(3):280-7.
7. Yusuf S, Rangarajan S, Teo K, Islam S, Li W, Liu L et al. Cardiovascular risk and events in 17 low-, middle-, and high-income countries. *N Engl J Med.* 2014;371(9):818-27.
8. Solomon A, Stanwix AE, Castaneda S, Llorca J, Gonzalez-Juanatey C, Hodgkinson B et al. Points to consider in cardiovascular disease risk management among patients with rheumatoid arthritis living in South Africa, an unequal middle income country. *BMC Rheumatol.* 2020;4:42.
9. Krishnamurthi RV, Ikeda T, Feigin VL. Global, regional and country-specific burden of ischaemic stroke, intracerebral haemorrhage and subarachnoid haemorrhage: A systematic analysis of the global burden of disease study 2017. *Neuroepidemiology.* 2020;54(2):171-9.
10. Jacob MA, Ekker MS, Allach Y, Cai M, Aarnio K, Arauz A et al. Global differences in risk factors, etiology, and outcome of ischemic stroke in young adults-a worldwide meta-analysis: The goal initiative. *Neurology.* 2022;98(6):e573-e88.
11. Owolabi M, Johnson W, Khan T, Feigin V. Effectively combating stroke in low-and middle-income countries: Placing proof in pragmatism—the Lancet Neurology Commission. *J Stroke Med.* 2018;1(1):65-7.

12. Langhorne P, O'Donnell MJ, Chin SL, Zhang H, Xavier D, Avezum A et al. Practice patterns and outcomes after stroke across countries at different economic levels (INTERSTROKE): An international observational study. *Lancet*. 2018;391(10134):2019-27.
13. Namale G, Kamacooko O, Kinengyere A, Yperzeele L, Cras P, Ddumba E et al. Risk factors for hemorrhagic and ischemic stroke in sub-Saharan Africa. *J Trop Med*. 2018;2018:4650851.
14. O'Donnell MJ, Chin SL, Rangarajan S, Xavier D, Liu L, Zhang H et al. Global and regional effects of potentially modifiable risk factors associated with acute stroke in 32 countries (INTERSTROKE): A case-control study. *Lancet*. 2016;388(10046):761-75.
15. Abboud H, Labreuche J, Arauz A, Bryer A, Lavados PG, Massaro A et al. Demographics, socio-economic characteristics, and risk factor prevalence in patients with non-cardioembolic ischaemic stroke in low- and middle-income countries: The optic registry. *Int J Stroke*. 2013;8 Suppl A100:4-13.
16. Cappuccio FP, Miller MA. Cardiovascular disease and hypertension in sub-Saharan Africa: Burden, risk and interventions. *Intern Emerg Med*. 2016;11:299-305.
17. Statistics South Africa. Mid-Year Population Estimates 2024 (P0302). Available from: <https://www.statssa.gov.za/publications/P0302/P03022024.pdf>. Accessed on 8 July 2025.
18. Benjamin LA, Bryer A, Emsley HCA, Khoo S, Solomon T, Connor MD. HIV infection and stroke: Current perspectives and future directions. *Lancet Neurol*. 2012;11(10):878-90.
19. Kroon L, van Zyl DG, Schutte CM, Smit C, Hiesgen J. Risk factors for stroke in HIV-positive and negative patients in Pretoria, South Africa. *J Stroke Cerebrovasc Dis*. 2021;30(8):105929.
20. Zimba S, Nutakki A, Chishimba L, Chomba M, Bahouth M, Gottesman RF et al. Risk factors and outcomes of HIV-associated stroke in Zambia. *AIDS*. 2021;35(13):2149-55.
21. Abdallah A, Chang JL, O'Carroll CB, Musubire A, Chow FC, Wilson AL et al. Stroke in human immunodeficiency virus-infected individuals in sub-Saharan Africa (SSA): A systematic review. *J Stroke Cerebrovasc Dis*. 2018;27(7):1828-36.
22. Benjamin LA, Corbett EL, Connor MD, Mzinganjira H, Kampondeni S, Choko A et al. HIV, antiretroviral treatment, hypertension, and stroke in Malawian adults: A case-control study. *Neurology*. 2016;86(4):324-33.
23. Walker RW, Jusabani A, Aris E, Gray WK, Unwin N, Swai M et al. Stroke risk factors in an incident population in urban and rural Tanzania: A prospective, community-based, case-control study. *Lancet Glob Health*. 2013;1(5):e282-e8.

24. Gutierrez J, Albuquerque ALA, Falzon L. HIV infection as vascular risk: A systematic review of the literature and meta-analysis. *PLoS One*. 2017;12(5):e0176686.
25. Achwoka D, Mutave R, Oyugi JO, Achia T. Tackling an emerging epidemic: The burden of non-communicable diseases among people living with HIV/AIDS in sub-Saharan Africa. *Pan Afr Med J*. 2020;36(1).
26. Rahbar MH, Medrano M, Diaz-Garelli F, Gonzalez Villaman C, Saroukhani S, Kim S et al. Younger age of stroke in low-middle income countries is related to healthcare access and quality. *Ann Clin Transl Neurol*. 2022;9(3):415-27.
27. Rêgo A, Nannoni S, Scherz A, Eskandari A, Salerno A, Pereira M et al. Undiagnosed major risk factors in acute ischaemic stroke patients: Frequency, profile, stroke mechanisms and outcome. *Eur J Neurol*. 2023;31(1):e16011.
28. Sung SF, Lai ECC, Wu DP, Hsieh CY. Previously undiagnosed risk factors and medication nonadherence are prevalent in young adults with first-ever stroke. *Pharmacoepidemiol Drug Saf*. 2017;26(12):1458-64.
29. Matz K, Keresztes K, Tatschl C, Nowotny M, Dachenhausen A, Brainin M et al. Disorders of glucose metabolism in acute stroke patients: An underrecognized problem. *Diabetes Care*. 2006;29(4):792-7.
30. Bugnicourt JM, Flament M, Guillaumont MP, Chillon JM, Leclercq C, Canaple S et al. Predictors of newly diagnosed atrial fibrillation in cryptogenic stroke: A cohort study. *Eur J Neurol*. 2013;20(10):1352-9.
31. So-Armah K, Benjamin LA, Bloomfield GS, Feinstein MJ, Hsue P, Njuguna B et al. HIV and cardiovascular disease. *Lancet HIV*. 2020;7(4):e279-e93.
32. Ford N, Calmy A, Mills EJ. The first decade of antiretroviral therapy in Africa. *Global Health*. 2011;7:1-6.
33. Castelli F, Pietra V, Diallo I, Schumacher RF, Simpore J. Antiretroviral (ARV) therapy in resource-poor countries: What do we need in real life? *Open AIDS J*. 2010;4:28-32.
34. Rao VR, Sas AR, Eugenin EA, Siddappa NB, Bimonte-Nelson H, Berman JW et al. HIV-1 clade-specific differences in the induction of neuropathogenesis. *J Neurosci*. 2008;28(40):10010-6.
35. 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;60(4):351-8.

36. Friis-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio Monforte A et al. Cardiovascular disease risk factors in HIV patients - Association with antiretroviral therapy. Results from the DAD study. *AIDS*. 2003;17(8):1179-93.
37. Ryom L, Lundgren JD, El-Sadr W, Reiss P, Kirk O, Law M et al. Cardiovascular disease and use of contemporary protease inhibitors: The D:A:D international prospective multicohort study. *Lancet HIV*. 2018;5(6):e291-e300.
38. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab*. 2007;92(7):2506-12.
39. Mdodo R, Frazier EL, Dube SR, Mattson CL, Sutton MY, Brooks JT et al. Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: Cross-sectional surveys. *Ann Intern Med*. 2015;162(5):335-44.
40. Vos AG, Barth RE, Klipstein-Grobusch K, Tempelman HA, Devillé WL, Dodd C et al. Cardiovascular disease burden in rural Africa: Does HIV and antiretroviral treatment play a role? Baseline analysis of the Ndlovu cohort study. *J Am Heart Assoc*. 2020;9(7):e013466.
41. Dillon DG, Gurdasani D, Riha J, Ekoru K, Asiki G, Mayanja BN et al. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: A systematic review and meta-analysis. *Int J Epidemiol*. 2013;42(6):1754-71.
42. Ekoru K, Young EH, Dillon DG, Gurdasani D, Stehouwer N, Faurholt-Jepsen D et al. HIV treatment is associated with a twofold higher probability of raised triglycerides: Pooled analyses in 21 023 individuals in sub-Saharan Africa. *Glob Health Epidemiol Genom*. 2018;3:e7.
43. Fourie C, Schutte A, Smith W, Kruger A, Van Rooyen J. Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans. *Atherosclerosis*. 2015;240(1):154-60.
44. Clark SJ, Gómez-Olivé FX, Houle B, Thorogood M, Klipstein-Grobusch K, Angotti N et al. Cardiometabolic disease risk and HIV status in rural South Africa: Establishing a baseline. *BMC Public Health*. 2015;15:1-9.
45. 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.
46. Bearden DR, Omech B, Rulaganyang I, Sesay SO, Kolson DL, Kasner SE et al. Stroke and HIV in Botswana: A prospective study of risk factors and outcomes. *J Neurol Sci*. 2020;413:116806.

47. Mosepele M, Hemphill LC, Palai T, Nkele I, Bennett K, Lockman S et al. Cardiovascular disease risk prediction by the American College of Cardiology (ACC)/American Heart Association (AHA) Atherosclerotic Cardiovascular Disease (ASCVD) risk score among HIV-infected patients in sub-Saharan Africa. *PLoS One*. 2017;12(2):e0172897.
48. Tahir IS, Vos AG, Damen JA, Barth RE, Tempelman HA, Grobbee DE et al. Comparative performance of cardiovascular risk prediction models in people living with HIV. *South Afr J HIV Med*. 2022;23(1):1395.
49. Emsley HC, Hopkins SJ. Acute ischaemic stroke and infection: Recent and emerging concepts. *Lancet Neurol*. 2008;7(4):341-53.
50. Kearns A, Gordon J, Burdo TH, Qin X. HIV-1-associated atherosclerosis: Unraveling the missing link. *J Am Coll Cardiol*. 2017;69(25):3084-98.
51. Worm SW, Sabin C, Weber R, Reiss P, El-Sadr W, Dabis F et al. Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: The data collection on adverse events of anti-HIV drugs (D:A:D) study. *J Infect Dis*. 2010;201(3):318-30.
52. Hsue PY. Mechanisms of cardiovascular disease in the setting of HIV infection. *Can J Cardiol*. 2019;35(3):238-48.
53. Durstenfeld MS, Hsue PY. Mechanisms and primary prevention of atherosclerotic cardiovascular disease among people living with HIV. *Curr Opin HIV AIDS*. 2021;16(3):177-85.
54. Connor MD. Carotid artery disease in sub-Saharan Africa: A marker of epidemiological and stroke transition. *Neuroepidemiology*. 2012;38(2):120-1.
55. Connor MD, Modi G, Warlow CP. Differences in the nature of stroke in a multiethnic urban South African population: The Johannesburg Hospital Stroke Register. *Stroke*. 2009;40(2):355-62.
56. Stein JH, Currier JS, Hsue PY. Arterial disease in patients with human immunodeficiency virus infection: What has imaging taught us? *JACC Cardiovasc Imaging*. 2014;7(5):515-25.
57. Grunfeld C, Delaney JA, Wanke C, Currier JS, Scherzer R, Biggs ML et al. Preclinical atherosclerosis due to HIV infection: Carotid intima-medial thickness measurements from the Fram study. *AIDS*. 2009;23(14):1841-9.

58. Hsue PY, Ordovas K, Lee T, Reddy G, Gotway M, Schnell A et al. Carotid intima-media thickness among human immunodeficiency virus-infected patients without coronary calcium. *Am J Cardiol.* 2012;109(5):742-7.
59. Lorenz M, Stephan C, Harmjanz A, Staszewski S, Buehler A, Bickel M et al. Both long-term HIV infection and highly active antiretroviral therapy are independent risk factors for early carotid atherosclerosis. *Atherosclerosis.* 2008;196(2):720-6.
60. Krikke M, Arends J, Van Lelyveld S, Hoepelman A, Visseren F. Greater carotid intima media thickness at a younger age in HIV-infected patients compared with reference values for an uninfected cohort. *HIV Medicine.* 2017;18(4):275-83.
61. van Vonderen MG, Smulders YM, Stehouwer CD, Danner SA, Gundy CM, Vos F et al. Carotid intima-media thickness and arterial stiffness in HIV-infected patients: The role of HIV, antiretroviral therapy, and lipodystrophy. *J Acquir Immune Defic Syndr.* 2009;50(2):153-61.
62. Schoffelen AF, De Groot E, Tempelman HA, Visseren FL, Hoepelman AI, Barth RE. Carotid intima-media thickness in mainly female HIV-infected subjects in rural South Africa: Association with cardiovascular but not HIV-related factors. *Clin Infect Dis.* 2015;61(10):1606-14.
63. Gleason Jr RL, Caulk AW, Seifu D, Parker I, Vidakovic B, Getenet H et al. Current efavirenz (EFV) or ritonavir-boosted lopinavir (LPV/r) use correlates with elevated markers of atherosclerosis in HIV-infected subjects in Addis Ababa, Ethiopia. *PloS One.* 2015;10(4):e0117125.
64. Volpe GE, Tang AM, Polak JF, Mangili A, Skinner SC, Wanke CA. Progression of carotid intima-media thickness and coronary artery calcium over 6 years in an HIV-infected cohort. *J Acquir Immune Defic Syndr.* 2013;64(1):51-7.
65. Hanna DB, Guo M, Bůžková P, Miller TL, Post WS, Stein JH et al. HIV infection and carotid artery intima-media thickness: Pooled analyses across 5 cohorts of the NHLBI HIV-CVD collaborative. *Clin Infect Dis.* 2016;63(2):249-56.
66. Hultén E, Mitchell J, Scally J, Gibbs B, Villines TC. HIV positivity, protease inhibitor exposure and subclinical atherosclerosis: A systematic review and meta-analysis of observational studies. *Heart.* 2009;95(22):1826-35.
67. Nonterah EA, Boua PR, Klipstein-Grobusch K, Asiki G, Micklesfield LK, Agongo G et al. Classical cardiovascular risk factors and HIV are associated with carotid intima-media thickness in adults from sub-Saharan Africa: Findings from H3Africa AWI-Gen study. *J Am Heart Assoc.* 2019;8(14):e011506.

68. Mosepele M, Hemphill LC, Moloi W, Moyo S, Nkele I, Makhema J et al. Pre-clinical carotid atherosclerosis and sCD163 among virally suppressed HIV patients in Botswana compared with uninfected controls. *PLoS One*. 2017;12(6):e0179994.
69. Feinstein MJ, Kim J-H, Bibangambah P, Sentongo R, Martin JN, Tsai AC et al. Ideal cardiovascular health and carotid atherosclerosis in a mixed cohort of HIV-infected and uninfected Ugandans. *AIDS Res Hum Retroviruses*. 2017;33(1):49-56.
70. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the advisory board of the 3rd and 4th Watching the Risk symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis*. 2007;23(1):75-80.
71. Veller MG, Fisher CM, Nicolaides AN, Renton S, Geroulakos G, Stafford NJ et al. Measurement of the ultrasonic intima-media complex thickness in normal subjects. *J Vasc Surg*. 1993;17(4):719-25.
72. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzler M. Prediction of clinical cardiovascular events with carotid intima-media thickness: A systematic review and meta-analysis. *Circulation*. 2007;115(4):459-67.
73. Sadiq E, Woodiwiss A, Tade G, Norton G, Modi G. Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke patients in South Africa. *J Neurol Sci*. 2024;465:123186.
74. Roozen GVT, Vos AG, Tempelman HA, Venter WDF, Grobbee DE, Scheuermaier K et al. Cardiovascular disease risk and its determinants in people living with HIV across different settings in South Africa. *HIV Med*. 2020;21(6):386-96.
75. Kim HL, Kim SH. Pulse wave velocity in atherosclerosis. *Front Cardiovasc Med*. 2019;6:41.
76. O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens*. 2002;15(5):426-44.
77. Benjamin LA, Bryer A, Lucas S, Stanley A, Allain TJ, Joeke E et al. Arterial ischemic stroke in HIV: Defining and classifying etiology for research studies. *Neurol Neuroimmunol Neuroinflamm*. 2016;3(4):e254.
78. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of ORG 10172 in Acute Stroke Treatment. *Stroke*. 1993;24(1):35-41.

79. 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;376(9735):112-23.
80. Tipping B, de Villiers L, Wainwright H, Candy S, Bryer A. Stroke in patients with human immunodeficiency virus infection. *J Neurol Neurosurg Psychiatry*. 2007;78(12):1320-4.
81. Benjamin LA, Allain TJ, Mzinganjira H, Connor MD, Smith C, Lucas S et al. The role of human immunodeficiency virus-associated vasculopathy in the etiology of stroke. *J Infect Dis*. 2017;216(5):545-53.
82. Zimba S, Ntanda PM, Lakhi S, Atadzhanov M. HIV infection, hypercoagulability and ischaemic stroke in adults at the University Teaching Hospital in Zambia: A case-control study. *BMC Infect Dis*. 2017;17(1):354.
83. Dawson J, Béjot Y, Christensen LM, De Marchis GM, Dichgans M, Hagberg G et al. European Stroke Organisation (ESO) guideline on pharmacological interventions for long-term secondary prevention after ischaemic stroke or transient ischaemic attack. *Eur Stroke J*. 2022;7(3):I-XLI.
84. Gutierrez J, Goldman J, Dwork AJ, Elkind MS, Marshall RS, Morgello S. Brain arterial remodeling contribution to nonembolic brain infarcts in patients with HIV. *Neurology*. 2015;85(13):1139-45.
85. Gutierrez J, Menshawy K, Gonzalez M, Goldman J, Elkind MS, Marshall R et al. Brain large artery inflammation associated with HIV and large artery remodeling. *AIDS*. 2016;30(3):415-23.
86. Tipping B, de Villiers L, Candy S, Wainwright H. Stroke caused by human immunodeficiency virus-associated intracranial large-vessel aneurysmal vasculopathy. *Arch Neurol*. 2006;63(11):1640-2.
87. Chetty R, Batitang S, Nair R. Large artery vasculopathy in HIV-positive patients: Another vasculitic enigma. *Hum Pathol*. 2000;31(3):374-9.
88. Bamford JM. Classical lacunar syndromes. *Stroke syndromes*, 2nd ed. Cambridge, UK: The Press Syndicate of the University of Cambridge. 2001:583-9.
89. Connor MD, Lammie GA, Bell JE, Warlow CP, Simmonds P, Brett RD. Cerebral infarction in adult AIDS patients: Observations from the Edinburgh HIV Autopsy Cohort. *Stroke*. 2000;31(9):2117-26.

90. Soontornniyomkij V, Umlauf A, Chung SA, Cochran ML, Soontornniyomkij B, Gouaux B et al. HIV protease inhibitor exposure predicts cerebral small vessel disease. *AIDS*. 2014;28(9):1297-306.
91. Fugate JE, Lyons JL, Thakur KT, Smith BR, Hedley-Whyte ET, Mateen FJ. Infectious causes of stroke. *Lancet Infect Dis*. 2014;14(9):869-80.
92. Nagel MA, Cohrs RJ, Mahalingam R, Wellish MC, Forghani B, Schiller A et al. The varicella-zoster virus vasculopathies: Clinical, CSF, imaging, and virologic features. *Neurology*. 2008;70(11):853-60.
93. Forbes HJ, Williamson E, Benjamin L, Breuer J, Brown MM, Langan SM et al. Association of herpesviruses and stroke: Systematic review and meta-analysis. *PLoS One*. 2018;13(11):e0206163.
94. Murala S, Nagarajan E, Bollu PC. Infectious causes of stroke. *J Stroke Cerebrovasc Dis*. 2022;31(4):106274.
95. Gebo KA, Kalyani R, Moore RD, Polydefkis MJ. The incidence of, risk factors for, and sequelae of herpes zoster among HIV patients in the highly active antiretroviral therapy era. *J Acquir Immune Defic Syndr*. 2005;40(2):169-74.
96. Richter ER, Dias JK, Gilbert II JE, Atherton SS. Distribution of herpes simplex virus type 1 and varicella zoster virus in ganglia of the human head and neck. *J Infect Dis*. 2009;200(12):1901-6.
97. Gilden D, Kleinschmidt-DeMasters B, Wellish M, Hedley-Whyte E, Rentier B, Mahalingam R. Varicella-zoster virus, a cause of waxing and waning vasculitis: The New England Journal of Medicine case 5-1995 revisited. *Neurology*. 1996;47(6):1441-6.
98. Nagel M, 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;77(4):364-70.
99. Marais G, Naidoo M, McMullen K, Stanley A, Bryer A, van der Westhuizen D et al. Varicella-zoster virus reactivation is frequently detected in HIV-infected individuals presenting with stroke. *J Med Virol*. 2022;94(6):2675-83.
100. Bobryshev YV, Cherian SM, Inder SJ, Lord RS, Tran D. Identification of HIV-1 in the aortic wall of AIDS patients. *Atherosclerosis*. 2000;152(2):529-30.
101. Feinstein MJ, Hsue PY, Benjamin LA, Bloomfield GS, Currier JS, Freiberg MS et al. Characteristics, prevention, and management of cardiovascular disease in people living with HIV: A scientific statement from the American Heart Association. *Circulation*. 2019;140(2):e98-e124.

102. Walker NF, Scriven J, Meintjes G, Wilkinson RJ. Immune reconstitution inflammatory syndrome in HIV-infected patients. *HIV AIDS (Auckl)*. 2015;7:49-64.
103. Johnson TP, Nath A. New insights into immune reconstitution inflammatory syndrome of the central nervous system. *Curr Opin HIV AIDS*. 2014;9(6):572-8.
104. O'Carroll CB, Barrett KM. Cardioembolic stroke. *Continuum: lifelong learning in neurology*. 2017;23(1):111-32.
105. Sadiq E, Woodiwiss A, Norton G, Modi G. Cardioembolic stroke in an HIV endemic region: Underdiagnosed and severe. *BMJ Neurol Open*. 2024;6(2).
106. Kamel H, Healey JS. Cardioembolic stroke. *Circ Res*. 2017;120(3):514-26.
107. Glezeva N, Gallagher J, Ledwidge M, O'Donoghue J, McDonald K, Chipolombwe J et al. Heart failure in sub-Saharan Africa: Review of the aetiology of heart failure and the role of point-of-care biomarker diagnostics. *Trop Med Int Health*. 2015;20(5):581-8.
108. Gallagher J, McDonald K, Ledwidge M, Watson CJ. Heart failure in sub-Saharan Africa. *Card Fail Rev*. 2018;4(1):21-4.
109. Lin HJ, Wolf PA, Kelly-Hayes M, Beiser AS, Kase CS, Benjamin EJ et al. Stroke severity in atrial fibrillation. The Framingham Study. *Stroke*. 1996;27(10):1760-4.
110. Grinspoon SK, Fitch KV, Overton ET, Fichtenbaum CJ, Zanni MV, Aberg JA et al. Rationale and design of the Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE). *Am Heart J*. 2019;212:23-35.
111. Grinspoon SK, Fitch KV, Zanni MV, Fichtenbaum CJ, Umbleja T, Aberg JA et al. Pitavastatin to prevent cardiovascular disease in HIV infection. *N Engl J Med*. 2023;389(8):687-99
112. Gnonlonfoun D, Adjien K, Adoukonou T. Human immunodeficiency virus infection (HIV), stroke severity and mortality predictive indicator in Centre National Hospitalier et Universitaire-Hubert Koutoukou Maga (CNHU-HKM) Cotonou, Benin. *Afr J Neurol Sci*. 2013;32(2):14-21.

Appendix A: Ethics Clearance Certificates

University of the Witwatersrand Human Research Ethics Committee Clearance Certificates



R14/49 Dr Eitzaz Sadiq and Dr Harold Majane et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140429

NAME:
(Principal Investigator)

Dr Eitzaz Sadiq and Dr Harold Majane et al

DEPARTMENT:

Neurosciences
Charlotte Maxeke Johannesburg Academic Hospital

PROJECT TITLE:

Risk Predictors for Stroke in Human Immunodeficiency
Virus Infected South Africans

DATE CONSIDERED:

25/04/2014

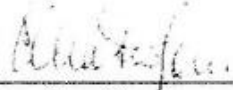
DECISION:

Approved unconditionally

CONDITIONS:

SUPERVISOR:

APPROVED BY:



Professor P Cleaton-Jones, Co-Chairperson, HREC (Medical)

DATE OF APPROVAL:

06/06/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.



R14/49 Professors AJ Woodiwiss & G Norton; Dr E Sadiq

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M190688**

NAME: Professors AJ Woodiwiss & G Norton; Dr E Sadiq

(Principal Investigator)

DEPARTMENT: School of Physiology
Medical School
University

PROJECT TITLE: Risk predictors for stroke in Human Immunodeficiency
Virus-infected South Africans

DATE CONSIDERED: Ad hoc

DECISION: Approved unconditionally

CONDITIONS: Renewal of M140429

SUPERVISOR: Not applicable

APPROVED BY:



Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2019/07/05

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.



R49 Professor AJ Woodiwiss and Dr ME Sadiq

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M240682

NAME: Professor AJ Woodiwiss and Dr ME Sadiq **DEGREE:** N/A
(Principal Investigators)

DEPARTMENT: School of Physiology
Cardiovascular Pathophysiology & Genomics RU
Faculty of Health Sciences

TITLE: *Risk predictors for stroke in Human Immunodeficiency Virus-
infected South Africans*

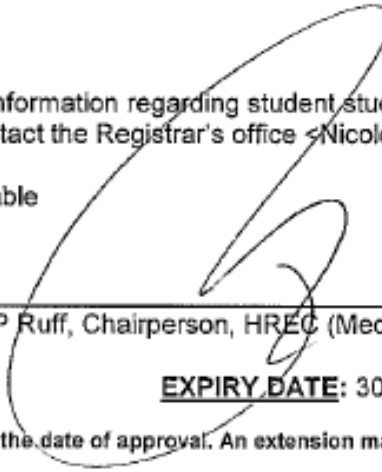
DATE CONSIDERED: Ad hoc

DECISION: Approved unconditionally

CONDITIONS: Renewal of M19/06/88 - expired on 04 July 2024

NOTE: If contact information regarding student study participants is required,
please contact the Registrar's office <Nicoleen.Potgieter@wits.ac.za>

SUPERVISORS: Not applicable

APPROVED BY: 
Professor P Ruff, Chairperson, HREC (Medical)

DATE OF APPROVAL: 31 July 2024

EXPIRY DATE: 30 July 2029

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

Appendix B: Similarity Report (Turnitin)

Turnitin Submission ID: 2587411147

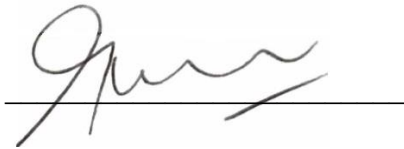
Turnitin Originality Report		
Processed on: 13-Feb-2025 9:11 AM SAST		
ID: 2587411147		
Word Count: 19291		
Submitted: 1		
Integrated Chapter 20250210.docx By Mohammad Sadiq	Similarity Index 15%	Similarity by Source Internet Sources: 7% Publications: 16% Student Papers: 0%

4% match (Internet from 30-Aug-2024) https://neurologyopen.bmj.com/content/bmjno/6/2/e000592.full.pdf
3% match (Eitzaz Sadiq, Alexander von Klemperer, Angela Woodiwiss, Gavin Norton, Girish Modi. "Stroke as the index presentation of traditional cardiovascular risk factors and Human Immunodeficiency Virus in a South African population", Journal of Stroke and Cerebrovascular Diseases, 2024) Eitzaz Sadiq, Alexander von Klemperer, Angela Woodiwiss, Gavin Norton, Girish Modi. "Stroke as the index presentation of traditional cardiovascular risk factors and Human Immunodeficiency Virus in a South African population", Journal of Stroke and Cerebrovascular Diseases, 2024
3% match (Eitzaz Sadiq, Angela Woodiwiss, Grace Tade, Gavin Norton, Girish Modi. "Lack of impact of HIV status on carotid intima media thickness in a cohort of stroke patients in South Africa", Journal of the Neurological Sciences, 2024) Eitzaz Sadiq, Angela Woodiwiss, Grace Tade, Gavin Norton, Girish Modi. "Lack of impact of HIV status on carotid intima media thickness in a cohort of stroke patients in South Africa", Journal of the Neurological Sciences, 2024
2% match (Eitzaz Sadiq, Sarah Katzew, Jeremy Nel, Grace Tade, Angela Woodiwiss, Gavin Norton, Girish Modi. "Stroke, HIV and the immune reconstitution inflammatory syndrome in the absence of opportunistic infections", Journal of the Neurological Sciences, 2024) Eitzaz Sadiq, Sarah Katzew, Jeremy Nel, Grace Tade, Angela Woodiwiss, Gavin Norton, Girish Modi. "Stroke, HIV and the immune reconstitution inflammatory syndrome in the absence of opportunistic infections", Journal of the Neurological Sciences, 2024
1% match (Internet from 03-Aug-2024) https://neurologyopen.bmj.com/content/6/2/e000592.full
1% match (Internet from 20-Oct-2020) https://journals.lww.com/jhypertension/Fulltext/2008/12000/Is_prehypertension_an_independent_predictor_of.7.as
1% match (Eitzaz Sadiq, Angela Woodiwiss, Grace Tade, Gavin Norton, Girish Modi. "HIV status and carotid intima-media thickness", Journal of the Neurological Sciences, 2024) Eitzaz Sadiq, Angela Woodiwiss, Grace Tade, Gavin Norton, Girish Modi. "HIV status and carotid intima-media thickness", Journal of the Neurological Sciences, 2024
1% match (Internet from 03-Sep-2024) https://discovery.researcher.life/topic/times-greater-risk/3099535?page=1&topic_name=Times+Greater+Risk
1% match (Internet from 08-May-2024) https://wiredspace.wits.ac.za/server/api/core/bitstreams/a28adf1a-d4ff-4b56-ab70-bdd5f5263e5c/content

Supervisor Declaration RE: Similarity Report

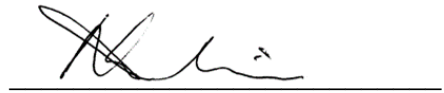
We, the supervisors of this PhD, are satisfied with the 15% Similarity Index reflecting on the Turn-it-in Report.

All sources with >1% similarity are due to the self-citation of the five published papers stemming from this work. This is expected and acceptable in any thesis using the “Publication” or “Integrated” format of PhD which was selected.

A handwritten signature in black ink, appearing to read 'Girish Modi', written over a horizontal line.

Professor Girish Modi

Date: 16/7/2025

A handwritten signature in black ink, appearing to read 'Angela Woodiwiss', written over a horizontal line.

Professor Angela Woodiwiss

Date: 16/7/2025

Appendix C: The Published Papers

The five published papers and their supplemental data are attached in the following order:

Paper 1: Sadiq E, von Klemperer A, Woodiwiss A, Norton G, Modi G. Stroke as the index presentation of traditional cardiovascular risk factors and human immunodeficiency virus in a South African population. *Journal of Stroke and Cerebrovascular Diseases*. 2024; 33(2):107529.

Paper 2: Sadiq E, Woodiwiss A, Tade G, Nel J, Norton G, Modi G. The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients. *HIV Medicine*. 2025;1-10.

Paper 3: Sadiq E, Woodiwiss A, Tade G, Norton G, Modi G. Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke patients in South Africa. *Journal of the Neurological Sciences*. 2024;465:123186.

Paper 3 Response to Letter to the Editor:

Sadiq E, Woodiwiss A, Tade G, Norton G, Modi G. HIV status and carotid intima-media thickness. *Journal of the Neurological Sciences*. 2024;466.

Paper 4: Sadiq E, Katzew S, Nel J, Tade G, Woodiwiss A, Norton G, Modi G. Stroke, HIV and the Immune Reconstitution Inflammatory Syndrome in the absence of opportunistic infections. *Journal of the Neurological Sciences*. 2024;457:122880.

Paper 5: Sadiq E, Woodiwiss A, Norton G, Modi G. Cardioembolic Stroke in an HIV endemic region: underdiagnosed and severe. *BMJ Neurology Open*. 2024;6(2):e000592.

Paper 1

Stroke as the index presentation of traditional cardiovascular risk factors and human immunodeficiency virus in a South African population.

Sadiq E, von Klemperer A, Woodiwiss A, Norton G, Modi G.

Journal of Stroke and Cerebrovascular Diseases. 2024; 33(2):107529.



Stroke as the index presentation of traditional cardiovascular risk factors and Human Immunodeficiency Virus in a South African population

Eitzaz Sadiq^{a,*}, Alexander von Klemperer^a, Angela Woodiwiss^b, Gavin Norton^b, Girish Modi^a

^a Division of Neurology, Department of Neurosciences, School of Clinical Medicine and Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg 2193, South Africa

^b Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

ARTICLE INFO

Keywords:

Stroke
Index
Cardiovascular risk factors
Undiagnosed
Dyslipidaemia
Human Immunodeficiency Virus

ABSTRACT

Objectives: We sought to identify what proportion of each cardiovascular risk factor and Human Immunodeficiency Virus (HIV) was first diagnosed at the time of stroke, compared to those that were diagnosed prior to the event, and to explore if this had any impact on the severity of stroke.

Methods: Adult patients presenting with a new stroke to a quaternary hospital in Johannesburg between 2014 and 2017 were prospectively recruited. Patients were investigated for undiagnosed traditional cardiovascular risk factors (hypertension, diabetes mellitus, dyslipidaemia, atrial fibrillation, obesity and smoking), as well as HIV infection. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS).

Results: 346 patients were included. Stroke was the index presentation for at least one risk factor in 199 (57.5 %) patients. Dyslipidaemia was newly diagnosed in 76.0 % of all dyslipidaemics (95 out of 125). Newly-diagnosed dyslipidaemia was associated with a more severe neurological deficit (Median NIHSS of 12 (8-16) vs 7 (4-12), $p=0.0007$) and younger age on presentation (53 (44-63) years vs 62 (51-71) years, $p=0.02$) as compared to previously-diagnosed dyslipidaemia.

Conclusions: More than half of patients had previously undiagnosed modifiable risk factors at the time of their stroke. Dyslipidaemia was undiagnosed in a very high proportion, and this was associated with a higher stroke severity and younger age of presentation.

Introduction

Stroke is the second leading cause of mortality worldwide, behind only ischemic heart disease.¹ The burden of disease falls disproportionately on lower and lower-middle income countries (LMICs) such as South Africa, with 80 % of the world's burden of stroke-related deaths and 90 % of its disability adjusted life years.^{2,3} Stroke patients in LMICs have poorer access to diagnostic and treatment modalities and greater severity of stroke,⁴ and as such have higher 3-month mortality than those in high-income countries.⁵ Despite this disproportionately large burden of disease, LMICs have access to fewer resources to both prevent and treat stroke.⁶

Large multinational studies affirm that traditional cardiovascular risk factors (RFs) are responsible for the majority of the population risk for stroke.^{4,7-9} These include hypertension (HPT), diabetes mellitus (DM), dyslipidaemia, atrial fibrillation (AF), obesity and smoking. Evidence suggests that the increased burden of stroke in LMICs may in part be due to worse control of modifiable RFs and reduced access to healthcare.¹⁰ In order to address the increasing incidence and burden of disease due to stroke, detailed analysis of the identification and control of modifiable stroke RFs, individualised to specific regions and countries, is required.

It is inappropriate to consider stroke risk factors in South Africa without discussing the contribution of Human Immunodeficiency Virus

Abbreviations: AF, atrial fibrillation; AI, artificial intelligence; ART, antiretroviral therapy; BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; Dx, diagnosed; ELISA, enzyme-linked immunosorbent assay; HDL, high density lipoprotein cholesterol; HIV, Human Immunodeficiency Virus; HPT, hypertension; ICH, intracerebral haemorrhage; IQR, interquartile range; IS, ischaemic stroke; LDL, low density lipoprotein cholesterol; LMICs, lower-middle income countries; MAP, mean arterial pressure; NIHSS, National Institutes of health stroke scale; PLWH, people living with Human Immunodeficiency Virus; RF, risk factor; SSA, Sub-Saharan Africa; TOD, target organ damage; US\$, United States Dollar.

* Corresponding author.

E-mail address: eitzaz.sadiq@wits.ac.za (E. Sadiq).

<https://doi.org/10.1016/j.jstrokecerebrovasdis.2023.107529>

Received 29 June 2023; Received in revised form 5 December 2023; Accepted 8 December 2023

Available online 16 December 2023

1052-3057/© 2023 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

(HIV). As of 2020, it was estimated that 7.8 million South Africans are living with HIV, making it the country with the most people living with HIV (PLWH) in the world.¹¹ HIV has been shown to independently contribute to stroke risk, with HIV positive patients having higher incidence of stroke and at younger ages than those who are HIV negative.^{12–15} There is now good evidence to suggest that HIV may be considered an independent risk factor for stroke.^{15–18} In Sub-Saharan Africa, this increased stroke risk in PLWH appears to be independent of traditional RFs¹⁵ though some recent studies have shown a similar traditional RF profile in PLWH and HIV negative stroke patients.¹⁹

Problem statement

It is clear that traditional RFs and HIV contribute to a large number of strokes in South Africa. It is however unclear how well these conditions are screened for and managed in the public healthcare sector, with its enormous resource constraints and very large patient load.

We thus sought to identify what proportion of each RF was first diagnosed at the time of stroke, compared to those that were diagnosed prior to the event, and to explore if this had any impact on stroke severity. The identification of potential shortcomings in RF screening and diagnosis is the first step in implementing strategies to decrease and control the RF burden which has been shown to contribute to major cardiovascular disease such as stroke.

Methods

This cross-sectional study took place at the Charlotte Maxeke Johannesburg Academic Hospital, a 1000-bed quaternary-level hospital in Johannesburg, South Africa, from August 2014 to November 2017 (40 months).

We recruited consecutive patients aged 18 years and older who presented with a new stroke. Stroke was defined as per the World Health Organization case definition: “a clinical syndrome consisting of rapidly developing clinical signs of focal (at times global) disturbance of cerebral function lasting >24 h or leading to death with no apparent cause other than a vascular origin”.²⁰ Exclusion criteria were the diagnosis of meningitis based on cerebrospinal fluid examination, or the presence of intracranial mass lesions on radiographic imaging, or other stroke mimics. Full informed consent was obtained as per the Helsinki Declaration. Ethics approval was granted by the University of the Witwatersrand Human Research Ethics Committee (Certificate number M140429, renewed as M190688).

All patients were assessed and examined by the same specialist neurologist (ES), who then co-ordinated and interpreted all relevant investigations. A detailed clinical history and review of medical records was used to establish the presence of a pre-diagnosed RF. Computed tomography scans were performed on all patients. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS).

HPT was defined as having 3 or more blood pressure readings greater than 140/90 mm Hg beyond the first 72 h post-stroke, with or without the presence of the following target organ damage (TOD): hypertensive retinopathy on direct ophthalmoscopy, left ventricular hypertrophy on electrocardiogram, echocardiogram or chest X-ray, or renal dysfunction (defined as estimated glomerular filtration rate less than 60 ml/min/1.73m² with no other cause). Acute post-stroke elevated blood pressure (BP) was not used as diagnostic for HPT due to the well-described transient increase in BP seen acutely post-stroke.²¹ Atrial fibrillation was diagnosed on standard 12-lead electrocardiography according to the criteria in the 2014 AHA/ACC/HRS Guideline.²²

DM was defined as a fasting glucose ≥ 7.0 mmol/L, or a random glucose ≥ 11.1 mmol/L, or an HbA1c > 6.5 %. Patients with anaemia were not diagnosed as diabetic solely on HbA1c measurement, to avoid false positives.²³

Dyslipidaemia was defined as a total cholesterol ≥ 5.2 mmol/L, and/or Low Density Lipoprotein Cholesterol (LDL) ≥ 3.3 mmol/L.²⁴ Blood for

lipogram assessment was taken in a non-fasting state. Current evidence suggests that for cardiovascular risk assessment, non-fasting total cholesterol, and LDL are equivalent to a fasting sample.^{24–27} Acute post-stroke transient decreases in cholesterol levels were accounted for by taking blood for lipograms within 24 h of the stroke onset, where possible, and by not considering a low post-stroke High Density Lipoprotein Cholesterol (HDL) to be diagnostic for dyslipidaemia.^{28–30}

Obesity was defined as a body mass index (BMI) of ≥ 30 kg/m². This was obtained by measuring the patient's height using a stadiometer and the weight using a calibrated mechanical column scale where BMI = weight $\div$ height². This was not possible in patients unable to stand unaided.

HIV testing (ELISA) was performed in all consenting patients.

Statistical analysis

Data was analysed using Excel (Microsoft) and Graphpad Prism (Graphpad Software V9.5). Continuous variables were evaluated for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. Chi square, Fisher's exact test and Mann-Whitney statistical tests were then performed for parametric and non-parametric variables where appropriate. Data are reported as median with interquartile ranges (IQR) unless otherwise specified. Only available data was analysed. Missing data was not imputed. P values of less than 0.05 were considered significant.

Results

346 patients presenting with stroke met inclusion criteria. (Fig. 1) Basic demographic data is shown in Table 1. The proportion of male to female patients was exactly 1:1, by random chance. Most patients were black African, with the proportions of different ethnicities being in keeping with the national population of South Africa.¹¹ 309 patients (89.3 %) had ischaemic stroke (IS), with 37 (10.7 %) having intracerebral haemorrhage (ICH).

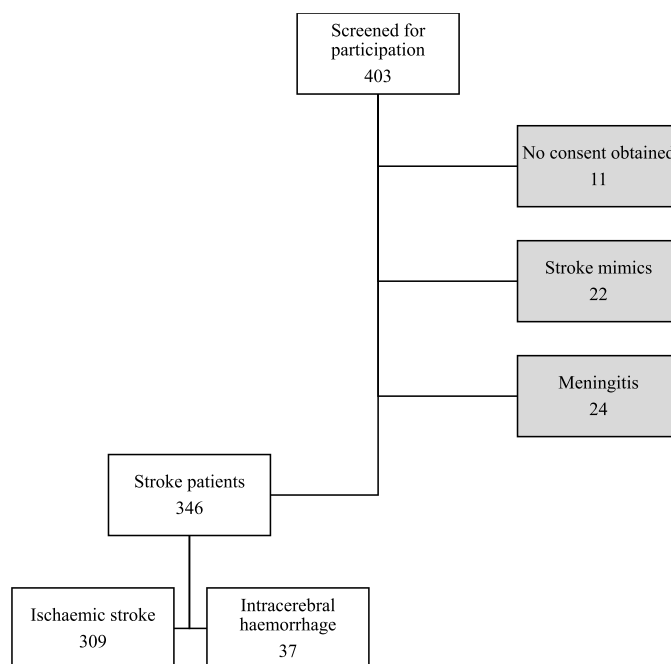


Fig. 1. Study flowchart
Blocks shaded in grey indicate patients not included in the study.

Table 1
Demographic characteristics of included patients.

Patients Included		N (%)
Age		346
Sex	Median (IQR)	52 (42-63)
	Male	173 (50)
	Female	173 (50)
Ethnicity	African	273 (78.9)
	Caucasian	45 (13.0)
	Mixed	17 (4.9)
	Indian/Asian	11 (3.2)
HIV Status	Positive	87 (25.1)
	Negative	234 (67.6)
	Unknown	25 (7.2)
Traditional RFs	HPT	229 (66.2)
	Dyslipidaemia (n=339)	125 (36.9)
	Obesity (n= 215)	76 (35.3)
	Smoking	91 (26.3)
	DM	73 (21.1)
	Atrial fibrillation	24 (6.9 %)

DM: diabetes mellitus, HIV: Human Immunodeficiency Virus, HPT: hypertension, IQR: interquartile range

Cardiovascular risk factors

The commonest RF was HPT, which was present in 66.2 % of the cohort, followed by dyslipidaemia in 36.9 % of the 339 patients for whom blood lipid profiles were available (Table 1) The median total cholesterol was 4.4 (3.7 - 5.1) mmol/L, LDL 2.7 (2.1 - 3.4) mmol/L, HDL 1.1 (0.9 - 1.4) mmol/L and triglycerides 1.0 (0.8 - 1.41) mmol/L.

Of those patients who had body mass index measured (n = 215), 35.3 % were clinically obese with BMI > 30 kg/m² (Table 1). Amongst this group the median BMI was 35.4 kg/m² (32.1 - 40.1 kg/m²) as compared to 27.9 kg/m² (24.0 - 32.9 kg/m²) for the cohort as a whole.

73 patients (21.1 %) had diabetes mellitus, of which only 2 (2.7 %) had Type 1 DM, whilst the remaining 71 patients had Type 2 DM. Amongst diabetic patients the median HbA1c was 7.9 % (6.9 - 10.9 %). Atrial fibrillation was detected in 24 patients (6.9 %).

25.1 % of patients were HIV positive, which was significantly higher than the expected HIV prevalence rate in the general South African population of 13.1 %¹¹ (χ^2 , p <0.0001). 7.2 % of patients refused testing for HIV or were unable to consent.

Out of the total of 346 stroke patients, 44.2 % had one known RF, whilst 27.2 % and 9.2 % of the population were known with two and three RFs respectively (Fig. 2A).

Stroke as the index presentation for risk factors

We further analysed those patients for whom the following RFs were newly diagnosed on admission: HPT, DM, dyslipidaemia, atrial fibrillation (AF) as well as HIV (as a non-traditional RF). Obesity and smoking are difficult to define as “newly diagnosed” RFs and were thus not analysed further.

In our cohort, stroke was the index presentation for at least 1 RF in 199 patients (57.5 %) (Fig. 2B). Amongst those patients with newly-diagnosed RFs on presentation, 72.4 % were newly-diagnosed with one new RF, 24.6 % were diagnosed with two and 3.0 % had three new RFs diagnosed.

Of the 281 patients known with RFs prior to their stroke, 162 (57.7 %) were newly diagnosed with one or more additional RFs on presentation with stroke (Fig. 2C).

The greatest proportion of newly-diagnosed patients compared to those with a pre-existing diagnosis was in dyslipidaemia (76.0 % of dyslipidaemics were newly diagnosed as such) followed by AF (50 %), DM (39.7 %) and HIV (37.9 %). HPT was newly-diagnosed in only 20.5

% of hypertensive patients. These proportional differences were statistically significant (Fig. 2D, χ^2 , p <0.0001).

The proportion of patients with newly diagnosed HPT and HIV was higher in males compared to females. There was no sex difference in newly diagnosed DM, dyslipidaemia and AF (Table 2).

Newly-diagnosed dyslipidaemia is associated with younger age and more severe stroke

Patients newly diagnosed with dyslipidaemia were significantly younger and had a higher NIHSS on presentation than those who were previously-diagnosed (Table 3). Those patients previously-diagnosed with dyslipidaemia were all on statin treatment, and had lower total and LDL cholesterol. BMI was similar between the groups.

Table 4 shows the relationships between RF and NIHSS on univariate regression analysis and on multivariate regression analyses. Univariate regression analyses revealed that a new diagnosis of dyslipidaemia as well as smoking were the only RFs having a significant effect on NIHSS. Multivariate linear regression models including age and all RFs with p≤0.25 on univariate regressions (sex, smoking, DM, HIV and newly diagnosed dyslipidaemia) demonstrated the only significant effect on NIHSS to be from those with newly diagnosed dyslipidaemia. Similarly multivariate regression models including age and all RF's with p≤0.25 on univariate analysis, but including all patients with dyslipidaemia (and not only those who were newly diagnosed as such), revealed the diagnosis of dyslipidaemia (known and newly diagnosed combined) as having no effect on NIHSS. This confirms that it is newly diagnosed dyslipidaemia (and not all dyslipidaemia) which influences stroke severity. Smoking and DM were the only 2 RFs affecting stroke severity in this analysis. The coefficient for smoking indicates a lower stroke severity in smokers (Table 4).

Newly diagnosed hypertension is associated with greater odds of ICH

A greater proportion of patients with ICH had HPT than patients with IS (97.3 % vs 62.46 %, Fischer's exact test, p <0.0001). Patients being diagnosed for the first time with HPT (n = 47) had a greater proportion of ICH than those patients (n=182) with known HPT (25.53 % vs 13.19 %, Odds Ratio 2.57 (1.00 - 4.81), Fishers exact test, p =0.045).

In comparing newly-diagnosed and established hypertensive patients (Table 5), the newly diagnosed patients had higher admission blood pressures than the patients who were already known with HPT. The age of presentation was similar between the groups.

Whilst the median NIHSS score for patients with ICH was greater than that for patients with IS (14¹⁰⁻²⁰ vs 11⁷⁻¹⁶, p =0.0074), being newly-diagnosed with HPT was not associated with a higher NIHSS score than being known with the diagnosis (Table 5).

We found no significant difference between the two groups when comparing any individual markers of hypertensive heart disease, or renal dysfunction (Table 4). Patients with established HPT were more likely to have 1 of the above features of TOD present than those newly diagnosed (Odds ratio 2.1 (1.1-3.9), p = 0.03). However, it was still notable that 44.7 % of newly diagnosed hypertensive patients had evidence of TOD, other than the stroke (Table 5).

With regards to AF, there was no difference in age, sex, other RFs or stroke severity between those known with AF and those newly diagnosed (Table S1 in online supplement). All known AF patients were found to be sub-therapeutically anticoagulated at the time of stroke.

New diagnosis of HIV on presentation with stroke had a higher median age than those with pre-existing diagnosis

Of the 87 patients who were HIV positive, 33 were newly diagnosed (37.9 %). Patients with newly diagnosed HIV were older than those with established HIV, and more likely to be male (Table 6). There were no differences in other RFs, NIHSS or BMI.

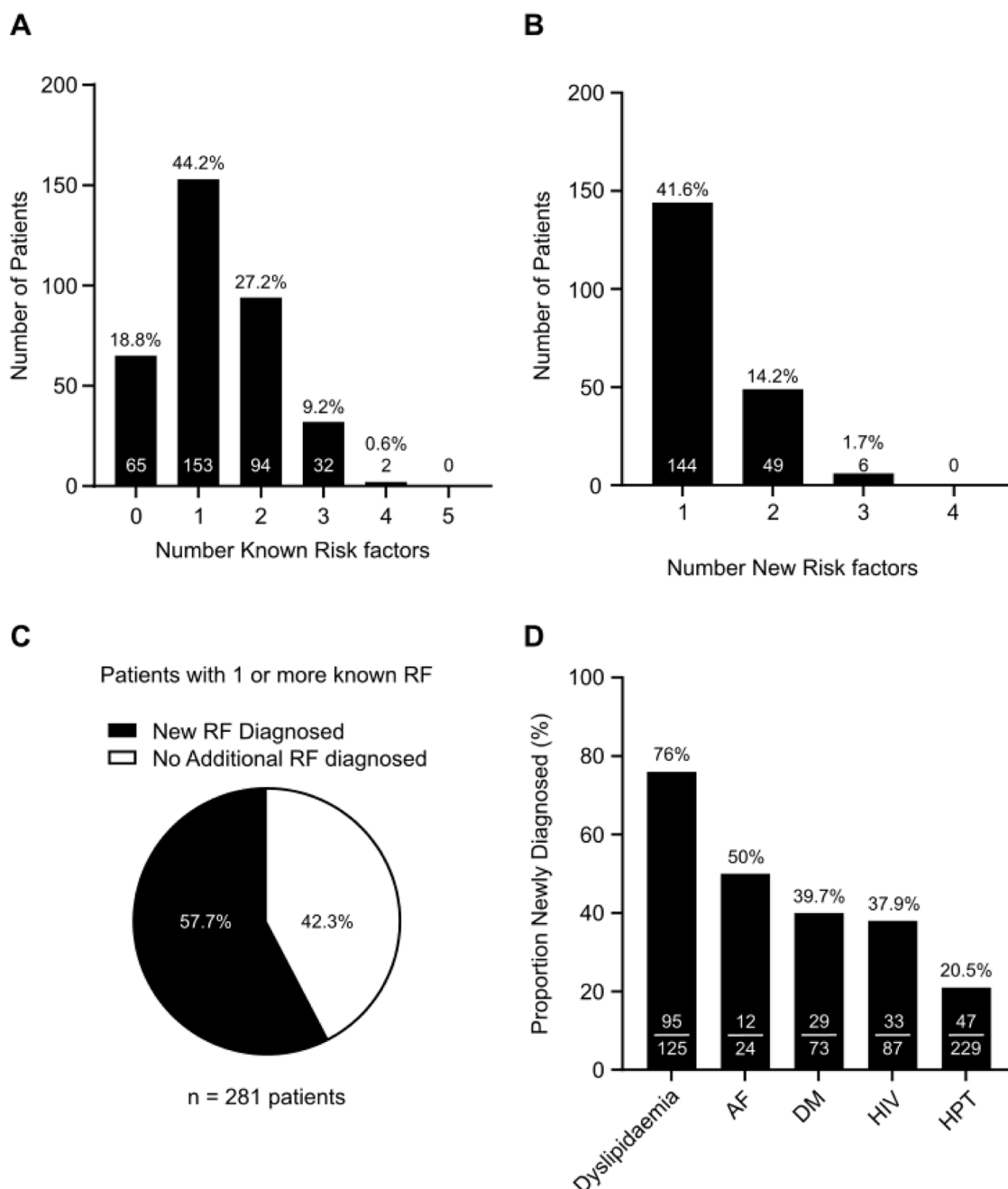


Fig. 2. Stroke is the index presentation for a number of risk factors

A Bar chart showing the number of previously identified cardiovascular risk factors (RF) within the patient population. Included risk factors are : Atrial fibrillation (AF), Diabetes Mellitus (DM), dyslipidaemia, obesity, infection with Human Immunodeficiency Virus (HIV), hypertension (HPT) and smoking. Chart shows absolute number of patients (bottom) and percentage of the cohort (top) with each number of RF. **B** Bar chart showing the number of newly diagnosed risk factors. Included risk factors are : AF, DM, Dyslipidemia, HPT and HIV. Chart shows absolute number of patients (bottom) and percentage of total population (top) with each number of newly identified RF. **C** Pie Chart showing the proportion of patients (as percentage) with 1 or more known risk factor who were diagnosed with additional risk factors on presentation with stroke. **D** Bar chart showing the proportion of patients with each RF for whom stroke was the index presentation for that RF.

Newly diagnosed diabetic patients were younger than those with a pre-existing diagnosis

Newly diagnosed diabetic patients were younger on presentation than those with a known prior diagnosis (Table 7). There was no significant difference in HbA1c between newly diagnosed and existing diabetic patients with pre-diagnosed patients with DM having a median HbA1C of 9.0 %. There was also no difference in other RFs, NIHSS, BMI or sex between the two groups.

Discussion

The identification and management of cardiovascular RFs remains the most valuable tool in stroke prevention at a community level.

From our cohort of 346 stroke patients seen at a major treatment centre in Johannesburg over a 40-month period, we found stroke to be the index presentation of at least one RF in 199 patients (57.5 % of our cohort). Over a quarter of these had two or more new RFs identified which may have contributed to the stroke pathogenesis. It is unknown whether these patients had ever sought healthcare in the past. However,

Table 2

Comparison of newly diagnosed RF between males and females.

RF	Proportion of each RF which is newly diagnosed		P-value
	Males	Females	
HPT	30/114 (26.3 %)	17/115 (14.8 %)	0.03
DM	17/40 (42.5 %)	12/33 (36.4 %)	0.59
Dyslipidaemia	45/58 (77.6 %)	50/67 (74.6 %)	0.70
Atrial fibrillation	3/10 (30.0 %)	9/14 (64.3 %)	0.11
HIV	21/43 (48.8 %)	12/44 (27.3 %)	0.04

DM: diabetes mellitus, HIV: Human Immunodeficiency Virus, HPT: hypertension, RF: Risk factor

Table 3

Comparison between newly diagnosed and known dyslipidaemic patients.

	Newly Diagnosed n = 95 (IQR)	Known Dyslipidaemia n = 30 (IQR)	P value
Age (years)	53 (44 - 63)	62 (51 - 71)	0.02
Male (%)	47.4	43.3	0.70
HPT (%)	26.3	16.7	0.28
DM (%)	27.4	20.0	0.42
Smoking (%)	17.9	40.0	0.012
Total Cholesterol (mmol/L)	5.4 (5.1 - 6.2)	3.8 (3.2 - 4.7)	<0.0001
LDL (mmol/L)	3.7 (3.4 - 4.3)	2.2 (1.7 - 3.0)	<0.0001
BMI (kg/m ²)	n = 62 29.5 (25.4 - 35.6)	n = 17 29.7 (26.8 - 34.1)	0.64
Obesity (%)	n = 62 43.6	n = 17 41.2	0.86
NIHSS	12 (8-16)	7 (4-12)	0.0007

BMI: Body Mass Index, DM: diabetes mellitus, HPT: hypertension, LDL: Low Density Lipoprotein, NIHSS: National Institutes Of Health Stroke Scale

of the 281 patients with at least 1 established RF, who are thus assumed to have accessed healthcare, 57.7 % had an additional RF identified on presentation with stroke. We do not know how many of these RFs were previously screened for (negatively) at the healthcare centre the patient was accessing. If they were not screened, this may represent a relatively easily accessible, high cardiovascular risk population which will potentially benefit greatly from the identification and treatment of all major RFs before they lead to stroke and other cardiovascular disease.

Our findings of a significantly younger age in patients with newly diagnosed dyslipidaemia and DM (when compared to those with a pre-existing diagnosis) is of great interest. Such diseases are considered “diseases of the elderly”, thus younger individuals may be less likely to be screened for them. On the contrary, our finding of a higher median age in those with newly diagnosed HIV compared to those with a pre-

stroke HIV diagnosis may indicate how HIV screening may be neglected in an older population, who may be considered to be at lower risk for acquiring the disease. Male patients had a significantly higher proportion of undiagnosed HPT and HIV when compared to female patients. Previous studies have suggested that males are less likely than females to seek healthcare, particularly for screening purposes.³¹ Our described age and sex differences are indicative of potential shortcomings in the screening of RFs and may assist in the targeting of screening towards specific demographics for particular diseases.

Dyslipidaemia was the most commonly undiagnosed RF. A striking 76 % of dyslipidaemics were only diagnosed as such on presentation with a stroke. This likely directly reflects the deficiencies of dyslipidaemia screening in South Africa at primary healthcare level, despite the South African Dyslipidaemia Guidelines recommending screening for dyslipidaemia in all adults from the age of 40 years.²⁴ A recent cohort from rural South Africa diagnosed 2860 participants with dyslipidaemia, of which only 30 (1 %) were aware of their diagnosis prior to

Table 5

Comparison of newly diagnosed and established hypertensive patients.

	Newly Diagnosed HPT n = 47 (IQR)	Established HPT n = 182 (IQR)	P-value
Age (years)	56 (45-64)	57(49-69)	0.15
Male (%)	63.8	46.2	0.03
DM (%)	21.2	30.8	0.20
Dyslipidaemia (%)	34.0	43.7	0.23
Smoking (%)	27.7	25.3	0.74
Obesity (%)	30.4	43.9	0.23
Presentation Systolic BP (mmHg)	184 (167 - 199)	156 (135-188)	<0.0001
Presentation Diastolic BP (mmHg)	116 (102 - 133)	94 (82 - 114)	<0.0001
Presentation MAP (mmHg)	141 (128 - 154)	115 (100 - 137)	<0.0001
Presentation Heart rate (bpm)	89 (75 - 103)	90 (78 - 100)	0.90
NIHSS	12 (7-15)	11(7.75-17)	0.95
Any evidence of TOD	21 (44.7 %)	114 (62.6 %)	0.03
Features of HHD on Echo	n=33 13 (39.4 %)	n=128 57 (44.5 %)	0.69
Decreased EF on Echo	n = 32 3 (9.4 %)	n=127 27 (21.3 %)	0.20
Atrial fibrillation	0 (0 %)	6 (3.3 %)	0.35
Renal dysfunction	n = 45 11 (24.4 %)	n=173 57 (32.9 %)	0.37

BP: Blood pressure, Echo: Echocardiogram EF: Ejection Fraction, HHD: Hypertensive Heart Disease, HPT: Hypertension, MAP: Mean arterial pressure, IQR: Interquartile range, NIHSS: National Institutes of Health Stroke Scale, TOD: Target organ damage

Table 4

Risk factors (RF) associated with NIHSS on univariate and multivariate regression analyses.

RF	Univariate regression (n=346**)		Multivariate regression*† (n=114**)		Multivariate regression*†† (n=314**)	
	β-Coefficient±SEM	P value	β-Coefficient±SEM	P value	β-Coefficient±SEM	P value
Age	-0.014±0.020	0.51	-0.069±0.039	0.078	-0.016±0.023	0.487
Sex	0.896±0.632	0.16	1.438±1.099	0.194	0.201±0.704	0.776
Smoking	-2.042±0.712	0.004	-2.643±1.341	0.051	-2.116±0.799	0.009
HPT	-0.397±0.670	0.55	-	-	-	-
Newly Dx HPT	-0.069±0.956	0.94	-	-	-	-
DM	1.195±0.775	0.12	1.933±1.261	0.128	1.720±0.836	0.041
Newly Dx DM	0.491±1.411	0.73	-	-	-	-
Dyslipidaemia	-1.126±0.657	0.09	-	-	-1.155±0.774	0.088
Newly Dx Dyslipid	3.879±1.181	0.001	3.281±1.350	0.017	-	-
BMI	-0.013±0.045	0.77	-	-	-	-
HIV	0.835±0.730	0.25	-1.068±1.323	0.421	0.908±0.675	0.241
Newly Dx HIV	0.210±1.307	0.87	-	-	-	-
Atrial fibrillation	-0.958±1.247	0.44	-	-	-	-
Newly Dx Atrial fibrillation	1.750±2.538	0.50	-	-	-	-

BMI: Body Mass Index, DM: diabetes mellitus, Dx: diagnosed, HIV: Human Immunodeficiency Virus, HPT: hypertension,

*Age and only those RF where $p \leq 0.25$; †Newly diagnosed dyslipidaemia included in model; ††Dyslipidaemia included in model; significant RF are shown in bold type

**Differences in sample size are due to missing data regarding lipograms (n=7) and HIV status (n=25 did not consent to testing)

Table 6

Comparison of newly diagnosed and known HIV patients.

	Newly Diagnosed HIV n = 33 (IQR)	Known HIV n = 54 (IQR)	P- value
Age (years)	47 (37.5 - 55.5)	39.6 (31-49.25)	0.02
Male (%)	63.6	40.7	0.04
HPT (%)	10.0	15.8	0.59
DM (%)	9.1	3.7	0.29
Dyslipidaemia (%)	24.2	34.6	0.31
Smoking (%)	36.4	18.5	0.07
BMI (kg/m ²)	n = 22 26.6 (23.3 - 29.6)	n = 36 27.0 (22.5 - 32.4)	0.66
NIHSS	12 (8 - 18)	12.5 (8 - 17.3)	0.95

BMI: Body mass index, **HIV:** Human Immunodeficiency Virus, **NIHSS:** National Institutes of Health Stroke Scale

Table 7

Comparison of newly diagnosed and known diabetic patients.

	Newly Diagnosed n = 29 (IQR)	Known Diabetes n = 44 (IQR)	P- value
Age (years)	57 (48 - 65)	61 (55 - 74)	0.02
Male (%)	58.6	52.3	0.59
HPT (%)	79.3	97.7	0.009
Dyslipidaemia (%)	34.5	51.2	0.16
Smoking (%)	34.5	22.7	0.27
BMI (kg/m ²)	n = 17 29.9 (24.5 - 38.7)	n = 29 31.7 (28.7 - 35.6)	0.37
HbA1c (%)	n = 28 7.5 (6.9 - 9.8)	n = 43 9.0 (7.2 - 11.0)	0.18
Fasting glucose (mmol/L)	n = 10 8.0 (6.2 - 9.8)	n = 11 10.2 (7.3 - 16.0)	0.10
NIHSS	14 (9 - 16)	11.5 (8 - 17.8)	0.73

BMI: Body mass index, **HbA1c:** Glycated Haemoglobin, **NIHSS:** National Institutes of Health Stroke Scale

recruitment.³² We acknowledge that it is not possible to screen all individuals for dyslipidaemia in South Africa due to the scarcity of resources and an overburdened public healthcare system. We thus require a more pragmatic, focussed approach to dyslipidaemia screening. A multicentre South African study revealed a prevalence of dyslipidaemia of 76.7 % in 614 patients with HPT and DM.³³ A targeted dyslipidaemia screening strategy beginning with patients already accessing healthcare for the treatment of other RFs is thus likely to detect large numbers of previously undiagnosed dyslipidaemics in a high-risk population.

Our most striking finding was that those with previously undiagnosed dyslipidaemia at the time of stroke had more severe stroke and at a younger age. To our knowledge, this has not been described before. It provides compelling evidence of the detrimental consequences of a poorly screened RF and emphasises the urgent need for intervention. It is unclear why it is only in dyslipidaemia that we noted this phenomenon, and not in HPT, DM or HIV. The 30 patients pre-diagnosed with dyslipidaemia had a median total (4.4mmol/L), HDL (1.1mmol/L) and LDL (2.7mmol/L) cholesterol within the broad targets of the South African Dyslipidaemia Guidelines,²⁴ suggesting that once diagnosed, the dyslipidaemia management appears to be adequate. All of the patients with known dyslipidaemia were on statin treatment prior to the stroke. Previous studies have described pre-stroke statin therapy being associated with lower stroke severity.³⁴⁻³⁶ Whether our described difference in stroke severity and age of stroke is related to the cardiovascular-protective effects of statins cannot be concluded from our data. More important however is our extrapolation that poor community-level screening and diagnosis of dyslipidaemia has catastrophic consequences on stroke morbidity.

In keeping with much preceding literature, the most common RF amongst stroke patients seen in our cohort was HPT.^{2,8} However only 20.5 % of our patients with HPT were newly-diagnosed at the time of

stroke. In the non-stroke literature, one large multicentre trial found that 47 % were unaware of their diagnosis of HPT.³⁷ This figure is even higher elsewhere in Sub-Saharan Africa (SSA), where a systematic review revealed a figure up to 70 % in this region.³⁸ A recent South African study revealed 52 out of 99 (52.5 %) of HPT patients were only diagnosed on presentation with stroke, however the methodology for HPT diagnosis was not clearly described.¹³ Our relatively low proportion of newly diagnosed HPT was unexpected. There was no clear methodological limitation or bias which may explain this finding, and our described methods of diagnosing HPT in an acute stroke patient were very unlikely to represent under diagnosis of HPT. It is our hope that this data represents an effective HPT screening program in our urban community. The lack of age difference between our known HPT and newly-diagnosed HPT groups may also be indicative of a screening program which appears to be equally detecting patients of all ages, although the previously noted sex-discrepancy suggests that males may require more targeting. Undiagnosed HPT was found to be associated with higher odds of having an ICH. Even though ICH had a higher median NIHSS than IS, this did not translate to a significantly greater stroke severity in those who were newly diagnosed with HPT compared to those with known HPT. Of concern was our finding that 44.7 % of newly diagnosed hypertensive patients already had other evidence of TOD on presentation with stroke, suggesting a prolonged period of undiagnosed and thus untreated HPT. Our relatively low prevalence of AF (6.9 %) is likely reflective of the young age of our stroke population (median 52 years).

Our high proportion of previously undiagnosed DM (39.7 %) was in keeping with previous non-stroke studies in the South African population.^{39,40} This remains a major concern for a common disease such as DM, with an easily accessible, inexpensive, and simple method of screening.

The past 2 decades in South Africa have seen the implementation and rollout of the world's largest antiretroviral therapy (ART) program.⁴¹ Our finding of approximately 40 % of patients with HIV only being diagnosed on presentation with stroke was very concerning. While a recent systematic review of stroke in SSA revealed stroke to be the presenting symptom of HIV in 42 to 57 % of patients,¹⁵ the papers referenced in this review were both from South Africa from the early 2000's, when the ART rollout program had just commenced, and not yet widely implemented.^{42,43} The lack of distinct improvement in this figure over the past 15 years, despite the massive ART rollout program, is of great concern and warrants further investigation. As mentioned, the significantly older age of the newly-diagnosed HIV patients, and the higher proportion of undiagnosed HIV in males compared to females may identify particular population groups to target in HIV screening.

To our knowledge this is the first study from South Africa looking 'backwards' at stroke patients' missed RFs, in an attempt to identify gaps in RF screening pathways. A recent study from Zambia reported similarly high proportions of newly-diagnosed traditional RFs (20 % of HPT, 43 % of DM, 91 % of dyslipidaemia) but only 12 % of HIV patients were first diagnosed on presentation with stroke.⁴⁴ In 2019, South Africa reported an annual health expenditure of US\$ 546 per capita, compared to US\$ 69 per capita in Zambia.⁴⁵ One would thus expect RF screening in South Africa to be far better, particularly from an urban community in the economic hub of the region, Johannesburg. The identification of characteristic patient profiles which are particularly poorly screened for specific RFs may assist to remedy this.

Our data is surprisingly similar to a recent study from Switzerland, where 69.7 % of 1125 stroke patients with no previous RFs were diagnosed with a new RF, of which dyslipidaemia (61.4 %) was the most frequent.⁴⁶ This is despite the vast differences in healthcare resources and access between Switzerland and South Africa. However patients who already had pre-existing RFs (n=3229) had far fewer newly diagnosed RFs than in our cohort (HPT in 8.6 %, DM in 4.1 %), although dyslipidaemia was still newly diagnosed in 37.9 %. This may indicate that patients who are accessing healthcare for a pre-existing RF are

being effectively screened for other RFs, in contrast to our findings. Of note is that the median age of 70 years (IQR 15.2) was almost 20 years older than our cohort. Other studies from Taiwan,⁴⁷ Austria⁴⁸ and France⁴⁹ also indicate that the high proportion of undiagnosed RFs at the time of stroke is indeed a global challenge.

Limitations

A limitation of our study was its hospital-based nature, including only strokes severe enough to warrant presentation to hospital i.e. at the extreme end of the spectrum of target organ damage from cardiovascular RFs. It was not possible to assess stroke patients that were not admitted, but managed at home, and importantly we could not assess the overall community-based RF screening and RF control of the non-stroke “healthy” population. It is also noted that education level and residence in an urban compared to rural setting impacts on knowledge of cardiovascular RFs.⁵⁰ Our study was conducted in a large centre in the city of Johannesburg, which may bias patient selection in this regard. However our high rates of undiagnosed RFs despite being in this urban setting likely does not bode well for the efficacy of a similar screening and management program in more isolated, resource-constrained rural healthcare facilities with poorly educated communities. Our use of non-fasting lipograms may have resulted in the over-diagnosis of dyslipidaemia. However, there is evidence to suggest that non-fasting total and LDL cholesterol are equivalent to a fasting sample for cardiovascular risk assessment.^{24–27} We elected to use slightly higher total and LDL cholesterol cut-off values than in many other SSA stroke studies as an attempt to mitigate for potential over-diagnosis. We also did not consider a low post-stroke HDL cholesterol to be solely diagnostic for dyslipidaemia, to account for the described transient post-stroke decrease in cholesterol levels.^{28–30} Our inability to measure weight and height in patients unable to stand unaided led to missing BMI data in patients with higher stroke severities.

Future directions

Our novel findings on the detrimental effects of poor RF diagnosis, particularly with regards to dyslipidaemia, paves the way for future local research in the form of prospective community-based studies aimed at motivating for policy change on RF screening.

We demonstrated that over 50 % of individuals with pre-existing RFs were diagnosed with a further RF on presentation with stroke. These patients, who are likely already attending healthcare facilities, are the simplest to screen for other RFs and possibly will benefit the most, considering their already elevated cardiovascular risk profile. We recommend a lower threshold for screening such patients for further RFs at a primary healthcare level.

Our findings of a younger age in newly-diagnosed DM and dyslipidaemia, and older age in newly-diagnosed HIV, when compared to with those with previously-diagnosed RFs, suggest that target ages for screening may also need to be revised. Sex differences, with poorer pre-stroke diagnosis of HPT and HIV in males compared to females, may also assist in identifying specific populations to target for screening. Our data points to the need for urgent public health interventions in the screening and diagnosis of RFs in order to best prevent catastrophic cardiovascular outcomes such as stroke.

Conclusions

More than half of our cohort had at least one RF only diagnosed on presentation with stroke, of which dyslipidaemia was the commonest. Newly diagnosed dyslipidaemia was associated with more severe stroke, and at a younger age than those with a pre-existing diagnosis.

CRedit authorship contribution statement

Eitzaz Sadiq: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alexander von Klemperer:** Writing – review & editing, Writing – original draft, Software, Formal analysis. **Angela Woodiwi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gavin Norton:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Girish Modi:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Eitzaz Sadiq was supported by the Discovery Foundation Academic Fellowship Award (Grant Reference number 035747), allowing for dedicated research time as a clinician scientist. The funder played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. There is thus no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jstrokecerebrovasdis.2023.107529](https://doi.org/10.1016/j.jstrokecerebrovasdis.2023.107529).

References

- World Health Organisation. *The Top 10 Causes of Death*. World Health Organisation; 2019 [Available from: <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>. Accessed on 6 May 2021].
- Collaborators GBDS. Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol.* 2021;20(10):795–820.
- Krishnamurthi RV, Ikeda T, Feigin VL. Global, regional and country-specific burden of ischaemic stroke, intracerebral haemorrhage and subarachnoid haemorrhage: a systematic analysis of the global burden of disease study 2017. *Neuroepidemiology.* 2020;54(2):171–179.
- Langhorne P, O'Donnell MJ, Chin SL, et al. Practice patterns and outcomes after stroke across countries at different economic levels (INTERSTROKE): an international observational study. *Lancet.* 2018;391(10134):2019–2027.
- Jacob MA, Ekker MS, Allach Y, et al. Global differences in risk factors, etiology, and outcome of ischemic stroke in young adults: a worldwide meta-analysis: the GOAL initiative. *Neurology.* 2022;98(6):e573–e588.
- Owolabi M, Johnson W, Khan T, et al. Effectively combating stroke in low- and middle-income countries: placing proof in pragmatism—the Lancet neurology Commission. *J Stroke Med.* 2018;1(1):65–67.
- Namale G, Kamacooko O, Kinengyere A, et al. Risk factors for hemorrhagic and ischemic stroke in Sub-Saharan Africa. *J Trop Med.* 2018;2018, 4650851.
- O'Donnell MJ, Chin SL, Rangarajan S, et al. Global and regional effects of potentially modifiable risk factors associated with acute stroke in 32 countries (INTERSTROKE): a case-control study. *Lancet.* 2016;388(10046):761–775.
- Abboud H, Labreuche J, Arauz A, et al. Demographics, socio-economic characteristics, and risk factor prevalence in patients with non-cardioembolic ischaemic stroke in low- and middle-income countries: the OPTIC registry. *Int J Stroke.* 2013;8 Suppl(A100):4–13.
- Rahbar MH, Medrano M, Diaz-Garelli F, et al. Younger age of stroke in low-middle income countries is related to healthcare access and quality. *Ann Clin Transl Neurol.* 2022;9(3):415–427.
- Statistics South Africa. Mid year Population Estimates 2021 (P0302) [Available from: <https://www.statssa.gov.za/publications/P0302/P03022021.pdf>. Accessed on 17 September 2022].
- Benjamin LA, Bryer A, Emsley HCA, et al. HIV infection and stroke: current perspectives and future directions. *Lancet Neurol.* 2012;11(10):878–890.
- Kroon L, van Zyl DG, Schutte CM, et al. Risk factors for stroke in HIV-positive and negative patients in Pretoria, South Africa. *J Stroke Cerebrovasc Dis.* 2021;30(8), 105929.
- Zimba S, Nutakki A, Chishimba L, et al. Risk factors and outcomes of HIV-associated stroke in Zambia. *AIDS.* 2021;35(13):2149–2155.

15. Abdallah A, Chang JL, O'Carroll CB, et al. Stroke in Human Immunodeficiency Virus-infected Individuals in Sub-Saharan Africa (SSA): a systematic review. *J Stroke Cerebrovasc Dis.* 2018;27(7):1828–1836.
16. Benjamin LA, Corbett EL, Connor MD, et al. HIV, antiretroviral treatment, hypertension, and stroke in Malawian adults: a case-control study. *Neurology.* 2016; 86(4):324–333.
17. Walker RW, Jusabani A, Aris E, et al. Stroke risk factors in an incident population in urban and rural Tanzania: a prospective, community-based, case-control study. *Lancet Glob Health.* 2013;1(5):e282–e288.
18. Gutierrez J, Albuquerque ALA, Falzon L. HIV infection as vascular risk: a systematic review of the literature and meta-analysis. *PLoS ONE.* 2017;12(5): e0176686.
19. Mbonde AA, Chang J, Musubire A, et al. An analysis of stroke risk factors by HIV serostatus in Uganda: implications for stroke prevention in sub-Saharan Africa. *J Stroke Cerebrovasc Dis.* 2022;31(7), 106449.
20. Hatano S. Experience from a multicentre stroke register: a preliminary report. *Bull World Health Organ.* 1976;54(5):541–553.
21. Qureshi AI. Acute hypertensive response in patients with stroke: pathophysiology and management. *Circulation.* 2008;118(2):176–187.
22. January CT, Wann LS, Alpert JS, et al. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. *J Am Coll Cardiol.* 2014;64(21):e1–e76.
23. Florkowski C. HbA1c as a diagnostic test for diabetes mellitus - reviewing the evidence. *Clin Biochem Rev.* 2013;34(2):75–83.
24. Klug E, Raal FJ, Marais AD, et al. South African dyslipidaemia guideline consensus statement: 2018 update A joint statement from the South African Heart Association (SA Heart) and the Lipid and Atherosclerosis Society of Southern Africa (LASSA). *S Afr Med J.* 2018;108(11b):973–1000.
25. Langsted A, Nordestgaard BG. Nonfasting versus fasting lipid profile for cardiovascular risk prediction. *Pathology.* 2019;51(2):131–141 (Phila).
26. Nordestgaard BG. A test in context: lipid profile, fasting versus nonfasting. *J Am Coll Cardiol.* 2017;70(13):1637–1646.
27. Di Angelantonio E, Sarwar N, Perry P, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA.* 2009;302(18):1993–2000.
28. Barth JH, Jackson BM, Farrin AJ, et al. Change in serum lipids after acute coronary syndromes: secondary analysis of SPACE ROCKET study data and a comparative literature review. *Clin Chem.* 2010;56(10):1592–1598.
29. Kargman DE, Tuck C, Berglund L, et al. Lipid and lipoprotein levels remain stable in acute ischemic stroke: the Northern Manhattan Stroke Study. *Atherosclerosis.* 1998; 139(2):391–399.
30. Perovic E, Mrdjen A, Harapin M, et al. Short term changes of serum lipids in acute ischemic stroke. *Clin Lab.* 2016;62(11):2107–2113.
31. Drammeh B, Medley A, Dale H, et al. Sex differences in HIV testing - 20 PEPFAR-supported Sub-Saharan African countries, 2019. *MMWR Morb Mortal Wkly Rep.* 2020;69(48):1801–1806.
32. Reiger S, Jardim TV, Abrahams-Gessel S, et al. Awareness, treatment, and control of dyslipidemia in rural South Africa: the HAALSI (Health and Aging in Africa: a Longitudinal Study of an INDEPTH Community in South Africa) study. *PLoS ONE.* 2017;12(10), e0187347.
33. Masilela C, Adeniyi OV, Benjeddou M. Prevalence, patterns and determinants of dyslipidaemia among South African adults with comorbidities. *Sci Rep.* 2022;12(1): 337.
34. Choi JC, Lee JS, Park TH, et al. Effect of pre-stroke statin use on stroke severity and early functional recovery: a retrospective cohort study. *BMC Neurol.* 2015;15:120.
35. Martínez-Sánchez P, Fuentes B, Martínez-Martínez M, et al. Treatment with statins and ischemic stroke severity: does the dose matter? *Neurology.* 2013;80(19): 1800–1805.
36. Deplanque D, Masse I, Lefebvre C, et al. Prior TIA, lipid-lowering drug use, and physical activity decrease ischemic stroke severity. *Neurology.* 2006;67(8): 1403–1410.
37. Chow CK, Teo KK, Rangarajan S, et al. Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. *JAMA.* 2013;310(9):959–968.
38. Ataklte F, Ergou S, Kaptoge S, et al. Burden of undiagnosed hypertension in sub-Saharan Africa: a systematic review and meta-analysis. *Hypertension.* 2015;65(2): 291–298 (Dallas, Tex : 1979).
39. Adedokun AO, Ter Goon D, Owolabi EO, et al. Prevalence, awareness, and determinants of type 2 diabetes mellitus among commercial taxi drivers in buffalo city metropolitan municipality South Africa: a cross-sectional survey. *Medicine.* 2019;98(9):e14652 (Baltimore).
40. Erasmus RT, Soita DJ, Hassan MS, et al. High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: baseline data of a study in Bellville, Cape Town. *S Afr Med J.* 2012;102(11 Pt 1):841–844.
41. UNAIDS South Africa Factsheet 2020 [Available from: <https://www.unaids.org/en/regionscountries/countries/southafrica>. Accessed on 17 September 2022.
42. Mochan A, Modi M, Modi G. Stroke in black South African HIV-positive patients: a prospective analysis. *Stroke.* 2003;34(1):10–15.
43. Tipping B, de Villiers L, Wainwright H, et al. Stroke in patients with Human Immunodeficiency Virus infection. *J Neurol Neurosurg Psychiatry.* 2007;78(12): 1320–1324.
44. Nutakki A, Chomba M, Chishimba L, et al. Risk factors and outcomes of hospitalized stroke patients in Lusaka, Zambia. *J Neurol Sci.* 2021;424, 117404.
45. World Health Organization Global Health Expenditure database 2022 [Available from: <http://apps.who.int/nha/database>. Accessed on 4 April 2023.
46. Rêgo A, Nannoni S, Scherz A, et al. Undiagnosed major risk factors in acute ischaemic stroke patients: frequency, profile, stroke mechanisms and outcome. *Eur J Neurol.* 2023. <https://doi.org/10.1111/ene.16011> [Internet]. Epub 31 July 2023 [Available from: Accessed on 25 November.
47. Sung SF, Lai ECC, Wu DP, et al. Previously undiagnosed risk factors and medication nonadherence are prevalent in young adults with first-ever stroke. *Pharmacoepidemiol Drug Saf.* 2017;26(12):1458–1464.
48. Matz K, Keresztes K, Tatschl C, et al. Disorders of glucose metabolism in acute stroke patients: an underrecognized problem. *Diabetes Care.* 2006;29(4):792–797.
49. Bugnicourt JM, Flament M, Guillaumont MP, et al. Predictors of newly diagnosed atrial fibrillation in cryptogenic stroke: a cohort study. *Eur J Neurol.* 2013;20(10): 1352–1359.
50. Boateng D, Wekesah F, Browne JL, et al. Knowledge and awareness of and perception towards cardiovascular disease risk in sub-Saharan Africa: a systematic review. *PLoS ONE.* 2017;12(12), e0189264.

Table S1: Comparison of newly diagnosed and known atrial fibrillation patients.

	Newly Diagnosed AF n = 12 (IQR)	Known AF n = 12 (IQR)	P-value
Age (years)	69.5 (67.0-74.5)	65.5 (58.0-71.5)	0.39
Male (%)	25.0	58.3	0.11
HPT (%)	83.3	83.3	1.00
DM (%)	16.7	41.7	0.19
Dyslipidaemia (%)	41.7	33.3	0.50
Obesity (%)	57.1 (n=7)	71.4 (n=7)	0.58
Smoking (%)	16.7	8.3	0.54
NIHSS	12.0 (7.0-15.5)	12.0 (3.5-15.5)	0.67

AF: atrial fibrillation, **IQR:** Interquartile range, **NIHSS:** National Institutes of Health Stroke Scale

Paper 2

The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients.

Sadiq E, Woodiwiss A, Tade G, Nel J, Norton G, Modi G.

HIV Medicine. 2025;1-10.

ORIGINAL ARTICLE

The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients

Eitzaz Sadiq¹  | Angela Woodiwiss² | Grace Tade² | Jeremy Nel³ | Gavin Norton² | Girish Modi¹

¹Division of Neurology, Department of Neurosciences, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

²Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

³Division of Infectious Diseases, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Correspondence

Eitzaz Sadiq, Division of Neurology, Department of Neurosciences, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193, Johannesburg, South Africa.
Email: eitzaz.sadiq@wits.ac.za

Funding information

Discovery Foundation Academic Fellowship

Abstract

Background: HIV-associated vasculopathy is known to cause stroke in people living with HIV (PLWH). The role of atherosclerosis is unclear. We assessed the aetiology of vasculopathy in PLWH and tested the utility of markers of subclinical atherosclerosis to distinguish atherosclerotic (AV) from non-atherosclerotic vasculopathy (NAV).

Methods: This cross-sectional study recruited PLWH with stroke at a hospital in Johannesburg, South Africa, from 2014 to 2017. Patients with meningitis were excluded. Cerebrospinal fluid (CSF) was tested for multi-viral polymerase chain reaction, including varicella zoster virus (VZV). Once an aetiological category was assigned, carotid intima-media thickness (cIMT) and aortic pulse wave velocity (PWV) were compared in AV and NAV, and to predetermined thresholds for subclinical atherosclerosis (cIMT ≥ 0.70 mm, PWV ≥ 10.00 m/s).

Results: Some 28/85 PLWH (32.9%) vs. 9/109 (8.3%, $p < 0.0001$) people without-HIV had vasculopathy on computed tomography angiography. Only four PLWH had AV. Compared with NAV ($n = 11$), those with AV were older (50.0 ± 4.1 vs. 39.2 ± 9.2 years, $p = 0.04$) and had more cardiovascular risk factors (median 2.0 [IQR 1.5–2.5] vs. 0.0 [IQR 0.0–1.0], $p = 0.02$). cIMT in AV was higher than in NAV (1.01 ± 0.07 mm [$n = 4$] vs. 0.63 ± 0.04 mm [$n = 9$], $p < 0.001$). All with AV had cIMT and PWV above the predetermined thresholds, while all except one with NAV were below. We found evidence of VZV in eight PLWH and HIV-associated vasculitis in six.

Conclusions: Vasculopathy in PLWH in our region appears to be predominantly non-atherosclerotic. cIMT and PWV were useful adjuncts in distinguishing AV from NAV. Despite excluding meningitis, VZV was implicated in a large proportion, emphasizing the likely underdiagnosis of this treatable infection. We thus recommend CSF VZV testing in all PLWH with stroke.

KEYWORDS

atherosclerosis, HIV, stroke, varicella zoster, vasculopathy

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2025 The Author(s). *HIV Medicine* published by John Wiley & Sons Ltd on behalf of British HIV Association.

INTRODUCTION

HIV-associated vasculopathy has been identified as a cause of stroke in up to a third of cases in people living with HIV (PLWH) [1, 2]. It is a broad term, encompassing abnormalities of blood vessels which may include an atherosclerotic vasculopathy (AV), non-atherosclerotic vasculopathy (NAV), HIV-associated vasculitis and small vessel disease (SVD) [3].

AV and NAV both affect large- to medium-sized vessels, resulting in stenotic lesions. However in the case of NAV, there is no histopathological evidence of atheroma [4–6]. The clinical differentiation of AV from NAV is based predominantly on the patient's age (>45 years), the presence of traditional cardiovascular risk factors (TRFs) or the presumed presence of atherosclerosis elsewhere in the body [3]. There are, however, no definitive radiological or clinical features to distinguish the two entities, nor is there any biomarker which has been tested in this setting.

Carotid intima media thickness (cIMT) is a well-recognized and validated surrogate marker for atherosclerosis in the cardiovascular system [7]. The presence of an increased cIMT is associated with an increased risk of cardiovascular disease, including stroke [7, 8]. Similarly, aortic pulse wave velocity (PWV) non-invasively measures large artery stiffness and is a marker of subclinical arteriosclerosis and atherosclerosis [9]. Hence, cIMT and PWV measurements may assist in objectively differentiating AV and NAV in PLWH.

There is insufficient evidence to suggest that atherosclerosis is a major contributor to HIV-related stroke in sub-Saharan Africa (SSA). On the contrary, opportunistic infections have been shown to contribute to 23%–55% of strokes in PLWH, often on the basis of a vasculitis [1]. Varicella zoster virus (VZV) reactivation in particular has a known association with stroke in PLWH [10–13]. From our large cohort of stroke patients from the epicentre of the world's HIV pandemic, we investigated the pathogenesis of HIV-associated vasculopathy and determined the roles of atherosclerosis and VZV in this disease process. We also assessed the utility of cIMT and PWV to distinguish AV from NAV.

METHODS

This cross-sectional study took place at the Charlotte Maxeke Hospital in Johannesburg South Africa, from August 2014 to November 2017.

We recruited consecutive patients (age ≥ 18 years) with ischaemic stroke (using the World Health Organization case definition) [14]. Patients diagnosed with

meningitis on cerebrospinal fluid (CSF) examination were excluded (see [Supporting Information Methods](#)).

All patients were assessed by the same specialist neurologist (ES). Stroke aetiology classification was guided by a consensus paper on HIV-related stroke classification [3]. TRFs (i.e., hypertension [HT], diabetes mellitus [DM], dyslipidaemia and obesity) were defined as detailed in the [Supporting Information Methods](#), together with the full protocol of laboratory investigations. HIV testing (enzyme-linked immunosorbent assay [ELISA]) was performed in all consenting patients. A suppressed viral load (VL) was defined as <50 copies/mL. A lumbar puncture was performed in all PLWH if not contraindicated. Cerebrospinal fluid (CSF) was examined for routine biochemistry, microscopy and a multi-viral polymerase chain reaction (PCR) panel, including VZV (see [Supporting Information Methods](#)).

Computed tomography (CT) scanning of the brain was performed in all patients and was examined by a specialist radiologist and neurologist (ES). All patients had carotid ultrasound performed by a specialist radiologist, assessing for occlusive atherosclerotic plaque. CT angiography (aortic arch, neck and brain) was performed in all patients with no clearly identified cause for the stroke. The cardiac evaluation was performed by a specialist cardiologist (detailed in the [Supporting Information Methods](#)).

Additional carotid Doppler studies to assess cIMT were performed using high-resolution B-mode ultrasound, and large artery stiffness was indexed from aortic PWV using applanation tonometry. The cIMT and PWV examinations were performed by a single operator (blinded to the HIV status of the patients) who had undergone dedicated training in the relevant techniques, which are described elsewhere and in the [Supporting Information Methods](#) [15, 16]. The operator was not part of the patient's clinical management team and was blinded to the aetiological category which had been already assigned to the patient. No changes were made to the classification after cIMT and PWV measurements. A carotid $\text{IMT} \geq 0.7$ mm and a $\text{PWV} \geq 10.00$ m/s were considered suggestive of subclinical atherosclerosis as per previously published data [7, 9, 17, 18].

Definitions of vasculopathies

Only patients with vascular abnormalities on angiography were classified as vasculopathy. These included the presence of monofocal or multifocal occlusions, alternating constrictions and dilatations ('beading') or aneurysmal/dolichoectatic changes in the intracranial or extracranial vessels. In order to diagnose AV we required the visualization of atherosclerotic plaque causing a $>50\%$ occlusion of

TABLE 1 Comparing traditional risk factors and stroke aetiology in people living with HIV and people-without-HIV.

Parameter	PLWH (<i>n</i> = 85)	People-without-HIV (<i>n</i> = 109)	<i>P</i> -value
Age (years)	43.4 ± 12.5	45.0 ± 10.5	0.33
Male (%)	49.4	47.7	0.81
HT (%)	43.5	60.6	0.018
DM (%)	5.9	22.9	0.001
Dyslipidaemia (%)	24.7	30.3	0.39
Smoking (%)	25.9	18.4	0.21
Obesity (%)	25.8 (16/62)	34.6 (28/81)	0.26
Median number of TRFs (IQR)	1.0 (0–2)	2.0 (1–2)	0.02
Stroke aetiology			
Cardioembolic (%)	20.0	30.3	<0.0001
SVD (%)	16.5	18.4	
Vasculopathy (%)	32.9	8.3	
Other (%)	4.7 ^a	6.4 ^b	
Undetermined (%)	25.9	36.7	

Abbreviations: DM, diabetes mellitus; HT, hypertension; IQR, interquartile range; SVD, small vessel disease; TRFs, traditional cardiovascular risk factors; VZV, varicella zoster virus.

^aIncluding recreational drug usage (*n* = 2), VZV with normal angiography (*n* = 2).

^bIncluding recreational drug usage (*n* = 3), hypercoagulable/hyperviscosity syndrome (*n* = 4).

the internal carotid (ICA) or common carotid artery (CCA) ipsilateral to the stroke on the ultrasound studies performed by the radiologist. As our objective was to test the utility of cIMT and PWV to distinguish AV and NAV, we did not use these measurements in the diagnostic workup of the patient but only compared them once a diagnostic category was allocated. HIV-associated vasculitis was diagnosed in the setting of angiographic features of vasculitis without an identified causative opportunistic infection [3]. SVD was defined as the presence of a traditional lacunar syndrome with an infarct <20 mm in size [3].

Statistical analysis

Data were analysed using Statistica Ver14.0.0.15, TIBCO Software Inc. Continuous variables were evaluated for normality using Shapiro–Wilk and Kolmogorov–Smirnov tests. Chi square, Fisher's exact test, Student's *t*-test and Mann–Whitney statistical tests were performed for parametric and non-parametric variables where appropriate. Continuous data are reported as mean ± SD unless otherwise specified. Missing data were not imputed. *P*-values <0.05 were considered significant.

Informed consent was obtained as per the Declaration of Helsinki [19]. Ethics approval was granted by the University of the Witwatersrand Human Research Ethics Committee (Certificates M140429/M190688/M240682).

RESULTS

A total of 85 PLWH with ischaemic stroke were recruited and compared with 109 age- and sex-matched people-without-HIV with ischaemic stroke recruited during the same time period (Figure S1). PLWH had less HT, DM and median number of TRFs than people-without-HIV, with a higher prevalence of vasculopathy (Table 1). Some 82/85 (96.5%) PLWH underwent angiography; the remaining three patients presented with unequivocal cardioembolic strokes.

Vasculopathy in PLWH

Twenty-eight PLWH (32.9%) had vascular abnormalities on angiography. Two distinct types of vasculopathy were identified: a large vessel monofocal occlusion (*n* = 16) and a multifocal vasculitis (*n* = 12) (Figure 1).

Monofocal occlusion

Some 11/16 PLWH (68.7%) had no evidence of atherosclerosis (NAV). Only four PLWH had AV, while one PLWH with monofocal occlusion of their left MCA had a recent zoster rash and was thus categorized separately as monofocal occlusion secondary to VZV-vasculitis (Figure 1). PLWH with AV were older than those with NAV, had

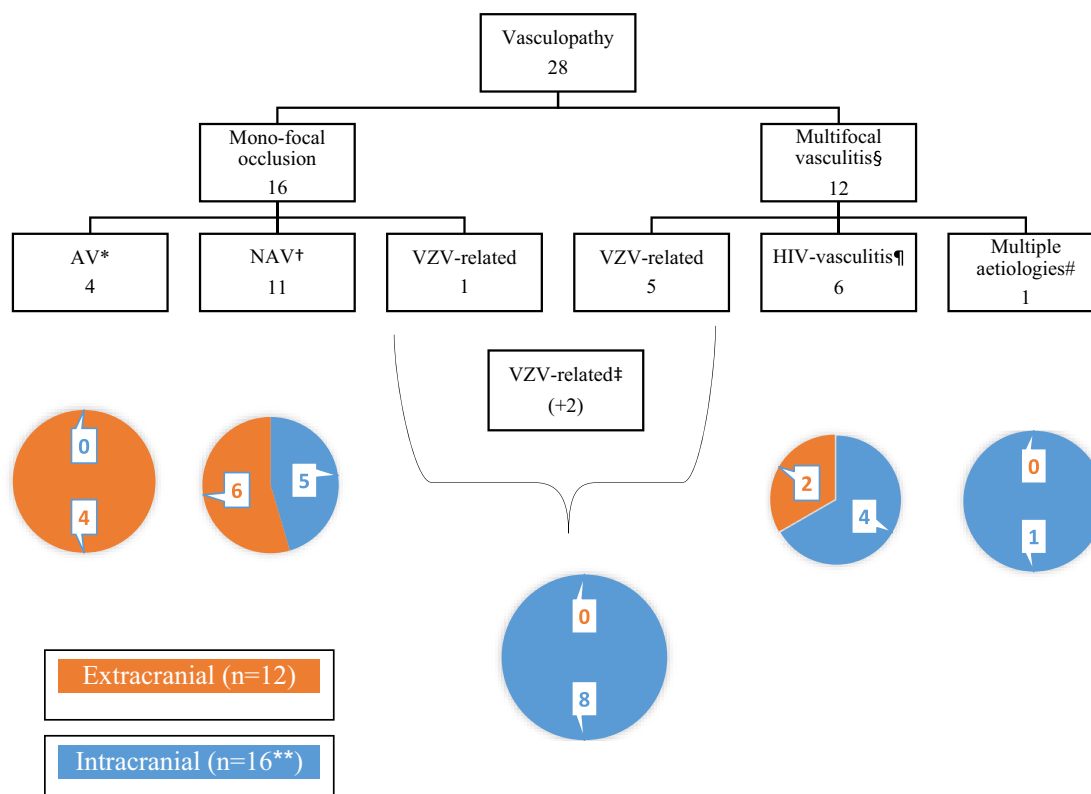


FIGURE 1 The aetiology of vasculopathy in people living with HIV (PLWH). *All four patients showed extracranial stenosis at the carotid bulb/proximal internal carotid artery (ICA). †The location was intracranial in five (all middle cerebral artery, MCA), and extracranial in six (common carotid artery (CCA) in three, ICA in two and vertebral artery in one). ‡Two patients with non-lacunar small vessel strokes also had evidence of recent VZV infection (not classified as ‘vasculopathy’ as angiography was normal). §The involved intracranial vessels included the bilateral ICA, MCA and/or anterior cerebral arteries (ACA). ¶The intracranial blood vessels involved included the ICA, MCA and ACA, while the two patients with extracranial multifocal vasculitis had involvement of the CCA and ICA, or the ICA alone (which extended partially intracranially). #One patient with multifocal vasculitis had multiple plausible aetiologies for stroke (recreational drug usage and neurosyphilis). This patient (43 years old, CD4 470 cells/ul, VL12000 copies/mL, antiretroviral therapy naïve) had bilateral irregularly beaded MCAs, and a saccular intracranial ICA aneurysm. This was the only patient in the cohort who had clear aneurysmal changes. **Excluding the two patients described in ‡ above. AV, atherosclerotic vasculopathy; NAV, non-atherosclerotic vasculopathy; VZV, varicella zoster virus.

more TRFs and a higher mean cIMT. PWV was only possible in one patient but was also markedly elevated (Table 2).

We then tested the utility of cIMT and PWV in differentiating AV and NAV. All patients with NAV had a cIMT below the threshold of 0.70 mm, while all of those with AV were above this threshold (Figure 2a). One outlier with NAV had a PWV of 11.1 m/s (a 34-year-old with no TRFs and cIMT of 0.67 mm) (Figure 2b). However the overall mean was 6.45 ± 1.77 m/s, with no other NAV patient having a PWV over 8.2 m/s.

Multifocal vasculitis

A multifocal large vessel vasculitis was noted in 12 patients (Figure 1). VZV was implicated in five of these (three were CSF VZV PCR-positive, while two had recent cutaneous zoster infection, but negative CSF VZV PCR). There was

also evidence of recent cutaneous zoster infection in one patient with monofocal MCA occlusion, and a further two patients with non-lacunar small vessel strokes. As these two patients had normal angiography, we elected not to classify them as ‘vasculopathy’, even though a normal CT angiogram does not exclude a small vessel vasculitis. VZV was thus potentially implicated in 21.4% (6/28) of PLWH with confirmed vasculopathy on angiography, or 26.7% (8/30) of those with presumed vasculopathy (Figure 1). PLWH with VZV-related stroke had a lower mean CD4 count compared with PLWH with non-VZV related stroke and were younger, with a lower body mass index (BMI) and fewer TRFs (Table 3). While 5/8 VZV patients were antiretroviral therapy (ART)-exposed, only one had VL-suppression. The cIMT and PWV were lower in VZV-related stroke patients (Table 3); however, multivariate regressions revealed these differences to be due to the difference in age, and not VZV status (Table S1).

TABLE 2 Comparison of atherosclerotic and non-atherosclerotic vasculopathy in people living with HIV.

Parameter	Atherosclerotic vasculopathy (AV) (<i>n</i> = 4)	Non-atherosclerotic vasculopathy (NAV) (<i>n</i> = 11)	<i>P</i> -value
Age (years)	50.0 ± 4.1	39.2 ± 9.2	0.04
Male (%)	100.0	36.4	0.03
HT (%)	75.0	18.2	0.04
DM (%)	0.0	0.0	1.00
Dyslipidaemia (%)	25.0	9.1	0.42
Smoking (%)	100.0	9.1	0.001
Obesity (%)	0.0 (<i>n</i> = 2)	12.5 (<i>n</i> = 7)	0.60
Median number of TRFs (IQR)	2 (1.5–2.5)	0 (0–1)	0.02
CD4 (cells/μL)	474.5 ± 255.3	351.2 ± 312.5	0.49
Median VL (copies/mL) (IQR)	2200 (0–192 800) (<i>n</i> = 3)	2156 (0–25 476)	0.87
ART-exposed (%)	50.0	45.5	0.88
Median ART duration (months) (IQR)	102 (60–144) (<i>n</i> = 2)	10.0 (4–60)	0.10
VL suppressed (%)	50.0	60.0	0.81
cIMT (mm)	1.01 ± 0.07 (<i>n</i> = 4)	0.63 ± 0.04 (<i>n</i> = 9)	<0.001
PWV (m/s)	12.60 (<i>n</i> = 1)	6.80 ± 2.14 (<i>n</i> = 8)	0.04

Abbreviations: ART, antiretroviral therapy; AV, atherosclerotic vasculopathy; cIMT, carotid intima-media thickness; DM, diabetes mellitus; HT, hypertension; IQR, interquartile range; NAV, non-atherosclerotic vasculopathy; PWV, pulse wave velocity; TRFs, traditional cardiovascular risk factors; VL, viral load.

In six PLWH with multifocal vasculitis the aetiology was unknown and was thus categorized as ‘HIV-associated vasculitis’ after the exclusion of autoimmune, inflammatory and other infective vasculitides (detailed in the [Supporting Information Methods](#)). The remaining patient with multifocal vasculitis had multiple plausible aetiologies (detailed in Figure 1). There was no angiographic evidence of a small vessel vasculitis in any patient. When comparing VZV-vasculitis to HIV-associated vasculitis, patients with VZV had a lower age, lower CD4 count and lower mean BMI, but no difference in VL or ART usage (Table 3). The PWV was higher in HIV-associated vasculitis, but below the pre-defined cut-off value for atherosclerosis (Table 3).

Comparison of patients with intracranial and extracranial vasculopathy revealed no differences in demographics, TRFs, CD4, VL, ART exposure, cIMT or PWV (Table S2). We then compared NAV (*n* = 11) to multifocal vasculitis (*n* = 12) under the assumption that there is no proven association between atherosclerosis and multifocal vasculitis. There were no differences in the above variables between the two groups (Table S2).

Small vessel disease

SVD was identified in 14 PLWH. Compared with non-SVD patients (*n* = 71), they had a higher mean age (53.6 ± 8.4 vs. 41.4 ± 12.2 years, *p* < 0.001), greater TRF prevalence and higher mean cIMT (0.78 ± 0.12

vs. 0.66 ± 0.17 mm, *p* = 0.03) (Table S3). Multivariate regressions revealed the difference in cIMT to be due to age, and not TRF burden (*b* = 0.00817, SE = 0.0014, *p* < 0.001, *n* = 64). PWV was similar in the two groups. SVD patients had a higher mean CD4, despite similar ART usage. Comparison of SVD in PLWH and people-without-HIV revealed no difference in age, sex, TRFs, cIMT or PWV (Table S3).

Effect of ART on cIMT and PWV

Some 40/85 PLWH (47.1%) were on ART at the time of their stroke. 37/40 were on a regimen containing emtricitabine, tenofovir and efavirenz. The three patients on protease-inhibitor-containing regimens had no evidence of an atherosclerosis-mediated aetiology (two with multifocal vasculitis, and one with cardioembolism from a metallic heart valve, all with normal cIMT and PWV measurements). There was no difference in demographics, TRFs, mean CD4, cIMT or PWV in the ART-exposed compared with ART-naïve patients (Table S4).

Vasculopathy in people-without-HIV

Some 9/109 people-without-HIV (8.3%) had vasculopathy, compared with 32.9% of PLWH (*p* < 0.001, Table 1). Three had extracranial AV, while six had intracranial multifocal vasculitis of large/medium sized vessels.

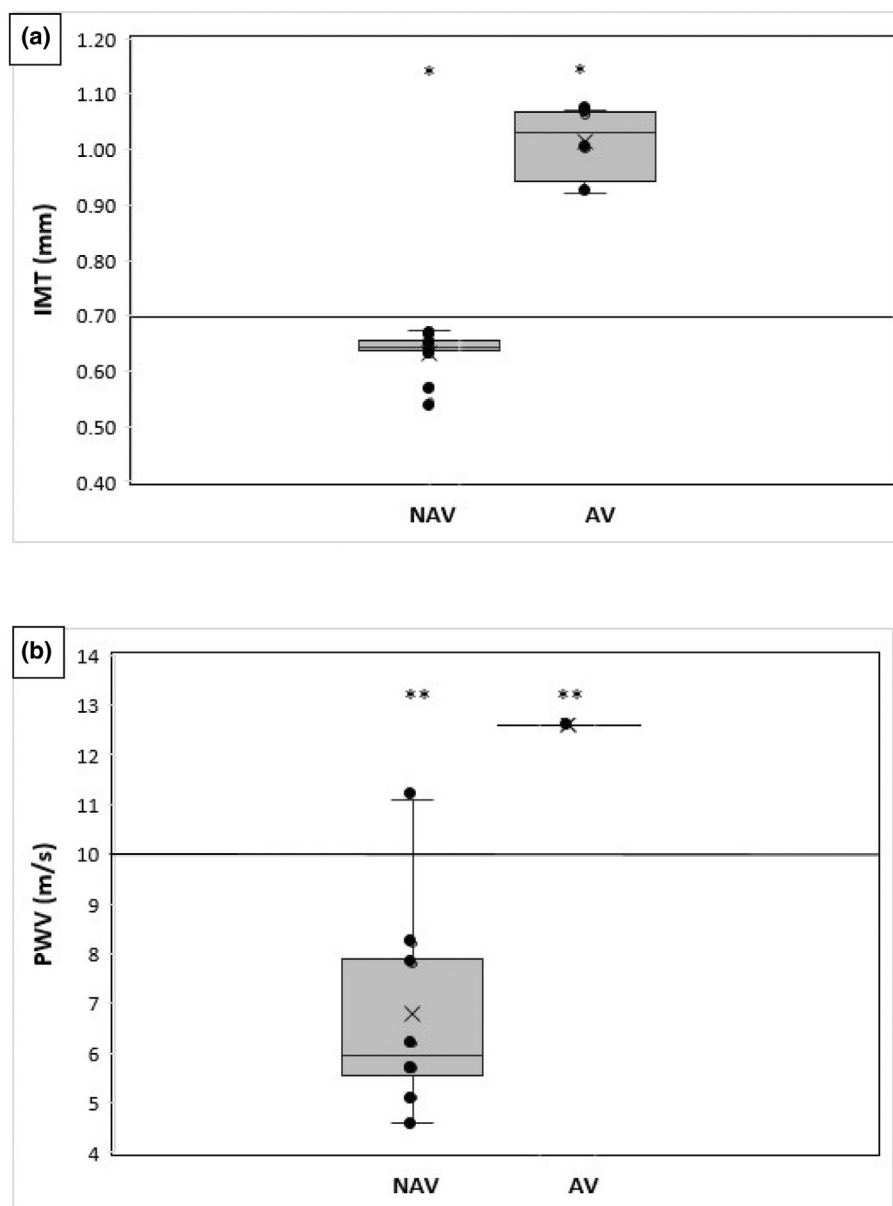


FIGURE 2 Box plots of (A) carotid intima media thickness (cIMT) and (B) aortic pulse wave velocity (PWV) to distinguish atherosclerotic (AV) from non-atherosclerotic vasculopathy (NAV). Comparison of cIMT and PWV to the predefined cut-off values (horizontal lines, cIMT = 0.70 mm, PWV = 10.00 m/s) revealed that all patients with NAV had a cIMT below the threshold, while all of those with AV were above this threshold. One outlier with NAV had a PWV of 11.1 m/s. * $p < 0.001$, ** $p = 0.04$, (Student's t -test). PWV was only possible in one patient with AV (12.60 m/s).

The aetiology was vasculitis secondary to systemic autoimmune disease ($n = 3$), Takayasu's arteritis ($n = 2$) and postpartum vasculitis ($n = 1$). There were no people-without-HIV with monofocal NAV.

DISCUSSION

We investigated the aetiology of vasculopathy in PLWH and explored the role of atherosclerosis in one of the largest PLWH stroke studies from SSA [1]. We described the

presence of vasculopathy in almost one-third of strokes in PLWH, compared with 8.3% of age and sex-matched people-without-HIV. AV was uncommon in PLWH (4.7% of all strokes). We demonstrated that measurements of cIMT and PWV correlated well with the clinico-radiological distinction of AV from NAV, and no evidence of subclinical atherosclerosis in NAV was shown. Despite the exclusion of meningitis from our cohort, we still implicated VZV in approximately one-quarter of PLWH with vasculopathy. This points to the likely under-diagnosis of this condition in patients who appeared to

TABLE 3 Comparisons of people living with HIV with varicella zoster virus (VZV)-related stroke, non-VZV related stroke and HIV-associated vasculitis.

Parameter	VZV-related (<i>n</i> = 8)	Non-VZV related (<i>n</i> = 71) ^a	<i>P</i> -value (vs. VZV)	HIV-associated vasculitis (<i>n</i> = 6)	<i>P</i> -value (vs. VZV)
Age (years)	28.5 ± 4.7	45.7 ± 12.1	<0.001	35.0 ± 4.9	0.03
Male (%)	37.5	52.1	0.43	33.3	0.65
Median number of TRFs (IQR)	0.0 (0.0–1.0)	1.0 (0.0–2.0)	0.01	1.0 (1.0–1.0)	0.12
BMI (kg/m ²)	19.7 ± 3.4 (<i>n</i> = 6)	27.8 ± 6.1 (<i>n</i> = 51)	0.003	28.5 ± 5.3 (<i>n</i> = 5)	0.01
CD4 (cells/μL)	92.9 ± 77.1	357.0 ± 201.5	<0.001	287.3 ± 217.6	0.04
Median VL (copies/mL) (IQR)	101 079 (1112–292 548)	1600 (0–44 331)	0.06	0 (0–19 200)	0.19
ART-exposed (%)	62.5	45.1	0.35	50.0	0.53
Median ART duration (months) (IQR)	8 (4–10) (<i>n</i> = 5)	36 (5–60) (<i>n</i> = 26)	0.24	60 (13–108)	0.18
VL suppressed (%)	20.0	74.2	0.02	100.0	0.07
cIMT (mm)	0.56 ± 0.05 (<i>n</i> = 7)	0.71 ± 0.17 (<i>n</i> = 54)	0.02	0.63 ± 0.08 (<i>n</i> = 3)	0.12
PWV (m/s)	5.30 ± 1.10 (<i>n</i> = 5)	8.11 ± 2.77 (<i>n</i> = 42)	0.03	7.25 ± 0.30 (<i>n</i> = 4)	0.01

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; cIMT, carotid intima-media thickness; IQR, interquartile range; PWV, pulse wave velocity; TRFs, traditional cardiovascular risk factors; VL, viral load.

^aThis excludes the six patients with HIV-associated vasculitis analysed in the following columns.

have had infections reasonably excluded by means of normal basic CSF parameters.

Two distinct angiographic phenotypes of vasculopathy were noted: a monofocal occlusion of a single blood vessel or a multifocal vasculitis.

Monofocal vessel occlusion is mostly non-atherosclerotic

AV had a very low prevalence in PLWH, and it appears to be driven by TRFs, and not by any HIV-related factors including ART. This low AV prevalence may be partially ascribed to the low mean age of our cohort. However, historical data from SSA have also demonstrated a low prevalence of large vessel atherosclerotic stroke, both in PLWH and people-without-HIV [1, 20]. A study from Malawi ascribed 23/64 (38%) of strokes in PLWH to HIV-associated vasculopathy. Of these, seven were judged to be atherosclerotic (10.9% of strokes in PLWH) [2]. The criteria used to distinguish AV from NAV were clearly defined; however, the distinction was highly reliant on patient age and the presence of only one TRF. For example a patient with age >45 years (or age ≤45 years with

exposure to a single TRF) with a previous transient ischaemic attack was considered as 'clinical history suggestive of atherosclerosis', and diagnosed with 'probable AV'. While this is plausible in a high TRF-prevalence population, it may be presumptive in the setting of HIV-related stroke in SSA, where the role of TRFs and atherosclerosis is yet to be established. A history of a previous cardiovascular event does not necessarily equate to the presence of atherosclerosis. cIMT or PWV was not reported in this or in other previous SSA studies.

Our findings of non-atherosclerotic monofocal vessel occlusions (NAV) in 11 patients were of particular interest. Our clinico-radiological diagnosis of NAV correlated with cIMT and PWV measurements, which were significantly lower than those in AV, and well below the predefined cut-off values for subclinical atherosclerosis. There have been very few descriptions of similar findings in the literature, and detailed angiographic descriptions or reports on cIMT and PWV are lacking [1, 21]. While the extracranial arteries are relatively easily accessible to Doppler examination for atherosclerotic plaque, the assessment of intracranial atherosclerosis remains a challenge. Our data show that intracranial monofocal occlusion of a large vessel is not associated with surrogate markers of subclinical atherosclerosis.

The almost identical TRF profile, cIMT and PWV between patients with NAV and those with multifocal vasculitis (including VZV, which has no known association with atherosclerosis) again implies that the described monofocal occlusive vasculopathy (i.e., NAV) is unlikely to be atherosclerotic in nature. It is unknown whether they are part of a spectrum ranging from monofocal occlusion to multifocal vasculitis, or distinct disease entities, but both appear to be independent of atherosclerosis.

Multifocal vasculitis: VZV is an underdiagnosed cause

Angiographic features of multifocal vasculitis have been described previously, often secondary to opportunistic infections or meningitis. VZV in particular has a known association with stroke [10–12]. Our most noteworthy finding was the identification of VZV in a large number of vasculopathy-related strokes in PLWH, despite us having excluded all patients with meningitis from our cohort. This critical observation suggests that VZV may be associated with stroke by causing a vasculitis in the absence of any CSF response and thus may be overlooked in patients with a normal basic CSF examination. The cutaneous features of VZV are not always present and may precede stroke by over 2 years [10]. We also did not have the availability of testing for VZV IgG on CSF, which is known to be more sensitive than PCR [10, 13]. The true prevalence of VZV-associated strokes is thus likely to be higher than what we have detected, and the index of suspicion needs to remain high to detect this potentially treatable cause of stroke. The vital clinical implication is that based on these findings, we recommend testing for VZV in the CSF of all PLWH with stroke, even if the basic CSF examination is within normal parameters. Whether or not treating VZV in this context will prevent ongoing vessel damage and reduce future stroke risk is unproven, but sufficiently plausible to consider antiviral treatment.

The vasculopathy associated with VZV was exclusively intracranial, with five of six causing a multifocal vasculitis, with one intracranial monofocal occlusion. Previous descriptions of VZV-related stroke indicate that any vessel size may be affected [10]. We failed to demonstrate any patients with small vessel vasculitis on CT angiography. Our patients with VZV-related stroke had evidence of greater immunocompromise than non-VZV-related stroke, which is in keeping with most opportunistic infections.

HIV-related vasculitis was diagnosed in 21.4% of vasculopathy patients. This is a diagnosis of exclusion, after ruling out other infectious and non-infectious causes of vasculitis. Our differences in patient profiles in HIV-associated vasculitis

when compared with VZV-vasculopathy suggests that these are likely distinct clinical entities. HIV-associated vasculitis occurs in less severely immunocompromised patients and thus appears less likely to be related to opportunistic infections, compared with VZV, which was seen in more profoundly immunocompromised PLWH. VZV reactivation has previously also been shown to be more common in patients with more advanced immunocompromise [10, 13].

We observed with interest how monofocal occlusion was more prevalent extracranially, with multifocal vasculitis more prevalent intracranially. However we did not demonstrate any differences in demographics, TRFs or HIV-related factors between intracranial and extracranial vasculopathy. This is in contrast to a previous description of extracranial vasculopathy occurring in patients with higher CD4 counts than those with intracranial vasculopathy [5]. The presence or absence of atherosclerosis was not commented on. Aneurysmal changes were also described in this case series; however, we found only one patient with an aneurysm (accompanying vasculitic changes in other intracerebral vessels).

Small vessel disease has a high TRF burden

SVD was an uncommon cause of stroke in PLWH (16.5%; Table 1). These patients' greater age and higher TRF burden (with elevated mean cIMT) suggests that the process of SVD stroke appears to be primarily TRF-driven, and possibly incidental to HIV (with similar SVD patient profiles in PLWH and people-without-HIV). Whether SVD is truly an HIV-associated vasculopathy is uncertain. The normal PWV measurements were expected, as PWV is a measure of large artery stiffness, not SVD [9, 18].

The study found a lower prevalence of TRFs in PLWH stroke patients compared with age- and sex-matched people-without-HIV with stroke, in accordance with most recent SSA data [22–26]. Thus, while TRFs do play a role in certain stroke types (AV and SVD), the majority of stroke in PLWH in our setting appears to be independent of TRFs and atherosclerosis, even after the exclusion of meningitis. This is in stark contrast to studies from high-income countries (HICs) [27–30]. Our findings may represent a low-middle-income country still in transition from one with infection-mediated to TRF-mediated cardiovascular disease in PLWH [20, 21, 31]. We also noted no increased TRF prevalence nor any difference in cIMT or PWV in the ART-exposed group compared with the ART-naïve PLWH. This supports previous data suggesting that stroke in PLWH in SSA does not appear to be related to ART-induced metabolic side effects, as opposed to in HICs [1, 24, 30]. Our low rates of protease inhibitor usage may be partly responsible for this.

Clinical implications

There are potential clinical implications of our findings of vasculopathy with a low atherosclerosis burden. Current risk-prediction tools target atherosclerosis-mediated stroke and have been shown to be inaccurate predictors of stroke risk in PLWH [32, 33]. To our knowledge, this is the first study reporting on markers of subclinical atherosclerosis in PLWH with stroke in SSA. While cIMT and PWV are admittedly surrogate measures, they are both well-validated and highly sensitive markers of subclinical atherosclerosis, making it unlikely that we have missed significant subclinical disease [7–9]. If future studies confirm that stroke in PLWH in SSA is largely unrelated to atherosclerosis, a paradigm shift in our investigation and management may be needed. This includes a higher index of suspicion for subclinical infections despite their apparent exclusion, as we have demonstrated with VZV.

Limitations of our study included small sample sizes and the lack of a PLWH non-stroke control group, which precluded direct comparisons of cIMT and PWV data. Our low AV prevalence did not permit for age-matching within the PLWH cohort and also precluded well-powered comparative cIMT and PWV analyses with other stroke aetiologies. We thus chose to analyse cIMT and PWV according to internationally accepted cut-off values for atherosclerosis; however, we acknowledge that these have not been extensively tested in PLWH in our population. We chose to utilize cut-off values on the lower end of normality, to increase the sensitivity of the analysis. While the cIMT and PWV operator was blinded to the patient's HIV status, it was not possible to blind the specialist neurologist and radiologist, which may have biased other assessments. The addition of other markers of atherosclerosis, such as arterial calcification on CT, would have been of interest, however this data was unavailable. We acknowledge that the angiographic features of monofocal occlusion may also be observed in patients with emboli from a proximal source. This was considered unlikely due to normal angiography of the proximal vasculature and normal cardiac investigations.

CONCLUSIONS

We have shown that vasculopathy contributed to almost a third of strokes in young South African PLWH. A largely non-atherosclerotic pathogenesis is suggested, and cIMT and PWV have been shown to be useful tools in distinguishing AV from NAV.

Despite excluding meningitis from our cohort and the non-availability of optimal diagnostic assays, we still implicated VZV in a large number of these vasculopathies, emphasizing the likely underdiagnosis of this potentially

treatable infection. We thus recommend testing for VZV in the CSF of all PLWH with strokes and maintaining a very high index of suspicion for covert VZV infection (even in the absence of CSF pleocytosis or recent skin rash).

Our findings regarding VZV emphasize that HIV-related stroke in SSA still appears to have a strong link with opportunistic infections. The appropriate treatment of HIV is therefore of major importance for primary stroke prevention, as opposed to the targeting of TRFs and atherosclerosis, of which we have found minimal evidence. Our findings are in stark contrast to data from HICs with their high atherosclerosis burden, and emphasizes the importance of region-specific research to guide the local management and prevention of HIV-related stroke.

AUTHOR CONTRIBUTIONS

ES: Conceptualisation, study design, investigation, data acquisition, data analysis, writing and editing. AW: Conceptualisation, study design, data analysis, supervision, writing and editing. GT: Investigation, data acquisition. JN: Writing and editing. GN: Conceptualisation, study design, supervision and editing. GM: Conceptualisation, study design, supervision and editing.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Nthabiseng Chaane for assistance with angiography.

FUNDING INFORMATION

ES was supported by the Discovery Foundation Academic Fellowship Award (Ref. 035747). The funder played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data will be made available to qualified researchers upon reasonable request.

ETHICS STATEMENT

Ethics approval was granted by the University of the Witwatersrand Human Research Ethics Committee (Certificates M140429/M190688/M240682).

ORCID

Eitzaz, Sadiq  <https://orcid.org/0000-0003-1801-3638>

REFERENCES

1. Abdallah A, Chang JL, O'Carroll CB, et al. Stroke in human immunodeficiency virus-infected individuals in sub-Saharan

- Africa (SSA): a systematic review. *J Stroke Cerebrovasc Dis*. 2018;27(7):1828-1836.
2. Benjamin LA, Allain TJ, Mzinganjira H, et al. The role of human immunodeficiency virus-associated vasculopathy in the etiology of stroke. *J Infect Dis*. 2017;216(5):545-553.
 3. Benjamin LA, Bryer A, Lucas S, et al. Arterial ischemic stroke in HIV: defining and classifying etiology for research studies. *Neurol Neuroimmunol Neuroinflamm*. 2016;3(4):e254.
 4. Tipping B, de Villiers L, Candy S, Wainwright H. Stroke caused by human immunodeficiency virus-associated intracranial large-vessel aneurysmal vasculopathy. *Arch Neurol*. 2006;63(11):1640-1642.
 5. Tipping B, de Villiers L, Wainwright H, Candy S, Bryer A. Stroke in patients with human immunodeficiency virus infection. *J Neurol Neurosurg Psychiatry*. 2007;78(12):1320-1324.
 6. Gutierrez J, Goldman J, Dwork AJ, Elkind MS, Marshall RS, Morgello S. Brain arterial remodeling contribution to nonembolic brain infarcts in patients with HIV. *Neurology*. 2015;85(13):1139-1145.
 7. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation*. 2007;115(4):459-467.
 8. Stein JH, Currier JS, Hsue PY. Arterial disease in patients with human immunodeficiency virus infection: what has imaging taught us? *J Am Coll Cardiol Img*. 2014;7(5):515-525.
 9. Kim HL, Kim SH. Pulse wave velocity in atherosclerosis. *Front Cardiovasc Med*. 2019;6:41.
 10. Nagel MA, Cohrs RJ, Mahalingam R, et al. The varicella zoster virus vasculopathies: clinical, CSF, imaging, and virologic features. *Neurology*. 2008;70(11):853-860.
 11. Forbes HJ, Williamson E, Benjamin L, et al. Association of herpesviruses and stroke: systematic review and meta-analysis. *PLoS One*. 2018;13(11):e0206163.
 12. Murala S, Nagarajan E, Bollu PC. Infectious causes of stroke. *J Stroke Cerebrovasc Dis*. 2022;31(4):106274.
 13. Marais G, Naidoo M, McMullen K, et al. Varicella-zoster virus reactivation is frequently detected in HIV-infected individuals presenting with stroke. *J Med Virol*. 2022;94(6):2675-2683.
 14. Hatano S. Experience from a multicentre stroke register: a preliminary report. *Bull World Health Organ*. 1976;54(5):541-553.
 15. Norton GR, Majane OH, Maseko MJ, et al. Brachial blood pressure-independent relations between radial late systolic shoulder-derived aortic pressures and target organ changes. *Hypertension*. 2012;59(4):885-892.
 16. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness consensus (2004–2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis*. 2007;23(1):75-80.
 17. Veller MG, Fisher CM, Nicolaides AN, et al. Measurement of the ultrasonic intima-media complex thickness in normal subjects. *J Vasc Surg*. 1993;17(4):719-725.
 18. O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens*. 2002;15(5):426-444.
 19. World Medical Association Declaration of Helsinki. ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194.
 20. Connor MD. Carotid artery disease in sub-Saharan Africa: a marker of epidemiological and stroke transition. *Neuroepidemiology*. 2012;38(2):120-121.
 21. Ransley G, Zimba S, Gadama Y, Saylor D, Benjamin L. Trends and clinical characteristics of HIV and cerebrovascular disease in low- and middle-income countries (LMICs) between 1990 and 2021. *Curr HIV/AIDS Rep*. 2022;19:548-565.
 22. Benjamin LA, Corbett EL, Connor MD, et al. HIV, antiretroviral treatment, hypertension, and stroke in Malawian adults: a case-control study. *Neurology*. 2016;86(4):324-333.
 23. Bearden DR, Omech B, Rulaganyang I, et al. Stroke and HIV in Botswana: a prospective study of risk factors and outcomes. *J Neurol Sci*. 2020;413:116806.
 24. Kroon L, van Zyl DG, Schutte CM, Smit C, Hiesgen J. Risk factors for stroke in HIV-positive and-negative patients in Pretoria, South Africa. *J Stroke Cerebrovasc Dis*. 2021;30(8):105929.
 25. Zimba S, Nutakki A, Chishimba L, et al. Risk factors and outcomes of HIV-associated stroke in Zambia. *AIDS*. 2021;35(13):2149-2155.
 26. Corbett C, Brey N, Pitcher RD, et al. Prevalence and characteristics of HIV-associated stroke in a tertiary hospital setting in South Africa. *Neurology*. 2022;99(9):e904-e915.
 27. Hsue PY. Mechanisms of cardiovascular disease in the setting of HIV infection. *Can J Cardiol*. 2019;35(3):238-248.
 28. Durstenfeld MS, Hsue PY. Mechanisms and primary prevention of atherosclerotic cardiovascular disease among people living with HIV. *Curr Opin HIV AIDS*. 2021;16(3):177-185.
 29. Bogorodskaya M, Chow FC, Triant VA. Stroke in HIV. *Can J Cardiol*. 2019;35(3):280-287.
 30. Feinstein MJ, Hsue PY, Benjamin LA, et al. Characteristics, Prevention, and Management of Cardiovascular Disease in People Living With HIV: A Scientific Statement from the American Heart Association. *Circulation*. 2019;140(2):e98-e124.
 31. Solomon A, Stanwix AE, Castaneda S, et al. Points to consider in cardiovascular disease risk management among patients with rheumatoid arthritis living in South Africa, an unequal middle income country. *BMC Rheumatol*. 2020;4:42.
 32. Mosepele M, Hemphill LC, Palai T, et al. Cardiovascular disease risk prediction by the American College of Cardiology (ACC)/American Heart Association (AHA) Atherosclerotic Cardiovascular Disease (ASCVD) risk score among HIV-infected patients in sub-Saharan Africa. *PLoS One*. 2017;12(2):e0172897.
 33. Triant VA, Perez J, Regan S, et al. Cardiovascular risk prediction functions underestimate risk in HIV infection. *Circulation*. 2018;137(21):2203-2214.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sadiq E, Woodiwiss A, Tade G, Nel J, Norton G, Modi G. The role of atherosclerosis in HIV-associated vasculopathy in young South African stroke patients. *HIV Med*. 2025;1-10. doi:10.1111/hiv.13764

Supplementary Methods

Definitions of Traditional Risk Factors

Hypertension was defined as having 3 or more blood pressure readings greater than 140/90 mm Hg beyond the first 72 hours post-stroke, with or without the presence of target organ damage (hypertensive retinopathy on direct ophthalmoscopy, left ventricular hypertrophy on electrocardiogram, echocardiogram or chest X-ray, or renal dysfunction with no other clear cause). Acute post-stroke elevated blood pressure (BP) was not used as diagnostic for HPT due to the well-described transient increase in BP seen acutely post-stroke.¹

Diabetes mellitus was defined as a fasting glucose ≥ 7.0 mmol/l, or a random glucose ≥ 11.1 mmol/l, or an HbA1c $> 6.5\%$.

Dyslipidaemia was defined as a total cholesterol ≥ 5.2 mmol/L, and/or low density lipoprotein cholesterol (LDL) ≥ 3.3 mmol/L. Blood for lipogram assessment was taken in a non-fasting state. Current evidence suggests that for cardiovascular risk assessment, non-fasting total cholesterol, and LDL are equivalent to a fasting sample.^{2,3} Acute post-stroke transient decreases in cholesterol levels were accounted for by taking blood for lipograms within 24 hours of the stroke onset, where possible, and by not considering a low post-stroke high density lipoprotein cholesterol (HDL) to be diagnostic for dyslipidaemia.⁴

Obesity was defined as a body mass index (BMI) of ≥ 30 kg/m². This was obtained by measuring the patient's height using a stadiometer and the weight using a calibrated mechanical column scale where BMI = weight $\div$ height². This was not possible in patients unable to stand unaided.

A patient was considered antiretroviral therapy (ART)-exposed if they were currently on ART for a period of ≥ 2 weeks.

Definition of meningitis

CSF protein >0.45g/l, OR serum:CSF glucose ratio <0.5 OR pleocytosis (lymphocytes >5cells/mm³ or polymorphonuclear cells >0cells/mm³)

Laboratory Investigations

1. Blood

- Full blood count, urea, creatinine
- Fasting glucose, HbA1c, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides.
- Vasculitis screen: erythrocyte sedimentation rate, C-reactive protein, syphilis serology (RPR and TPHA), hepatitis studies (Hepatitis A, B, C), antinuclear antibodies, extractable nuclear antigens, anti-double stranded DNA antibodies, rheumatoid factor, anti-neutrophil cytoplasmic antibodies, serum angiotensin converting enzyme
- Coagulation screen: PT, INR, D-dimer, Protein C, Protein S, anti-thrombin 3, fibrinogen, antiphospholipid antibodies
- HIV ELISA. If HIV ELISA positive, CD4 and viral load (VL) were measured.

2. Cerebrospinal fluid (CSF) analysis

- Protein, glucose (with simultaneous plasma glucose), microscopy, cryptococcal latex antigen, India Ink test, Gene Xpert and culture for TB, bacteria and fungi.
- CSF polymerase chain reaction (PCR) testing for varicella zoster virus (VZV), herpes simplex virus, cytomegalovirus and Epstein Barr virus

- Syphilis tests (VDRL and/or FTA-Abs) were performed on CSF if blood syphilis serology (TPHA) was positive

Cardiac evaluation (performed by the specialist cardiologist on call)

In all patients:

- Full cardiovascular clinical examination
- 12-lead electrocardiogram (ECG)
- Transthoracic echocardiography

Where clinically indicated:

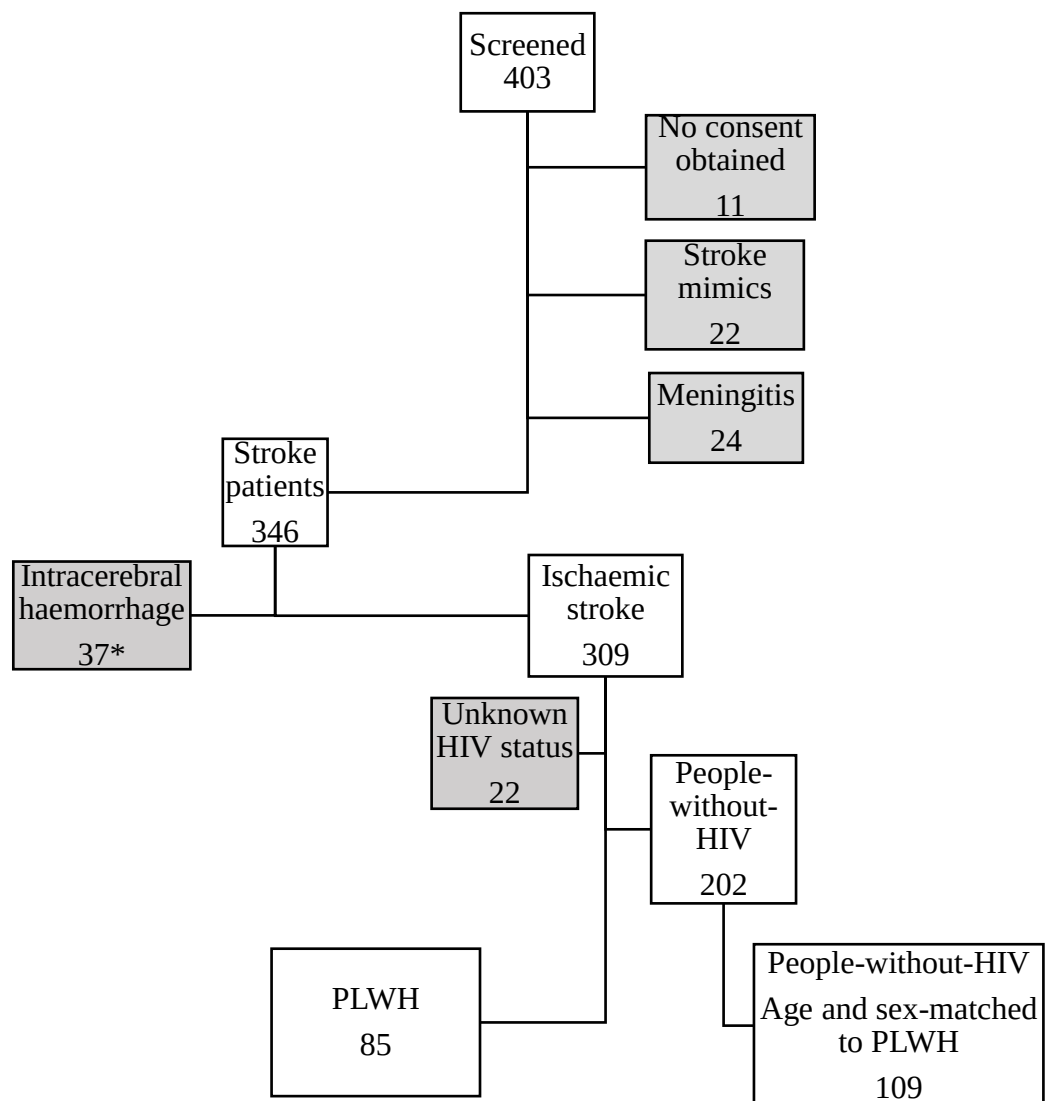
- Trans-oesophageal echocardiography
- Bubble test
- 24 hour Holter ECG

Techniques for the measurement of Carotid intima-media thickness and aortic pulse wave velocity

Carotid Doppler studies were performed using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington) employing a linear array 7.5 MHz probe. Carotid intima-thickness, plaque and stenosis were assessed. Images of at least 1cm length of the far wall of the distal portion of the right common carotid artery (CCA) from an optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal (ICA) and external carotid artery (ECA) are visualized simultaneously) at least 1 cm proximal to the flow divider were obtained. cIMT measurements were determined using semi-automated border-detection and quality control software. All images were saved electronically, allowing for later quality control and re-evaluation if necessary.

Large artery stiffness was indexed from aortic pulse wave velocity (PWV). After participants had rested for 15 minutes in the supine position, sequential recordings of the arterial pressure waveform at the carotid and femoral arteries were determined using applanation tonometry. A high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas), interfaced with a computer utilizing SphygmoCor software, version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) was used. Arterial pressure waveform recordings were discarded when the systolic or diastolic variability of consecutive waveforms exceeded 5%, or when the amplitude of the pulse wave signal was less than 80 mV. To determine aortic PWV, distances from the suprasternal notch to the carotid sampling site (distance A) and from the suprasternal notch to the femoral artery (distance B) were measured. Pulse wave velocity distance was calculated as distance B minus distance A. Pulse transit time, calculated as the mean time difference between sites A and B was determined from the average of 10 consecutive beats. Aortic PWV was calculated as the ratio of the distance in metres to the transit time in seconds.

Figure S1: Study flowchart



Blocks shaded in grey indicate patients not included in the study

*Only 2 patients with intracerebral haemorrhage were PLWH, thus further analyses were only conducted in ischaemic stroke patients

Table S1: Factors associated with cIMT and PWV on multivariate regression analyses

	cIMT (n=61)			PWV (n=47)		
	b	SE of b	p-value	b	SE of b	p-value
Age	0,002	5,288	<0.001	0,087	0.033	0.01
Sex	0,034	-1,070	0.29	-0.108	0.750	0.89
VZV Status	0,059	-0,040	0.97	-1.161	1.336	0.39
Number of TRFs	0,019	-0,250	0.80	0.145	0.414	0.73

cIMT, Carotid intima media thickness; PWV, pulse wave velocity; TRFs, traditional risk factors; VZV, varicella zoster virus

Table S2: Comparing TRFs and HIV-related factors between different locations and types of vasculopathy

	Intracranial NAV (n=5)	Extracranial NAV (n=6)	p-value	Intracranial vasculopathy (n=16)*	Extracranial vasculopathy (n=8) †	p-value	Monofocal NAV‡ (n=11)	Multifocal vasculitis‡ (n=12)	p-value
Age	39.4 ± 8.2	39.0 ± 10.7	0.95	34.0 ± 8.0	38.3 ± 9.1	0.26	39.2 ± 9.2	32.5 ± 6.8	0.06
Male (%)	40.0	33.3	0.65	31.3	37.5	0.55	36.4	25.0	0.55
HT (%)	20.0	16.7	0.73	18.8	12.5	0.60	18.2	16.7	0.92
DM (%)	0.0	0.0	1.00	0.0	0.0	1.00	0.0	0.0	1.00
Dyslipidaemia (%)	0.0	16.7	0.55	12.5	25.0	0.41	9.1	16.7	0.59
Smoking (%)	0.0	16.7	0.55	12.5	25.0	0.41	9.1	25.0	0.31
Obesity (%)	25.0 (n=4)	0.0 (n=4)	0.50	16.7	16.7	1.00	12.5	22.2	0.60
BMI (kg/m²)	27.3 ± 6.6 (n=4)	25.0 ± 5.0 (n=4)	0.59						

Median No of TRFs (IQR)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	1.00	0.5 (0.0-1.0)	0.5 (0-1.5)	0.74	0 (0-1)	1 (0-1)	0.37
CD4 (cells/ul)	260.0 ± 152.0	427.2 ± 401.9	0.41	232.4 ± 182.1	379.0 ± 355.5	0.19	351.2 ± 235.0	312.5 ± 189.5	0.29
Median VL (copies/ml) (IQR)	452 (82- 3860 (0-24300) 25476)	1.00	452 (0- 19200 (0- 102000) 156000)	0.57	2156 (0- 12000 (0- 25476) 157096)	0.53			
ART-exposed (%)	60.0	33.3	0.39	68.8	25.0	0.06	45.5	58.3	0.54
% with VL Suppressed	33.3	100.0	0.30	45.5	100.0	0.27	60.0	42.9	0.56
cIMT (mm)	0.66 ± 0.02 (n=4)	0.61 ± 0.05 (n=5)	0.10	0.61 ± 0.07 (n=11)	0.61 ± 0.04 (n=6)	0.84	0.63 ± 0.04 (n=9)	0.59 ± 0.07 (n=7)	0.17
PWV (m/s)	6.90 ± 2.88 (n=4)	6.70 ± 1.53 (n=4)	0.91	6.24 ± 2.08 (n=10)	6.80 ± 1.20 (n=6)	0.56	6.80 ± 2.14 (n=8)	6.43 ± 1.10 (n=7)	0.69

ART, antiretroviral therapy; cIMT, carotid intima-media thickness; DM, diabetes mellitus; HT, hypertension; IQR, interquartile range; NAV, non-atherosclerotic vasculopathy; PWV, pulse wave velocity; TRFs, traditional risk factors; VL, viral load

*Includes NAV (n=5), HIV-associated vasculitis (n=4), VZV-related with abnormal angiography (n=6), and vasculopathy of multiple possible aetiologies (n=1)

†Includes NAV (n=6), HIV-associated vasculitis (n=2). Excludes AV (n=4).

‡ Intra-and extracranial combined

Table S3: Comparisons of SVD strokes in PLWH to non-SVD strokes in PLWH, and to SVD strokes in People-without-HIV

	PLWH with SVD (n=14)	PLWH with Non-SVD strokes (n=71)	P-value (PLWH SVD vs non-SVD)	People- without-HIV with SVD (n=20)	P-value SVD (PLWH vs People- without- HIV)
Age(years)	53.6±8.4	41.4±12.2	<0.001	48.5±7.4	0.07
Male(%)	50.0	49.3	0.96	60.0	0.56
HT(%)	100.0	32.4	<0.001	95.0	0.40
DM(%)	21.4	2.8	0.007	35.0	0.39
Dyslipidaemia(%)	57.1	26.1	0.02	45.0	0.49
Smoking(%)	7.1	29.6	0.08	20.0	0.30
Obesity(%)	57.1	19.1	0.03	46.7	0.34
Median No of TRFs (IQR)	2 (2-3)	1(0–2)	<0.001	2 (2-3)	1.00
cIMT(mm)	0.78±0.12(n=11)	0.66±0.17(n=51)	0.03	0.83±0.11(n=15)	0.28
PWV(m/s)	8.14±3.03 (n=7)	7.71±2.63(n=44)	0.69	8.73±2.51(n=15)	0.64
CD4 (cells/μL)	436.9±156.8	306.4±210.7	0.04	-	-

Median VL	191(0–	2840(0–	0.33	-	-
(copies/ml)	22433)	77158)			
(IQR)					
ART-	42.9	47.9	0.73	-	-
exposed(%)					
VL	83.3	66.7	0.42	-	-
suppressed(%)					

ART, antiretroviral therapy; cIMT, carotid intima-media thickness; DM, diabetes mellitus; HT, hypertension; IQR, interquartile range; PWV, pulse wave velocity; SVD, small vessel disease; TRFs, traditional risk factors; VL, viral load

Table S4: Comparing TRFs and HIV-related factors between ART-exposed and ART-naïve PLWH

	ART (n = 40)	ART-naïve (n = 45)	p-value
Age (years)	42.7±11.9	43.9±13.1	0.65
Male (%)	45.0	53.3	0.44
HT (%)	30.0	55.6	0.018
DM (%)	2.5	8.9	0.21
Dyslipidaemia (%)	27.5	22.2	0.57
Smoking (%)	17.5	33.3	0.096
Obesity (%)	29.4	21.4	0.47
Median No of TRFs (IQR)	1.0 (0.0 – 2.0)	1.0 (1.0 – 2.0)	0.12
CD4 (cells/ul)	349.4±238.1	302.7±173.9	0.32
Median VL (copies/ml) (IQR)	0 (0 – 382)	67264 (20200 – 207000)	<0.001
cIMT (mm)	0.67 ± 0.18 (n=29)	0.69 ± 0.14 (n=33)	0.58
PWV (m/s)	7.51 ± 2.75 (n=27)	8.05 ± 2.60 (n=24)	0.46

ART, antiretroviral therapy; cIMT, carotid intima-media thickness; DM, diabetes mellitus; HT, hypertension; IQR, interquartile range; PWV, pulse wave velocity; TRFs, traditional risk factors; VL, viral load

References

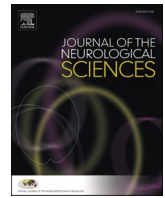
1. Qureshi AI. Acute hypertensive response in patients with stroke: Pathophysiology and management. *Circulation*. 2008;118:176-187
2. Klug E, Raal FJ, Marais AD, Smuts CM, Schamroth C, Jankelow D, et al. South African Dyslipidaemia Guideline Consensus Statement: 2018 Update a joint statement from the South African Heart Association (SA Heart) and the Lipid and Atherosclerosis Society of Southern Africa (LASSA). *S Afr Med J*. 2018;108:973-1000
3. Langsted A, Nordestgaard BG. Nonfasting versus fasting lipid profile for cardiovascular risk prediction. *Pathology*. 2019;51:131-141
4. Perovic E, Mrdjen A, Harapin M, Simundic AM. Short term changes of serum lipids in acute ischemic stroke. *Clinical laboratory*. 2016;62:2107-2113

Paper 3

Lack of impact of HIV status on carotid intima-media thickness in a cohort of stroke patients in South Africa

Sadiq E, Woodiwiss A, Tade G, Norton G, Modi G.

Journal of the Neurological Sciences. 2024;465:123186.



Clinical short communication

Lack of impact of HIV status on carotid intima media thickness in a cohort of stroke patients in South Africa

Eitzaz Sadiq^{a,*}, Angela Woodiwiss^b, Grace Tade^b, Gavin Norton^b, Girish Modi^a^a Division of Neurology, Department of Neurosciences, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa^b Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

ARTICLE INFO

Keywords:

HIV
Stroke
Atherosclerosis
Intima media thickness
Biomarker
Africa

ABSTRACT

Introduction: People living with HIV (PLWH) are at increased risk for cardiovascular disease. Carotid intima media thickness (cIMT) is a validated surrogate marker of atherosclerosis, and an accurate predictor of future cardiovascular events. It is uncertain whether HIV potentiates stroke risk through atherosclerosis in Sub-Saharan Africa and what effect HIV status has on cIMT. We sought to investigate the relationship between HIV status and cIMT in stroke patients in a region that is burdened with dual epidemics of HIV and stroke in the young.

Methods: Consecutive patients with new onset ischaemic stroke were recruited from a quaternary-level hospital in Johannesburg, South Africa, from August 2014 to November 2017. Patients were assessed for the presence of traditional cardiovascular risk factors and HIV infection, and investigated for stroke aetiology. cIMT was measured using high resolution B-mode ultrasound following standardized techniques.

Results: 168 patients were included in the study, of which 62 (36.9%) were PLWH. Mean cIMT was higher in HIV-uninfected patients when compared to PLWH (0.79 ± 0.19 mm vs 0.69 ± 0.18 mm, $p = 0.0021$). However after adjusting for age, sex, hypertension, diabetes mellitus, smoking, total cholesterol, body mass index and stroke aetiology, there was no difference in mean cIMT between the groups (0.76 ± 0.16 mm vs 0.73 ± 0.17 mm, $p = 0.29$). Regression models revealed the determinants of cIMT to be age ($p < 0.0001$), hypertension ($p = 0.0098$) and total cholesterol ($p = 0.005$), while the determinants of increased cIMT (≥ 0.70 mm) were only age ($p < 0.0001$) and hypertension ($p = 0.0002$).

Conclusion: HIV status had no effect on cIMT in our cohort of stroke patients. The main determinants of cIMT were age and hypertension.

1. Introduction

People living with HIV (PLWH) are twice as likely to develop cardiovascular disease, including stroke, compared to uninfected individuals. [1] Proposed mechanisms for this include accelerated atherosclerosis secondary to chronic inflammation and immune activation in an aging population, as well as the atherogenic effects of traditional cardiovascular risk factors (TRFs) and the metabolic side effects of longstanding antiretroviral therapy (ART), particularly protease inhibitors (PIs). [2]

Carotid intima media thickness (cIMT) has been widely investigated and validated as a surrogate marker of atherosclerosis and is regarded as

an accurate predictor of future cardiovascular events. [3] Its non-invasive, inexpensive technique makes it a valuable risk prediction tool. Evidence for the association between HIV status and cIMT is not conclusive, although data from high-income countries (HICs) suggest a moderately higher cIMT in PLWH when compared to uninfected controls, after adjusting for TRFs. [4]

Over 80% of the world's PLWH reside in low-to-middle income countries (LMICs), particularly in Sub-Saharan Africa (SSA). [5] South Africa itself is home to the greatest number of PLWH in the world (8.5 million people). [6] While data from HICs, based on a very small proportion of the world's PLWH population, is often extrapolated globally due to a paucity of local data, it is not always applicable. It is unknown

* Corresponding author at: Division of Neurology, Department of Neurosciences, University of the Witwatersrand Medical School, 7 York Road, Parktown, 2193 Johannesburg, South Africa.

E-mail addresses: eitzaz.sadiq@wits.ac.za (E. Sadiq), angela.woodiwiss@wits.ac.za (A. Woodiwiss), oluwatosin.tade@wits.ac.za (G. Tade), physiology@health.wits.ac.za (G. Norton), girish.modi@wits.ac.za (G. Modi).

<https://doi.org/10.1016/j.jns.2024.123186>

Received 21 June 2024; Received in revised form 12 August 2024; Accepted 13 August 2024

Available online 14 August 2024

0022-510X/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

what effect HIV status has on cIMT in a LMIC population where stroke patients are much younger, have fewer TRFs, less ART-exposure and often have alternative, non-atherosclerotic stroke aetiologies when compared to those in HICs. [7,8]

We thus sought to investigate the relationship between HIV status and cIMT in stroke patients from a LMIC at the epicentre of the world's HIV epidemic, and to establish what the determinants of cIMT are in this population.

2. Methods

This cross-sectional study took place at the Charlotte Maxeke Johannesburg Academic Hospital, a 1000-bed quaternary-level hospital in Johannesburg, South Africa, from August 2014 to November 2017 (40 months).

We recruited consecutive patients aged 18 years and older who presented with a new onset ischaemic stroke. Stroke was defined as per the World Health Organization case definition. [9] Exclusion criteria were the diagnosis of meningitis based on cerebrospinal fluid examination, evidence of opportunistic infections, the presence of intracranial mass lesions on radiographic imaging, or other stroke mimics. Full informed consent was obtained as per the Helsinki Declaration. Ethics approval was granted by the University of the Witwatersrand Human Research Ethics Committee (Certificate number M140429, renewed as M190688).

All patients were assessed and examined by the same specialist neurologist (ES), who co-ordinated and interpreted all relevant investigations and assigned a stroke aetiology according to the Trial of Org 10,172 in Acute Stroke (TOAST) Classification. [10]

Hypertension (HT) was defined as having 3 or more blood pressure readings $>140/90$ mmHg beyond the first 72 h post-stroke, with or without the presence of the following target organ damage: hypertensive retinopathy on direct ophthalmoscopy, left ventricular hypertrophy on electrocardiogram, echocardiogram or chest X-ray, or renal dysfunction with no other cause. Acute post-stroke elevated blood pressure (BP) was not used as diagnostic for HT due to the well-described transient increase in BP seen acutely post-stroke. [11]

Diabetes mellitus (DM) was defined as a fasting glucose ≥ 7.0 mmol/L, or a random glucose ≥ 11.1 mmol/L, or an HbA1c $> 6.5\%$. Patients with anaemia were not diagnosed as diabetic solely on HbA1c measurement, to avoid false positives. [12]

Dyslipidaemia was defined as a total cholesterol ≥ 5.2 mmol/L, and/or low density lipoprotein cholesterol (LDL) ≥ 3.3 mmol/L. [13] Blood for lipogram assessment was taken in a non-fasting state. Current evidence suggests that for cardiovascular risk assessment, non-fasting total cholesterol, and LDL are equivalent to a fasting sample. [13–16] Acute post-stroke transient decreases in cholesterol levels were accounted for by taking blood for lipograms within 24 h of the stroke onset, where possible, and by not considering a low post-stroke high density lipoprotein cholesterol (HDL) to be diagnostic for dyslipidaemia. [17–19]

Body mass index (BMI) was obtained by measuring the patient's height using a stadiometer and the weight using a calibrated mechanical column scale where $BMI = \text{weight} \div \text{height}^2$. This was not possible in patients unable to stand unaided.

Investigations for stroke aetiology included CT and/or MRI of the brain, CT angiography of the brain, neck and aortic arch, 12-lead electrocardiogram, transthoracic and/or transoesophageal echocardiogram and the full protocol of laboratory investigations as described in the online supplement (Appendix A). HIV testing (ELISA) was performed in all consenting patients, and all PLWH had a CSF examination unless contra-indicated.

cIMT was measured by carotid doppler studies which were performed by a single operator using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc., Bothell, Washington) employing a linear array 7.5 MHz probe as described previously. [20] Images of at least 1 cm length of the far wall of the distal portion of the right common

carotid artery from an optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualized simultaneously) at least 1 cm proximal to the flow divider were obtained. cIMT measurements were determined using semi-automated border-detection and quality control software. A total of 6 separate measurements (3 on each side) were averaged to a single mean cIMT. All images were saved electronically, allowing for later quality control and re-evaluation if necessary. A cIMT ≥ 0.70 mm was considered to be pathological, and suggestive of sub-clinical atherosclerosis, as per previously published data. [21,22] Patients whose cIMT data were of insufficient quality were excluded from the study.

2.1. Statistical methods

Data was analysed using SAS software, version 9.4 (The SAS Institute, Cary, NC). Continuous variables were evaluated for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. To compare data between groups, unpaired Student's *t*-test, Chi squared test, and Mann Whitney *U* test were then performed for parametric and non-parametric variables where appropriate. To identify the determinants of cIMT, univariate and multivariate regression analysis was performed. Logistic regression analysis was performed to identify the determinants of increased cIMT. Continuous data were reported as mean $\pm$ SD for parametric, or median and interquartile range for non-parametric data. Only available data was analysed. Missing data was not imputed. *P* values < 0.05 were considered significant.

3. Results

168 patients were included in the study. (See Fig. 1 for study flow-chart). HIV-uninfected patients ($n = 106$, 63.1%) were older and had more traditional risk factors (HT, DM, higher total cholesterol and higher BMI) than PLWH ($n = 62$, 36.9%). (Table 1). The only other baseline difference between the two groups was in stroke aetiology (higher proportion of strokes of 'other' aetiology in PLWH). (Table 1).

The mean cIMT (Fig. 2A, HIV-uninfected: 0.79 ± 0.19 mm; PLWH: 0.69 ± 0.18 mm, $p = 0.0021$), and proportion with increased cIMT (Table 1) were higher in HIV-uninfected patients. However after adjusting for age (Fig. 2B, HIV-uninfected: 0.76 ± 0.15 mm; PLWH: 0.74 ± 0.15 mm, $p = 0.35$), and adjusting for age, sex, HT, DM, BMI, smoking, total cholesterol and stroke aetiology (Fig. 2C), there was no difference in cIMT between HIV-uninfected and PLWH (0.76 ± 0.16 mm vs 0.73 ± 0.17 mm, $p = 0.29$).

Multivariate linear regression analyses revealed that the only determinants of cIMT were age, HT and total cholesterol, (Table 2), while multivariate logistic regression analyses showed that the only determinants of an increased cIMT (≥ 0.70 mm) were age and HT. (Table 2 and Fig. 3). HIV status had no effect on cIMT. Similar results were noted with regression models which excluded stroke aetiology, or included dyslipidaemia instead of total cholesterol, or used median number of risk factors instead of HT, DM, smoking, dyslipidaemia and BMI individually (with and without stroke aetiology) in the model (see Appendix B - Table S1).

3.1. Effect of ART

Of the 62 PLWH, 29 were on ART at the time of stroke. 27 of these were on a fixed-dose combination comprising emtricitabine, tenofovir and efavirenz, while only 2 patients were on a PI-containing regimen. There were no differences in demographics, TRFs, and mean CD4 between ART-exposed and ART-naïve PLWH. (Table 3) Mean cIMT was also similar between the 2 groups. (Table 3). On multivariate regression analyses, the main determinants of cIMT in PLWH were age and sex (Table 4), while age and HT were the main determinants of increased cIMT (≥ 0.70 mm) (Table 5). ART usage was neither associated with

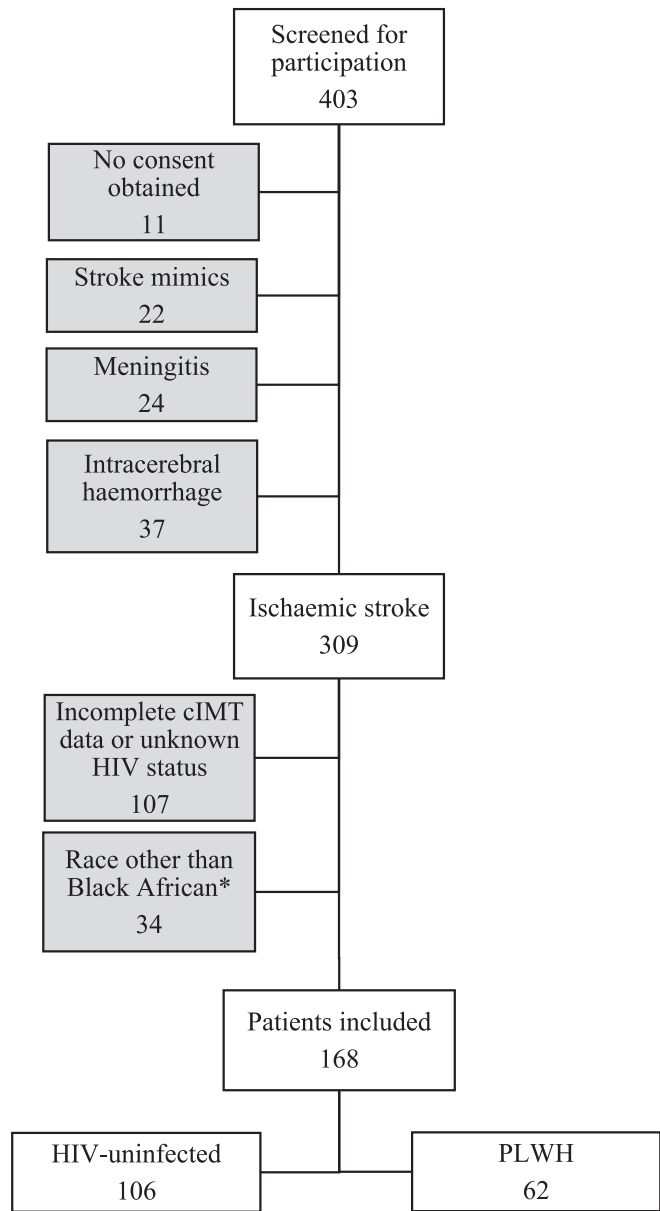


Fig. 1. Study flowchart. cIMT, carotid intima media thickness; PLWH, people living with HIV. Groups excluded from the study are shaded in grey. *In order to mitigate for differences in cIMT reference values in different races, we only analysed the most common racial group (Black African).

cIMT nor an increased IMT in PLWH (Tables 4 and 5).

4. Discussion

We have demonstrated in stroke patients that mean cIMT was similar in PLWH and HIV-uninfected after adjusting for demographics, TRFs and stroke aetiology. The main determinants of cIMT were age and HT, with a lesser role played by total cholesterol. HIV status had no effect on cIMT in our cohort of stroke patients from the epicentre of the world’s HIV epidemic. Previous studies on the effect of HIV status on cIMT have yielded inconsistent results. While some have shown evidence of an increased cIMT in PLWH when compared to uninfected controls [23–27], others have shown no association [28–32] while others have only shown associations in certain groups of patients, including certain age categories. [33]

Table 1
Comparing traditional risk factors and cIMT in PLWH and HIV-uninfected stroke patients.

	PLWH (n = 62)	HIV-uninfected (n = 106)	P value
Age (years)	42.8 ± 12.2	51.8 ± 15.6	0.0002
Male (%)	51.6	43.4	0.30
HT (%)	43.6	65.1	0.01
DM (%)	4.8	22.6	0.002
Dyslipidaemia (%)	29.5 (n = 61)	37.5 (n = 104)	0.30
Total cholesterol (mmol/L)	4.09 ± 0.91 (n = 61)	4.41 ± 1.11 (n = 104)	0.06
LDL cholesterol (mmol/L)	2.60 ± 0.84 (n = 60)	2.78 ± 0.93 (n = 104)	0.21
BMI (kg/m ²)	27.5 ± 6.4 (n = 50)	30.5 ± 8.8 (n = 96)	0.03
Smoking (%)	27.4	17.0	0.11
Stroke aetiology			0.0002
Cardioembolic (%)	16.1	31.1	
Large vessel atherosclerosis (%)	6.5	3.7	
Small vessel disease (%)	17.7	20.8	
Undetermined (%)	25.8	36.8	
Other (%)	33.9*	7.6**	
Increased cIMT (≥0.70 mm) (%)	35.5	67.9	<0.0001

BMI, body mass index; DM, diabetes mellitus; HT, hypertension; LDL, low density lipoprotein cholesterol; PLWH, people living with HIV. * including non-atherosclerotic vasculopathy (n = 11), HIV-associated vasculitis (n = 6), stroke due to recreational drug usage (n = 2) and non-lacunar small vessel vasculopathy (n = 2). ** including autoimmune vasculitis (n = 5), stroke due to hypercoagulable state (n = 2) and stroke due to recreational drug usage (n = 1).

A meta-analysis of 26 studies [4], and another large study [23] not part of this prior meta-analysis, have suggested that the TRF-independent effects of HIV on cIMT are modest at best (0.02 to 0.06 mm higher in PLWH). The meta-analysis reported evidence of publication bias (p = 0.001), with smaller studies being more likely to report an increased cIMT in PLWH when compared to larger studies, which mostly showed minimal or no effect. [4] There was also significant heterogeneity between studies (I² = 86.5%) and evidence of confounding. Strikingly, most of this data comes from HICs, where the profile of PLWH differs markedly from LMICs. PLWH in HICs are generally older, with more TRFs and exposure to ART for longer periods when compared to PLWH in LMICs. [34] They also have higher proportions of recreational drug users and men who have sex with men than cohorts from LMICs. A large multinational study from 4 African countries (Burkina Faso, Kenya, Ghana and South Africa) examined cIMT in 8872 patients and reported an inverse association of HIV status with cIMT (β = −8.86 95% CI −15.7 to −2.03). [35] No convincing explanation was offered by the authors for this unexpected finding but may again be indicative of a distinct patient profile in SSA in contrast to HICs. Other cohorts from SSA all reported no effect of HIV status on cIMT. [28–31,36,37] Importantly, all of these studies have been in non-stroke patients, which makes our study unique. To our knowledge there is no previously published data on cIMT in PLWH who have suffered a stroke. A comprehensive literature search on cIMT, HIV and stroke revealed only 2 publications of potential relevance, both of which used stroke as an outcome measure. Sarfo et al. [38] prospectively followed up 255 ART-exposed Ghanaian PLWH for 12 months, during which time 3 PLWH had a stroke. Carotid bulb IMT was independently associated with stroke occurrence (aHR 12.23 (1.28–117.07). However there were no HIV negative controls, thus no inferences can be made on the effect of HIV on cIMT and stroke outcomes. The metabolic side effects of PIs and subsequent atherogenesis are well described. [2,39,40] We showed that ART usage had no impact on cIMT in stroke patients. This is likely as a result of the very infrequent use of PI’s in our region. The aforementioned meta-analysis from regions

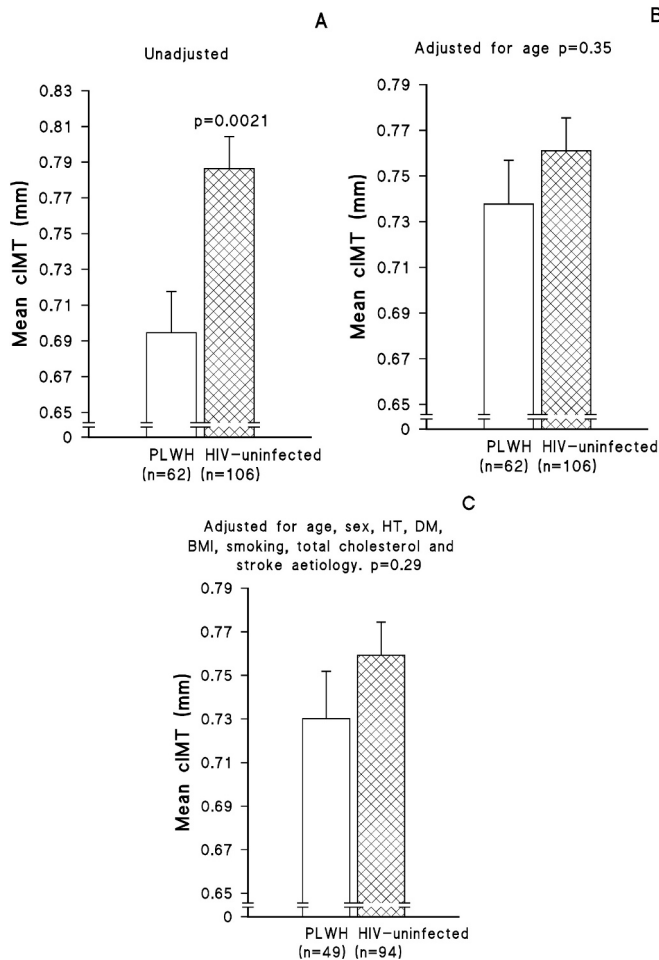


Fig. 2. Bar graphs comparing mean cIMT in PLWH and HIV-uninfected stroke patients with adjustments. BMI, body mass index; cIMT, carotid intima media thickness; DM, diabetes mellitus; HT, hypertension; PLWH, people living with HIV.

Table 2
Multivariate regression analyses showing determinants of cIMT and increased cIMT.

Variable	Determinants of cIMT (n = 143)		Determinants of increased cIMT (≥ 0.70 mm) (n = 143)	
	β -coeff. $\pm$ SEM	p-value	β -coeff. $\pm$ SEM	p-value
HIV status	-0.028 $\pm$ 0.027	0.29	-0.844 $\pm$ 0.607	0.17
Age	0.005 $\pm$ 0.001	<0.0001	0.122 $\pm$ 0.030	<0.0001
Sex	-0.033 $\pm$ 0.029	0.24	0.076 $\pm$ 0.660	0.91
HT	0.076 $\pm$ 0.029	0.0098	2.348 $\pm$ 0.631	0.0002
DM	0.033 $\pm$ 0.034	0.35	2.287 $\pm$ 1.219	0.06
Total cholesterol	0.035 $\pm$ 0.012	0.005	0.393 $\pm$ 0.314	0.21
BMI	-0.001 $\pm$ 0.002	0.49	-0.012 $\pm$ 0.043	0.79
Smoking	0.049 $\pm$ 0.035	0.16	0.707 $\pm$ 0.856	0.41
Stroke aetiology	-0.004 $\pm$ 0.009	0.61	-0.315 $\pm$ 0.223	0.16

BMI, body mass index; cIMT, carotid intima media thickness; DM, diabetes mellitus; HT, hypertension. n = 143 (limited by BMI and total cholesterol data).

with higher PI usage also did not report any association between PI usage and cIMT, [4] while a subsequent meta-analysis reported increased cIMT in those on ART and PIs, but only between the ages of 20 and 40 years. [41] Again, the conflicting data illustrates the complexities and uncertainties on the topic.

Stroke in PLWH in SSA is associated with infection in 23–55%. [7] We thus excluded all PLWH with opportunistic infections from our

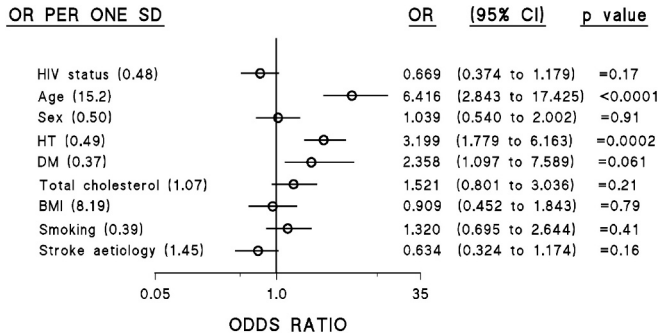


Fig. 3. Determinants of increased cIMT (≥ 0.70 mm). Odds ratios per one standard deviation of each determinant are shown.

Table 3
Comparing risk factors, cIMT and CD4 in ART-exposed vs ART-naïve PLWH.

	ART-exposed (n = 29)	ART-naïve (n = 33)	p-value
Age (years)	41.9 $\pm$ 10.7	43.6 $\pm$ 13.5	0.58
Male (%)	48.3	54.6	0.62
HT (%)	34.5	51.5	0.18
DM (%)	0.0	9.1	0.10
Dyslipidaemia (%)	28.6 (n = 28)	30.3 (n = 33)	0.88
Total cholesterol (mmol/L)	4.23 $\pm$ 0.92 (n = 28)	3.97 $\pm$ 0.91 (n = 33)	0.27
BMI (kg/m ²)	28.1 $\pm$ 7.3 (n = 27)	26.7 $\pm$ 5.2 (n = 23)	0.44
Smoking (%)	20.7	33.3	0.27
cIMT (mm)	0.67 $\pm$ 0.18	0.72 $\pm$ 0.18	0.33
CD4 (cells/ul)	385 $\pm$ 255	304 $\pm$ 190	0.17

BMI, body mass index; cIMT, carotid intima media thickness; DM, diabetes mellitus; HT, hypertension; PLWH, people living with HIV.

Table 4
Determinants of cIMT in PLWH.

Variable	Univariate regression (n = 62 ^a)		Multivariate regression ^b (n = 59 ^b)	
	β -coeff. $\pm$ SEM	p-value	β -coeff. $\pm$ SEM	p-value
Age	0.008 $\pm$ 0.002	<0.0001	0.005 $\pm$ 0.002	0.014
Sex	-0.098 $\pm$ 0.045	0.033	-0.088 $\pm$ 0.043	0.045
HT	0.140 $\pm$ 0.043	0.002	0.061 $\pm$ 0.046	0.19
DM	0.088 $\pm$ 0.108	0.42		
Total cholesterol (n = 61)	0.064 $\pm$ 0.025	0.012	0.034 $\pm$ 0.025	0.17
BMI (n = 50)	0.005 $\pm$ 0.004	0.24		
Smoking	0.027 $\pm$ 0.052	0.60		
CD4 count (n = 60)	0.00023 $\pm$ 0.00010	0.033	0.00007 $\pm$ 0.00009	0.44
ART usage	-0.046 $\pm$ 0.046	0.33		

ART, antiretroviral therapy; BMI, body mass index; cIMT, carotid intima media thickness; DM, diabetes mellitus; HT, hypertension; PLWH, people living with HIV.

^a differences in sample size are due to missing data on total cholesterol (n = 1) and CD4 (n = 2).

^b including variables with p < 0.10 on univariate regression.

cohort, as infection-mediated stroke is unlikely to be related to cIMT, and inclusion of these patients may have resulted in an underestimation of HIV's effect on cIMT. Despite this, our only determinants of cIMT were TRFs. This corroborates with previous non-stroke data from both HICs [3,33,42] and SSA. [29,35,43,44]

It is possible that HIV may confer an increased cardiovascular risk by affecting vessels in a manner which is not detectable on cIMT measurement ie. a non-atherosclerotic vasculopathy. [7,44] If this is indeed

Table 5
Determinants of increased cIMT (≥ 0.70 mm) in PLWH.

Variable	Univariate regression (n = 62 ^a)		Multivariate regression ^b (n = 47 ^a)	
	β -coeff. $\pm$ SEM	p-value	β -coeff. $\pm$ SEM	p-value
Age	0.026 $\pm$ 0.004	<0.0001	0.017 $\pm$ 0.005	0.0028
Sex	-0.171 $\pm$ 0.122	0.17		
HT	0.487 $\pm$ 0.108	<0.0001	0.305 $\pm$ 0.122	0.017
DM	0.678 $\pm$ 0.274	0.016	0.381 $\pm$ 0.293	0.20
Total cholesterol (n = 61)	0.156 $\pm$ 0.066	0.021	0.003 $\pm$ 0.066	0.97
BMI (n = 50)	0.021 $\pm$ 0.010	0.046	0.009 $\pm$ 0.009	0.35
Smoking	0.078 $\pm$ 0.138	0.57		
CD4 count (n = 60)	0.00053 $\pm$ 0.00027	0.052	-0.00015 $\pm$ 0.00025	0.54
ART usage	-0.084 $\pm$ 0.123	0.50		

ART, antiretroviral therapy; BMI, body mass index; cIMT, carotid intima media thickness; DM, diabetes mellitus; HT, hypertension, PLWH, people living with HIV.

^a differences in sample size are due to missing data on total cholesterol (n = 1) and CD4 (n = 2).

^b including variables with p < 0.10 on univariate regression.

the case, the undisputed value of cIMT in cardiovascular risk prediction may be compromised in our setting, the implications of which would include the need to develop and test alternative risk prediction tools specific to PLWH in SSA. [45,46]

Our findings are likely reflective of the different profile of stroke patients in PLWH in SSA when compared to HICs, with a low atherosclerotic burden despite the exclusion of infections. The aetiology of stroke in our cohort was predominantly non-atherosclerotic, with only 6.5% of PLWH fulfilling the TOAST criteria for large vessel atherosclerotic stroke. It is possible that if we repeated our analyses only in patients with atherosclerotic stroke, the results would differ. Our prevalence of atherosclerotic stroke was too low to perform such analyses.

Besides being the first study to our knowledge to report on the impact of HIV status on cIMT in stroke patients in SSA, other strengths of our study include the use of standardized cIMT measurement methodology which was performed by a single operator, eliminating inter-observer bias, as well as our comprehensive workup for stroke aetiology, and subsequent adjustment for this in the analyses. Limitations included the lack of a non-stroke PLWH group to compare cIMT in PLWH with and without stroke. Our inability to measure BMI in patients unable to stand resulted in some missing data, which may have biased against those with severe strokes in relevant analyses. Selection bias may have played a role as patients were recruited from a quaternary-level hospital, where more atypical stroke patients are more likely to be referred. While we showed no impact of HIV on cIMT in our cohort of stroke patients, our findings may not be generalizable to other populations with a higher atherosclerotic burden. This serves to further highlight the differences in stroke in PLWH between HICs and LMICs and underlines the importance of region-specific research.

5. Conclusion

HIV status had no effect on cIMT in a cohort of stroke patients in a LMIC region with a high prevalence of HIV. The main determinants of cIMT in this cohort were age and hypertension.

Funding

ES was supported by the Discovery Foundation Academic Fellowship Award (Ref 035747). The funder played no role in the study design; in

the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

CRedit authorship contribution statement

Eitzaz Sadiq: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Angela Woodiwiss:** Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Grace Tade:** Project administration, Methodology, Investigation, Data curation. **Gavin Norton:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization. **Girish Modi:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

None

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jns.2024.123186>.

References

[1] A.S. Shah, D. Stelzle, K.K. Lee, et al., Global burden of atherosclerotic cardiovascular disease in people living with HIV: systematic review and meta-analysis, *Circulation* 138 (11) (2018) 1100–1112.

[2] M.S. Durstenfeld, P.Y. Hsue, Mechanisms and primary prevention of atherosclerotic cardiovascular disease among people living with HIV, *Curr. Opin. HIV AIDS* 16 (3) (2021) 177–185.

[3] J.H. Stein, J.S. Currier, P.Y. Hsue, Arterial disease in patients with human immunodeficiency virus infection: what has imaging taught us? *J. Am. Coll. Cardiol. Img.* 7 (5) (2014) 515–525.

[4] E. Hultén, J. Mitchell, J. Scally, et al., HIV positivity, protease inhibitor exposure and subclinical atherosclerosis: a systematic review and meta-analysis of observational studies, *Heart* 95 (22) (2009) 1826–1835.

[5] WorldHealthOrganisation, HIV statistics, globally and by WHO region [Available from]: <https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv-strategic-information/hiv-data-and-statistics>, 2023. Accessed on 19/01/2024.

[6] StatisticsSouthAfrica, Mid-year population Estimates (P0302) [Available from]: <https://www.statssa.gov.za/publications/P0302/MidYear2022.pdf>, 2022. Accessed on 19/01/2024.

[7] A. Abdallah, J.L. Chang, C.B. O’Carroll, et al., Stroke in human immunodeficiency virus-infected individuals in sub-Saharan Africa (SSA): a systematic review, *J. Stroke Cerebrovasc. Dis.* 27 (7) (2018) 1828–1836.

[8] E.P. Hyle, B.M. Mayosi, K. Middelkoop, et al., The association between HIV and atherosclerotic cardiovascular disease in sub-Saharan Africa: a systematic review, *BMC Public Health* 17 (1) (2017) 954.

[9] S. Hatano, Experience from a multicentre stroke register: a preliminary report, *Bull. World Health Organ.* 54 (5) (1976) 541–553.

[10] H.P. Adams Jr., B.H. Bendixen, L.J. Kappelle, et al., Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of org 10172 in acute stroke treatment, *Stroke* 24 (1) (1993) 35–41.

[11] A.I. Qureshi, Acute hypertensive response in patients with stroke: pathophysiology and management, *Circulation* 118 (2) (2008) 176–187.

[12] C. Florkowski, HbA1c as a diagnostic test for diabetes mellitus - reviewing the evidence, *Clin. Biochem. Rev.* 34 (2) (2013) 75–83.

[13] E. Klug, F.J. Raal, A.D. Marais, et al., South African dyslipidaemia guideline consensus statement: 2018 update a joint statement from the south African heart association (SA heart) and the lipid and atherosclerosis Society of Southern Africa (LASSA), *S. Afr. Med. J.* 108 (11b) (2018) 973–1000.

[14] A. Langsted, B.G. Nordestgaard, Nonfasting versus fasting lipid profile for cardiovascular risk prediction, *Pathology* 51 (2) (2019) 131–141.

[15] B.G. Nordestgaard, A test in context: lipid profile, fasting versus nonfasting, *J. Am. Coll. Cardiol.* 70 (13) (2017) 1637–1646.

[16] E. Di Angelantonio, N. Sarwar, P. Perry, et al., Major lipids, apolipoproteins, and risk of vascular disease, *Jama* 302 (18) (2009) 1993–2000.

[17] J.H. Barth, B.M. Jackson, A.J. Farrin, et al., Change in serum lipids after acute coronary syndromes: secondary analysis of SPACE ROCKET study data and a comparative literature review, *Clin. Chem.* 56 (10) (2010) 1592–1598.

[18] D.E. Kargman, C. Tuck, L. Berglund, et al., Lipid and lipoprotein levels remain stable in acute ischemic stroke: the northern Manhattan stroke study, *Atherosclerosis* 139 (2) (1998) 391–399.

- [19] E. Perovic, A. Mrdjen, M. Harapin, et al., Short term changes of serum lipids in acute ischemic stroke, *Clin. Lab.* 62 (11) (2016) 2107–2113.
- [20] P.J. Touboul, M.G. Hennerici, S. Meairs, et al., Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the advisory board of the 3rd and 4th watching the risk symposium, 13th and 15th European stroke conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006, *Cerebrovasc. Dis.* 23 (1) (2007) 75–80.
- [21] M.G. Veller, C.M. Fisher, A.N. Nicolaides, et al., Measurement of the ultrasonic intima-media complex thickness in normal subjects, *J. Vasc. Surg.* 17 (4) (1993) 719–725.
- [22] M.W. Lorenz, H.S. Markus, M.L. Bots, et al., Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis, *Circulation* 115 (4) (2007) 459–467.
- [23] C. Grunfeld, J.A. Delaney, C. Wanke, et al., Preclinical atherosclerosis due to HIV infection: carotid intima-media thickness measurements from the FRAM study, *AIDS* 23 (14) (2009) 1841–1849.
- [24] P.Y. Hsue, K. Ordovas, T. Lee, et al., Carotid Intima-media thickness among human immunodeficiency virus-infected patients without coronary calcium, *Am. J. Cardiol.* 109 (5) (2012) 742–747.
- [25] M. Lorenz, C. Stephan, A. Harmjan, et al., Both long-term HIV infection and highly active antiretroviral therapy are independent risk factors for early carotid atherosclerosis, *Atherosclerosis* 196 (2) (2008) 720–726.
- [26] M. Krikke, J. Arends, S. Van Lelyveld, et al., Greater carotid intima media thickness at a younger age in HIV-infected patients compared with reference values for an uninfected cohort, *HIV Med.* 18 (4) (2017) 275–283.
- [27] M.G. van Vonderen, Y.M. Smulders, C.D. Stehouwer, et al., Carotid intima-media thickness and arterial stiffness in HIV-infected patients: the role of HIV, antiretroviral therapy, and lipodystrophy, *JAIDS J. Acquir. Immune Deficien. Synd.* 50 (2) (2009) 153–161.
- [28] A.G. Vos, R.E. Barth, K. Klipstein-Grobusch, et al., Cardiovascular disease burden in rural Africa: does HIV and antiretroviral treatment play a role? Baseline analysis of the Ndlovu cohort study, *J. Am. Heart Assoc.* 9 (7) (2020) e013466.
- [29] A.F. Schoffelen, E. De Groot, H.A. Tempelman, et al., Carotid intima media thickness in mainly female HIV-infected subjects in rural South Africa: association with cardiovascular but not HIV-related factors, *Clin. Infect. Dis.* 61 (10) (2015) 1606–1614.
- [30] C. Fourie, A. Schutte, W. Smith, et al., Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans, *Atherosclerosis* 240 (1) (2015) 154–160.
- [31] R.L. Gleason Jr., A.W. Caulk, D. Seifu, et al., Current Efavirenz (EFV) or ritonavir-boosted lopinavir (LPV/r) use correlates with elevate markers of atherosclerosis in HIV-infected subjects in Addis Ababa, Ethiopia, *PLoS One* 10 (4) (2015) e0117125.
- [32] G.E. Volpe, A.M. Tang, J.F. Polak, et al., Progression of carotid intima-media thickness and coronary artery calcium over 6 years in an HIV-infected cohort, *JAIDS J. Acquir. Immune Deficien. Synd.* 64 (1) (2013) 51–57.
- [33] D.B. Hanna, M. Guo, P. Bůžková, et al., HIV infection and carotid artery intima-media thickness: pooled analyses across 5 cohorts of the NHLBI HIV-CVD collaborative, *Clin. Infect. Dis.* 63 (2) (2016) 249–256.
- [34] G. Ransley, S. Zimba, Y. Gadama, et al., Trends and clinical characteristics of HIV and cerebrovascular disease in low- and middle-income countries (LMICs) between 1990 and 2021, *Curr. HIV/AIDS Rep.* 19 (6) (2022) 548–565.
- [35] E.A. Nonterah, P.R. Boua, K. Klipstein-Grobusch, et al., Classical cardiovascular risk factors and HIV are associated with carotid intima-media thickness in adults from sub-Saharan Africa: findings from H3Africa AWI-gen study, *J. Am. Heart Assoc.* 8 (14) (2019) e011506.
- [36] M. Mosepele, L.C. Hemphill, W. Moloi, et al., Pre-clinical carotid atherosclerosis and sCD163 among virally suppressed HIV patients in Botswana compared with uninfected controls, *PLoS One* 12 (6) (2017) e0179994.
- [37] M.J. Feinstein, J.-H. Kim, P. Bibangamah, et al., Ideal cardiovascular health and carotid atherosclerosis in a mixed cohort of HIV-infected and uninfected Ugandans, *AIDS Res. Hum. Retrovir.* 33 (1) (2017) 49–56.
- [38] F.S. Sarfo, B. Norman, L. Appiah, et al., Factors associated with incidence of stroke and heart failure among people living with HIV in Ghana: evaluating vascular event risk while on long-term antiretroviral suppressive therapy (EVERLAST) study, *J. Clin. Hypertens. (Greenwich)* 23 (6) (2021) 1252–1259.
- [39] C.A. Dimala, H. Blencowe, Association between highly active antiretroviral therapy and selected cardiovascular disease risk factors in sub-Saharan Africa: a systematic review and meta-analysis protocol, *BMJ Open* 7 (3) (2017).
- [40] S.W. Worm, C. Sabin, R. Weber, et al., Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D: A: D) study, *J. Infect. Dis.* 201 (3) (2010) 318–330.
- [41] D. Sun, Y. Wu, Y. Yuan, et al., Is the atherosclerotic process accentuated under conditions of HIV infection, antiretroviral therapy, and protease inhibitor exposure? Meta-analysis of the markers of arterial structure and function, *Atherosclerosis* 242 (1) (2015) 109–116.
- [42] M.J. Blaauw, M.A. Berrevoets, W.A. Vos, et al., Traditional cardiovascular risk factors are stronger related to carotid intima-media thickness than to presence of carotid plaques in people living with HIV, *J. Am. Heart Assoc.* 12 (20) (2023) e030606.
- [43] M. Owolabi, A. Agunloye, E. Umeh, et al., Can common carotid intima media thickness serve as an indicator of both cardiovascular phenotype and risk among black Africans? *Eur. J. Prev. Cardiol.* 22 (11) (2015) 1442–1451.
- [44] G.V.T. Roozen, A.G. Vos, H.A. Tempelman, et al., Cardiovascular disease risk and its determinants in people living with HIV across different settings in South Africa, *HIV Med.* 21 (6) (2020) 386–396.
- [45] M. Mosepele, L.C. Hemphill, T. Palai, et al., Cardiovascular disease risk prediction by the American College of Cardiology (ACC)/American Heart Association (AHA) atherosclerotic cardiovascular disease (ASCVD) risk score among HIV-infected patients in sub-Saharan Africa, *PLoS One* 12 (2) (2017) e0172897.
- [46] I.S. Tahir, A.G. Vos, J.A. Damen, et al., Comparative performance of cardiovascular risk prediction models in people living with HIV, *S. Afr. J. HIV Med.* 23 (1) (2022).

Appendix A

Supplementary Methods

Laboratory investigations

1. Blood

- a. Full blood count, urea, creatinine
- b. Fasting glucose, HbA1c, total cholesterol, low-density lipoprotein (LDL), uric acid
- c. Vasculitis screen: erythrocyte sedimentation rate, C-reactive protein, syphilis serology (RPR and TPHA), hepatitis studies (Hepatitis A, B, C), antinuclear antibodies, extractable nuclear antigens, anti-double stranded DNA antibodies, rheumatoid factor, anti-neutrophil cytoplasmic antibodies, serum angiotensin converting enzyme
- d. Coagulation screen: PT, INR, D-dimer, Protein C, Protein S, anti-thrombin 3, fibrinogen, antiphospholipid antibodies
- e. HIV ELISA. If HIV ELISA positive, CD4 and VL were measured.

2. Cerebrospinal fluid (CSF) analysis

- a. Protein, glucose (with simultaneous plasma glucose), microscopy, cryptococcal latex antigen, India Ink test, Gene Xpert and culture for TB, bacteria and fungi.
- b. CSF polymerase chain reaction (PCR) testing for varicella zoster virus (VZV), herpes simplex virus, cytomegalovirus and Epstein Barr virus
- c. Syphilis tests (VDRL and/or FTA-Abs) were performed on CSF if blood syphilis serology (TPHA) was positive



Letter to the Editor

HIV status and carotid intima-media thickness

ARTICLE INFO

Keywords:

Ultrasound

Carotid artery

Surrogate marker

Carotid intima-media thickness

Human Immunodeficiency Virus

Dear Editor,

Sadiq et al. published in a recent issue a study titled, "Lack of impact of HIV status on carotid intima media thickness in a cohort of stroke patients in South Africa" [1]. The authors wrote, "People living with HIV (PLWH) are twice as likely to develop cardiovascular disease, including stroke, compared to uninfected individuals. Proposed mechanisms for this include accelerated atherosclerosis secondary to chronic inflammation and immune activation in an aging population, as well as the atherogenic effects of traditional cardiovascular risk factors (TRFs) and the metabolic side effects of longstanding antiretroviral therapy (ART), particularly protease inhibitors (PIs)" [1]. As surrogate marker for pre-clinical atherosclerosis the authors used the carotid intima-media thickness (cIMT) measured by high resolution B-mode ultrasound [1]. The study included 168 patients, 62 (36.9 %) were PLWH. The study found that "after adjusting for age, sex, hypertension, diabetes mellitus, smoking, total cholesterol, body mass index and stroke aetiology, there was no difference in mean cIMT between the groups (0.76 ± 0.16 mm vs 0.73 ± 0.17 mm, $p = 0.29$). Regression models revealed the determinants of cIMT to be age ($p < 0.0001$), hypertension ($p = 0.0098$) and total cholesterol ($p = 0.005$), while the determinants of increased cIMT (≥ 0.70 mm) were only age ($p < 0.0001$) and hypertension ($p = 0.0002$)" [1]. The authors concluded that "HIV status had no effect on cIMT in our cohort of stroke patients" [1]. As to their cIMT measurements the authors wrote, "Images of at least 1 cm length of the far wall of the distal portion of the right common carotid artery ...at least 1 cm proximal to the flow divider were obtained.... A total of 6 separate measurements (3 on each side) were averaged to a single mean cIMT" [1]. From the methodological description it is not clear on which side cIMT measurement occurred. The authors state first that images "of the right common carotid artery" were obtained only to finish by writing that "6 separate measurements (3 on each side)" were averaged [1]. Importantly to mind that cIMT values differ significantly between the right and left side, with higher values on the left side [2,3]. Rodríguez Hernández et al. showed a significant difference between both sides (left, 0.75 ± 0.11 mm; right, 0.71 ± 0.11 mm; $P < 0.001$) [3]. The authors explained this side- difference in cIMT with a higher cross-sectional area of the intima-media complex and a higher flow velocity at the left side [3]. Importantly, Rodríguez Hernández et al. found a significantly

higher incidence of non-lacunar cerebrovascular stroke at the left side [3]. They concluded that left side predilection for cerebrovascular events potentially related to increased hemodynamic stress and intimal damage as reflected by a significantly increased left CCA-IMT [3]. Therefore, this critical point which side (right/left CCA?) was used for cIMT measurements in the Sadiq et al. study needs clarification [1]. Importantly also to mind that a one-site measurement, namely far wall of the CCA, may well coincide with a normal CA segment, but be still within an atherosclerotic altered vessel: atherosclerosis presents asymmetrically [4,5]; therefore, a multi-site/composite (bilateral) measurement including e.g. both walls (far/near) of different CA segments (CCA, bifurcation, internal CA) will arguably capture more accurately the overall state of the artery, provide a more precise cIMT and be a better predictor for cardiovascular disease [6–8]. From the methodological description it appears further that in the Sadiq et al. study [1] cIMT measurement was not synchronized, as recommended, with the cardiac cycle (i.e. end-diastolic phase) [2]. Average differences between the two cardiac phases of 0.037 mm to 0.041 mm are reported [9,10]. Without synchronization measurement occur randomly in both cardiac phases, rendering the cIMT measures of the subjects in the Sadiq et al. cohort [1] incomparable. In conclusion: CIMT is a non-invasive, cost-effective and safe method to evaluate pre-clinical atherosclerosis. However, as surrogate marker of atherosclerosis cIMT is equally a delicate biomarker as sub-millimetric differences will suffice to categorize subjects into different cIMT categories. In the Sadiq et al. study a cIMT cut-off ≥ 0.70 mm was considered pathological [1]. A meticulous measurement protocol needs to be in place and the pro and cons of the different measurement methods need discussing [2,6]. Only in this way the reader can fully evaluate and analyze the results. Considering these multiple issues in cIMT data gathering of the Sadiq et al. study, the cIMT results and conclusions "HIV status had no effect on cIMT in a cohort of stroke patients" [1] should be considered with due caution.

Funding

No funding was received.

<https://doi.org/10.1016/j.jns.2024.123268>

Received 15 September 2024; Accepted 6 October 2024

Available online 9 October 2024

0022-510X/© 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.



Data access statement

Not applicable.

Ethics statement

Not applicable.

Author contribution

Christian Saleh wrote and revised manuscript.

Declaration of competing interest

Nothing to declare.

References

- [1] E. Sadiq, A. Woodiwiss, G. Tade, G. Norton, G. Modi, Lack of impact of HIV status on carotid intima media thickness in a cohort of stroke patients in South Africa, *J. Neurol. Sci.* (2024 Oct) 465, <https://doi.org/10.1016/j.jns.2024.123186>.
- [2] P.-J. Touboul, M.G. Hennerici, S. Meairs, H. Adams, P. Amarenco, N. Bornstein, et al., Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European stroke conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011, *Cerebrovasc. Dis.* 34 (4) (2012) 290–296.
- [3] S.A. Rodríguez Hernández, A.A. Kroon, M.P.J. van Boxtel, W.H. Mess, J. Lodder, J. Jolles, et al., Is there a side predilection for cerebrovascular disease? *Hypertension* 42 (1) (2003 Jul) 56–60.
- [4] Kolaszyńska O, Lorkowski J. Symmetry and asymmetry in atherosclerosis. *Int. J. Occup. Med. Environ. Health.* 36(6):693–703.
- [5] P. Tajik, R. Meijer, R. Duivenvoorden, S.A.E. Peters, J.J. Kastelein, F.J. Visseren, et al., Asymmetrical distribution of atherosclerosis in the carotid artery: identical patterns across age, race, and gender, *Eur. J. Prev. Cardiol.* 19 (4) (2012 Aug) 687–697.
- [6] M.L. Bots, G.W. Evans, W.A. Riley, D.E. Grobbee, Carotid intima-media thickness measurements in intervention studies: design options, progression rates, and sample size considerations: a point of view, *Stroke* 34 (12) (2003 Dec) 2985–2994.
- [7] A.I. del Sol, K.G. Moons, M. Hollander, A. Hofman, P.J. Koudstaal, D.E. Grobbee, et al., Is carotid intima-media thickness useful in cardiovascular disease risk assessment? The Rotterdam Study, *Stroke* 32 (7) (2001 Jul) 1532–1538.
- [8] D.H. O'Leary, J.F. Polak, R.A. Kronmal, T.A. Manolio, G.L. Burke, S.K. Wolfson, Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults, *N. Engl. J. Med.* 340 (1) (1999 Jan) 14–22.
- [9] J.F. Polak, C. Johnson, A. Harrington, Q. Wong, D.H. O'Leary, G. Burke, et al., Changes in carotid intima-media thickness during the cardiac cycle: the multi-ethnic study of atherosclerosis, *J. Am. Heart Assoc.* 1 (4) (2012 Aug) e001420.
- [10] J.F. Polak, A. Meisner, M.J. Pencina, P.A. Wolf, R.B. D'Agostino, Variations in common carotid artery intima-media thickness (cIMT) during the cardiac cycle: implications for cardiovascular risk assessment, *J. Am. Soc. Echocardiogr.* 25 (9) (2012 Sep) 1023–1028.

Christian Saleh*
Basel, Switzerland

* Corresponding author.
E-mail address: chs12us75010@yahoo.com.

Paper 3: Response to Letter to Editor

HIV status and carotid intima-media thickness.

Sadiq E, Woodiwiss A, Tade G, Norton G, Modi G.

Journal of the Neurological Sciences. 2024;466



Response to letter to the Editor

HIV status and carotid intima-media thickness

ARTICLE INFO

Keywords:

HIV
Stroke
Atherosclerosis
Intima media thickness
Biomarker
Africa

We read the letter from Christian Saleh with interest and appreciate the insights provided. We would like to clarify the queries raised regarding carotid intima-media thickness (cIMT) measurement techniques. cIMT was measured bilaterally, with 3 measurements on each side averaged to a mean cIMT, according to guidelines [1], as described in our paper [2]. We acknowledge the confusion created by our typographical error earlier in the manuscript, which should have read “Images of at least 1cm length of the far wall of the distal portion of the right and left common carotid artery...”.

While we agree that there are likely side differences in cIMT, the technique we used was in keeping with the accepted guidelines [1], which indicate that IMT values from the left and right side can be averaged. Most importantly, the same technique was used to assess cIMT in people living with HIV (PLWH) and HIV-uninfected patients.

Regarding multi-site measurements, we agree that this may be more sensitive to detect atherosclerotic changes; however whether HIV differentially affects different sites would be a question for future research.

Regarding ECG gating – although the IMT was not measured together with an ECG, images were taken at the minimal carotid artery diameter. This is in keeping with guidelines: “The optimal diameter should be obtained during diastole by automatic cine-loop detection or by looking for the minimal diameter during the cardiac cycle” [1].

Interestingly, the paper by Polak et al [3] quoted by Saleh in his letter, only measured right-sided cIMT, while in another quoted paper [4], means of the measurements of left and right IMT were analysed [4]. It is unknown if the differences in IMT between systole and diastole are the same between the left and the right. In addition, in both of these papers images were only taken at one site. In essence, the papers quoted by Saleh in his letter criticising our measurement technique, have used the same measurement technique as we have. Moreover, most longitudinal studies similarly report on mean measurements of left and right IMT, and often from only one site [5,6].

In conclusion, our cIMT measurement technique was in keeping with standard guidelines. Furthermore, as the identical technique was used in PLWH and HIV-infected patients, any potential limitations were unlikely to have compromised our findings.

Grant support

ES was supported by the Discovery Foundation Academic Fellowship Award (Ref 035747). The funder played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

CRediT authorship contribution statement

Eitzaz Sadiq: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing. **Angela Woodiwiss:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Writing – review & editing. **Grace Tade:** Investigation, Methodology, Project administration, Writing – review & editing. **Gavin Norton:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **Girish Modi:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

None.

References

- [1] P.-J. Touboul, M. Hennerici, S. Meairs, H. Adams, P. Amarenco, N. Bornstein, et al., Mannheim carotid intima-media thickness and plaque consensus (2004–2006–2011) an update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th european stroke conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011, Cerebrovasc. Dis. 34 (2012) 290–296.
- [2] E. Sadiq, A. Woodiwiss, G. Tade, G. Norton, G. Modi, Lack of impact of hiv status on carotid intima media thickness in a cohort of stroke patients in South Africa, J. Neurol. Sci. 465 (2024) 123186.
- [3] J.F. Polak, C. Johnson, A. Harrington, Q. Wong, D.H. O’Leary, G. Burke, et al., Changes in carotid intima-media thickness during the cardiac cycle: the multi-ethnic study of atherosclerosis, J. Am. Heart Assoc. 1 (2012) e001420.
- [4] J.F. Polak, A. Meisner, M.J. Pencina, P.A. Wolf, R.B. D’Agostino, Variations in common carotid artery intima-media thickness during the cardiac cycle:

<https://doi.org/10.1016/j.jns.2024.123269>

Received 1 October 2024; Accepted 6 October 2024

Available online 9 October 2024

0022-510X/© 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.



- implications for cardiovascular risk assessment, *J. Am. Soc. Echocardiogr.* 25 (2012) 1023–1028.
- [5] M.W. Lorenz, H.S. Markus, M.L. Bots, M. Rosvall, M. Sitzer, Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis, *Circulation* 115 (2007) 459–467.
- [6] C. Cuspidi, C. Sala, F. Provenzano, M. Tadic, E. Gherbesi, G. Grassi, et al., Metabolic syndrome and subclinical carotid damage: a meta-analysis from population-based studies, *J. Hypertens.* 36 (2018) 23–30.

Eitzaz Sadiq^{a,*}, Angela Woodiwiss^b, Grace Tade^b, Gavin Norton^b,
Girish Modi^a

^a Division of Neurology, Department of Neurosciences and Department of Internal Medicine, School of Clinical Medicine, South Africa

^b Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

* Corresponding author at: Division of Neurology, Department of Neurosciences, University of the Witwatersrand Medical School, 7 York Road, Parktown, 2193 Johannesburg, South Africa.

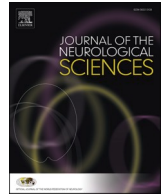
E-mail addresses: eitzaz.sadiq@wits.ac.za (E. Sadiq), angela.woodiwiss@wits.ac.za (A. Woodiwiss), oluwatosin.tade@wits.ac.za (G. Tade), physiology@health.wits.ac.za (G. Norton), girish.modi@wits.ac.za (G. Modi).

Paper 4

Stroke, HIV and the Immune Reconstitution Inflammatory Syndrome in the absence of opportunistic infections.

Sadiq E, Katzew S, Nel J, Tade G, Woodiwiss A, Norton G, Modi G.

Journal of the Neurological Sciences. 2024;457:122880.



Clinical short communication

Stroke, HIV and the Immune Reconstitution Inflammatory Syndrome in the absence of opportunistic infections

Eitzaz Sadiq^{a,*}, Sarah Katzew^b, Jeremy Nel^b, Grace Tade^c, Angela Woodiwiss^c, Gavin Norton^c, Girish Modi^a^a Division of Neurology, Department of Neurosciences, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa^b Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa^c Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

ARTICLE INFO

Keywords:

Stroke
HIV
Antiretroviral therapy
Immune reconstitution inflammatory syndrome
IRIS
South Africa

ABSTRACT

Introduction: Stroke in people living with HIV (PLWH) has been described to occur soon after the initiation of antiretroviral therapy (ART) possibly related to the Immune Reconstitution Inflammatory Syndrome (IRIS). We sought to investigate whether there was a temporal association between stroke and recent ART initiation in the absence of opportunistic infections (OIs), and to identify risk factors for this.**Methods:** This cross-sectional study recruited PLWH with new-onset stroke at a hospital in Johannesburg, South Africa, from 2014 to 2017, excluding all patients with OIs. Patients were assessed for ART duration, CD4 count, HIV viral load, inflammatory markers and cardiovascular risk factors.**Results:** 77 PLWH were recruited, of which 35 were on ART at the time of stroke. Of the patients with confirmed ART duration ($n = 28$), 9 (32.1%) had a stroke within the first 6 months of starting ART (crude incidence rate of 0.73 cases per patient year). In the period beyond 6 months, 19 strokes occurred (crude incidence rate of 0.21 cases per patient year), translating to a 3.5 times greater risk in the first 6 months ($p = 0.0002$). There were no clearly identified risk factors when comparing those who had strokes in the first 6 months to those after 6 months and ART-naïve patients.**Conclusion:** Almost a third of strokes in PLWH may be related to IRIS, with a crude incidence rate 3.5 times higher in the first 6 months following ART-initiation compared to beyond 6 months. This appears to be independent of OIs. Risk factors are unclear.

1. Introduction

The rollout of antiretroviral therapy (ART) has been key in reducing HIV-associated mortality, morbidity and opportunistic infections (OIs). However, many people living with HIV (PLWH) in South Africa and other resource-limited settings still initiate ART at late stages with established advanced immunodeficiency. (1) Approximately 10–20% of PLWH, particularly those with low baseline CD4 counts and/or high HIV viral loads, may experience an immune reconstitution inflammatory syndrome (IRIS) following ART initiation. (2) This is a dysregulated, hyper-inflammatory response that is usually directed at opportunistic

infections, almost always occurs within 6 months of ART initiation, and may cause considerable morbidity and mortality. (2)

Neurological complications of immune reconstitution represent the most debilitating form of IRIS, conferring a high mortality and significant morbidity in the form of permanent neurological deficit in up to 50% of cases. (3) *Mycobacterium tuberculosis*, *Cryptococcus neoformans* and JC virus account for the majority of cases of central nervous system (CNS)-IRIS. (3)

It has been suggested that CNS-IRIS may occur in the absence of OIs. Proposed mechanisms for this relatively novel concept include a dysregulated and destructive excessive immune response to occult OIs or HIV-associated proteins. Residual virus in the CNS and persistent release

* Corresponding author at: Division of Neurology, Department of Neurosciences, University of the Witwatersrand Medical School, 7 York Road, Parktown, 2193, Johannesburg, South Africa.

E-mail addresses: eitzaz.sadiq@wits.ac.za (E. Sadiq), sarahkatzew@gmail.com (S. Katzew), Jeremy.nel@wits.ac.za (J. Nel), oluwatosin.tade@wits.ac.za (G. Tade), angela.woodiwiss@wits.ac.za (A. Woodiwiss), physiology@health.wits.ac.za (G. Norton), girish.modi@wits.ac.za (G. Modi).

<https://doi.org/10.1016/j.jns.2024.122880>

Received 30 November 2023; Received in revised form 5 January 2024; Accepted 6 January 2024

Available online 8 January 2024

0022-510X/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Non-standard Abbreviations and Acronyms

ART	Antiretroviral therapy
cIMT	Carotid intima-media thickness
IRIS	Immune reconstitution inflammatory syndrome
NIHSS	National Institute of Health Stroke Scale
OI	Opportunistic infection
PLWH	People living with HIV
PWV	Pulse wave velocity
TRF	Traditional cardiovascular risk factor

of HIV-associated proteins from infected cells may occur even in the face of viral suppression. (3) The possibility of an autoimmune aggression towards self-antigens has also been proposed, but lacks sufficient evidence. (4)

HIV has been shown to be a significant and independent risk factor for stroke. (5,6) Over 50% of previously described PLWH with stroke in SSA are ART-naïve. (5) In Sub-Saharan Africa (SSA), infection is implicated in 23–55% of stroke in PLWH. (5) There have been case reports describing stroke as an IRIS phenomenon, all of which were associated with an OI; namely cryptococcal meningitis, (7) varicella zoster (8) and cytomegalovirus. (9) All of these pathogens are known to cause strokes, and thus the reports of them causing stroke in the setting of an IRIS are not surprising. Recently a group from Malawi described 64 PLWH with stroke, of whom 25% had their event within 6 months of initiating ART. Most of these patients had no evidence of OIs. (10) Subsequent cohorts have produced conflicting results, casting some doubt over whether stroke can be a manifestation of IRIS in the absence of specific opportunistic infections. (11–13)

Atherosclerosis in PLWH may be partially related to HIV-related inflammation and endothelial dysfunction. It is possible that this process may be acutely accelerated in the presence of an aberrant immune response, as is seen in IRIS. Whether or not this translates to an increased stroke risk is unknown. The utility of surrogate markers of subclinical atherosclerosis, including carotid intima-media thickness (cIMT) and aortic pulse wave velocity (PWV) have never been tested in this setting.

We sought to investigate whether there was a temporal association between stroke and recent ART initiation among PLWH in the absence of OIs, and whether risk factors for stroke and markers of atherosclerosis differed between those with “early” stroke (within 6 months of ART initiation) compared to “late” stroke and those PLWH who were not on ART.

2. Methods

This cross-sectional study took place at the Charlotte Maxeke Johannesburg Academic Hospital, a 1000-bed quaternary-level hospital in Johannesburg, South Africa, from August 2014 to November 2017.

We recruited consecutive patients aged 18 years and older who presented within 48 h of a new onset stroke. Stroke was defined as per the World Health Organization case definition. Exclusion criteria were the presence of OIs, including on cerebrospinal fluid (CSF) examination (elevated protein, a low serum:CSF glucose ratio, pleocytosis or positive viral PCR), the presence of intracranial mass lesions on radiographic imaging, or other stroke mimics. Full informed consent was obtained as per the Declaration of Helsinki. Ethics approval was granted by the University of the Witwatersrand Human Research Ethics Committee (Certificate number M140429, renewed as M190688).

All patients were assessed and examined by the same specialist neurologist (ES). Traditional cardiovascular risk factors (TRFs) were defined as described in Appendix A (Supplementary material). Stroke aetiology was assigned following the interpretation of the following investigations by ES and the treating physicians: electrocardiogram,

transthoracic echocardiography, carotid artery doppler studies, CT brain and CT angiography of the brain, neck and aortic arch, as well as the laboratory investigations detailed in Appendix B (Supplementary material). Vasculopathy was diagnosed in patients with intra- or extracranial angiographic abnormalities with no alternate explanation. The type of vasculopathy was labelled as atherosclerotic vasculopathy (AV) in the presence of large vessel occlusion with the additional ultrasound visualisation of atherosclerotic plaque causing a > 50% occlusion of the internal or common carotid artery ipsilateral to the stroke. In the absence of this plaque, non-atherosclerotic vasculopathy (NAV) was diagnosed. HIV-associated vasculitis was diagnosed in the setting of the classic angiographic features of vasculitis without an identified causative opportunistic infection, while small vessel disease (SVD) was defined as the presence of a traditional clinical lacunar syndrome with an infarct <20 mm in size. (14)

HIV testing (ELISA) was performed in all consenting patients. A suppressed VL was defined as a VL of <50 copies/ml. A lumbar puncture was performed in all PLWH provided no contra-indication was present. Cerebrospinal fluid (CSF) was examined as per the protocol in Appendix B (Supplementary material). Stroke severity was graded according to the National Institutes of Health Stroke Scale (NIHSS). (15) The patient's duration of ART was recorded as “Unconfirmed” if there was no available written or electronic proof of the initiation date from a healthcare provider. Carotid intima-media thickness (cIMT) and aortic pulse wave velocity (PWV) were measured as markers of subclinical atherosclerosis. The methods are detailed in Appendix B (Supplementary material).

2.1. Statistical methods

Data was analysed using Statistica Ver 14.0.0.15, TIBCO Software Inc. Continuous variables were evaluated for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. Chi square, ANOVA and Kruskal-Wallis statistical tests were then performed for parametric and non-parametric variables where appropriate. Continuous data were reported as mean $\pm$ SD for parametric, or median and interquartile range for non-parametric data. Proportions were compared using the z-test. *P* values <0.05 were considered significant.

3. Results

107 PLWH were screened for participation in the study, of which 30 were excluded as illustrated in Fig. 1. The mean age of the 77 included participants was 44.9 ± 12.0 years, and 50.7% were male.

35/77 (45.4%) of the PLWH were on ART at the time of their stroke. 32 of these were on a fixed dose combination comprising emtricitabine, tenofovir and efavirenz, while 3 patients were on a protease-inhibitor containing regimen. The median duration of ART was 36 months (IQR 5.5–66 months). The 42 ART-naïve patients were either newly diagnosed with HIV on this presentation ($n = 30$), were in the process of ART initiation ($n = 4$), or had previously refused ART ($n = 8$).

When compared to ART-naïve patients, there was no increased prevalence of TRFs in the ART-exposed group (Table S1 – Appendix C Supplementary material). Hypertension was actually more prevalent in the ART-naïve group (59.5% vs 34.3%, $p = 0.03$, Table S1). There was no difference in mean CD4, stroke severity or markers of subclinical atherosclerosis (cIMT and PWV) between the 2 groups (Table S1 – Appendix C Supplementary material).

The duration of ART was known and verified in 28 of the patients on ART, of which 9 (32.1%) had been on ART for ≤ 6 months at the time of their stroke. (Fig. 1, Fig. 2 and Fig. S1 – Appendix C supplementary material).

The crude incidence rate for the first 6 months post-ART initiation was 0.73 strokes per one person year (Table 1), compared to 0.21 per one person year for the period beyond 6 months post-ART initiation (Table 1). This difference was statistically significant (z-test, $p < 0.0005$), (Appendix D - Supplementary material) and translated to a 3.5

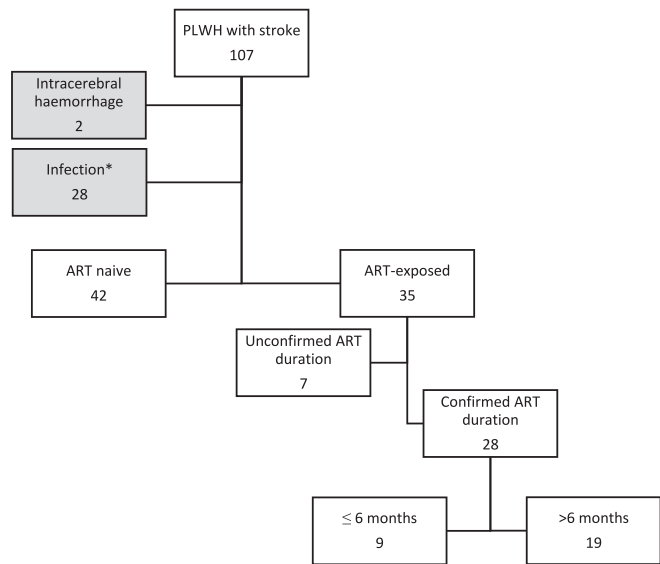


Fig. 1. Flowchart of PLWH presenting with acute stroke.
*Including meningitis ($n = 20$) and varicella zoster virus vasculopathy ($n = 8$). Groups excluded from the study are shaded in grey.
ART, antiretroviral therapy; PLWH, people living with HIV. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

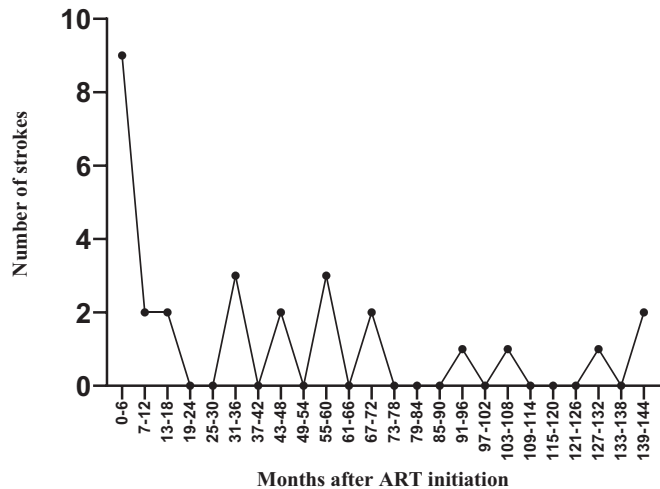


Fig. 2. Number of strokes according to duration of ART.

times greater crude incidence rate for ART duration ≤ 6 months when compared to ART duration > 6 months (Table 1).

The group of patients with a stroke while on ART for ≤ 6 months (the “early” group) was compared to those on ART for > 6 months (the “late” group), and showed no difference in demographics, TRFs, CD4, VL, stroke severity or markers of subclinical atherosclerosis (Table 2). The blood markers detailed in Table S2 (Appendix C Supplementary material) also showed no significant difference between the two groups. These included white cell count, haemoglobin, creatinine, CRP, ESR,

angiotensin-converting enzyme and uric acid. (Table S2).

Comparison of the early group to the ART-naïve revealed a lower prevalence of hypertension in the early group, with no other significant differences in the other variables in Table 1, nor in blood markers (Table S2) We did not however have data on nadir CD4 counts.

Stroke aetiology was similar across the groups. (Table 2). 2 out of the 9 patients (22.2%) in the early group were diagnosed with non-atherosclerotic vasculopathy. One of these was intracranial (middle cerebral artery, with CD4 354 cells/uL, VL 452 copies/ml), and the other extracranial (internal carotid artery, with CD4 1168 cells/uL, VL suppressed). Both patients with cardioembolic stroke in the early group had dilated cardiomyopathy secondary to HIV. 1 patient had small vessel disease secondary to chronic uncontrolled hypertension and 1 had a stroke secondary to recreational drug usage (listed as “Other” in Table 2). Of those with atherosclerotic vasculopathy ($n = 4$), 2 were on ART > 6 months, and 2 were ART-naïve. None of these patients were exposed to protease inhibitors. Small sample sizes in the ART groups precluded further meaningful analyses comparing stroke aetiologies across the groups.

4. Discussion

We demonstrated that almost one-third of strokes in PLWH on ART in our cohort occurred in the first 6 months of initiating ART. The crude incidence rate for stroke in this period was 3.5 times higher when compared to the period > 6 months after ART initiation. This was despite the exclusion of all overt infections. We found no clear difference in demographics, TRFs, HIV-related factors, blood markers or surrogate markers of atherosclerosis between those who had a stroke within 6 months after starting ART and those whose strokes occurred later, outside the possible window for an IRIS reaction.

It is postulated that IRIS may result in stroke by either unmasking a latent infection, or possibly due to an immune response to HIV particles or even self-antigens. (3) A landmark case-control study from Malawi reported that the highest risk of stroke in PLWH was within 6 months of ART initiation (adjusted OR 15.6, 95% CI 4.21–46.6, $p < 0.001$). (6) Out of 64 PLWH with stroke, 16 (25%) fell into this category. These patients had a median CD4 of 92 cells/mm³, with lower VLs than other categories. Nadir CD4 counts were not reported. In contrast, we did not detect any difference in CD4 counts between those in the early group and the late group or ART-naïve patients. This may be due to our exclusion of infections, which generally occur at lower CD4 counts. Our high prevalence of possible IRIS-related stroke despite the exclusion of infection supports a role for a non-infectious inflammatory mechanism. This corroborates with the Malawian findings of HIV-associated vasculopathy being the commonest aetiology of stroke in the IRIS group (9 of 16), with only 3 of the patients’ aetiology being attributed to opportunistic infections. (10) We did not detect a higher prevalence of vasculopathy (or any other specific aetiology) in our early group, although our small sample size limited comparisons.

Other small HIV stroke studies in SSA have reported promising yet varying results on the role of IRIS, none of which specifically excluded OIs. A Zambian study reported almost a third of strokes in PLWH (17/48, 29%) occurred soon after ART initiation, however they incorporated a longer time frame (1 year post ART initiation) and had large amounts of missing data on immunological and virological parameters. (13). A study from Cape Town, South Africa reported that 39.3% (22/56) of ART-exposed PLWH with stroke may be related to IRIS, most of which

Table 1
Comparing crude incidence rates for stroke in the periods ≤ 6 months and > 6 months post ART-initiation.

Time period	Stroke cases	Total person-time at risk (months)*	Cases per patient year (Crude incidence rate)	Risk ratio
≤ 6 months	9	147.25	0.73	3.5
> 6 months	19	1090	0.21	

* Sum of the total number of months from ART initiation to stroke (see Appendix D - Supplementary methods for details).

Table 2

Comparing traditional cardiovascular risk factors, HIV-related factors and stroke aetiology of those on ART for ≤ 6 months (“Early” group) to those on ART for > 6 months (“Late” group), and to the ART-naïve PLWH.

	ART-naïve (n = 42)	≤ 6 months ART (“Early”) (n = 9)	> 6 months ART (“Late”) (n = 19)	p-value
Age (years)	45.2 $\pm$ 12.5	45.7 $\pm$ 11.3	48.5 $\pm$ 10.5	0.60
Male (%)	52.4	77.8	47.4	0.30
Hypertension (%)	59.5*	11.1*	47.4	0.03*
Diabetes mellitus (%)	9.5	0.0	5.3	0.56
Dyslipidaemia (%)	23.8	36.8	22.2	0.54
Smoking (%)	31.0	22.2	26.3	0.84
Obesity (%)	23.1 (n = 26)†	25.0 (n = 8)†	40.0 (n = 15)†	0.50
Median number of TRFs (IQR)	1 (1–2)	0 (0–1)	2 (1–2)	0.16
Median ART duration (months) (IQR)	–	4 (2–5)	60 (36–96)	0.0004
CD4 (cells/ μ L)	323 $\pm$ 164 (n = 38)†	394 $\pm$ 325	424 $\pm$ 198	0.16
Median VL (copies/ml) (IQR)	45,015 (19200–192,800) (n = 30)†	0 (0–49)	0 (0–0)	< 0.0001
% with VL suppression	–	77.8	84.2	0.68
NIHSS	12.3 $\pm$ 5.9	11.2 $\pm$ 4.3	12.9 $\pm$ 5.6	0.77
cIMT (mm)	0.71 $\pm$ 0.14 (n = 31)†	0.72 $\pm$ 0.23 (n = 8)†	0.72 $\pm$ 0.20 (n = 14)†	0.99
PWV (m/s)	8.26 $\pm$ 2.61 (n = 22)†	7.76 $\pm$ 2.69 (n = 7)†	8.35 $\pm$ 3.05 (n = 13)†	0.89
Stroke Aetiology				
Cardioembolic	9 (21.4%)	2 (22.2%)	4 (21.1%)	0.75
Atherosclerotic Vasculopathy	2 (4.8%)	0 (0.0%)	2 (10.5%)	
Non-atherosclerotic vasculopathy	6 (14.3%)	2 (22.2%)	2 (10.5%)	
HIV-associated vasculitis	3 (7.1%)	0 (0.0%)	2 (10.5%)	
Small vessel disease	8 (19.0%)	1 (11.1%)	5 (26.3%)	
Undetermined	13 (31.0%)	3 (33.3%)	4 (21.1%)	
Miscellaneous	1 (2.4%)	1 (11.1%)	0 (0.0%)	

ART, antiretroviral therapy; cIMT, carotid intima media thickness; IRIS, Immune Reconstitution Inflammatory Syndrome; IQR, interquartile range; NIHSS, National Institute of Health Stroke Scale; PWV, pulse wave velocity; TRFs, traditional risk factors; VL, viral load.

* p-value = 0.01 “Early” group vs ART-naïve.

† Indicates the number of patients for whom data was available (in the event of missing data).

occurred within 6 months of restarting ART after an unspecified period of defaulting treatment (11). The stroke aetiologies and presence of OI's were not reported in relation to IRIS. It should also be noted that not all recent SSA stroke studies have detected any role for IRIS, including one from Pretoria, South Africa, which reported that none of their 32 PLWH with stroke fulfilled the criteria for IRIS. (12) The fact that the findings on this potentially preventable phenomenon have not been consistently observed emphasises its complexity and has opened a space that certainly merits further investigation.

Ours is the first study to describe a high prevalence of possible IRIS-related strokes having reasonably excluded infections, and the first to explore the role of atherosclerosis in this setting. We hypothesized that atherosclerosis, as an inflammation-mediated pathology, may be accelerated in patients with an aberrant immune response as is seen in IRIS. However we found no evidence of this, with no increased prevalence of atherosclerotic vasculopathy, nor any difference in markers of subclinical atherosclerosis (cIMT and PWV) in our early group when compared to both the late group and ART-naïve patients. This points towards IRIS-induced accelerated atherosclerosis being an unlikely stroke mechanism in these patients, though our study was not adequately powered to provide a definitive conclusion.

Other possibilities to explain an association between ART initiation and early stroke may include the effects of occult OIs which may have remained undetected despite our stringent efforts to exclude them. We noticed this with varicella zoster virus (VZV), which was implicated in causing stroke in the absence of any cerebrospinal fluid pleocytosis or biochemical abnormality except a positive CSF VZV PCR in 8 of the 28 patients that were excluded from this cohort. It is possible that other infections went undetected despite our efforts. The direct metabolic side effects of ART itself are unlikely to be contributing to stroke risk in our cohort as very few patients were on protease inhibitors (which are most commonly implicated). Furthermore, one would expect the risk of this to continue with time, and not be concentrated in the first few months after ART initiation, as was demonstrated here.

4.1. Limitations

We were unable to identify any risk predictors for early stroke following ART initiation. Our non-availability of data on nadir CD4 counts was a missed opportunity to identify if those with more profound pre-ART immunosuppression were most at risk. Our small sample size (compounded by information bias due to missing data on exact ART duration) limited the power of many comparisons, including our inability to adjust the reported crude incidence rates for potential confounders. Selection bias may also have played a role as patients were recruited from a quaternary-level hospital, where more atypical stroke patients are more likely to be referred.

5. Conclusion

Almost a third of strokes in PLWH may potentially be related to IRIS, with a crude incidence rate 3.5 times higher in the first 6 months following ART-initiation compared to beyond 6 months. This appears to be independent of OIs, nor with any evidence of atherosclerosis. The risk factors and underlying mechanisms are unclear and warrant further investigation in order to identify which patients are at greatest risk for this catastrophic outcome following ART initiation.

Grant support

ES was supported by the Discovery Foundation Academic Fellowship Award. The funder played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

CRediT authorship contribution statement

Eitzaz Sadiq: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing. **Sarah Katzew:** Writing – original draft, Writing – review & editing. **Jeremy Nel:** Formal analysis, Validation, Visualization, Writing – original draft,

Writing – review & editing. **Grace Tade:** Data curation, Investigation, Methodology, Project administration, Writing – review & editing. **Angela Woodiwiss:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Writing – review & editing. **Gavin Norton:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **Girish Modi:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

None

Appendix. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jns.2024.122880>.

References

- [1] T.G. Fuge, G. Tsourtos, E.R. Miller, Risk factors for late linkage to care and delayed antiretroviral therapy initiation among adults with HIV in sub-Saharan Africa: a systematic review and meta-analyses, *Int. J. Infect. Dis.* 122 (2022) 885–904.
- [2] N.F. Walker, J. Scriven, G. Meintjes, et al., Immune reconstitution inflammatory syndrome in HIV-infected patients, *HIV/AIDS (Auckland, NZ)*. 7 (2015) 49–64.
- [3] T.P. Johnson, A. Nath, New insights into immune reconstitution inflammatory syndrome of the central nervous system, *Curr. Opin. HIV AIDS* 9 (6) (2014) 572–578.
- [4] G. Martin-Blondel, P. Delobel, A. Blancher, et al., Pathogenesis of the immune reconstitution inflammatory syndrome affecting the central nervous system in patients infected with HIV, *Brain J. Neurol.* 134 (Pt 4) (2011) 928–946.
- [5] A. Abdallah, J.L. Chang, C.B. O'Carroll, et al., Stroke in human immunodeficiency virus-infected individuals in sub-Saharan Africa (SSA): a systematic review, *J. Stroke Cerebrovasc. Dis.* 27 (7) (2018) 1828–1836.
- [6] L.A. Benjamin, E.L. Corbett, M.D. Connor, et al., HIV, antiretroviral treatment, hypertension, and stroke in Malawian adults: a case-control study, *Neurology*. 86 (4) (2016) 324–333.
- [7] J.P. Ellis, N. Kalata, E.C. Joeles, et al., Ischemic stroke as a complication of cryptococcal meningitis and immune reconstitution inflammatory syndrome: a case report, *BMC Infect. Dis.* 18 (1) (2018) 1–5.
- [8] S. Teo, D. Raha, D. Warren, et al., Central nervous system–immune reconstitution inflammatory syndrome presenting as varicella zoster virus-mediated vasculitis causing stroke, *Int. J. STD AIDS* 25 (9) (2014) 683–685.
- [9] A.M. Anderson, J.A. Fountain, S.B. Green, et al., Human immunodeficiency virus-associated cytomegalovirus infection with multiple small vessel cerebral infarcts in the setting of early immune reconstitution, *J. Neuro-Oncol.* 16 (2) (2010) 179–184.
- [10] L.A. Benjamin, T.J. Allain, H. Mzinganjira, et al., The role of human immunodeficiency virus-associated vasculopathy in the etiology of stroke, *J. Infect. Dis.* 216 (5) (2017) 545–553.
- [11] C. Corbett, N. Brey, R.D. Pitcher, et al., Prevalence and characteristics of HIV-associated stroke in a tertiary hospital setting in South Africa, *Neurology*. 99 (9) (2022) e904–e915.
- [12] L. Kroon, D.G. van Zyl, C.M. Schutte, et al., Risk factors for stroke in HIV-positive and-negative patients in Pretoria, South Africa, *J. Stroke Cerebrovasc. Dis.* 30 (8) (2021) 105929.
- [13] S. Zimba, A. Nutakki, L. Chishimba, et al., Risk factors and outcomes of HIV-associated stroke in Zambia, *Aids*. 35 (13) (2021) 2149–2155.
- [14] L.A. Benjamin, A. Bryer, S. Lucas, et al., Arterial ischemic stroke in HIV: defining and classifying etiology for research studies, *Neurol. Neuroimmunol. Neuroinflamm.* 3 (4) (2016) e254.
- [15] P. Lyden, T. Brott, B. Tilley, et al., Improved reliability of the NIH stroke scale using video training. NINDS TPA stroke study group, *Stroke*. 25 (11) (1994) 2220–2226.

Appendix A: Definitions of Traditional Risk Factors

Hypertension was defined as having 3 or more blood pressure readings greater than 140/90 mm Hg beyond the first 72 hours post-stroke, with or without the presence of target organ damage (hypertensive retinopathy on direct ophthalmoscopy, left ventricular hypertrophy on electrocardiogram, echocardiogram or chest X-ray, or renal dysfunction with no other clear cause). Acute post-stroke elevated blood pressure (BP) was not used as diagnostic for HPT due to the well-described transient increase in BP seen acutely post-stroke. (1)

Diabetes mellitus was defined as a fasting glucose ≥ 7.0 mmol/l, or a random glucose ≥ 11.1 mmol/l, or an HbA1c $> 6.5\%$. Patients with anaemia were not diagnosed as diabetic solely on HbA1c measurement, to avoid false positives.(2)

Dyslipidaemia was defined as a total cholesterol ≥ 5.2 mmol/L, and/or low density lipoprotein cholesterol (LDL) ≥ 3.3 mmol/L.(3) Blood for lipogram assessment was taken in a non-fasting state. Current evidence suggests that for cardiovascular risk assessment, non-fasting total cholesterol, and LDL are equivalent to a fasting sample.(3-6) Acute post-stroke transient decreases in cholesterol levels were accounted for by taking blood for lipograms within 24 hours of the stroke onset, where possible, and by not considering a low post-stroke high density lipoprotein cholesterol (HDL) to be diagnostic for dyslipidaemia.(7-9)

Obesity was defined as a body mass index (BMI) of ≥ 30 kg/m². This was obtained by measuring the patient's height using a stadiometer and the weight using a calibrated mechanical column scale where BMI = weight $\div$ height². This was not possible in patients unable to stand unaided.

Appendix B

Supplementary Methods

Laboratory investigations

1. Blood

- a. Full blood count, urea, creatinine
- b. Fasting glucose, HbA1c, total cholesterol, low-density lipoprotein (LDL), uric acid
- c. Vasculitis screen: erythrocyte sedimentation rate, C-reactive protein, syphilis serology (RPR and TPHA), hepatitis studies (Hepatitis A, B, C), antinuclear antibodies, extractable nuclear antigens, anti-double stranded DNA antibodies, rheumatoid factor, anti-neutrophil cytoplasmic antibodies, serum angiotensin converting enzyme
- d. Coagulation screen: PT, INR, D-dimer, Protein C, Protein S, anti-thrombin 3, fibrinogen, antiphospholipid antibodies
- e. HIV ELISA. If HIV ELISA positive, CD4 and VL were measured.

2. Cerebrospinal fluid (CSF) analysis

- a. Protein, glucose (with simultaneous plasma glucose), microscopy, cryptococcal latex antigen, India Ink test, Gene Xpert and culture for TB, bacteria and fungi.
- b. CSF polymerase chain reaction (PCR) testing for varicella zoster virus (VZV), herpes simplex virus, cytomegalovirus and Epstein Barr virus
- c. Syphilis tests (VDRL and/or FTA-Abs) were performed on CSF if blood syphilis serology (TPHA) was positive

Markers of atherosclerosis

Carotid Doppler studies were performed using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington) employing a linear array 7.5 MHz probe as described previously.(10) Carotid intima-thickness, plaque and stenosis were assessed. Images of at least 1cm length of the far wall of the distal portion of the right common carotid artery (CCA) from an optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal (ICA) and external carotid artery (ECA) are visualized

simultaneously) at least 1 cm proximal to the flow divider were obtained. cIMT measurements were determined using semi-automated border-detection and quality control software. All images were saved electronically, allowing for later quality control and re-evaluation if necessary. A carotid IMT ≥ 0.7 mm was considered to be pathological, and suggestive of subclinical atherosclerosis, as per previously published data.(11, 12)

Large artery stiffness was indexed from aortic pulse wave velocity (PWV). Aortic PWV was measured as described elsewhere.(13) After participants had rested for 15 minutes in the supine position, sequential recordings of the arterial pressure waveform at the carotid and femoral arteries were determined using applanation tonometry. A high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas), interfaced with a computer utilizing SphygmoCor software, version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) was used. Arterial pressure waveform recordings were discarded when the systolic or diastolic variability of consecutive waveforms exceeded 5%, or when the amplitude of the pulse wave signal was less than 80 mV. To determine aortic PWV, distances from the suprasternal notch to the carotid sampling site (distance A) and from the suprasternal notch to the femoral artery (distance B) were measured. Pulse wave velocity distance was calculated as distance B minus distance A. Pulse transit time, calculated as the mean time difference between sites A and B was determined from the average of 10 consecutive beats. Aortic PWV was calculated as the ratio of the distance in metres to the transit time in seconds. A PWV above the threshold of ≥ 10.00 m/s was considered as suggestive of increased large artery stiffness, as per previously published data.(14, 15)

The cIMT and PWV examinations were performed by a single operator who had undergone dedicated training in the relevant technique. The operator was blinded to the aetiological category which had been already assigned to the patient and no changes were made to the classification after cIMT and PWV measurements.

References (Appendix A and B)

1. Qureshi AI. Acute hypertensive response in patients with stroke: pathophysiology and management. *Circulation*. 2008;118(2):176-187.
2. Florkowski C. HbA1c as a Diagnostic Test for Diabetes Mellitus - Reviewing the Evidence. *Clin Biochem Rev*. 2013;34(2):75-83.
3. Klug E, Raal FJ, Marais AD, et al. South African dyslipidaemia guideline consensus statement: 2018 update A joint statement from the South African Heart Association (SA Heart) and the Lipid and Atherosclerosis Society of Southern Africa (LASSA). *S Afr Med J*. 2018;108(11b):973-1000.
4. Langsted A, Nordestgaard BG. Nonfasting versus fasting lipid profile for cardiovascular risk prediction. *Pathology*. 2019;51(2):131-141.
5. Nordestgaard BG. A Test in Context: Lipid Profile, Fasting Versus Nonfasting. *J Am Coll Cardiol*. 2017;70(13):1637-1646.
6. Di Angelantonio E, Sarwar N, Perry P, et al. Major lipids, apolipoproteins, and risk of vascular disease. *Jama*. 2009;302(18):1993-2000.
7. Barth JH, Jackson BM, Farrin AJ, et al. Change in serum lipids after acute coronary syndromes: secondary analysis of SPACE ROCKET study data and a comparative literature review. *Clin Chem*. 2010;56(10):1592-1598.
8. Kargman DE, Tuck C, Berglund L, et al. Lipid and lipoprotein levels remain stable in acute ischemic stroke: the Northern Manhattan Stroke Study. *Atherosclerosis*. 1998;139(2):391-399.
9. Perovic E, Mrdjen A, Harapin M, et al. Short Term Changes of Serum Lipids in Acute Ischemic Stroke. *Clinical laboratory*. 2016;62(11):2107-2113.
10. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis*. 2007;23(1):75-80.
11. Veller MG, Fisher CM, Nicolaides AN, et al. Measurement of the ultrasonic intima-media complex thickness in normal subjects. *J Vasc Surg*. 1993;17(4):719-725.
12. Lorenz MW, Markus HS, Bots ML, et al. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation*. 2007;115(4):459-467.

13. Norton GR, Majane OH, Maseko MJ, et al. Brachial blood pressure-independent relations between radial late systolic shoulder-derived aortic pressures and target organ changes. *Hypertension (Dallas, Tex : 1979)*. 2012;59(4):885-892.
14. O'Rourke MF, Staessen JA, Vlachopoulos C, et al. Clinical applications of arterial stiffness; definitions and reference values. *American journal of hypertension*. 2002;15(5):426-444.
15. Kim HL, Kim SH. Pulse Wave Velocity in Atherosclerosis. *Frontiers in cardiovascular medicine*. 2019;6:41.

Appendix C: Supplementary Data

Table S1: Comparing TRFs and HIV-related factors between ART-exposed and ART-naïve PLWH

	ART (n=35)	ART-naïve (n=42)	p-value
Age (years)	44.6±11.6	45.2±12.5	0.82
Male (%)	48.6	52.4	0.74
Hypertension (%)	34.3	59.5	0.03
Diabetes mellitus (%)	2.9	9.5	0.24
Dyslipidaemia (%)	28.6	23.8	0.64
Smoking (%)	20.0	31.0	0.28
Obesity (%)	33.3	23.1	0.40
Median No of TRFs (IQR)	1 (0-2)	1 (1-2)	0.28
CD4 (cells/ul)	381.9±235.6	322.9±163.7 (n=38)	0.22
Median VL (copies/ml) (IQR)	0 (0-38) (n=34)	45015 (19200- 192800) (n=30)	<0.001
cIMT (mm)	0.70±0.20 (n=26)	0.72±0.14 (n=31)	0.74
PWV (m/s)	7.83±2.73 (n=24)	8.26±2.61 (n=22)	0.59
NIHSS	7.8±2.7	8.3±5.9	0.48

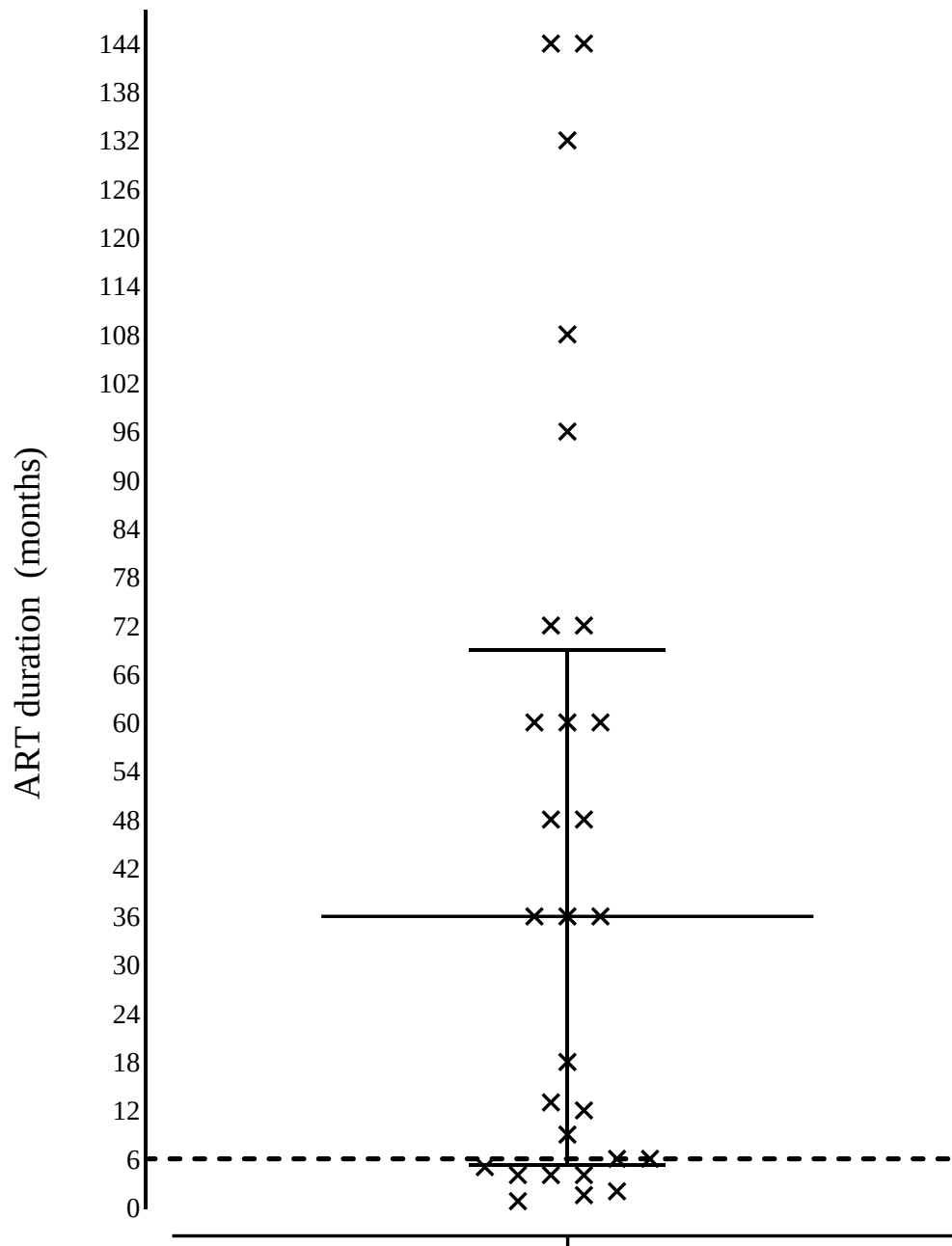
ART, antiretroviral therapy; **cIMT**, carotid intima media thickness; **IQR**, interquartile range; **NIHSS**, National Institute of Health Stroke Scale; **PWV**, pulse wave velocity; **TRFs**, traditional risk factors; **VL**, viral load

Table S2: Comparing selected blood markers in PLWH on ART for ≤6 months to those on ART for >6 months, and to the ART-naïve PLWH

	ART-naïve (n=42)	≤6 months ART ("Early") (n=9)	>6 months ART ("Late") (n=19)	p- valu e
White cell count (x 10⁹/L)	5.8 (4.6-7.8)	5.6 (5.4-8.3)	6.7 (5.6-10.4)	0.10
Haemoglob in (g/dL)	14.6 (12.9-15.7)	12.8 (12.3-14.3)	13.2 (12.1-15.9)	0.37
Creatinine (umol/L)	80.0 (68.0-94.0)	68.5 (35.5-75.0)	77.5 (70.0- 101.0)	0.13
Albumin (g/L)	38.0 (35.0-41.0)	38.0 (35.0-41.0)	39.0 (37.0-42.0) (n=17)	0.94
Uric Acid (mmol/L)	0.35 (0.30-0.41) (n=29)	0.29 (0.21-0.36)	0.35 (0.24-0.44)	0.84
ESR (mm/hr)	35.0 (20.0-70.0) (n=33)	79.0 (23.0-91.0) (n=7)	19.5 (11.5-78.5) (n=16)	0.29
CRP (mg/L)	12.0 (9.0-26.0)	17.0 (9.0-27.0)	16.0 (9.0-37.0)	0.80
ACE (ug/L)	21.0 (8.0-43.0) (n=30)	30.0 (22.0–59.0)	30.0 (9.0-62.0)	0.28

ACE, angiotensin converting enzyme; **CRP**, C-reactive protein; **ESR**, erythrocyte sedimentation rate

Figure S1: Scatterplot indicating the spread of strokes according to ART duration (n=28)



Legend

Long horizontal line indicates median duration (36 months)

Short horizontal lines indicate interquartile range (5.5 – 66 months)

Dotted line is drawn at 6 months post ART-initiation (9 strokes in this time period)

Appendix D

Supplementary Methods: Table 1 Calculations

Table 1: Comparing crude incidence rates for stroke in the periods ≤ 6 months and >6 months post ART-initiation

	Cases	Total person-time at risk *(months)	Cases per patient year	Risk ratio
1 st 6 months	9	$33.25 + (19 \times 6) = 147.25$	$9/147.25 \times 12 = 0.73$	$0.73/0.21 = 3.47$
After 6 months	19	$1204 - (19 \times 6) = 1090$	$19/1090 \times 12 = 0.21$	

*Total Person-time at risk was calculated as follows:

A. First 6 months

1. Sum of the total number of months from ART initiation to stroke (33.25)
2. Plus the 19 patients from the group who stroked after 6 months who have also gone through that first 6 month time period (without having a stroke)

B. After 6 months

1. Sum of the total number of months from ART initiation to stroke (1204)
2. Less the 19 patients who were counted under (A) above

Comparison of two proportions using z-test

Difference between 2 proportions

$$0.73 - 0.21 = 0.52$$

Overall proportion

$$\frac{9 + 19}{147.25/12 + 1090/12}$$

$$= 28/12.27 + 90.83$$

$$= 0.27$$

$$\mathbf{SEM} = \sqrt{0.27(1-0.27)(1/12.27 + 1/90.83)}$$

$$=\sqrt{0.27 \times 0.73 \times 0.092}$$

$$=0.135$$

$$\mathbf{Z \text{ test} = difference/SEM}$$

$$0.52/0.135 = 3.85$$

Look up in table: $1 - 0.9998 = 0.0002$ therefore $p\text{-value} < 0.0005$

Paper 5

Cardioembolic Stroke in an HIV endemic region: underdiagnosed and severe.

Sadiq E, Woodiwiss A, Norton G, Modi G.

BMJ Neurology Open. 2024;6(2):e000592.

Cardioembolic stroke in an HIV endemic region: underdiagnosed and severe

Eitzaz Sadiq ¹, Angela Woodiwiss,² Gavin Norton,² Girish Modi¹

To cite: Sadiq E, Woodiwiss A, Norton G, *et al.* Cardioembolic stroke in an HIV endemic region: underdiagnosed and severe. *BMJ Neurology Open* 2024;**6**:e000592. doi:10.1136/bmjno-2023-000592

► Additional supplemental material is published online only. To view, please visit the journal online (<https://doi.org/10.1136/bmjno-2023-000592>).

Received 21 November 2023
Accepted 11 July 2024



© Author(s) (or their employer(s)) 2024. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

¹Division of Neurology, Department of Neurosciences, University of the Witwatersrand Johannesburg Faculty of Health Sciences, Johannesburg, South Africa

²Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, University of the Witwatersrand Johannesburg Faculty of Health Sciences, Johannesburg, South Africa

Correspondence to

Dr Eitzaz Sadiq;
eitzaz.sadiq@wits.ac.za

ABSTRACT

Background and objectives Cardioembolic stroke (CES) appears to be a rare cause of stroke (4%–9%) in people living with HIV (PLWH) in sub-Saharan Africa (SSA). However, due to limited access to diagnostic resources, this may be an underestimate. It is also unclear which cardiac pathologies are the major contributors to CES in this region. We sought to determine the prevalence and aetiology of CES in PLWH and to determine whether there are any differences compared with HIV negative stroke patients.

Methods This cross-sectional study recruited PLWH with new-onset stroke at a quaternary-level hospital in Johannesburg, South Africa, from 2014 to 2017, and compared them to age-matched and sex-matched HIV negative stroke patients. Comprehensive investigations were performed to determine the underlying stroke aetiology, including electrocardiography, echocardiography, CT angiography and cerebrospinal fluid examination.

Results 85 PLWH with ischaemic stroke were recruited and compared with 109 HIV negative controls. CES was identified in 17/85 (20.0%) of PLWH. These patients had more severe strokes than PLWH with non-CES (National Institutes of Health Stroke Scale score 14.9 ± 6.7 vs 11.7 ± 5.4 , $p=0.04$). Cardiomyopathy was the predominant cardiac pathology in PLWH (76.4% vs 45.5% in HIV negative, $p=0.04$) while valvulopathy was more common in HIV negative patients (42.4% vs 11.8% in PLWH, $p=0.03$). Arrhythmia ($n=1$) and ischaemic heart disease ($n=1$) were uncommon in PLWH.

Conclusion CES is underdiagnosed in SSA and is more severe than non-CES. The identification of cardiomyopathy as the predominant underlying cardiac pathology may assist to target resources towards its detection using accessible cost-effective biomarkers.

INTRODUCTION

Cardioembolic stroke (CES) has been reported to be a relatively rare cause of stroke in people living with HIV (PLWH) in sub-Saharan Africa (SSA), causing only 4%–9% of all strokes.^{1 2} However, due to severely limited resources, a comprehensive cardiovascular assessment including echocardiography is seldom possible in stroke patients in this region.¹ It is thus likely that the true prevalence of CES is higher than previously reported.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Cardioembolic stroke (CES) appears to be a rare cause of stroke in people living with HIV (PLWH) in sub-Saharan Africa, although the lack of access to diagnostic resources may be partially responsible for an underestimated prevalence.

WHAT THIS STUDY ADDS

⇒ 20% of strokes in PLWH in this study were due to CES. These strokes were more severe than those with non-CES. HIV-related cardiomyopathy was the most common aetiology.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Resources need to be channelled towards cardiac diagnostics in PLWH with stroke, with a particular emphasis on detecting cardiomyopathy.

HIV is associated with cardiac diseases that may predispose to CES, including cardiomyopathy (CM), ischaemic heart disease, valvular heart disease and arrhythmias.^{3 4} While the incidence of CM has decreased with antiretroviral therapy (ART),³ it remains a major cause of morbidity and mortality in regions with poor ART coverage and has been shown to be responsible for up to 38% of cardiac disease in PLWH in SSA.^{3 5} The risk of ischaemic heart disease is almost doubled by HIV infection in studies from high-income countries (HICs).⁴ However, it appears to be less common in low-middle-income countries (LMICs), with a prevalence of only 2.4% in a large South African study.⁵ Valvular heart disease in PLWH in HICs is commonly secondary to intravenous recreational drug use-related infective endocarditis.⁶ However, intravenous drug usage is less common in LMICs, with rheumatic heart disease being the predominant cause of valvulopathy in these regions.⁷ Finally, CES in PLWH may also be related to cardiac arrhythmia, with an increased prevalence of atrial fibrillation (AF) noted in PLWH in HICs.⁸

Problem statement

Due to a paucity of data from LMICs, it is unclear which cardiac pathologies are largely responsible for CES in PLWH in SSA. Extrapolation of data from HICs may not be appropriate due to the vast differences in ART coverage, patient profiles and cardiac pathologies as described above. In this study, we sought to determine the prevalence and aetiology of CES in PLWH in a LMIC at the epicentre of the world's HIV epidemic and to determine whether there are any differences compared with HIV negative stroke patients.

METHODS

This cross-sectional study took place at the Charlotte Maxeke Johannesburg Academic Hospital, a 1000-bed quaternary-level hospital in Johannesburg, South Africa, from August 2014 to November 2017 (40 months).

We recruited consecutive patients aged 18 years and older who presented within 48 hours of a new-onset stroke. Stroke was defined as per the WHO case definition.⁹ Exclusion criteria were the presence of meningitis on cerebrospinal fluid (CSF) examination (elevated protein, a low serum:CSF glucose ratio or pleocytosis), the presence of intracranial mass lesions on radiographic imaging or other stroke mimics. Full informed consent was obtained as per the Declaration of Helsinki.¹⁰

All patients were assessed and examined by the same specialist neurologist (ES). Stroke aetiology was classified according to the Trial of Org 10172 in Acute Stroke (TOAST) Classification.¹¹ Stroke severity was graded according to the National Institutes of Health Stroke Scale (NIHSS).¹²

The cardiac evaluation included a full cardiovascular clinical examination, a 12-lead ECG and transthoracic echocardiography. 24-hour Holter ECG and transoesophageal echocardiography were performed where clinically indicated, particularly in patients with large vessel stroke of undetermined aetiology after standard investigations. These were performed and interpreted by the specialist cardiologist on call, who then assigned the cardiac aetiological category most likely to be responsible for the stroke. The classification of the aetiology of CM was based on the 2013 American College of Cardiology Foundation/American Heart Association (ACCF/AHA) Guideline for the Management of Heart Failure.¹³ Cardiac wall motion abnormalities due to ischaemic heart disease were categorised separately from CM. Patients with definite cardiac pathology but not meeting the TOAST criteria for CES were categorised as 'Stroke of undetermined aetiology'.

The full protocol of laboratory investigations for underlying stroke aetiology is available in the online supplemental file. HIV testing (ELISA) was performed in all consenting patients. Those found to be HIV positive subsequently had their CD4 count and HIV viral load (VL) measured. A suppressed VL was defined as a VL of less than 50 copies/mL. A lumbar puncture was performed in all PLWH, and those HIV negative were

clinically indicated, provided no contraindication was present. CSF was examined as per the protocol in the online supplemental file.

CT scan of the brain was performed in all patients. MRI of the brain and angiography (CT or MRI angiogram) was performed if clinically indicated, particularly if there was no clearly identified cause for the stroke from the preceding investigations, or a vasculitis or vasculopathy was suspected. Angiography included the aortic arch, neck and brain. Carotid stenosis was assessed by means of carotid doppler studies of the distal common carotid artery. All radiological investigations were examined by a specialist radiologist and the same specialist neurologist (ES).

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Statistical methods

Data were analysed by using Statistica V.14.0.0.15, TIBCO Software. Continuous variables were evaluated for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. χ^2 , Fisher's exact test, Student's t-test, Mann-Whitney, Pearson correlation coefficient and Spearman correlation coefficient statistical tests were then performed on parametric and non-parametric variables where appropriate. Continuous data are reported as mean $\pm$ SD unless otherwise specified. Missing data were not imputed. P values <0.05 were considered significant.

RESULTS

A total of 127 consecutive PLWH with stroke were screened for participation in the study. 40 were excluded due to the presence of abnormal basic CSF findings. As only two PLWH suffered from intracerebral haemorrhage, further analyses were only conducted in ischaemic stroke patients (n=85). These were compared with 109 age-matched and sex-matched HIV negative ischaemic stroke patients, recruited during the same time period.

Aetiology of stroke

When analysed according to HIV serostatus, the relative percentages of cardioembolic, atherosclerotic, small vessel and strokes of undetermined aetiology according to the TOAST criteria were similar in the two groups (table 1). However, the aetiology was classified as 'other' in a higher proportion of PLWH (p<0.001) (table 1). 40/45 (47%) of the PLWH were on ART at the time of stroke.

CES in PLWH

20% of strokes in PLWH (n=17) were due to a cardioembolic cause. (table 1). 76.4% of these (n=13) were secondary to CM (figure 1). The aetiologies of the CM are shown in figure 2 and were mostly secondary to HIV-related dilated CM. Valvular abnormalities were detected in 2 PLWH (both with rheumatic heart disease) while

Table 1 Comparing demographics and stroke aetiology (TOAST criteria) between PLWH and HIV negative patients

	PLWH (n=85)	HIV negative (n=109)	P value
Age (years)	43.4±12.5	45.0±10.5	0.33
Male (%)	49.4	47.7	0.81
TOAST aetiology			
Cardioembolic	17 (20.0%)	33 (30.3%)	0.10
Atherosclerotic	4 (4.7%)	3 (2.8%)	0.47
SVD	14 (16.5%)	20 (18.4%)	0.73
Undetermined	22 (25.9%)	40 (36.7%)	0.10
Other	28* (32.9%)	13† (11.9%)	<0.001

*including vasculopathy (definite and probable, n=26) and stroke due to recreational drug use (n=2).

†including vasculitis (n=6), hypercoagulable state (n=3), neurosyphilis (n=2), stroke due to recreational drug use (n=1) and hyperhomocystinaemia (n=1).

PLWH, people living with HIV; SVD, small vessel disease; TOAST, Trial of Org 10172 in Acute Stroke.

arrhythmia was identified in only 1 PLWH (a patient in their 70s known with a long-standing history of AF). Ischaemic heart disease was the likely source of CES in one PLWH, an ART-naïve patient in their late 50s with multiple traditional cardiovascular risk factors (TRFs). All CES patients were diagnosed as such by transthoracic echocardiography. Transoesophageal echocardiography or 24-hour Holter ECG, where performed, did not diagnose additional cardiac pathology which altered the patient's aetiological classification. None of those with

CM had any other significant concomitant cardiac disease on echocardiography.

PLWH with CES (n=17) were compared with PLWH with non-CES (n=68), which revealed a higher stroke severity in those with CES (table 2). Demographics including mean age and proportion of males were similar, as were the prevalences of all TRFs (table 2). HIV-related factors (CD4 count, VL and ART exposure) were also similar between the two groups (table 2).

Univariate and multivariate regression analyses confirmed that the NIHSS score was only influenced by whether the aetiology was CES or non-CES, and not by differences in age, sex or HT status (table 3).

CES in HIV negative patients

In HIV negative patients, 33/109 (30.3%) had CES (table 1). CM (45.5%) and valvulopathy (42.4%) were the most common aetiologies of CES in these patients (figure 1). When compared with PLWH, the relative prevalences of CM and valvulopathy differed significantly (p=0.04 and 0.03 respectively, figure 1). The aetiology of CM in HIV negative patients is shown in figure 2 and contrasted to PLWH.

There was no difference in NIHSS scores between CES and non-CES in HIV negative patients (table 2). The CES patients had a lower mean age and less TRFs (median number of TRFs and obesity) than non-CES (table 2).

Comparison of PLWH and HIV negative patients with CES revealed no difference in demographics, TRFs or stroke severity (table 2).

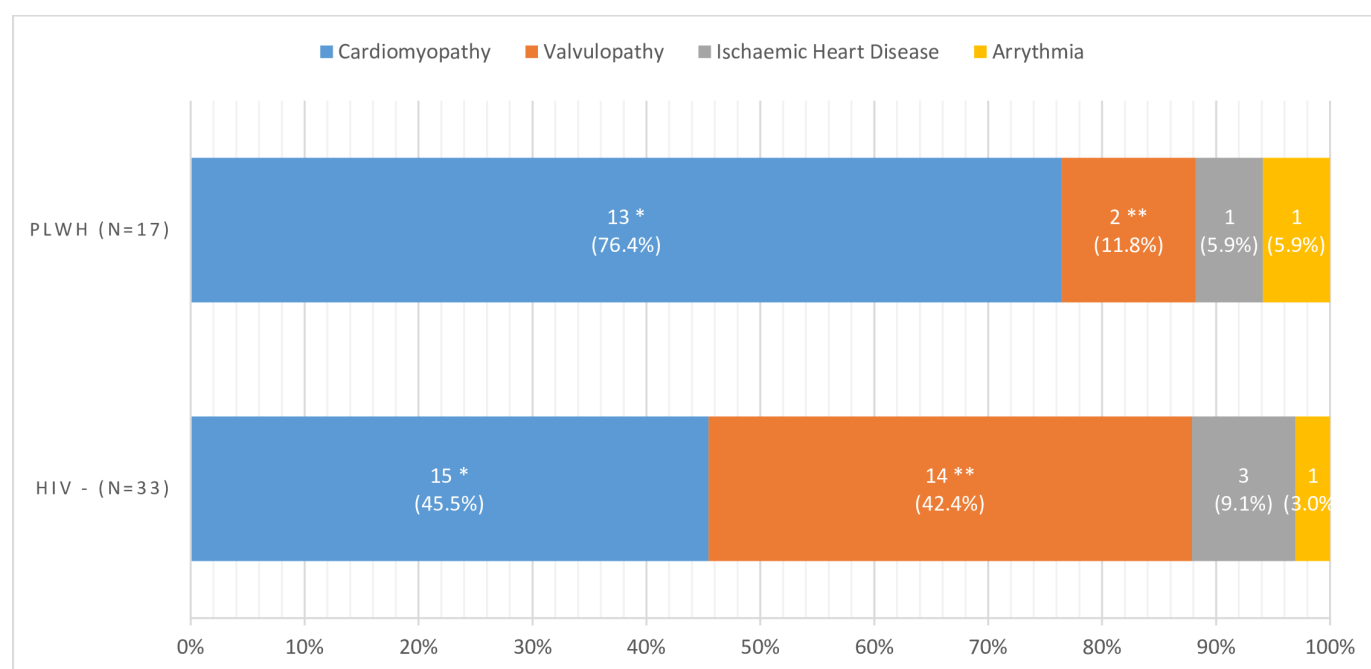


Figure 1 Aetiology of CES in PLWH compared with HIV negative patients. Statistically significant differences are denoted by * or **, where *p=0.04 (CM in PLWH vs HIV negative) and **p=0.03 (valvulopathy in PLWH vs HIV negative). CES, cardioembolic stroke; CM, cardiomyopathy; PLWH, people living with HIV.

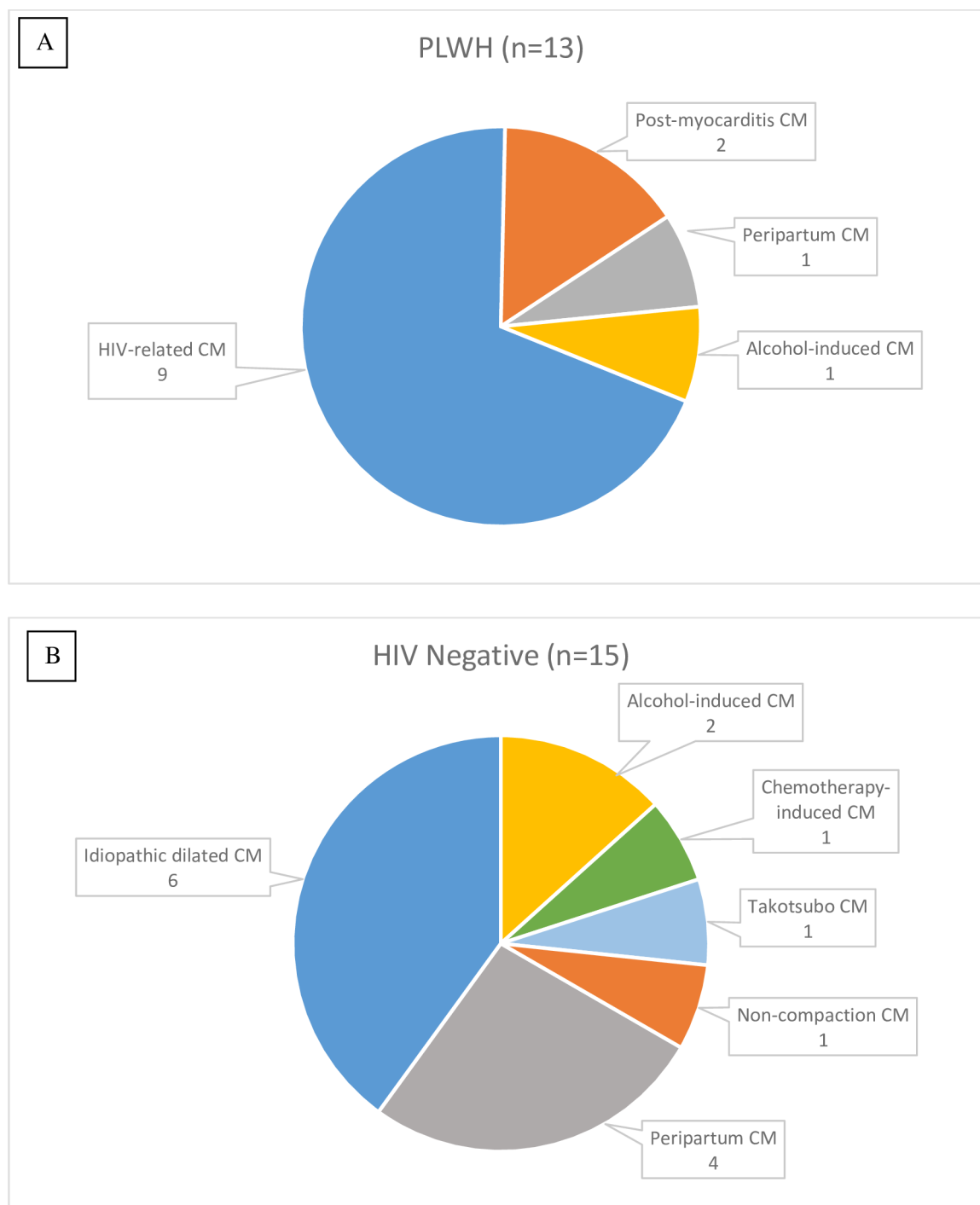


Figure 2 Aetiology of cardiomyopathy (CM) in PLWH (A) and HIV negative (B) patients. PLWH, people living with HIV.

Stroke of undetermined aetiology

Incomplete investigations were the most common reason for a stroke being classified as being of undetermined aetiology (47/62, 75.8%). There was no difference in the proportion of patients who had incomplete investigations in PLWH compared with HIV negative patients (16/22, 72.7% vs 31/40, 77.5%, $p=0.55$). Trans-thoracic echocardiography was not possible in only two patients while transoesophageal echocardiography (n=15) and 24-hour Holter ECG (n=8) were not possible in a larger number of patients. Six patients demised

prior to their full workup being completed. 19/22 PLWH with strokes of undetermined aetiology had large vessel strokes, of which 6 had prominent cardiac pathology but was insufficient to categorise them as CES as per the TOAST criteria.

Stroke severity

The mean NIHSS score did not differ between HIV negative and PLWH stroke patients overall (12.0 ± 5.9 vs 12.4 ± 5.8 , $p=0.68$). Besides the higher stroke severity in PLWH with CES as described above, there were no

Table 2 Comparing CES to non-CES in PLWH and HIV negative patients and comparing CES in PLWH to HIV negative patients

	PLWH (n=85)			HIV negative (n=109)			P value (CES in PLWH vs CES in HIV negative)
	CES (n=17)	Non-CES (n=68)	P value	CES (n=33)	Non-CES (n=76)	P value	
Age (years)	43.6±14.3	43.3±12.1	0.93	41.8±10.4	46.3±10.3	0.04	0.63
Male (%)	64.7	45.6	0.16	48.5	47.4	0.91	0.27
HT (%)	41.2	44.2	0.83	48.5	65.8	0.09	0.62
DM (%)	5.9	5.9	1.00	12.1	27.6	0.08	0.49
Dyslipidaemia (%)	23.5	25.0	0.90	24.2	32.9	0.37	0.96
Smoking (%)	23.5	26.5	0.80	15.2	19.7	0.57	0.47
Obesity (%)	23.1 (3/13)	26.5 (13/49)	0.80	19.2 (5/26)	41.9 (23/55)	0.046	0.78
Median no of TRFs (IQR)	1 (1–2)	1 (0–2)	0.77	1 (0–2)	2 (1–3)	0.01	1.00
NIHSS	14.9±6.7	11.7±5.4	0.04	11.9±6.7	12.1±5.6	0.87	0.15
CD4 (cells/μL)	353.2±157.6	318.4±220.1 (n=64)	0.54				
Median VL (IQR) (copies/ml)	1600 (0–131 540)	2200 (0–67 264)	0.77				
ART-exposed (%)	47.1	47.1	1.00				
Suppressed VL (%)	85.7	65.6	0.30				

.ART, antiretroviral therapy; CES, cardioembolic stroke; DM, diabetes mellitus; HT, hypertension; NIHSS, National Institute of Health Stroke Scale; PLWH, people living with HIV; VL, viral load.

other differences in stroke severity between the other aetiological categories in PLWH (data are available on request). In PLWH, there was no correlation between CD4 count and NIHSS ($r=-0.141$, $p=0.90$, $n=81$) or VL and NIHSS ($r_s=-0.098$, $p=0.41$, $n=72$). There was also no difference in NIHSS between those exposed to ART or not, or those with a suppressed VL or not (data are available on request).

DISCUSSION

Our cohort of 85 PLWH with ischaemic stroke is one of the largest from SSA and one of the most extensively

investigated.^{1 2} We showed that 20% of strokes in PLWH were of cardioembolic origin, of which 76.4% were secondary to CM. PLWH with CES suffered from more severe strokes than PLWH with non-CES.

Our prevalence of CES is higher than other PLWH cohorts in SSA and other LMICs (4%–9%).^{1 2} However, less than 60% of the participants in these studies had an ECG or echocardiography, compared with the detailed cardiac assessments performed in our cohort. Our higher prevalence of CES thus likely illustrates the desperate need for improved access to resources in order to adequately diagnose CES.

Table 3 Risk factors (RF) associated with NIHSS in PLWH on univariate and multivariate regression analyses

Univariate regression (n=85)			Multivariate regression* (n=85)	
RF	β-coefficient±SEM	P value	β-coefficient±SEM	P value
Age	−0.0263±0.0512	0.61	0.008±0.054	0.88
Sex	0.7686±1.2714	0.55	1.131±1.276	0.35
Smoking	−1.810±1.441	0.21		
HT	−2.040±1.265	0.11	−1.949±1.323	0.14
DM	0.488±2.707	0.86		
Dyslipidaemia	−1.844±1.463	0.21		
Obesity	−0.226±1.682	0.89		
CD4 (n=81)	−0.001±0.003	0.90		
VL (n=72)	−0.001±0.001	0.83		
ART exposure	1.103±1.271	0.39		
VL suppression (n=39)	0.592±1.951	0.76		
Non-CES vs CES	−3.221±1.544	0.04	−3.390±1.560	0.03

*Age, sex and only those RF where $p\leq 0.20$ on univariate analyses were included in the multivariate analysis. Significant RFs are shown in bold type. ART, antiretroviral therapy; CES, cardioembolic stroke; DM, diabetes mellitus; HT, hypertension; PLWH, people living with HIV; RF, risk factor; VL, viral load.

Previous SSA data on CES is very limited but does not appear to show any significant difference in the type of cardiac abnormalities when comparing PLWH to HIV negative CES patients.^{2 14–16} We found CM to be the most common pathology in PLWH while valvulopathy was more common in HIV negative patients. A study from a similar population to ours reported CM in six out of nine PLWH with CES but did not compare these to HIV negative patients.¹⁴ If it is indeed true that the source of CES in our PLWH population is more likely to be CM, this has the potential to guide a targeted search for the most likely sources of CES in a particular population. Our very low prevalence of arrhythmia (1 out of 17 PLWH with CES) adds further value to this model. We did not detect any further patients to have arrhythmia despite the use of 24-hour Holter ECG in most patients. This suggests that such resources may be better allocated towards detecting cardiac abnormalities such as CM, as opposed to an intensive search for arrhythmia in this stroke population. In regions such as SSA, the rational allocation of scarce diagnostic resources is of great importance.

A recent consensus paper presented an insightful diagnostic algorithm and defined the minimum set of recommended investigations for determining the aetiology of a stroke in PLWH.¹⁷ This included an ECG and transthoracic echocardiography as a minimum. Unfortunately, echocardiography in particular is an extremely scarce resource in SSA. It requires not only equipment, but more importantly, the technical expertise to perform and interpret the examination. Due to massive economic and healthcare system challenges, it is very unlikely that widespread access to echocardiography is a realistic solution to diagnosing CES in populations such as ours, with a high HIV burden. The use of alternative cost-effective biomarkers for CES which are more readily accessible and requiring less technical expertise may be the more practical solution.^{18 19} In order to guide the development and testing of these biomarkers, it is important to know which particular cardiac diseases are most prevalent in a particular stroke population, which appears to be CM in our PLWH population.

The clinical implication of a missed cardioembolic source is profound, as these patients are at very high risk of recurrent strokes if not treated with the appropriate anticoagulation. Data from AF studies have shown a greater than 70% reduction in recurrent CES using this strategy.²⁰ In the case of CM (or other causes of heart failure with reduced ejection fraction), there is also good evidence for stroke risk reduction with anticoagulation when used in the correct circumstances, particularly in those patients with a previous thromboembolic event of no alternative aetiology.²¹ This applies to a large proportion of the CES patients we have described. The missed opportunity to anticoagulate such patients may lead to recurrent strokes, with their associated additive morbidity and mortality.

Our findings of CES being more severe than non-CES in PLWH are alarming. CES stroke unrelated to HIV

infection has been shown to cause more severe stroke in large HIC studies.²² However, the majority of these strokes are in older patients with AF and multiple other TRFs.²³ This is a vastly different patient profile to our description, emphasising the novelty of our findings. To our knowledge, there have been no previous data examining the differences in stroke severity between different stroke aetiologies in PLWH in our region. We did not detect any difference in stroke severity in HIV negative CES patients when compared with non-CES, nor was there any significant difference in stroke severity across the other aetiological categories. It remains unclear what predisposes PLWH with CES to having more severe strokes and warrants further investigation. We did not detect a difference in stroke severity between PLWH and HIV negative stroke patients in keeping with most previous SSA studies reporting on stroke severity.^{1 24–26}

Limitations

The TOAST classification has limitations for classifying ischaemic stroke in a young PLWH population. Previous studies have shown a large proportion of patients falling into the ‘undetermined’ or ‘other’ categories due to alternative stroke aetiologies.¹⁷ Despite this, we chose to categorise according to this classification, to allow comparison with previous data. The consensus paper on aetiological classification of stroke in PLWH also agreed with criteria very similar to those in TOAST to diagnose CES in PLWH.¹⁷ We acknowledge that our exclusion of patients with meningitis may have resulted in a greater relative proportion of CES. We re-examined the published data from the five SSA studies which described the relative percentages of CES and stroke associated with infectious aetiology.^{1 14 15 24 27 28} After exclusion of the latter, the percentage of CES still ranged from 4% to 12%. The only exception was Tipping *et al*, where the relative contribution of CES rose to 21% once infections were excluded.¹⁴

While our overall prevalence of stroke of undetermined aetiology was within the international norm of approximately one-third of all strokes,²⁹ a large proportion of these were due to incomplete investigations. We describe a very low prevalence of arrhythmia; however, we could not perform a 24-hour Holter ECG in eight patients with stroke of undetermined aetiology, nor did we have access to any prolonged cardiac rhythm monitoring beyond 24 hours. Thus, it is possible that paroxysmal arrhythmia may have gone undetected in some of these patients. Similarly, transoesophageal echocardiography was not possible in 15 patients. While this missing data may have altered our reported aetiologies of CES, they also serve to illustrate that our reported prevalence of CES is also likely to be an underestimate.

CONCLUSION

With a detailed cardiac assessment, we found CES to be the likely stroke mechanism in 20% of strokes in PLWH, which is almost double that which has been previously

reported in SSA. The most common underlying cardiac pathology differed significantly between PLWH (CM) and HIV negative (valvulopathy). Alarming, we have shown PLWH with CES to have more severe strokes than those with non-cardioembolic aetiology. This, together with the intrinsically higher stroke recurrence risk in untreated CES, points to an urgent need to address the probable underdiagnosis of CES as a cause of stroke in PLWH in SSA.

Contributors ES: conception and design, data acquisition, data analysis, data interpretation, writing. AW: conception and design, data analysis, data interpretation, review and editing. GN: conception and design, data interpretation, review and editing. GM: conception and design, data interpretation, review and editing. ES is responsible for the overall content as guarantor.

Funding ES was supported by the Discovery Foundation Academic Fellowship Award (Reference number 035747).

Disclaimer The funder played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Competing interests No, there are no competing interests.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and ethics approval was granted by the University of the Witwatersrand Human Research Ethics Committee (certificate number M140429, renewed as M190688). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The data that support the findings of this study are available from the corresponding author on reasonable request.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

Eitzaz Sadiq <http://orcid.org/0000-0003-1801-3638>

REFERENCES

- 1 Ransley G, Zimba S, Gadama Y, et al. Trends and clinical characteristics of HIV and cerebrovascular disease in low- and middle-income countries (Imics) between 1990 and 2021. *Curr HIV/AIDS Rep* 2022;19:548–65.
- 2 Abdallah A, Chang JL, O'Carroll CB, et al. Stroke in human immunodeficiency virus-infected individuals in Sub-Saharan Africa (SSA): a systematic review. *J Stroke Cerebrovasc Dis* 2018;27:1828–36.
- 3 Manga P, McCutcheon K, Tsabedze N, et al. HIV and nonischemic heart disease. *J Am Coll Cardiol* 2017;69:83–91.
- 4 Vachiat A, McCutcheon K, Tsabedze N, et al. HIV and ischemic heart disease. *J Am Coll Cardiol* 2017;69:73–82.
- 5 Sliwa K, Carrington MJ, Becker A, et al. Contribution of the human immunodeficiency virus/acquired immunodeficiency syndrome epidemic to de novo presentations of heart disease in the heart of Soweto study cohort. *Eur Heart J* 2012;33:866–74.
- 6 Gebo KA, Burkey MD, Lucas GM, et al. Incidence of, risk factors for, clinical presentation, and 1-year outcomes of infective endocarditis in an urban HIV cohort. *J Acquir Immune Defic Syndr* 2006;43:426–32.
- 7 Nel SH, Naidoo DP. An echocardiographic study of infective endocarditis, with special reference to patients with HIV. *Cardiovasc J Afr* 2014;25:50–7.
- 8 Hsu JC, Li Y, Marcus GM, et al. Atrial fibrillation and atrial flutter in human immunodeficiency virus-infected persons: incidence, risk factors, and association with markers of HIV disease severity. *J Am Coll Cardiol* 2013;61:2288–95.
- 9 Hatano S. Experience from a multicentre stroke register: a preliminary report. *Bull World Health Organ* 1976;54:541–53.
- 10 World Medical Association. World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;310:2191–4.
- 11 Adams HP, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. definitions for use in a multicenter clinical trial. *TOAST Trial of Org 10172 in Acute Stroke Treatment Stroke* 1993;24:35–41.
- 12 Lyden P, Brott T, Tilley B, et al. Improved reliability of the NIH stroke scale using video training. ninds tpa stroke study group. *Stroke* 1994;25:2220–6.
- 13 WRITING COMMITTEE MEMBERS, Yancy CW, Jessup M, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines. *Circulation* 2013;128:e240–327.
- 14 Tipping B, de Villiers L, Wainwright H, et al. Stroke in patients with human immunodeficiency virus infection. *J Neurol Neurosurg Psychiatry* 2007;78:1320–4.
- 15 Mochan A, Modi M, Modi G. Stroke in black south african HIV-positive patients: a prospective analysis. *Stroke* 2003;34:10–5.
- 16 Longo-Mbenza B, Longokolo Mashi M, Lelo Tshikwela M, 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.
- 17 Benjamin LA, Bryer A, Lucas S, et al. Arterial ischemic stroke in HIV: defining and classifying etiology for research studies. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e254.
- 18 Glezeva N, Gallagher J, Ledwidge M, et al. Heart failure in sub-Saharan africa: review of the aetiology of heart failure and the role of point-of-care biomarker diagnostics. *Tropical Med Int Health* 2015;20:581–8.
- 19 Gallagher J, McDonald K, Ledwidge M, et al. Heart failure in Sub-Saharan Africa. *Card Fail Rev* 2018;4:21–4.
- 20 Aguilar MI, Hart R, Pearce LA. Oral anticoagulants versus antiplatelet therapy for preventing stroke in patients with non-valvular atrial fibrillation and no history of stroke or transient ischemic attacks. *Cochrane Database Syst Rev* 2007;CD006186.
- 21 Schäfer A, Flierl U, Bauersachs J. Anticoagulants for stroke prevention in heart failure with reduced ejection fraction. *Clin Res Cardiol* 2022;111:1–13.
- 22 Kamel H, Healey JS. Cardioembolic stroke. *Circ Res* 2017;120:514–26.
- 23 Lin H-J, Wolf PA, Kelly-Hayes M, et al. Stroke severity in atrial fibrillation. *Stroke* 1996;27:1760–4.
- 24 Zimba S, Ntanda PM, Lakhi S, et al. HIV infection, hypercoagulability and ischaemic stroke in adults at the university teaching hospital in Zambia: a case control study. *BMC Infect Dis* 2017;17:354.
- 25 Benjamin LA, Allain TJ, Mzinganjira H, et al. The role of human immunodeficiency virus-associated vasculopathy in the etiology of stroke. *J Infect Dis* 2017;216:545–53.
- 26 Heikinheimo T, Chimbayo D, Kumwenda JJ, et al. Stroke outcomes in Malawi, a country with high prevalence of HIV: a prospective follow-up study. *PLoS One* 2012;7:e33765.
- 27 Hoffmann M. Stroke in the young in South Africa—an analysis of 320 patients. *S Afr Med J* 2000;90:1226–37.
- 28 Benjamin LA, Corbett EL, Connor MD, et al. HIV, antiretroviral treatment, hypertension, and stroke in Malawian adults. *Neurology (ECRicon)* 2016;86:324–33.
- 29 Li L, Yiin GS, Geraghty OC, et al. Incidence, outcome, risk factors, and long-term prognosis of cryptogenic transient ischaemic attack and ischaemic stroke: a population-based study. *Lancet Neurol* 2015;14:903–13.

SUPPLEMENTARY METHODS

Laboratory Investigations

1. Blood

- Full blood count, urea, creatinine
- Fasting glucose, HbA1c, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides.
- Vasculitis screen: erythrocyte sedimentation rate, C-reactive protein, syphilis serology (RPR and TPHA), hepatitis studies (Hepatitis A, B, C), antinuclear antibodies, extractable nuclear antigens, anti-double stranded DNA antibodies, rheumatoid factor, anti-neutrophil cytoplasmic antibodies, serum angiotensin converting enzyme
- Coagulation screen: PT, INR, D-dimer, Protein C, Protein S, anti-thrombin 3, fibrinogen, antiphospholipid antibodies
- HIV ELISA. If HIV ELISA positive, CD4 and VL were measured.

2. Cerebrospinal fluid (CSF) analysis

- Protein, glucose (with simultaneous plasma glucose), microscopy, cryptococcal latex antigen, India Ink test, Gene Xpert and culture for TB, bacteria and fungi.
- CSF polymerase chain reaction (PCR) testing for varicella zoster virus (VZV), herpes simplex virus, cytomegalovirus and Epstein Barr virus
- Syphilis tests (VDRL and/or FTA-Abs) were performed on CSF if blood syphilis serology (TPHA) was positive

