

**A longitudinal investigation of the cardiovascular health
of a black South African cohort infected with the human
immunodeficiency virus**

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Thesis accepted in fulfilment of the requirements for the degree
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PREFACE

The article-format approved and recommended by North-West University has been chosen for this thesis. The layout of the thesis is as follows:

- **Chapter 1** includes background, literature review, problem statement, aim, objectives and hypotheses.
- **Chapter 2** comprises the procedure for the literature search for the narrative review study. It also consists of the study design and procedures, methodology and consistent with the protocol of the Prospective Urban and Rural Epidemiology (PURE) Study – the larger study for this PhD.
- **Chapter 3** consists of manuscript one titled: *A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls residing in Sub-Saharan Africa: a narrative review*. Published in the *Journal of Human Hypertension* (2020).
- **Chapter 4** is the second manuscript titled: *A 10-year follow-up study of demographic and cardiometabolic factors in HIV-infected South Africans*. Published in the *Cardiovascular Journal of Africa* (2019)
- **Chapter 5** includes the third manuscript titled: *Arterial structure and function in Africans infected with HIV for >5 years: longitudinal relationship with endothelial activation and cardiovascular risk markers*. This manuscript is under review in the journal of *HIV Medicine* (Reference number: HIV-OA-11-2020-5249).
- **Chapter 6** consists of the summary of the main findings, recommendations and concluding remarks.

The references for each manuscript are cited according to the author's guidelines for the particular journal in which the manuscript was either published or under review. For the rest of the thesis, the Vancouver referencing style was used. The reference list for each of the manuscripts and the rest of the thesis chapters are listed at the end of each chapter. References are listed in the order that they are cited in the text. A maximum of three authors are listed and in cases where there are more than three authors, the first three authors are recorded and then followed by et al.

AUTHOR CONTRIBUTIONS

Ms E Phalane

Ms Phalane conducted the initial literature search to propose the concept and design of each research article presented in this thesis. She was responsible for writing the PhD proposal and conducting an in-depth literature review. She completed the ethics application for this study as part of the larger Prospective Urban and Rural Epidemiology (PURE) study. She helped with the collection of data for the African-PREDICT and EndoAfrica study as a postgraduate student and retrieved files at the local clinics and hospital for the PURE study participants included in her study. As the first author of the research manuscripts, she performed all statistical analyses, interpreted the results of all analyses and prepared the thesis, including each manuscript for publication and conference proceedings.

Professor CMT Fourie

Professor Fourie, the main promoter for this study, provided supervision and guidance through all the stages of this study. She was responsible for the initial research planning and design as well as providing support for the statistical analyses, interpretation of results and critical evaluation of all three research manuscripts as well as finalising the thesis.

Professor AE Schutte and Professor CMC Mels

As my co-promoters, Professor Schutte and Professor Mels assisted with the planning and design of this study, provided an in-depth contribution towards the statistical analyses, interpretation of data and critical revising of all three manuscripts, as well as provided guidance for the entire thesis.

Professor IM Kruger

As a co-author for the third manuscript presented as Chapter 5, Professor Kruger assisted with the interpretation of statistical analyses, provided intellectual input and critically revised the article.

Below is the statement of all co-authors attesting to their contribution to the study and giving their approval for the three manuscripts to form part of this thesis.

Hereby, I declare that I authorised the above three manuscripts and that my contribution in this thesis as stated above, is an accurate representation of my actual role in the study. I also give my consent that the three manuscripts shall be published as part of the thesis of Edith Phalane.

Professor CMT Fourie

Professor AE Schutte

Professor CMC Mels

Professor IM Kruger

PUBLICATIONS, CONFERENCES AND OTHER ACTIVITIES RELATED TO THIS STUDY

Publications, conference preceding and other activities

Publications

- A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls residing in Sub-Saharan Africa: a narrative review. *Journal of Human Hypertension*. 2020; 34:692-7058. Please refer to an abstract in **Appendix C**.
- A 10-year follow-up study of demographic and cardiometabolic factors in HIV-infected South Africans. *Cardiovascular Journal of Africa*. 2019; 30: 1-10. Please refer to an abstract in **Appendix D**.

Conference Proceedings

- **Title:** Demographic and cardio-metabolic factors in HIV-infected South African cohort over 10 years at the: 12th International Symposium for Molecular Diagnostics, Graz, Austria, 31 May-02 June. **Poster presentation won best poster presentation.**
- **Title:** A 10-year follow-up study of demographic and cardiometabolic factors in HIV-infected South Africans was presented at the:
4th Southern African HIV Clinicians Society Conference held in Johannesburg, South Africa, 24-27 October. **Poster presentation** and
9th Southern African AIDS conference held in Durban, South Africa, 11-14 June 2019. **Oral presentation won the Clinical Excellence award.**
- **Title:** A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls residing in Sub-Saharan Africa: a narrative review, was presented at the 17th European AIDS conference, Basel, Switzerland, 6-9 November 2019. **Poster presentation.**

Other activities relating to this study

- Since the study aimed to contribute to a better understanding of CVD development, the researcher was actively involved in science communication and contributed to this regard by participating in the May Measurement Month (2016-2019) - a global initiative that raises awareness on the importance of

measuring blood pressure regularly. This included measuring the blood pressure of students/staff members of the North-West University and community members at Ikagang and rural areas Tzaneen, Limpopo Province. The contributions resulted in two publications which the researcher co-authored and titled as follows:

Angela J. Woodiwiss, Ruan Kruger, Gavin R. Norton, Aletta E. Schutte, Muhammed Vally, Daniel Da Silva Fernandes, Mohlabani Masiu Andrea Kolkenbeck-Ruh, Lebo F. Gafane-Matemane, Blessing O. Ahiante, **Edith Phalane**, Simone H. Crouch, Thomas Beaney, Xin Xia and Neil R. Poulter. May Measurement Month 2017: an analysis of blood pressure screening results in South Africa— Sub-Saharan Africa. *European Heart Journal Supplements* (2019) 21 (Supplement D), D104–D106.

Angela J. Woodiwiss, Ruan Kruger, Gavin R. Norton, Aletta E. Schutte, Caitlynd Myburgh, Benedicta Nkeh-Chungag, Constance R. Sewani-Rusike, Muhammed Vally, Erika Jones , Vernice Peterson, Justin Marsh, Lebo F. Gafane-Matemane, Blessing O. Ahiante, **Edith Phalane**, Thomas Beaney, Anca Chis Ster, Xin Xia and Neil R. Poulter. May Measurement Month 2018: an analysis of blood pressure screening results in South Africa. *European Heart Journal Supplements* (2020) 22 (Supplement H), H115–H118.

- The researcher also took part at the World AIDS Day and delivered health talks titled: *The cardiovascular risk in HIV-infected population (2018) and drug resistance and ART adherence (2019)* at the Potchefstroom hospital, North West Province. She also conducted a radio interview at Ikageng Community Radio station sharing the importance of a healthy lifestyle and screening for cardiovascular risk factors in both HIV-infected and the general population.
- As part of her continued contribution to science communication, the researcher was selected to participate in the Soapbox Science Cape Town (2019) – an initiative that brings together women scientist to educate the public on their research. She shared on a topic titled: “Keeping your heart healthy: five easy steps to follow” and this was featured on Quest Magazine. http://research.assaf.org.za/bitstream/handle/20.500.11911/133/Science_Outreach.pdf?sequence=61&isAllowed=y.
- The researcher was selected as a Session Chair at the 9th Southern African AIDS conference held in Durban, South Africa, 11-14 June 2019. Session title: Double trouble - Comorbidities & Coinfections.

- In 2018, the researcher was awarded the TATA Doctoral scholarship during the South African Women in Science Award in recognition of outstanding academic and research ability. The highlights of this study were featured on the Sowetan Newspaper and the website of the Department of Science and Technology.
- This study attracted funding from the National Research Foundation: Department of Science and Technology (NRF-DST) and TATA Africa Holdings.
- The researcher was selected among 268 young scientists globally to participate at the Global Young Scientist Summit (Biopolis, Singapore, 2020) in the field of science, technology, engineering and mathematics. Highlights of this study were featured in the book of abstract of the Global Young Scientist Summit.
- The researcher was selected among 600 young scientists to attend the Lindau Nobel Laureate Meetings in Germany, Lindau, 2018. Highlights of this study were featured in the book of abstract of the Lindau Nobel Laureate meeting. She was also selected to participate in a panel discussion alongside two Nobel Prize Winners, namely Peter Agre and Aaron Ciechanover to discuss “*Health Innovation in Africa: the way forward*” by the Global Perspective Initiative (GPI). The outcomes of this discussion were published in a booklet by the GPI and presented during the Grand Challenges Annual Meeting in Germany. <https://globalperspectives.org/en/publications/gp-policy-paper-health-innovation-in-africa/>
- The researcher was featured on the Women in Research blog featuring young women scientist participating at the 68th Lindau Nobel Laureates Meeting to increase the visibility of women in research. The detailed purpose of this is published in an article on the website of the Women in Research. <https://www.lindau-nobel.org/blog-women-in-research-at-lino18-edith-phalane-from-south-africa/>.
- The researcher was a winner for the Inspiring Fifty SA 2020 as a PhD candidate in the category of academics. This organisation showcases inspiring female role models in the field of science and technology.
- The researcher was featured on the Science Stars Magazine in celebration of women in science during Women’s month (August 2020). The highlights of this study are included in this online magazine. https://issuu.com/sciencestarsmagazine/docs/science_stars_wis_2020_-_for_issuu.

- The researcher was selected for the Black Women in Science fellowship (2019/2020) alongside other PhD candidates in South Africa in the field of science. Through the fellowship, the researcher completed training in scientific skills, sustainability project, business and entrepreneurship as well as science communication and branding.
- The researcher was profiled by the National Research Foundation in celebration of their 20th anniversary. The relevance of this study in South Africa as well as its contribution to the field of HIV and cardiovascular diseases. <https://www.nrf.ac.za/content/my-nrf-story-edith-phalane>.
- The researcher was selected as one of the North-West University's Early-career Women Scientist during the women's month in 2019. <http://www.nwu.ac.za/womens-month>.
- The researcher was selected by News24 as one of the 100 Young Mandelas of the future in the category of academics for outstanding performance and the relevance of this study was published on their website. <https://mandela100.news24.com/>.

SUMMARY

Title: A longitudinal investigation of the cardiovascular health of a black South African cohort infected with the human immunodeficiency virus

Motivation

The human immunodeficiency virus (HIV) continues to be a global health concern, with an estimation of 38 million people living with HIV. Out of these 38 million people living with HIV, 70% are residing in Sub-Saharan Africa (SSA) countries. Alongside the high burden of HIV infection, cardiovascular diseases (CVD) are also an important health concern in this region. The introduction of antiretroviral therapy (ART) has increased the life expectancy of HIV-infected individuals with consequential exposure to comorbidities such as hypertension, dyslipidaemia and diabetes mellitus as well as liver and renal disease – all known to be associated with an increased risk for the development of CVD. With 90% of South Africans living with HIV expected to be on ART by 2030, the prevalence of CVD risk factors is also expected to increase. Hence, it is vital to fully understand the impact of HIV, ART and conventional cardiovascular risk factors in the development of CVD in HIV-infected individuals. This understanding is currently limited by available evidence from longitudinal studies, especially in the SSA region. To better understand the long-term risk of developing CVD in people living with HIV, the cardiovascular health transition of HIV-infected Africans who are at risk of developing CVD was investigated for this study.

Aim

The central aim of this study was to investigate the longitudinal cardiovascular health of a black South African cohort infected with HIV and a literature review based on studies conducted in the SSA region.

Methods

The narrative review included a synthesis of studies done in SSA reporting on blood pressure (BP) and hypertension in HIV. PubMed and EBSCO Discovery Service were searched for manuscripts published up to May 2020. Ultimately, 19 studies were included that reported on estimates of BP and/or hypertension

prevalence in treated HIV-infected individuals versus untreated HIV-infected individuals and uninfected controls, residing in SSA countries.

The original papers of this study are based on the South Africa leg of the Prospective Urban and Rural Epidemiology (PURE) study, North West Province. Manuscript two and three, which form part of the PURE study, included 117 HIV-infected individuals and 131 uninfected controls and 126 HIV-infected and 126 uninfected controls, respectively. The study participants comprised of African men and women older than 35 years. Participation in the PURE study was voluntary and written informed consent was obtained from each participant.

Standard protocols and methods were used to gather all data and consisted of questionnaire data (demographic, lifestyle and medical history). Other data included HIV-related factors (ART use and duration, CD4 cell count), body composition and cardiovascular measurements (blood pressure, carotid femora pulse wave velocity, carotid intima-media thickness), as well as biochemical analyses of all relevant biomarkers used in the study.

Regarding statistical analyses, variables that were not normally distributed were log-transformed. Means and proportions were compared using the independent t-tests, dependent t-tests, Chi-square test, McNemar test or analysis of variance or covariance and repeated measure analysis. With manuscript three, partial and multiple regression analyses were used to investigate associations between the main dependent (cfPWV and cIMT) and independent (endothelial activation and cardiovascular risk markers) variables of interest. In all cases, $p \leq 0.05$ was used to indicate statistical significance.

Results of each manuscript

Manuscript one

A narrative review synthesis was conducted to determine the estimates of raised BP and hypertension in HIV-infected patients compared to untreated and HIV-uninfected controls from SSA. A total of 19 eligible studies that compared treated HIV-infected with untreated and/or HIV-uninfected controls were included. In studies comparing treated HIV-infected patients to uninfected controls, a large proportion of studies, reported lower BP 6,882 (56.30%) and hypertension prevalence 21,819 (79.2%) respectively, in the treated HIV-infected individuals than in HIV-uninfected controls. On the other hand, studies including 753 (6.16%) and

3,553 (12.9%) participants showed a higher BP and hypertension prevalence. Lastly, 4,588 (37.54%) and 2,180 (7.91%) participants showed no difference in BP and the prevalence of hypertension between the treated HIV-infected individuals and HIV-uninfected controls. Estimates of BP and hypertension prevalence were further compared for of treated versus untreated HIV-infected individuals. Studies including 5,757 (44.2%) and 4,547 (74.5%) participants reported lower BP and hypertension, respectively in treated compared to untreated HIV-infected individuals. On the other hand, studies including 200 (1.53%) and 598 (9.80%) participants showed higher BP and hypertension prevalence. Similar BP and hypertension prevalence was reported 7,073 (54.28%) and 959 (15.7%) participants respectively in the treated and untreated HIV-infected individuals.

Manuscript two

This manuscript profiled the demographic and cardiometabolic factors, indicators of inflammation and oxidative stress as well as liver and renal function of HIV-infected Africans and uninfected controls over 10 years. The HIV-infected individuals living with HIV for over 10 years presented with longitudinal increases in C-reactive protein ($p=0.002$), alanine transaminase ($p=0.006$) and gamma-glutamyl transferase ($p=0.046$) levels as well as estimated glomerular filtration rate (eGFR) ($p<0.001$). The HIV-infected individuals also showed increased high-density lipoprotein cholesterol (HDL-c) ($p<0.001$) and total cholesterol ($p<0.001$) levels and decreased triglycerides: HDL-c ($p=0.011$) levels. Low-density lipoprotein cholesterol (LDL-c) decreased in both groups ($p < 0.001$), although the decrease was relatively smaller in the HIV-infected individuals.

Manuscript three

In this manuscript, it was firstly determined whether individuals living with HIV for over five years have increased measures of arterial injury (carotid intima-media thickness (cIMT)) and large artery stiffness (carotid-femoral pulse wave velocity (cfPWV)) when compared to their HIV-uninfected counterparts. As a result, similar cIMT ($p=0.90$) and cfPWV ($p=0.35$) were reported in both groups. Secondly, it was determined whether baseline markers of endothelial activation and cardiovascular risk are associated with cIMT and cfPWV after five years. Although, levels of soluble intracellular adhesion molecule-1 (ICAM-1)

and soluble vascular cell adhesion molecule-1 (VCAM-1) were elevated in the HIV-infected group (all $p < 0.001$) at baseline, these markers did not associate with cIMT and cfPWV after five years. In multivariable-adjusted regression analysis cIMT associated positively with age ($\beta = 0.31$, $p = 0.002$) and triglycerides: high-density lipoprotein-cholesterol ($\beta = 0.23$; $p = 0.016$) in the HIV-infected group. Also, cfPWV was positively associated with mean arterial pressure (MAP) ($\beta = 0.28$; $p = 0.010$) in the HIV-infected group. In the HIV-uninfected group, cIMT was associated with sex ($\beta = 0.31$; $p = 0.004$) and glycated hemoglobin (HbA1c) ($\beta = 0.24$; $p = 0.026$) while cfPWV was associated with age ($\beta = 0.17$; $p = 0.049$), sex ($\beta = 0.29$; $p = 0.003$), MAP ($\beta = 0.31$; $p = 0.001$) and HbA1c ($\beta = 0.21$; $p = 0.041$).

General conclusion

A large proportion of studies conducted in SSA countries suggest that lower BP and hypertension prevalence is eminent in treated HIV-infected individuals compared to their uninfected and untreated HIV-infected controls. Despite this, deranged lipid profiles were reported accompanied by increased trajectories of inflammatory status, oxidative stress, liver enzymes and eGFR in HIV-infected individuals living with HIV for 10 years, proposing increased long-term risk for CVD. Traditional CV risk factors and, not elevated endothelial activation and HIV status, associated with measures of arterial injury and stiffness over time in HIV-infected people. This finding emphasises the need for HIV care in SSA settings where CVD is increasing due to epidemiological transition. Therefore, it is critical to screen for these CVD risk factors in HIV-infected individuals to reduce the possible burden of CVD burden in the future.

Keywords: Human immunodeficiency virus, cardiovascular diseases, hypertension, atherosclerosis, arterial stiffness, South Africa, Sub Saharan Africa.

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LIST OF ABBREVIATIONS

ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine transaminase
Ang I	Angiotensin I
Ang II	Angiotensin II
ART	Antiretroviral therapy
AST	Aspartate transaminase
AZT	Zidovudine
BMI	Body mass index
BP	Blood pressure
95%CI	95% confidence interval
CHD	Coronary heart disease
CARE	Cardiovascular AIDS Risk Evaluation
CKD	Chronic kidney disease
cIMT	Carotid intima-media thickness
cfPWV	Carotid-femoral pulse wave velocity
CKD-EPI	Chronic kidney disease epidemiology collaboration
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular diseases
DAD	Data Collection on Adverse Events on Anti-HIV Drugs
D4T	Stavudine
DBP	Diastolic blood pressure
DoH	Department of Health
Env	Envelope glycoprotein
EFV	Efavirenz
eGFR	Estimated glomerular filtration rate

FDC	Fixed-dose combination
FTC	Lamivudine
Gag	Group-specific antigen
GALT	Gut-associated lymphoid tissue
GGT	γ -glutamyltransferase
gp	Glycoprotein
HbA1c	Glycated haemoglobin
HBV	Hepatitis B virus
HDL-c	High-density lipoprotein-cholesterol
HIV	Human immunodeficiency virus
HIV-	HIV-uninfected
HIV+	HIV-infected
HIV+ ART-	HIV-infected ART-naive
HIV+ ART+	HIV-infected taking ART
HR	Heart rate
HT	Hypertension
ICAM-1	Intracellular adhesion molecule-
IDF	International Diabetes Federation
InSTI	Integrase inhibitors
LDL-c	Low-density lipoprotein-cholesterol
LPS	lipopolysaccharide
LPV/r	Lopinavir/ritonavir
LPV	Lopinavir
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
MetS	Metabolic syndrome
NCEP: ATP III	The National Cholesterol Education Program-Adult Treatment Panel III
NAFLD	Non-fatty liver disease

Nef	HIV-1 integrase factor
NRTI	Nucleoside reverse transcriptase inhibitors
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
N	Number
N/A	Not applicable
NCD	Non-communicable disease
NO	Nitric oxide
NS	Not significant
NVP	Nevirapine
Oz	Ounce
TDF	Tenofovir
3TC	Emtricitabine
PA	Physical activity
PI	Protease inhibitors
PhD	Doctor of philosophy
Pol	DNA polymerase,
PP	Pulse pressure
β	Regression coefficient
RAAS	Renin angiotensinogen-aldesterone system
Rev	Regulator of viral protein expression
RR	Risk ratio
RTV	Ritonavir
PURE	Prospective Urban and Rural Epidemiology study
Scd14	Soluble-CD14
SA	South Africa
SBP	Systolic blood pressure
SD	Standard deviation
SSA	Sub Saharan Africa

SMART	Strategies for Management of Anti-Retroviral Therapy
START	Strategic Timing of AntiRetroviral Treatment
Tat	Trans-activator of transcription
TC	Total cholesterol
TG	Triglycerides
TG: HDL-c ratio	Triglycerides: high-density lipoprotein-cholesterol ratio
TC: HDL-c ratio	Total cholesterol: high-density lipoprotein-cholesterol ratio
uACR	Urinary albumin-creatinine ratio
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low-density lipoprotein
Vpu	Viral protein U
Vpr	Viral protein R
Vif	Virion infectivity factor
WC	Waist circumference
WHO	World Health Organisation
Yrs	Years

Chapter 1
Background, Literature Review, Aim, Objectives and
Hypotheses

1. BACKGROUND

The burden of cardiovascular disease (CVD) is increasing globally [1] and is a major challenge in low-and-middle-income countries such as South Africa [2]. The driving force of CVD development includes risk factors such as obesity[3], hypertension [4], dyslipidaemia [5], diabetes mellitus[6], liver [7] and renal diseases [8] as well as lifestyle factors [9]. In addition to the traditional cardiovascular (CV) risk factors, infection with the human immunodeficiency virus (HIV) has also emerged as a risk factor for the development of CVD [10]. A systematic review and meta-analysis showed that the adjusted relative risk of CVD is 1.6 times greater in the HIV-infected population when compared to the uninfected population [11]. In the United States, the risk of developing acute myocardial infarction is approximately 50% greater in HIV-infected people after modifying for traditional CV risk factors [12].

By the end of 2019, it was estimated that 38 million people were living with HIV in the world and Sub-Saharan Africa (SSA) contributed about 59% of the HIV infections [13]. According to Statistics South Africa, South Africa continues to be one of the countries with the highest number of HIV infections, with approximately 7.97 million South Africans living with HIV [14] and 71% are on antiretroviral therapy (ART) [13]. With ART, the HIV-infected individuals are living longer and there has been a reduction in mortality associated with acquired immunodeficiency syndrome (AIDS)-opportunistic diseases [13]. Moreover, HIV is now a chronic manageable disease with the use of ART but has exposed a new set of challenges. These challenges include lipodystrophy [15, 16], lipid alterations [17, 18], hypertension [19, 20], diabetes mellitus [20, 21], inflammation [22], oxidative stress [23], endothelial dysfunction [24, 25], liver [26, 27] and renal disease [28] which are all risk factors for the development of CVD.

Hypertension is currently the major risk factor, leading to cardiovascular damage and complications including coronary artery disease, heart failure, stroke and renal disease [29] and is a growing problem in the HIV-infected population [30]. Indeed, the previous finding has shown an increased

risk for hypertension in people living with HIV compared to uninfected controls [31-33]. However, other studies have reported that blood pressure (BP) in HIV-infected people is either lower [34-36] or comparable [19, 37, 38] to their uninfected counterparts. Since hypertension has a marked impact on the aspects related to CVD development [30], it is important to clarify the hypertension rates in the HIV-infected population to adequately address CVD risk contributed by hypertension.

Atherosclerosis [39, 40] and arterial stiffness [41, 42] are reported more in the HIV-infected population than uninfected individuals. Moreover, atherosclerosis and arterial stiffness are highly predictive of cardiovascular events [43-46]. Factors related to HIV, conventional CV risk factors, inflammation and endothelial dysfunction have been suggested to play a fundamental role in the development of atherosclerosis and arterial stiffness [39, 42, 47, 48]. These observations have been largely reported in the Western population [40, 42, 49-51] than the African population [52, 53].

Several factors have been suggested to contribute to the development of CVD in HIV-infected individuals [47, 54]. These include the effects of the HIV infection itself, which results in chronic immune activation and inflammation [22]; traditional CV risk factors [55], as well as ART and its associated toxicities [47]. However, the pathogenesis of CVD development in the HIV-infected population is not fully understood. Although the SSA region is faced with a co-epidemic of HIV infection and CVD [4, 13], there is limited available data from longitudinal studies in this region on CVD development in HIV-infected individuals. Some of the cross-sectional studies done in the SSA region investigating CV risk in the HIV-infected individuals indicated an increase in CV risk profile as a result of HIV infection [34, 56] while others did not [57, 58]. Moreover, available studies have mainly consisted of HIV-infected individuals without an uninfected control group for a proper comparison of the CV risk profile [59, 60]. Following the above findings, it is therefore important to investigate the role of traditional CV risk factors, HIV infection and ART-associated toxicities in HIV-infected individuals – especially those living in SSA region, who may be at risk of CVD development.

This chapter entails the relevant literature overview on the possible role of HIV-related factors, cardio-metabolic factors, inflammation, oxidative stress and endothelial function as well as liver and renal function on the development of CVD in the HIV-infected population and how it compares to uninfected controls. Also, in this chapter, changes in arterial structure and function with HIV infection are discussed.

2. LITERATURE REVIEW

2.1 Discovery of HIV

The discovery of HIV was done in 1980 [61]. Later, groups of researchers were able to isolate and characterise HIV, showing a link between HIV and AIDS, supporting a causal link. Initially, the virus was given three different names including lymphadenopathy-associated virus, human T-cell leukaemia virus type III and AIDS-associated retrovirus. The naming of the virus was founded on the symptoms of the patients from whom the virus was isolated or on similarities to a known virus [61]. Eventually, in 1986, a community of scientists proposed the name HIV, which is how HIV is identified today. Transmission of can either be through unprotected sex, mother-to-child transmissions during birth, needle injection or contact with HIV-infected blood [62, 63].

2.2 HIV structure and subtypes

HIV is a retrovirus with a glycoprotein-rich envelope surrounding a nucleocapsid. The structural and regulatory proteins of HIV are divided for the virus to efficiently adapt to the human host, thus encouraging its replication and transmission [64]. The genome of HIV is made up of nine primary genes, namely group-specific antigen (Gag), DNA polymerase (Pol), Envelope glycoprotein (Env), trans-activator of transcription (Tat), a regulator of viral protein expression (Rev), viral protein U (Vpu), viral protein R (Vpr), Virion infectivity factor (Vif) and HIV integrase factor (Nef) [65]. The Gag-Pol precursor protein undergoes proteolytic cleavage to synthesise the matrix p17, capsid p24, nucleocapsids p9 and p6, reverse transcriptase, protease and integrase, all of which are the main structural constituents of the viral core. The Env undergoes proteolytic cleavage to produce the envelope glycoproteins (gp) 120 and gp41. The Vpu, Vpr, Vif and Nef are the accessory proteins and

Tat and Rev belong to regulatory proteins [64]. Among these viral proteins, gp120, Tat and Nef play a major role in the pathogenesis of cardiovascular diseases mainly through endothelial dysfunction [65].

According to the taxonomy of species, HIV belongs to the genus *Lentivirus* and it is divided into HIV-1 and HIV-2 [64]. The former HIV strain is responsible for most HIV infections globally. It is further shared into, three major groups of HIV strains, namely M (major group), O (an outlier group) and N (non-M/non-O group), all with a slightly varied genetic make-up. The HIV-1 Group M is the most studied strain and has extended all over the world and is responsible for the vast majority of the reported HIV infections [66]. This HIV-1 is further divided into three subtypes A, B and C [66, 67]. The HIV-1 subtype C strain is commonly found in Southern African countries, Ethiopia and India [66] while HIV-1 subtype A is the main causative strain in North Africa and North America. Subtype B is the third leading cause of HIV-1 infections mainly in America, Western Europe and Australia [66, 68]. The HIV-1 subtype C is more diverse and it can be transmitted by various routes [69] because it has numerous NF-kappa B sites [70, 71]. The nuclear factor (NF)-kappa B sites is associated with activation of HIV expression in cells and rapid HIV replication [72], which in turn are linked with increased risk for being infected with HIV due to high viral load and expression of HIV [73, 74]. The effects of the different HIV subtypes on the development of CVD in HIV-infected individuals are unknown.

2.3 Prevalence of HIV

According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) [13], 38.0 million people were living with HIV in the year 2019. Of the global infections, 19.2 million are attributable to women living with HIV. In the year 2019, 1.7 million were newly infected with HIV. However, new HIV infections have been reduced by 40% since the peak in the year 1997. The UNAIDS report also indicates that SSA countries contribute about 59% of the HIV infections globally, depicting a greater burden of HIV infections in this region [13]. South Africa is also not exempted, since the country has approximately 7.97 million people living with HIV [14]. Women of reproductive age are mostly affected by the HIV burden, with almost 4.30 million South African women living with HIV [14].

2.4 Antiretroviral therapy

The use of ART had increased tremendously, since its introduction in 1996 and its global scale up a decade later with free access for most people living with HIV [13]. At the end of 2019, 86% of the world's population living with HIV were initiated on ART [13]. South Africa has made tremendous contributions in addressing its HIV epidemic in recent years by introducing the use of ART. As such, South Africa has the world's largest ART-roll out programme, which has undergone further expansion with the implementation of test-and-treat guidelines and the Joint United Nations Programmes on HIV/AIDS (UNAIDS) 90-90-90 goals (**Figure 1**) [13]. Moreover, South Africa is largely funding these ART programmes from internal resources which places an enormous burden on the healthcare system [75]. In line with the 90-90-90 goals, South Africa has been able to achieve the first goal of having 90% of the population being aware of their HIV status, with 70% of those living with HIV initiated on ART and 64% being virally suppressed (**Figure 1**) [13]. The 90-90-90 goals are important in the light of CVD risk with the use of ART in the HIV-infected population. It may, therefore, be expected that the proportion of the HIV-infected population with CVD may increase as the use of ART increase and individuals become aware of their status.

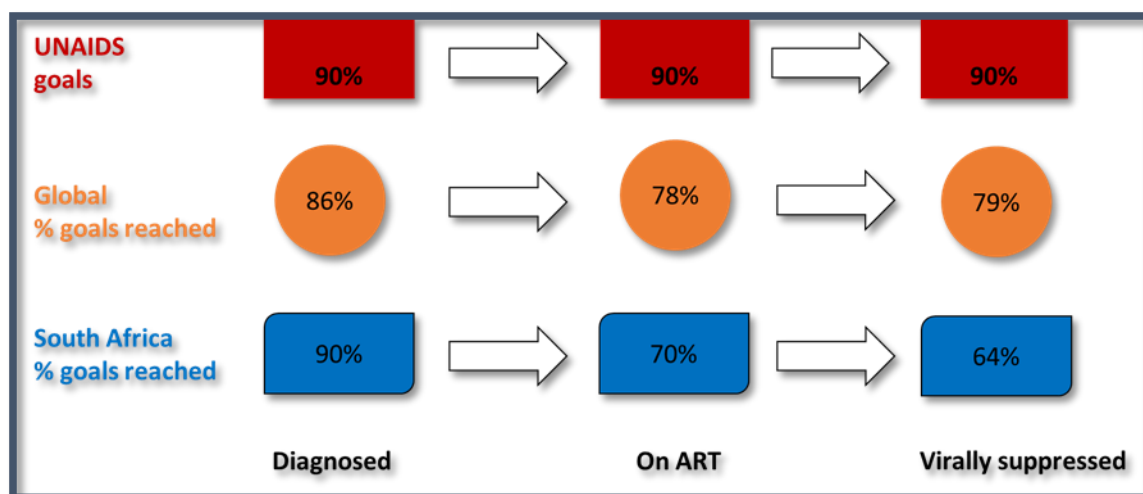


Figure 1. The percentage coverage reached in terms of the 90-90-90 goals globally and South Africa [13].

Different ART regimens are utilised in low, middle and high-income countries and since ART exert varying effects on CVD, it would be expected that the outcomes of the therapy may differ between countries. The guidelines for the management of HIV in South Africa adults have evolved from the past to the current [75]. Some of the noted changes include a shift from the use of three separate pills to the one-pill, fixed-dose combination (FDC) [76], comprehensive testing on drug resistance and Hepatitis B virus (HBV) co-infection as well as test-and-treat [75]. The guidelines are divided into first, second and third-line ART regimens (**Table 1**).

Table 1: South African guidelines for the management of human immunodeficiency virus (Department of Health) [75]

Antiretroviral therapy regimens 2020						
First-line regimen					Second-line regimens	
	NNRTI-based regimen		InSTI-based regimen for > 2 years		PI-based regimens for > 2 years	
Regimen	TDF + 3TC/FTC + EFV/NVP		TDF + 3TC/FTC + DTG		AZT/TDF + 3TC/FTC + LPV/r or ATV/r	
Resistance testing	Resistance test not required		Resistance test may be required under expert consultation		Resistance test required	
Resistance test results	Not applicable		Not applicable		No PI resistance	PI resistance
HBV co-infection status	HBV negative	HBV positive	HBV negative	HBV positive	HBV negative or positive	
New regimen	AZT+3TC/FTC+DTG	TDF + AZT + 3TC/FTC + DTG	AZT + 3TC/FTC + LPV/r	TDF + 3TC/FTC + LPV/r	Continue current regimen and address	Refer to the Third Line

	If DTG not suitable, AZT + 3TC/FTC + LPV/r	If DTG not suitable , TDF + 3TC + LPV/r			adherence. If intolerance to LPV/r is affecting adherence, discuss possible substitutions with an expert.	Committee. The regimen will be determined by the results of the resistance test
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NNRTI, non-nucleoside reverse transcriptase inhibitor; InSTI, integrase inhibitors; PI, protease inhibitor; HBV, Hepatitis B virus; TDF, Tenofovir; FTC, Emtricitabine; 3TC, Lamivudine; AZT, Zidovudine; NVP, Nevirapine; EFV, Efavirenz; DTG, dolutegravir; LPV/r, Lopinavir/ritonavir; AZT/r, atazanavir/ritonavir.

Once an individual is diagnosed with HIV, the HIV-infected individual is firstly initiated on the first line regimen followed by the second and third-line regimen in cases of immunological failure or multi-drug resistance. The first line regimen consists of non-nucleoside reverse transcriptase inhibitors (NNRTIs) and integrase inhibitors (InSTI) – based regimen while the second regimen is composed of protease inhibitors (PIs) –based regimen. Lastly, the third regimen may include PIs and is prescribed by an expert physician [75].

2.5 HIV-related factors and cardiovascular disease risk in people living with HIV

Factors such as ART and CD4 cell count which are associated with HIV may contribute significantly to the development of CVD in HIV-infected people [47]. Evidence from observational studies has shown that HIV infection is associated with an increased risk of acute myocardial infarction and coronary heart diseases (CHD) [46, 77-79]. Hsue et al [49] reported increased thickening of the intima-media in an HIV-infected Latino population compared to uninfected controls. Additionally, the HIV-infected population had higher frequencies of myocardial infarction compared to their age-matched uninfected counterparts [12]. The hazard ratio of 1.48 was determined for an association between myocardial infarction and being diagnosed with HIV after adjusting for sociodemographic and CV risk factors [12]. A meta-analysis assessing CV risk in an HIV-infected population projected that HIV infection is associated with a 61% greater relative risk of CVD endpoints such as ischemic heart diseases, myocardial infarction, heart attack and cerebrovascular events [11].

Depletion of CD4 cell is associated with a decline in immune function, especially from the gut-linked lymphoid tissues [80]. Compromised immune function in the spectra of HIV infection is important considering its consequent effect on immune activation and systemic inflammation [81], both of which are associated with the risk of developing CVD [82]. In particular, a link between noncalcified coronary artery plaques and reduced CD4 cell count was reported in HIV-infected people [83]. Noncalcified plaques in the HIV-infected population reflects an early stage of atherosclerosis and these plaques are more susceptible to rupture and thrombus formation, which in turn may lead to HIV-linked acute coronary syndrome [83]. Moreover, detectable viral load have been reported to be

significantly linked to CVD [84], stroke [85], acute myocardial infarction [12, 86] as well as non-AIDS outcomes [87].

Exposure to ART in HIV-infected people has presented new challenges, including cardio-metabolic challenges such as hypertension, dyslipidaemia [20], chronic inflammation [22], renal diseases [88] and liver diseases [89], which are all associated with increased risk for CVD development. This is observed in a study by Mbunkah who reported a higher prevalence of hypertension, dyslipidaemia and glucose levels in HIV-infected people on ART, than uninfected controls and those without ART [20]. Additionally, the development of premature atherosclerosis in HIV-infected individuals is increasing with exposure to ART, especially PIs [39, 40]. In particular, HIV-infected individuals treated with PIs have a higher prevalence of atherosclerosis compared to HIV-infected individuals ART-naïve [49]. Both ART and HIV infection may promote atherosclerosis through mechanisms involving alteration of the endothelial function, either directly or indirectly through cardio-metabolic risk factors [90, 91].

Nonetheless, HIV-infected people who had ART interruption had a 70% increased hazard risk ratio of CVD episodes [92]. Similarly, in the START study, early initiation of ART was associated with CVD events [93]. In a meta-analysis done by Islam et al [11], HIV-infected individuals taking ART had a 2.0 relative risk of developing CVD compared to their uninfected counterparts. Considering HIV-infected individuals with and without ART, HIV-infected individuals with ART had a 1.5 relative risk of developing CVD than HIV-infected without ART [11]. Findings from the large, multinational and observational study, Data Collection on Adverse Events on Anti-HIV Drugs (DAD), showed that PIs is an independent predictor of myocardial infarction [94]. Indeed, PIs such as lopinavir/ritonavir, indinavir and amprenavir/fosamprenavir have been linked to increased risk for myocardial infarction [95, 96]. Nucleoside reverse transcriptase inhibitors (NRTIs), including didanosine, also may increase myocardial infarction risk, although data are conflicting [97, 98].

2.6 Non-modifiable factors, CVD risk and HIV

2.6.1 Age

Increased age is an established risk factor for CVD in both the HIV-infected and uninfected population. Ageing is associated with deterioration in immune function and vascular changes which underlies various CVD risk factors such as hypertension and arterial stiffness [99, 100]. It was projected that by the year 2030, three out of every four HIV-infected people will be 50 years and older of age, over 80% of the HIV-infected population will have a minimum of one age-associated comorbidity and one-third will have a minimum of three age-associated comorbidities [101]. In HIV-infected individuals, HIV infection is accompanied by premature development of CVD when compared to the HIV-uninfected population [102]. Furthermore, in a systematic review including a European population, the overall incidence of CVD is approximately two-fold higher in HIV-infected individuals when compared to the HIV-uninfected individuals of the same age [103]. Findings from the Cardiovascular AIDS Risk Evaluation (CARE) study reported accelerated vascular ageing in HIV-infected people that 12 years more increased compared to the sequential age of their HIV-uninfected Americans [104].

It is suggested that the comorbidities in the HIV-infected people, do not occur simply as an after-effect of increased life-expectancy with ART use, but also due to rapid vascular ageing imposed by HIV and/or ART [99]. The suggested mechanism by which HIV induces premature vascular ageing includes accelerated immune senescence secondary to the exhaustion of immunological resources [100], oxidative stress, changes in arterial structure and function [105], ART toxicity (mitochondrial dysfunction and oxidative stress induced by thymidine analogues) [106], or even with PI toxicity [47, 107].

Among, HIV-infected adults living in France, the risk of myocardial infarction was higher compared to their age and sex-matched uninfected counterparts [108]. In another study, including the Western population, myocardial infarction increased from 0.27 per 1000 person-years in individuals of 25-30 years of age to 16.99 per 1000 person-years in individuals of 70 years of age [94]. Currier *et al.*

reported higher frequencies of CVD in HIV-infected population than uninfected population aged between 18 to 44 years [77]. In HIV-infected men between the age of 40-45 years, the crude risk for myocardial infarction, CHD and CVD outcome frequencies was 2.26, 3.11 and 3.65 per 1000 person-years, respectively [109]. While in those aged 60-65 years the crude risk increased to 6.53, 11.91 and 15.89 for myocardial infarction, CHD and CVD outcomes per 1000 person-years [109].

2.6.2 Sex

It is understood that men and women present with different levels of CVD risk, due to differences in body composition, BP and metabolic factors such as triglycerides (TG) as well as hormone [110, 111]. Oestrogen is considered to be cardioprotective in premenopausal women when compared to their age-matched men [112]. However, the protective role of oestrogen diminishes in menopause, hence the incidence and severity of CVD increases in women postmenopause [113]. While CVD risk in men develops linearly across the lifespan, women experience a sharp increase in CVD risk at the onset of menopause [127].

The relative risk for CVD morbidity in an age- and sex standardised study of HIV infected population from France was 2.7 times higher in women than men [108]. Existing evidence suggests that men have elevated BP when compared to women due to the protective effect of oestrogen, however, women in menopause have higher BP when compared to men [114, 115]. African women have a higher prevalence of obesity when compared to African men [116, 117] and have a marked impact on aspects related to CVD such as dyslipidaemia, diabetes mellitus [118, 119], hypertension and atherosclerosis [120]. It is important to highlight that in South Africa more women than men are living with HIV. In fact, over a fifth (4.3 million) of South African women between the ages of 15-49 years are living with HIV in South Africa [14]. Considering the role of HIV and ART on the risk of CVD, it may be postulated that more women will be exposed to CVD risk.

In sex-stratified models, the unadjusted AMI rates per 1000 person-years were higher for HIV patients among women (12.71 vs. 4.88 for HIV compared with non-HIV women), but not among men (10.48

vs. 11.44 for HIV compared with non-HIV men). The risk ratio (for HIV vs. non-HIV) were 2.98 (95% CI 2.33–3.75; $p < 0.0001$) for women and 1.40 (95% CI 1.16 –1.67; $p < 0.0003$) for men, adjusting for traditional CV risk factors [46]. Among HIV-infected men, 2% had a moderate predicted risk of CHD (10-year CHD risk, 15%–25%) and 17% had high predicted risk (10-year CHD risk of $\geq 25\%$ or diabetes). Among HIV-infected women, 2% had moderate predicted CHD risk and 12% had high predicted CHD risk [121].

2.7 Socioeconomic status, locality, CVD risk and HIV

Socioeconomic status is determined by using income, education [122] and employment level and it has been linked to CVD risks such as CHD and CVD mortality [123]. In particular, Low socioeconomic status measured by low income was associated with an increased probability of moderate-to-high risk for CHD [121]. It has reported that families with low-income purchase food items including saturated fats, sweets, sweetened beverages and spend less on fruit, vegetables, lean meat and fish [124]. The consumption of food, containing high content of fat and salt are negatively associated with increased risk of developing hypertension, especially in socioeconomically disadvantaged population, who are known to frequently eat this kind of food [122]. Low income and educational level have been linked to the risk of developing obesity [125]. However, a negative association between socioeconomic status and obesity have been observed in women with higher educational level and high income [126]. This observation is explained by access to healthy food and the modern examples of western society that impose women to make an effort to be thinner [127, 128]

South Africa is a developing country undergoing a rapid epidemiologic and demographic transition [129, 130]. With the transition, there is a shift from rural and traditional ways of living to adopt the westernised or urbanised living [131, 132]. The urban lifestyle is associated with unhealthy diets and lack of physical activity as well as access to tobacco product and alcohol use as a result of industrialisation and increased transportation [131, 133]. Urbanisation is therefore associated with increased emergence of CVD risk factors such as obesity and high BP [131, 134-136]. Additionally, a

South African survey reported a higher prevalence of people living with HIV in urban than rural areas and with lower socioeconomic status [137]. Hence, the colliding of the high HIV prevalence and epidemiological transition in these areas could increase the CVD risk of rural and urban dwellers living with HIV.

2.8 Modifiable factors, CVD risk and HIV

2.8.1 Alcohol use

Alcohol is a psychoactive substance with an addictive potential [138]. Alcohol use may be in a form of binge drinking (consumption of 5 and 4 standard drinks on the same occasion, for male and female, respectively), standard drinking (consist of 12 oz of beer or 5 oz of wine), heavy drinking (binge drinking on 5 or more days in the past month) [139]. Lastly, alcohol use disorder is a problematic pattern of alcohol use leading to clinically significant impairment or distress [139], with some studies reporting a higher prevalence of alcohol use in HIV infected individuals as compared to the HIV uninfected individuals and vice versa [140, 141]. Hazardous drinking (33.2% vs. 30.9%) and alcohol abuse (20.9% vs. 26.2%) was reported among men living with HIV and uninfected men, respectively [142]. Consumption of alcohol is common amongst people living with HIV, with previous reports indicating almost double rates of heavy drinking in HIV-infected people than the general population [143].

Alcohol consumption in treated HIV-infected individuals is associated with lower CD4 cell count and higher viral load [144]. Immune suppression may result in increased activation of the immune system, subsequent inflammation and increased end-organ damage, including acute myocardial infarction [145]. Moreover, the Veterans Aging Cohort Study including HIV-infected individuals reported that excessive drinking was independently associated with an increased prevalence of CVD, even after controlling for traditional CV risk factors, such as cholesterol or concurring diabetes mellitus [142].

Additionally, heavy alcohol use together with the HIV infection may promote gut microbial translocation -the leaking of bacteria from the intestine into the bloodstream, where they can stimulate

inflammatory and immune reactions that alter the function of the cardiovascular system [146]. Heavy alcohol consumption (above 60 grams per day) also is associated with an increased risk of hemorrhagic stroke (RR = 2.18, 95% CI = 1.48–3.20) [147]. This relationship may be facilitated by an alcohol-associated elevation in BP, which is an important risk factor for hemorrhagic stroke [148]. In addition to BP, dyslipidaemia and insulin resistance are commonly reported with the use of alcohol [149] and these factors are associated with increased risk of plaque formation leading to atherosclerosis and ultimately CVD risk [150, 151].

2.8.2 Smoking or the use of tobacco

Tobacco smoking consists of inhaling smoke from burning tobacco into the mouth and lungs. The type of product smoked is usually cigarettes, but can also include cigarillos, cigars, pipes or water pipes [152]. Tobacco smoking is one of the most important risk factors for CVD [153] and the prevalence of smoking among HIV-infected individuals is estimated at 70% [154]. The prevalence of smoking in the HIV-infected population ranges between 50-70% compared to 12% in the general people. Among HIV-infected individuals, prevalence rates of smoking range from 50% to 70% compared to 21% for the general population [155, 156]. In a South African population, smoking rates was 29% for HIV-infected people and 41.4% for uninfected people [24]. A median of 10 cigarettes was reported in both HIV-infected and uninfected population, but 18.0% of the HIV-infected individuals were less likely to use more than one pack of cigarettes daily compared to 26.9% of the uninfected counterparts [157].

The high smoking prevalence in this population might be due to poor socioeconomic status, psychological stress, discrimination and physical-mental distress caused by HIV and its treatment [154, 158, 159]. HIV-infected individuals who smoke have higher T-cell lymphocyte activation than HIV-infected individuals who do not smoke [160]. When compared to HIV-uninfected who do not smoke, the HIV-infected individuals who smoke showed higher microbial translocation (soluble-CD14 (s-CD14) and lipopolysaccharides (LPS)) which was directly correlated with CD4⁺ and CD8⁺ T-cell activation [160]. Monocytes and macrophages express a diverse pattern of recognition antigen

receptors and stimulation of these cells via the antigen receptors can promote inflammation and CVD [161]. Moreover, elevated plasma levels of s-CD14 are independently associated with coronary calcification and the extent of subclinical vascular disease in HIV-infected individuals on ART [162]. Thus, chronic innate immune activation may directly promote an increased thrombosis risk observed in HIV-infected individuals. Monocytes in the peripheral blood of HIV-infected individuals express high levels of tissue factor [163, 164] and these tissue factors are capable of initiating the extrinsic clotting pathways, potentially contributing to increased levels of D-dimers [165]. Coagulation markers such as D-dimers, have been associated with LPS, microbial translocation potentially leading to thrombosis and CVD risk [166, 167]. Smoking may also contribute to increased CVD risk by altering the elasticity of the large arteries and leading to arterial stiffness – a well-established all-cause CVD mortality [168]. In support of the role of smoking in CVD, termination of smoking was associated with the likelihood of a 40% decrease in myocardial infarction in the HIV-infected Danish population [169]. Furthermore, a follow-up report by DAD study reported a reduction in CVD outcomes with the duration of smoking cessation [170].

2.8.3 Diet or nutrition

A healthy and balanced nutritional diet is important for proper immune function [171]. A healthy diet constitutes more of fruits, vegetable and protein-rich food and less of saturated fats, salt and refined carbohydrates [172]. Diet and nutrition are linked to CV factors such diabetes mellitus, high BP and obesity- factors which are frequently reported in HIV-infected individuals prevalent and have also been reported as risk factors for coronary heart disease and stroke [173]. The relationship between dietary fats and CVD is recognised and it is considered to be facilitated via the atherogenic effects of total cholesterol (TC), TG and other lipid fractions [174]. Plasma TG has been directly associated with the risk of atherosclerotic vascular disease and thrombotic events [174]. In addition to dietary fats, the carbohydrate-rich diet has been shown to associate with increased risk for CVD development [173]. This relationship is mediated by the indirect mechanisms, such as the role of total energy and its effect on overweight and obesity; influence on central obesity; effects on lipid profile, particularly TG and effects on glycaemic control [173]. Carbohydrate food with a high-glycaemic index might

negatively impact glucose control, with associated changes in plasma lipids [175]. Moreover, high-carbohydrate diets tend to decrease the concentration of high-density lipoprotein-cholesterol (HDL-c) levels and elevate the fraction of small dense low-density lipoprotein-cholesterol (LDL-c), both of which are known to impact adversely on vascular disease [175]. This dyslipidaemic pattern is common with HIV and the exposure of ART in people living with HIV and is associated with coronary artery disease. A healthy and balanced diet is important in people living with HIV to complement the contribution of the ART for proper management of HIV disease [176].

2.9 Metabolic factors, CVD risk and HIV

2.9.1 Body composition

Body composition can be measured by several methods such as the measurement of waist circumference (WC) and hip circumference [177] which indicates abdominal obesity and gynoid obesity, respectively [178]. Measurement of WC of > 102 cm in men and > 88 cm in women is indicative of abdominal obesity [177]. Additionally, body mass index (BMI), is a reliable predictor of total fat and can therefore be utilised as an indicator of overweight and obesity [177]. The measurement of BMI is categorised into the following: underweight (< 18.5 kg/m²), normal (18.5 – 24.9 kg/m²), overweight (25.0 -29.9 kg/m²), obesity Class I (30.0 -34.9 kg/m²), obesity Class II (35.0 – 39.9 kg/m²) and obesity Class III (> 40 kg/m²) [179]. It is important to note that the BMI categories differ by ethnic population and although the African population do not have tailored BMI cut-off-values, the European BMI cut-off-values are used for this population [179].

Concerning HIV infection, lower WC, BMI and prevalence of obesity were seen in HIV-infected individuals compared to uninfected controls [20]. A possible explanation for this observation is the well-known association between HIV infection with wasting in the HIV-infected population. However, the Miami Cohort including the youth population reported similar BMI and WC for HIV-infected and uninfected African Americans [180]. With the use of ART, in particular PIs, lipodystrophy is prevalent in the HIV-infected population. This occurs as a result of factors such as mitochondrial toxicity, higher levels of oxidised LDL-c, lower HDL-c and altered glucose

metabolism. Lipodystrophy may present as either lipoatrophy, lipohypertrophy or a combination of both [15, 60, 181]. Lipodystrophy is associated with hypertension, diabetes mellitus – which are prevalent in HIV-infected people and are associated with myocardial infarction and coronary artery disease [118, 182].

A cross-sectional study done by Mbunkah et al [20] reported a higher percentage of abdominal obesity (43.5%) in HIV-infected on ART compared to those without ART (31.1%). A study done in Nigeria reported that the prevalence of obesity was 15.9% in the uninfected individuals, 3% in the untreated HIV-infected individuals and 8% in the HIV-infected individuals on ART [34]. Obesity is a major public health concern [199] and is suggested to indicate excessive body fat content concerning stature [177]. This condition may develop as a result of high circulating adipocytes, the release of cytokines and pro-inflammatory markers by adipose tissue [183]. More importantly, previous studies have linked obesity and the risk of CVD development. Obesity was associated with increased systemic inflammation in a six-year follow-up study of the Turkish population [184] and has a consequential impact on CVD, namely increased sympathetic activity [185, 186], BP [187], endothelial cell activation [188] and changes in the arterial structure and function [189]. It adds to the pathogenesis of hypertension [190], atherosclerosis [191], renal diseases [192] leading to an increased CVD burden. A longitudinal study including black South Africans indicated that abdominal obesity increases the five-year risk for developing metabolic and cardiovascular diseases [193]. A cross-sectional including 2230 HIV-infected individuals treated at HIV clinics in the township of Pretoria, South Africa reported the prevalence of overweight, obesity and high WC at 29.3%, 21.9% and 44% for women [194]. In men, the prevalence of overweight was 12.4% while 4.0% and 3.9% of the men had obesity and high WC, respectively [194]. From the studies reported it is evident that bodyweight parameters differ for ART-naïve HIV-infected and HIV-infected individuals on ART (**Figure 2**).

2.9.2 Lipid profile

Dyslipidaemia refers to abnormal lipid (hydrophobic fat molecules such as cholesterol and fatty acids) and lipoprotein (molecules consisting of lipids and apolipoproteins) concentrations in the blood [177].

High TC, low-density lipoprotein-cholesterol (LDL-c) is considered “bad” cholesterol and were identified as a CV risk factor [195] while HDL-c is the “good” cholesterol and considered as protective against CVD [196]. According to The National Cholesterol Education Program-Adult Treatment Panel III (NCEP: ATP III) guidelines, the following values are signs of dyslipidaemia: TC >4.9 mmol/L, LDL-c >3.0 mmol/L, HDL-c < 1.7 mmol/L [177]. Lipid alterations are consistent with both HIV infection and are associated with increased risk for CVD development such as atherosclerosis, coronary artery diseases [197]. People living with HIV commonly present with dyslipidaemia than uninfected controls [20, 198, 199].

A possible role of HIV infection on lipid metabolism is suggested by disturbances in the cytokine status and reduced lipid removal and elevated hepatic production of very low density-lipoprotein cholesterol (VLDL) [200]. Reduction in CD4 lymphocyte cell count is linked to serum TG increases and decrease in HDL-c [201], as a result, reduced clearance of the circulatory lipoproteins. Even though HDL-c particles can promote cholesterol efflux in HIV-infected persons [202], the loss of protection against atherosclerosis by these particles becomes evident at lower concentration [196]. The latter can be caused by the impairment of the cholesterol delivery through liver scavenger receptors because of the higher TG content that HDL-c has in HIV infection [203]. Dyslipidaemia induces deposition of lipids particles into the arterial wall, thus aggravating the process of atherosclerosis [204]. Moreover, the deposition of fats in the arteries leads to narrowing of the lumen, obstruction of blood supply leading to thrombo-embolic episodes such as stroke, transient ischemic attack, ischemic heart disease and pulmonary embolism [205].

In addition to the lipid profile abnormalities usually seen with HIV infection, ART elevates total cholesterol levels while high-density lipoprotein-cholesterol may increase or decrease depending on the ART regimen used [17, 206]. It is important to note that the ART classes as well as the individual ART drugs exert differing effects on lipid metabolism. In particular, PI-based regimens are associated with higher levels of TG, TC and LDL-c [207, 208]. The use of NNRTIs are associated with higher levels of TC and LDL-c and may induce a concomitant elevation in HDL-c [20, 197]. The PIs

promote higher TG levels by encouraging hepatic TG production through upregulation of mRNA production in the hepatic cells, causing hepatic accumulation of TG-rich lipoparticles [209]. These lipid alterations are further associated with lipogenesis, adipocyte differentiation and a decrease in hepatocyte clearance of chylomicron [208] and support further immune activation and inflammation [210].

In summary, it is evident from the literature that HIV and different ART regimens exert differing effects on lipid metabolism (**Figure 2**), which encourage the development of CVD. Given that ART regimens and HIV subtypes differ considerably by countries, it would be important to stratify the effect of lipid changes on CVD development in the SSA population where HIV-1 Subtype C and use of NNTIs and NRTIs is predominate.

2.9.3 Glucose metabolism

The clinical diagnosis of diabetes mellitus is based on the determination of cut-off points for glucose and/or glycated haemoglobin (HbA1c) [211]. According to the International Diabetes Federation (IDF), fasting glucose levels of between 5.0 – 5.6 mmol/l is categorised as normal while levels above this range lead to glucose abnormalities such as diabetes mellitus, impaired glucose tolerance and hyperglycemia [211]. Hyperglycemia as defined by fasting glucose of 5.6 mmol/l [211]. These glucose abnormalities may be a result of the suboptimal level of insulin to effectively facilitate the uptake of glucose into the skeletal muscles [212].

The HbA1c is used to determine concentrations of blood glucose over the long-term and is highly associated with longitudinal complications of type 2 diabetes mellitus as well as CVD risk in patients with diabetes [213]. The American Diabetes Association has endorsed an HbA1c cut-off value of $\geq 6.5\%$ as the criteria for diagnosing diabetes mellitus [211]. Diabetes mellitus is associated with a 2- to 4-fold greater risk of CHD than that observed in the population of persons without diabetes mellitus [214, 215]. Individuals with a moderate degree of glucose abnormalities such as impaired glucose tolerance and glycemia above the upper limit of normality have increased risk for atheromatous

lesions [214-216]. Disturbed glucose metabolism has previously been related to lipodystrophy, which includes the combination of lipoatrophy and abdominal lipohypertrophy [217]. Importantly, lipodystrophy, is frequently seen in HIV-infected individuals on PIs treatment and is associated with risk of CVD development [15, 208]. In HIV-infected individuals, lipodystrophy (associated with abnormal fat distribution, glucose intolerance, and hyperlipidaemia) [182] has been reported to present a risk for sub-clinical coronary atherosclerosis [15], heart disease, diabetes, and early hypercholesterolemia [218]. In populations with diabetes, LDL-c particles are glycated and glycation of LDL-c lengthens its half-life [219] and therefore increases the ability of the LDL-c to promote atherogenesis.

Another possible mechanism for altered glucose homeostasis in HIV includes genetic predisposition, disease-related body changes as well as metabolic effects associated with ART exposure [220]. The use of PI is associated with a decrease in the expression of glucose transporter (GLUT 4) and this decreased expression impairs insulin-mediated intake of glucose into the adipocytes [220, 221]. The elevated concentration of circulatory glucose may indicate persistent immune activation and inflammation in HIV-infected individuals on ART.

The studies reporting on diabetes in HIV-infected individuals are not consistent and glucose levels differ for those with and without ART (**Figure 2**). A cross-sectional study reported a similar prevalence in hyperglycaemia between the HIV infected individuals taking ART (12.7%), not taking ART (12.9%) and the uninfected controls (13%) [198]. This is supported by cross-sectional studies done in South Africa that reported similar glucose in HIV-infected individuals versus uninfected individuals [199] and similar HbA1c levels in HIV-infected individuals on ART than uninfected controls [24]. In the Women's Interagency HIV Study, HIV-infected women without ART exposure showed an incidence rate of 1.53/100 person-years in diabetes mellitus. Moreover, the incidence of diabetes mellitus was 2.50/100 person-years lower in PI-treated HIV-infected individuals compared to 2.89/100 person-years in HIV-infected individuals without PI-containing regimens [222]. In the Multicenter AIDS study, the incidence of type 2 diabetes mellitus was reported to be more than four

times higher in HIV-infected men using ART than in uninfected men [223]. In a Tanzanian population, the prevalence of diabetes mellitus (18.0 vs. 5.2%, $p=0.001$) and impaired glucose tolerance (14.0 vs. 2.0%, $p<0.001$) was higher in the HIV-infected population on ART ≥ 2 years than uninfected controls [224].

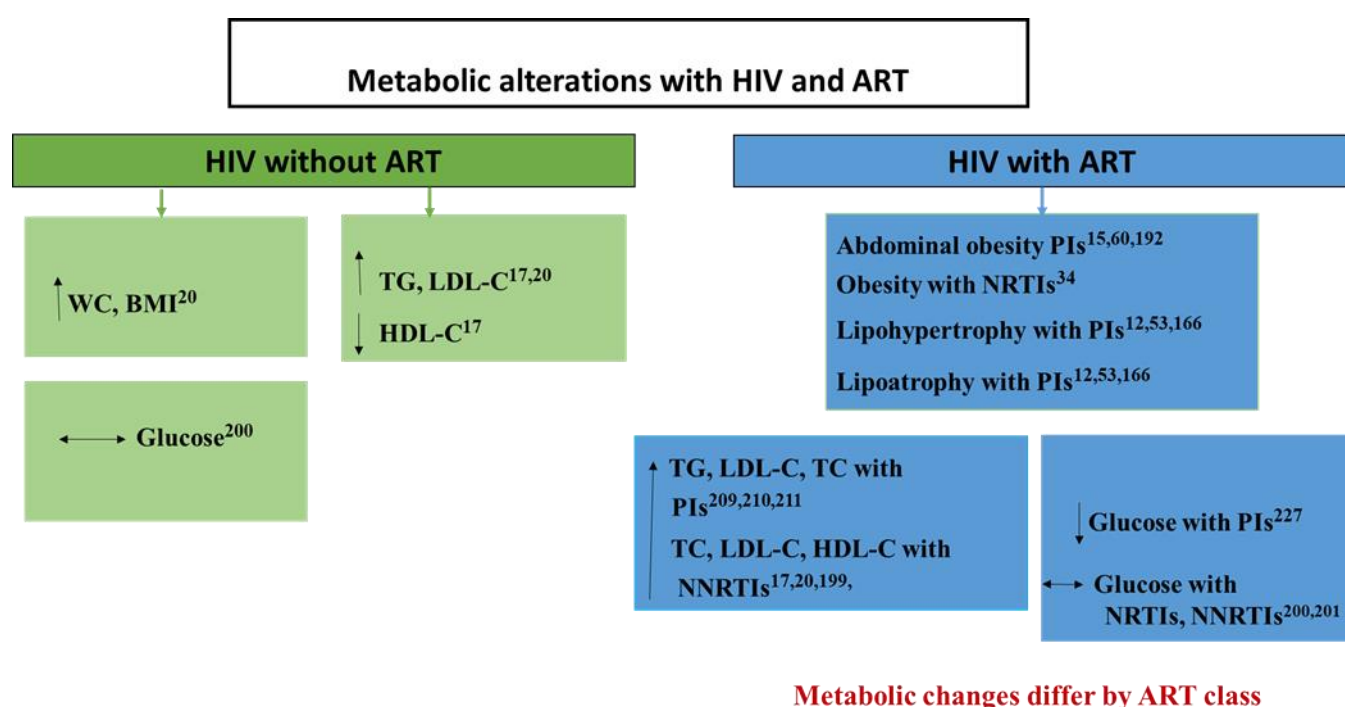


Figure 2. Summary of the cardio-metabolic alteration in HIV-infected individuals

HIV, human immunodeficiency virus; ART, antiretroviral therapy; BP, blood pressure; TG, triglycerides; TC, total cholesterol, LDL-c, Low-density lipoprotein-cholesterol; HDL-c, high-density lipoprotein cholesterol, HbA1c, glycated haemoglobin; PI, protease inhibitor; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, non-nucleoside reverse transcriptase.

2.10 Liver enzymes, CVD risk and HIV

The liver is a critical organ in the human body that is responsible for functions that help support metabolism, immunity, digestion, detoxification, vitamin storage among other functions [225]. Liver enzymes such as γ -glutamyltransferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [226] – all have been used as indicators of hepatic dysfunction and non-alcoholic fatty liver disease (NAFLD) [227]. The synthesis of ALT is mainly done by the liver as a

result of increased hepatic inflammation or injury, while AST is released by both the liver and muscle cells and rises with myocardial cell injury or hepatic dysfunction [228]. Concerning GGT, it is present in hepatocytes and biliary epithelial cells, renal tubules and pancreas [226]. Large increases of 50 U/l or above for GGT and 40 U/l or above for AST and ALT are indicative of hepatotoxicity [229, 230]. These enzymes have emerged as risk factors for CVD, although there have been differences in the relationship between specific liver enzymes and CVD [231]. A study reported that elevated ALT and AST, but not GGT levels were not associated with all-cause mortality and mortality from CVD in an American population, predominantly non-Hispanic white [232]. Lee et al [233] showed that elevated levels of ALT and AST are related to CVD mortality and all-cause mortality in a Korean population [234]. A meta-analysis using the British Women's Heart and Health Study explored the association of GGT and ALT with incident CHD, stroke and combined outcomes and reported that GGT, but not ALT, was related to incident cardiovascular events independent of alcohol use. Conversely, Kumutson et al [235] demonstrated that ALT is positively associated with stroke.

Increased ALT level is associated with reduced insulin sensitivity, adiponectin and glucose tolerance as well as increased free fatty acids and triglycerides – all of which are representative CVD risk [236]. A synergistic impact of NAFLD and metabolic syndrome have been reported on subclinical atherosclerosis. Moreover, NAFLD is associated with vascular inflammation, which may reflect rupture-prone vulnerable atherosclerotic plaques [237]. Visceral fat accumulation and metabolic syndrome are significantly associated with GGT and ALT concentrations [234, 238]. Possible pathogenic mechanisms have been suggested to explain the presence of atherosclerosis and increased CVD burden in patients with NAFLD, such as oxidative stress state due to steatosis-stimulated fatty-acid oxidation in the liver [239], the systemic release of pro-atherogenic molecules like tumour necrosis factor- α , interleukin-6 and oxidized LDL-c [240], insulin resistance [241] and macrophage activation [242]. People with NAFLD have increased atherosclerosis when compared with people with simple steatosis [243], supporting the atherogenic role of hepatic inflammation. Moreover, carotid intima-media thickness (cIMT) is a reliable index of subclinical atherosclerosis and a mirror of atherosclerosis progression in people with NAFLD [244]. Furthermore, NAFLD might stimulate

insulin resistance and dyslipidaemia (highlighted by overproduction of TG and cholesterol-rich remnant particles) leading to accelerated atherosclerosis [245, 246]. Dyslipidaemia represents a major risk factor in CVD and small, dense LDL in nonalcoholic steatohepatitis may increase the risk for atherosclerosis and CVD [247].

Liver diseases are becoming increasingly prominent with HIV infection [229] and are associated with a greater burden of hospitalisation, morbidity and mortality in HIV-infected individuals [248, 249]. Hepatotoxicity is an adverse effect derived from the use of ART and may challenge the treatment of HIV infection [27]. The possible pathogenic mechanism for hepatotoxicity in HIV infection includes direct ART toxicity, immune reconstitution in the presence of hepatitis C and B virus co-infection, hypersensitivity of the liver and mitochondrial toxicity [7]. The contribution of the individual ART regimen to the development of hepatotoxicity is not fully known [7]. However, studies propose that the use of NRTIs is associated with mitochondrial toxicity which indicates liver injury [250]. Another study reported that HIV-infected individuals on nevirapine-based regimes presented with high ALT at an earlier time compared to those who were on the efavirenz-based regimen [251]. In the latter study, no differences were seen in the incidence of hepatotoxicity between the two regimens [251]. Apart from ART, liver injury is also independently associated with older age [252], previous ART monotherapy and an increase in CD4 cell count [253, 254]. The association for liver injury with the increase in CD4 cell count is concerning, considering one of the UNAIDS goals which aim to have 90% of the world's population living with HIV having viral suppression.

2.11 Inflammation, CVD risk and HIV

Inflammation is a physiological process in response to injury or infection and involves the release and activation of leukocytes and the production of inflammatory cytokines [255] such as C-reactive protein (CRP). Although inflammation is beneficial, if this response is chronically activated it can have a detrimental effect such as ongoing immune activation and endothelial dysfunction [256]. This protein is synthesised in the liver and it is an acute-phase reactant, that is released by the hepatocytes and adipocytes upon induction by the interleukin-6 (IL-6) [257]. Guimarães et al [22] have classified

a high CRP level as >3 mg/l, while a normal level of CRP is classified as < 1 mg/l. This inflammatory marker has been used as a predictor of atherothrombotic episodes and CRP plays a critical role in the development of atherosclerosis [258, 259]. This notion is supported by that CRP may induce changes such as an increase in the expression of adhesion soluble molecules, recruitment of monocytes, ion channel modification and increased uptake of oxidised LDL-c, which lead to vascular alterations and increased risk for arterial stiffness [260-262]. Moreover, elevated concentrations of CRP predict increased risk for the development of peripheral disease [258], coronary events in both stable [263] and unstable angina pectoris [263, 264]. Another pathway by which CRP contributes to CVD risk is that CRP is strongly associated with the development of hypertension [265], obesity [266] and diabetes [267] - these factors are the major contributors to CVD development.

Chronic inflammation in HIV is induced as a result of persistent immune activation with resultant elevated levels of inflammatory markers [99]. The levels of CRP are upregulated in HIV-infected individuals and inversely associated with CD4 cell count [268]. The liver-synthesised CRP is implicated in the development of endothelial dysfunction, coronary vascular diseases [269] as well as increased risk for myocardial infarction in HIV-infected individuals [270]. Findings from a South African cohort indicated higher CRP in HIV infected individuals as compared to HIV uninfected individuals [199]. Systemic inflammation is a hallmark of HIV infection and even after long-term combination ART, some degree of low-grade inflammation persists. This low-grade inflammation has been suggested to contribute to the increased incidence of CVD and cardiovascular events in treated HIV-infected individuals [271, 272].

The persistence of the virus supports a chronic status of immune activation and inflammation leading to the increased production of pro-inflammatory cytokines [273], thymus dysfunction and immunosenescence [274]. In addition, persistent inflammation amplifies tissue damage in people living with HIV, particularly in the gastrointestinal tract, leading to gut microbial translocation – which further aggravates inflammation and is linked to CVD development [275]. Although ART can

restore the immunological function to a certain extent, low-grade inflammation is persistent with ART. Since inflammation is strongly associated with CVD risk in HIV-infected individuals than uninfected controls [22], it is expected that CVD attributable to the effects of chronic inflammation will continue to increase.

2.12 Oxidative stress, CVD risk and HIV

Under the normal physiological state, ROS concentrations are tightly controlled by the activity of antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase [276]. In the heart, ROS play a fundamental function in cell homeostasis when present at low concentrations, since they regulate multiple physiological signalling pathways and biological processes [277]. Once the homeostatic state is disturbed and ROS levels cannot be controlled, oxidative stress becomes apparent [278]. A disturbance in the balance of ROS and antioxidants such as GGT explain oxidative stress [278].

Most importantly, ROS and GGT are key hallmarks of CVD development [279]. The influence of ROS on endothelial molecules that can promote cell apoptosis and necrosis and therefore thrombosis makes oxidative stress a critical hallmark to a CVD and it is defined as its early causative factor [280, 281]. As such, ROS have been implicated in the signalling cascades leading to vascular pro-inflammatory and pro-thrombotic gene expression [280]. It is also a potent activator of matrix metalloproteinases (MMPs), which reflect the destabilization and rupture of carotid plaque [279]. In a South African study, ROS was positively related to SBP and PP in African men, suggesting that oxidative stress may contribute to the development of arterial stiffness and hypertension [282]. Also, decreased arterial compliance and increased peripheral vascular resistance were linked to oxidative damage independent of the presence of hypertension in a population of black South African teachers [283]. Oxidative stress in HIV occurs as results of deregulation of oxidative stress pathways with increased ROS synthesis and by causing mitochondrial dysfunction [284]. The production of ROS is enhanced by gp120, Nef and Tat proteins and reverse transcriptase via the cytochrome P450 2E1 by upregulating CYP2E1 expression and induction of spermine oxidase [284, 285]. The progression of

HIV infection is thought to be linked to oxidative stress [23, 286]. Mandas *et al* [287] reported that HIV infected individuals taking ART have elevated levels of serum peroxides and reduced antioxidant levels when compared to HIV infected individuals not taking ART and uninfected control.

In addition to ROS, GGT, a liver enzyme, has been noted as a marker of oxidative stress [288]. This liver enzyme is responsible for the maintenance of the glutathione homeostasis and normal redox status by breaking down extracellular glutathione and providing cysteine, the rate-limiting substrate, for intracellular de novo synthesis of glutathione [289, 290]. HIV-infected individuals have an elevated concentration of serum GGT when compared to uninfected controls [291], while ART seems to lower serum GGT in HIV-infected individuals [26]. It is suggested that GGT impacts LDL cholesterol oxidation to influence plaque progression and rupture [292]. The pathogenic role of GGT on CVD development may be independent or synergistic through its interaction with traditional CVD risk factors [292]. The pathophysiological mechanism is not clear, but the association between GGT and lipoproteins, suggests that LDL lipoprotein may convey GGT activity inside the atherosclerotic plaque [293]. The GGT-mediated oxidative stress may have a role in the development and vulnerability of plaque, such as plaque erosion and rupture, enhance plate aggregation and thrombosis [294]. Oxidative stress is a potent activator of matrix metalloproteinases (MMPs), which reflect the destabilization and rupture of carotid plaque [295].

Existing literature indicates that HIV infection and ART in combination or alone may trigger oxidative stress and further augment the pathogenesis of HIV infection with consequent outcomes such as rapid viral replication, inflammation and endothelial activation [23]. This inflammation and endothelial activation play a critical role in initiating vascular changes leading to atherogenesis.

2.13 Endothelial dysfunction, CVD risk and HIV

The endothelium is primarily responsible for homeostatic mechanisms, such as the maintenance of non-thrombotic vascular surfaces, regulation of vascular tone and immunomodulation [296]. Alteration in the endothelial function is elicited by CVD factors such as ageing, smoking,

hypercholesterolemia, hypertension, hyperglycemia [297, 298]. This leads to a chronic inflammatory process coupled with a loss of antithrombotic factors and an increase in vasoconstrictor and prothrombotic products, in addition to abnormal vasoreactivity, therefore elevating the risk of cardiovascular events [299].

Sustained endothelial activation may lead to endothelial dysfunction – a phenomenon that is commonly reported in HIV-infected individuals [320]. Endothelial dysfunction is, therefore, considered a key factor in atherosclerosis-related diseases and is present in all stages of atherosclerosis [300]. Activation of the endothelium as a result of the HIV proteins (gp120, Tat and Nef) increases the production of inflammatory cytokines, expression of monocyte chemoattractant-1 protein (MCP-1), soluble intercellular adhesion molecules-1 (sICAM-1) and soluble vascular adhesion molecules-1 (sVCAM-1), among others [158-160]. These soluble are considered reliable biomarkers of vascular dysfunction and atherosclerosis development [301, 302] and also contribute to the predictive value of traditional risk factors for CVD [303]. Additionally, endothelial dysfunction is associated with the progression of HIV disease and oxidised LDL-c levels in HIV-infected individuals [304] and endothelium of HIV-1 infected people is characterized by increased numbers of adherent leukocytes as compared to HIV-uninfected individuals [305]. Monocytes independently predict clinical CVD events [306], patients with acute coronary syndrome have increased percentages of both inflammatory and patrolling monocytes along with TNF expression [307].

Endothelial dysfunction in HIV infection may further be attenuated as results of the direct effect of HIV proteins on the endothelium [65], effects of vascular inflammation [255] due to systemic inflammatory cytokine or chemokine dysregulation and lipid alteration linked to HIV infection [197].

Studies have reported elevated levels of soluble adhesion molecules, such as soluble intracellular adhesion molecule (ICAM-1) and soluble vascular cell adhesion molecule (VCAM-1) in people living with HIV compared with the HIV-uninfected controls [24, 308]. In the context of ART exposure, these soluble adhesion molecules, especially ICAM tend to decrease with ART use and duration as

results of viral suppression and thus reducing the viral effects of HIV on the endothelium [309]. However, other studies argue that these adhesion molecules, increase despite the use of ART, especially with PIs and reverse transcriptase inhibitors, [310, 311] and are positively associated with a longer duration on ART [312].

2.14 Blood pressure, CVD risk and HIV

According to Guyton [313], BP is the force that the blood applies against a unit area of the vessel wall and it is taken in millimetres mercury (mmHg). A BP is measured using two parameters namely, systolic or diastolic BP. Systolic BP is taken during heartbeat while diastolic BP is measured between heartbeats. A normal systolic and diastolic BP is defined by 120 mmHg and/or 80 mmHg, respectively, whereas systolic and diastolic BP ≥ 140 mmHg and/or ≥ 90 mmHg and/or the use of antihypertensive medication defines hypertension [314].

Control of BP is dependent on the actions of the cardiovascular, neural, endocrine and renal systems and their derangement plays a role in the development of hypertension [315]. Maintenance of a normal BP is reliant on the balance between the cardiac output and peripheral vascular resistance which are regulated by the autonomic nervous system (ANS) [316]. Stimulation of the sympathetic nervous system induces secretion of catecholamine which increase cardiac output. Additionally, the increased retention of water and sodium by the renal tubules causes an elevation in blood volume and hence cardiac output. Stimulation of α -adrenergic receptors increases peripheral vascular resistance, which together with the high cardiac output results in elevated BP [317].

The nervous system contributes to BP control through the sympathetic and parasympathetic nervous systems, which regulate both cardiac output and vascular resistance [318]. The sympathetic nervous system can achieve both arteriolar constriction and vasodilation, while the parasympathetic nervous system contributes primarily to the regulation of cardiac functions [316]. Sympathetic activation causes prolonged BP increases via peripheral vasoconstriction, potentiation of cardiac contraction, reduction of venous capacitance and modulating the renal sodium and water excretion [319]. Another

pathway by which BP is controlled is via the renin-angiotensin-aldosterone system (RAAS). This system also plays an important function in cellular growth and arterial remodelling [320]. The RAAS is a vasoactive system that acts as a circulating endocrine system and a local tissue paracrine system. Renin converts the substrate angiotensinogen into angiotensin (Ang) I, which is further converted into Ang II by the angiotensin-converting enzyme [321, 322]. Importantly, Ang II is a potent vasoconstrictor and hence it causes an increase in BP. Additionally, Ang II induces the release of aldosterone from the zona glomerulosa of the adrenal gland, which causes a more increase in BP linked to the retention of sodium and water [315].

Structural and functional vascular abnormalities are involved in the pathophysiology of hypertension, leading to increased total peripheral resistance and arterial stiffening, respectively [315]. The endothelium has emerged as the key player in vascular homeostasis. As such, alteration in the endothelial function precedes endothelial dysfunction which is characterized by a reduced nitric oxide availability, contributing to raised BP [323]. Last but not least, genetic and environmental factors (such as obesity, physical inactivity and excessive salt intake) are associated with the risk of developing hypertension [324, 325]. The structural damage caused by hypertension to large and small arteries may lead to endothelial dysfunction, reduced vascular compliance, increased vascular stiffness, reduced lumen diameter and formation of atherosclerotic plaques [43].

Hypertension is a major risk factor for CVD such as coronary disease, left ventricular hypertrophy and heart diseases, cardiac arrhythmias including atrial fibrillation, stroke and renal failure [136, 326]. Moreover raised BP is associated with CVD events including stroke, myocardial infarction, heart failure, peripheral arterial disease and end-stage renal disease in various age and ethnic groups [327, 328]. An increase in BP is noted with ageing and it might be related to the greater CVD prevalence [329]. In that study, the onset of coronary heart disease was seen more in people with hypertension than those with normal BP. Moreover, BP was enhanced in individuals with angina pectoris [329]. Another pathway that hypertension, is linked to CVD risk is through BP mediated increase in arterial stiffness and vascular resistance, especially with ageing [330].

The mechanism involved in the development of hypertension in the context of HIV include factors such as microbial translocation, chronic inflammation, immune suppression, lipodystrophy, HIV-related renal diseases and the use of ART [30]. Concerning, microbial translocation, HIV infects CD4 T-cells in gut-associated lymphoid tissue (GALT) causing a disturbance in the body natural mucosal defence and passage of microbes into the systemic circulation [340, 341]. Markers of microbial translocation, LPS and sCD14, are associated with hypertension in HIV-infected people [331]. The former may induce hypertension via stimulating RAAS and promotion of Ang II and adhesion of leukocytes on the endothelium [332, 333]. Mitochondrial toxicity and microphage build in the cell as a result of PI exposure are associated with the production of ROS and which in turn induce vascular collagen deposition, arterial stiffness and kidney injury in genetically modified mice [334].

The increase in life expectancy due to ART in the HIV-infected population has resulted in age-related chronic diseases such as hypertension and consequential CVD [60, 335]. Hypertension is the leading cause of CV mortality in the general population globally and likely in the HIV-infected population [336]. A recent systematic review by Bigna et al [337] included 194 studies from 61 countries, with 396 766 HIV-infected individuals to determine the prevalence of hypertension in people living with HIV. The study reported that the burden of hypertension in HIV-infected was higher in SSA regions, which contributed to about 60% of all HIV-infected individuals with hypertension. The increase in hypertension prevalence globally was driven by SSA countries. In a prospective study of ART-naïve individuals, initiating ART at a public sector HIV clinic in South Africa between April 2004–2017, it was reported that 77 696 (22%) had hypertension at ART initiation [59]. In the latter study, the use of zidovudine+stavudine and nevirapine was associated with an increased risk of having hypertension compared to those on tenofovir or efavirenz. Additionally, a meta-analysis of data from around the globe reported that 35% of all HIV-infected individuals on ART have hypertension, compared to 30% of uninfected counterparts [338]. Fahme et al [30] reported that hypertension is frequently seen in the HIV-infected population and may be due to a combination of traditional risk factors, HIV-specific factors and ART exposure.

A systemic review and meta-analysis of studies including 29 755 individuals in the black population living in the SSA region, reported that the HIV-infected, ART-naive individuals had lower systolic and diastolic BP compared to the uninfected controls [60]. In the latter study, exposure to ART was not associated with BP [60]. Other studies reported inconsistent findings, with the hypertension prevalence in the HIV-infected populations either comparable to [19, 38], lower than [339, 340], or higher than [120, 341] their uninfected counterparts.

2.15 Arterial structure and function

2.15.1 Atherosclerosis, CVD risk and HIV

Atherosclerosis is characterized by plaque formation in the inner coronary artery walls and it is assessed using cIMT. Endothelial activation is an initial stage in process of atherogenesis [298]. In response to proatherogenic stimuli, the endothelium alters NO production, leading to disturbance in vascular tone, increases endothelial permeability, allowing the intake of lipids and expresses chemokines like monocyte chemoattractant protein-1 (MCP-1) and soluble adhesion molecule, which results in leukocyte trans-endothelial migration [298, 342, 343]. Further, in the intima, the monocytes differentiate into macrophages, expressing receptors that facilitate lipid uptake. During lipid uptake and accumulation, macrophages transform into foam cells. These foam cells initiate atherosclerotic lesions, which are later characterized by plaque formation [302]. The plaque can either be stable or unstable [344]. Stable plaques regress or are fixed or develop slowly whereas unstable plaques are complicated by erosion, fissure, rupture and cause stenosis, thrombosis, infarction [344].

Unstable plaque plays a critical role in promoting CVD risk. When the unstable plaque ruptures, contents of the plaque enter the circulating blood leading to thrombosis [345]. Atherosclerosis is characterised by the accumulation of fatty substances in the arteries, which can cause heart disease and stroke [294]. Atherosclerotic plaque is susceptible to rupture and when it does it causes blockage in the arteries that supply the heart and brain, leading to heart and stroke [151]. Pathologic thickening of the intima leads to fatty streak which forms fibrous cap atheromas and in turn plaques leading to sudden cardiac death [151].

The influence of HIV in atherosclerosis is mediated by the HIV proteins, namely gp120, Tat and Nef [302]. In vitro studies show that both gp120 and Tat, activate the expression of adhesion molecules [346] and Nef together with gp120 reduce the production of endothelial nitric oxide [347, 348]. Also, Nef increases inflammatory cytokine (TNF- α) release from the macrophages and support the secretion of MCP-1 from the endothelial cells [349, 350]. The MCP-1 is further stimulated by Tat protein to mediate monocyte transmigration into the endothelial cell [351]. Finally, Nef and gp120 may induce endothelial apoptosis, which could promote fibro-atheroma rupture/erosion, resulting in the formation of an acute thrombus [352, 353]. Moreover, HIV is marked by inflammation and endothelial injury, even beyond the use of ART [191, 297] – these markers are hallmark of the development of atherosclerosis [300, 354, 355]. Last but not the least, cardiovascular risk factors such as hypertension [356, 357], dyslipidaemia [20, 197] and diabetes [20, 36] are frequently observed in the HIV-infected population, the possible role of these comorbidities on CVD development has been previously reported [90, 104, 358] (**Figure 3**).

Previous studies have hypothesized an increased risk of atherosclerosis - reflected by cIMT, in people living with HIV [53] compared to their uninfected counterparts [40, 47, 50]. In a case-control study that compared HIV-infected individuals and uninfected controls matched for age and sex, Hsue et al [49] showed that HIV infection per se is independently associated with an increase in cIMT. In the latter study, it was also shown that the progression in carotid wall thickening occurs more rapidly in HIV-infected individuals than uninfected controls [49]. Likewise, Lorenz et al [40] and Maggi et al [39] reported higher cIMT in HIV-infected compared to uninfected controls residing in Europe.

On the other hand, Mosepele et al [52] found a similar cIMT in 208 HIV-infected individuals with viral suppression on ART and 224 uninfected controls living in the SSA region. Another cross-sectional study included HIV-infected individuals on ART (N=133) and ART-naive (N=53) and uninfected controls (N=54) to assess the correlation between HIV infection, ART use and risk of atherosclerosis. The study reported a cIMT > 1 mm for the treated HIV-infected (51.1%), ART-naive (39.6%) and uninfected controls (48.1%) but the prevalence was not significantly different in all the three groups [359].

Concerning ART, prospective data from a large international multi-cohort observational study, the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study, showed an increased relative risk for myocardial infarction in HIV-infected individuals on ART over seven years [360]. The PREVALEAT study [39] demonstrated that cIMT increased among HIV-infected individuals treated with PIs in comparison with ART-naïve individuals or HIV-infected individuals treated with NNRTI. Finally, in the AIDS Clinical Trial Group (AACTG 5078) trial, no correlation was found between the use of PIs, HIV infection and cIMT [361]. The potential proatherogenic effects of ART include leukocyte adhesion to the endothelial cell with the use of Efavirenz [362]. Abacavir is associated with heightened platelet reactivity [363] and ritonavir exerts its effect by reducing the cholesterol efflux from macrophages, reduce the synthesis of NO and endothelial cytotoxicity [364, 365]. Furthermore, PI promotes the accumulation of cholesterol in the macrophages [366].

2.15.2 Arterial stiffness, CVD risk and HIV

Arterial stiffness also referred to as arteriosclerosis, represents the stiffening of the arteries [192], which alters the dampening function of the large arteries [367]. Arterial stiffness collectively refers to the distensibility, compliance and elastic modulus of the arterial system [368]. A loss of arterial elasticity or an increase in arterial wall rigidity increases arterial stiffness, which alters the buffering role of the arterial system [368].

Arterial stiffness is a useful indicator of future CVD since changes in arterial stiffness are identified before the clinical manifestation of vascular diseases [369]. In particular, mounting evidence has shown that arterial stiffness is an independent risk factor for CVD morbidity and mortality in the general population [370], hypertensive individuals [43, 44], patients with renal disease [371] and with IGT [372]. The measurement of cfPWV is considered a gold standard for measuring arterial stiffness because it is non-invasive, reproducible and cost-effective [373]. A reference value of 10 m/s and above for cfPWV indicates arterial stiffness [373].

In the spectra of HIV disease, it is still debatable whether the virus itself or ART relates to increased arterial stiffness in people living with HIV. The effects of HIV on the arterial may be directly (HIV itself) or through indirect mechanism including inflammation and endothelial activation [374, 375] (**Figure 3**). Arterial stiffness may also be induced by the ART [42] as well as ART-associated comorbidities such as hypertension [51, 376], dyslipidaemia [377], diabetes [56, 376] and obesity [56]. Concerning HIV infection *per se*, arterial stiffness was reported to be higher in HIV infected individuals than uninfected controls [378], even when the control group are matched for age, sex and BP [375]. These researchers also found that HIV infection was an independent predictor of greater PWV in HIV-infected individuals [375]. In a cross-sectional study including age and sex-matched study population, PWV was higher in the HIV-infected ART-naïve individuals compared to treated HIV-infected individuals and uninfected controls [42].

In contrast, others indicated that HIV-infected individuals with and without ART presented with higher PWV than their uninfected counterparts [49, 379], while no difference was seen in arterial stiffness in HIV-infected South Africans (either on ART or not) compared to HIV-uninfected controls [24]. A cross-sectional study including Rwandan women assessed arterial stiffness using radial artery tonometry and the study reported no association between HIV infection and the arterial wave reflection. The Rwandan population were younger and had lower BP [380]. Monteiro et al [51] similar cfPWV in HIV-infected individuals ART (66% on NNRTIs and 87% on PIs) and uninfected individuals. In that study, PWV was positively associated with age, sex and BP [51]. Nonetheless, van Vonderen et al. [381] reported an increased stiffness in the femoral artery after 24 months of taking ART.

Overall, most studies on HIV infection and arterial stiffness included Western populations with fewer studies done on SSA populations. Moreover, most of the studies are cross-sectional, with a relatively smaller sample size. Cross-sectional studies do not fully explore the long-term association which are vital to understanding the complex association between HIV and CVD risk factors. Furthermore, the

interpretation of previous studies in HIV infection and arterial stiffness is not easy based on the different methods used to assess arterial stiffness.

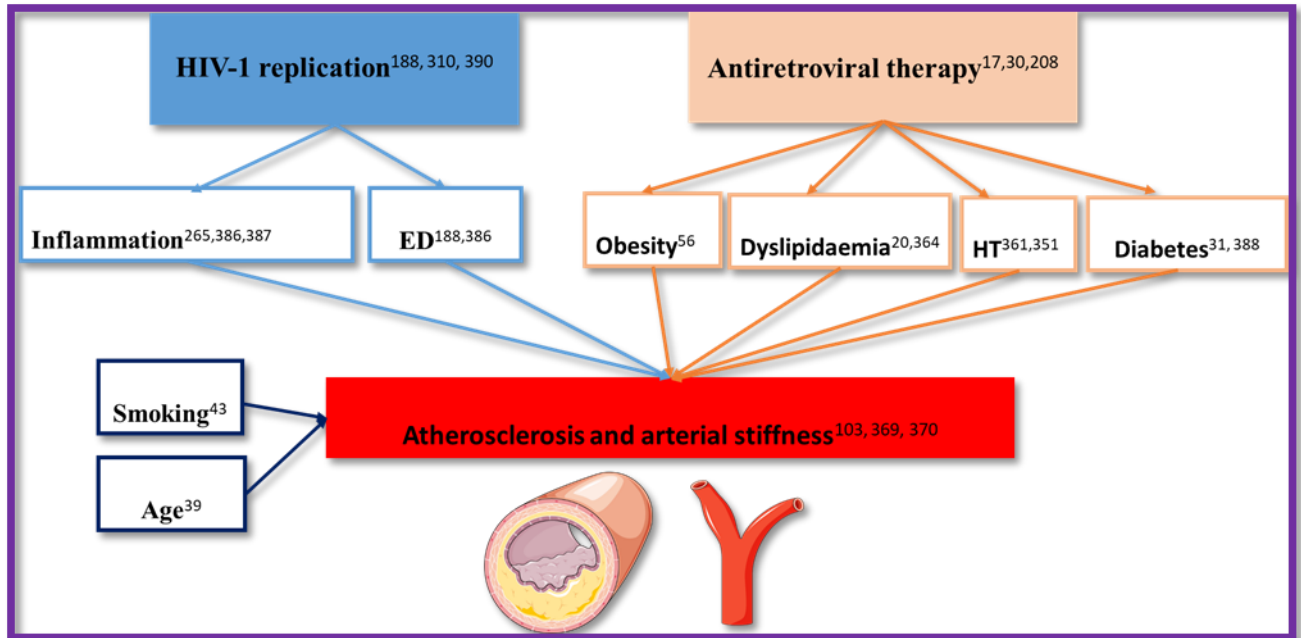


Figure 3. Factors contributing to atherosclerosis and arterial stiffness in HIV-infected individuals.

HIV-1, human immunodeficiency virus -1; ED, endothelial dysfunction; HT, hypertension. Images from Servier Medical Art.

2.16 Renal disease, CVD risk and HIV

Renal function can be measured using creatinine clearance (CrCl), estimated glomerular filtration rate (eGFR) and urinary albumin-creatinine ratio (uACR) [382]. Since eGFR is not easy to measure in practice, it can be calculated using the following equations, Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI). The former equation is widely used to measure and was originally developed in patients with chronic kidney diseases [190], as a result, it may overestimate the eGFR [22]. Alternatively, the CKD-EPI was designed and it is considered to be more accurate compared to the MDRD equation at eGFR of $>60\text{ml/min/1.73m}^2$ [383]. With the CKD-EPI equation an individuals' age, ethnicity and serum creatinine can be controlled [383]. The categories for uACR to define albuminuria are as follows: $<30\text{ mg/g}$ as normal-to-mildly increased, $30\text{--}300\text{ mg/g}$ moderately increased and $>300\text{ mg/g}$ as severely

increased [384]. The physiological functions of albumin include the transport of substances in the blood to its main target organs, maintenance of the pH and osmotic pressure of blood plasma [191]. Albuminuria reflects the presence of protein in the urine [385].

The combination of both microalbuminuria and a reduction in eGFR almost doubled the risk for all-cause mortality compared with individuals with either a reduction in eGFR or microalbuminuria among elderly individuals with diabetes [386]. Moreover, it was reported that a decrease in eGFR is associated with a 1.5- and 1.9-fold increased risk for CVD and mortality, respectively in type 2 diabetic individuals with renal complications [387]. In a prospective study including participants with type 2 diabetes, participants diagnosed with microalbuminuria had a 1.8-fold increased risk for CV mortality during 12 years of follow-up compared with participants with normal uACR [388]. In the Heart Outcomes Prevention Evaluation trial, the presence of microalbuminuria was independently associated with increased risk for CVD and mortality in individuals with and without diabetes [389].

The diagnosis of CVD is now prevalent in a population with chronic kidney diseases (CKD) and in turn, CKD is a significant risk factor for CVD [390]. Conventional risk factors (such as hypertension and diabetes mellitus) [389, 391] and specific CKD risk factors such as albuminuria, inflammation [392], immune activation [393], oxidative stress [394], endothelial dysfunction [395] causes remodelling of the myocardium and blood vessels [396]. This process may contribute to the development and progression of cardiomyopathy, atherosclerosis, arterial stiffness, [396] and calcification and subsequent ischemic heart disease, heart failure [397]. Kokubo et al [391] have shown that hypertension increases the incidence of CVD in people with chronic kidney disease (CKD) compared to people with normal kidney function. The prevalence of left ventricular hypertrophy (LVH) in patients with CKD is increased; especially in patients with an eGFR $<30\text{ml/min/1.73m}^2$ [398]. Renal diseases are commonly known to advance with ageing and are more prevalent in the elderly than younger people. Moreover, CKD is associated with hypertension and it increases CVD risk [399].

In the past, renal disease in HIV-infected individuals occurred as a result of HIV-associated nephropathy and HIV-immune complex renal disease [400]. Yet, in the post-ART era causes have shifted towards comorbid diseases such as hypertension, diabetes mellitus as well as effects of ART such as tenofovir- induced renal tubular toxicity [401]. However, considering HIV and its treatment, renal diseases are reported to manifest in the HIV-infected population independent of age [402]. Concerning ART, tenofovir and indinavir are known to be nephrotoxic and have many deleterious effects when used in combination with lopinavir/ritonavir [403].

Kidney diseases in HIV-infected individuals are commonly characterised by higher urinary protein excretion and elevated levels of creatinine [28]. A report of a study done in Tanzania, including HIV-infected individuals taking ART, highlighted that 25% of the HIV infected individuals have decreased eGFR and 72% have microalbuminuria [28]. People living with HIV have a 3-to-5 fold increase risk for kidney diseases compared to uninfected controls [404]. Another form of renal dysfunction is glomerular hyperfiltration ($\text{eGFR} \geq 140 \text{ ml/mm/1.73 m}^2$), which refers to an elevated glomerular filtration rate (GFR) to pathologically high levels [405]. Hyperfiltration generally accepted as a risk factor for proteinuria, in the presence of other metabolic and CV complications [406]. Some thymidine analogues (NRTIs and NNRTIs) are metabolised and excreted by the kidney, which can lead to nephrotoxicity and long-term use may cause acute kidney injury [250]. Indirect effects such as dyslipidaemia, diabetes mellitus and other metabolic complication are associated with hyperfiltration [407, 408].

From these observations, it may be expected that the prevalence of kidney diseases in HIV-infected individuals will increase in the future. This will in turn have significant consequences for the management of HIV-infected individuals since renal insufficiency affects ART drug clearance and the risk of ART toxicity [409].

It is evident from the literature that factors related to HIV, ART toxicities, cardio-metabolic factors (BP, lipid and glucose alteration), inflammation, oxidative stress, endothelial function coupled with

liver and renal dysfunction play a critical role in the development of CVD in the HIV-infected population. The factors contributing to increased CVD risk in HIV-infected individuals are summarised in **Figure 3** below.

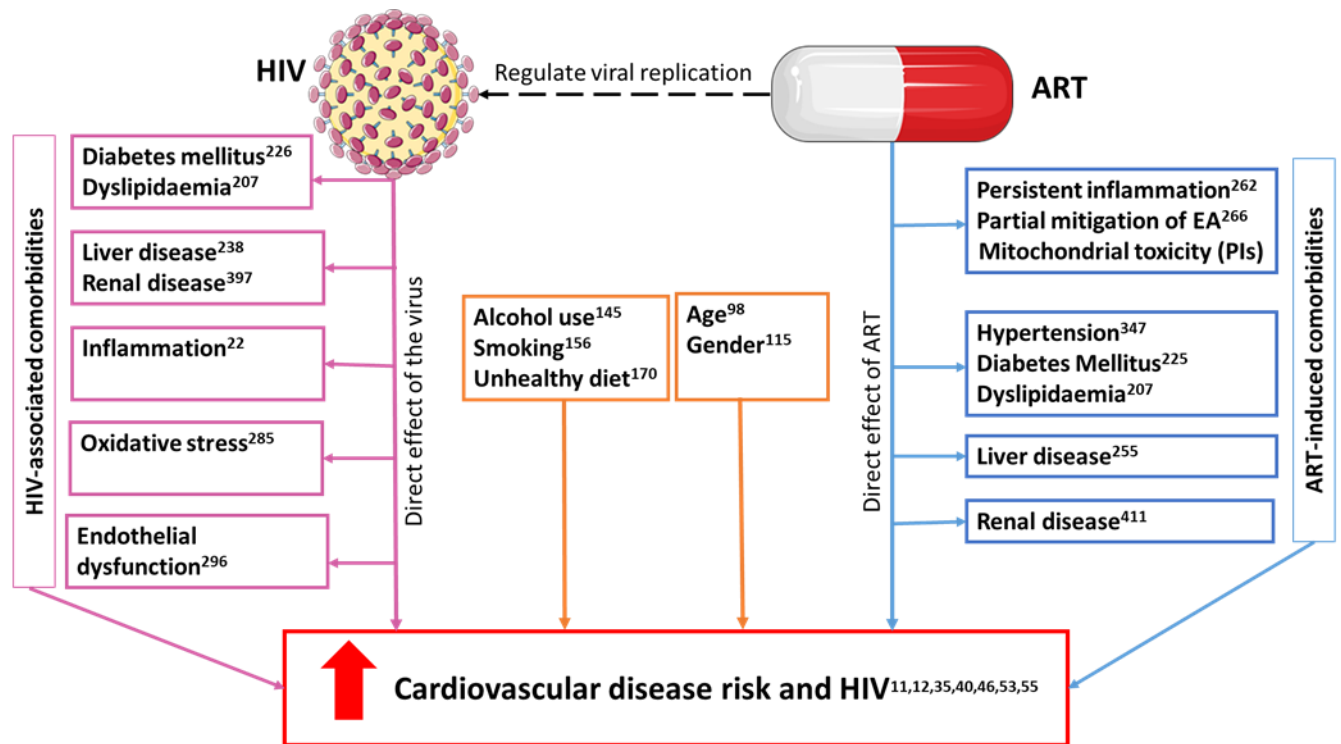


Figure 4. Summary of factors contributing to increased CVD risk in HIV-infected population.

HIV, human immunodeficiency virus; ART, antiretroviral therapy; BP, blood pressure; EA, endothelial activation; soluble intracellular adhesion molecule (ICAM-1) and soluble vascular cell adhesion molecule (VCAM-1).

3. PROBLEM STATEMENT AND MOTIVATION

Since CVD is one of the most important causes of morbidity and mortality in HIV-infected populations [46] and with South Africa accounting for the highest number of people living with HIV. As such, studies focusing on CVD risk factors and outcomes in this population are paramount. Our understanding of the mechanisms driving CVD in HIV infection remains limited, due to limited evidence from longitudinal data, particularly for the SSA population. People living with HIV seem to be at a higher risk for CVD as a result of complex interactions between HIV infection, ART, chronic inflammation, immune activation and traditional CVD risk factors (such as hypertension, dyslipidaemia and diabetes mellitus) [55]. Hypertension is commonly observed in HIV-infected

people on PI and is one of the major risk factor contributing to CVD development [11,20]. However, the studies reporting on hypertension in HIV-infected people remain controversial, especially in SSA settings [4,32]. HIV infection induces oxidative stress and inflammation – key mediators between endothelial dysfunction and changes in arterial structure and function as well as renal dysfunction [46]. Few studies report on the long-term association between measures of arterial structure and function, with endothelial activation and cardio-metabolic diseases in HIV infected individuals [25]. Existing studies are limited by cross-sectional designs and small sample sizes and was mostly done in Western populations [10, 410, 411]. Furthermore, HIV-1 subtypes may contribute to the differences in CVD risk factors and CVD development. In African countries, HIV subtype C is mostly evident whereas sub-type B is prevalent in Western countries [412]. The outcomes of these viral subtypes on the cardiovascular system are not clear. To better the understanding on the risk of long-term CVD development in the HIV-infected individuals living in SSA region, it is necessary to investigate the potential effect of the traditional risk factors, HIV-infection as well as ART-associated comorbidities on cardiovascular health.

This thesis was written in article format and therefore the main motivation for each of the three manuscripts was based on available findings on the CVD risk in the HIV-infected population. Detailed and motivations for these manuscripts are expressed in each of the three manuscripts (Chapters 3, 4 and 5) and Chapter 6 (Summary of the main findings and conclusions) of this thesis.

4. RESEARCH AIM, OBJECTIVES AND HYPOTHESES

4.1 Aim

The central aim of this study was to investigate the longitudinal cardiovascular health of a black South African cohort infected with HIV and review of the literature based on studies conducted in the SSA region.

4.2 Objectives and hypotheses

4.2.1 Manuscript one

Objectives

To review the relevant literature comparing estimates of BP and hypertension prevalence in treated HIV-infected patients compared to untreated and HIV uninfected controls in SSA countries.

Hypotheses

- BP estimates and hypertension prevalence will be higher in the treated HIV-infected patients compared to uninfected controls
- BP estimates and hypertension prevalence will be higher in the treated HIV-infected patients compared to untreated HIV-infected controls.

4.2.2 Manuscript two

Objectives

To profile and compare the cardio-metabolic factors (systolic and diastolic BP, WC, BMI, TC, LDL-c, HDL-c, TG, HbA1c and glucose levels), inflammation (CRP), oxidative stress (GGT) liver function (AST, ALT and GGT) and renal function (serum creatinine, uACR and eGFR) of HIV infected participants and HIV uninfected matched controls at baseline (2005) and follow-up (2015).

Hypotheses

- HIV-infected individuals will have higher systolic and diastolic BP readings than the HIV-uninfected controls from baseline to follow-up
- HIV-infected individuals will have a lower BMI and WC when compared to the uninfected controls as well as a decrease in BMI and WC over time.
- HIV-infected individuals will have higher TG, TC, LDL-c and HDL-c than HIV-uninfected controls at baseline (2005) and follow-up (2015) as well as greater increases in TG, TC, LDL-c and HDL-c over time.
- Plasma glucose and HbA1c will be similar in the HIV-infected group and uninfected controls at baseline and follow-up.

- HIV-infected patients will have higher CRP levels than uninfected controls at baseline and follow-up as well as a greater increase in CRP over time.
- HIV-infected patients will have higher GGT levels (as a marker of oxidative stress) than uninfected controls at baseline and follow-up as well as a greater increase in GGT over time
- Markers of liver function namely, GGT, AST and ALT will be higher at baseline and follow-up and will have a greater increase over time in the HIV-infected individuals than in uninfected controls.
- Renal function markers such as serum creatinine and uACR will be higher and eGFR lower in the HIV-infected individuals than in uninfected controls at baseline and follow-up. Serum creatinine and uACR will increase and eGFR decreases more over 10 years in the HIV-infected group than in the uninfected controls.

4.2.3 Manuscript three

Objectives

To determine whether measures of arterial structure (cIMT) and function (cfPWV) will be higher in the HIV-infected individuals living with HIV for more than five years compared to age, sex and locality matched uninfected controls.

To determine whether baseline endothelial activation (ICAM-1 and VCAM-1) at baseline is related to measures of arterial structure and function (cIMT and cfPWV) measured after 5 years in the HIV-infected and uninfected groups.

To determine whether baseline cardiovascular risk factors (CRP, GGT, HIV status, ART use, age, sex, BMI, mean arterial pressure, HbA1c, TG: HDL-c ratio and tobacco use) is related to measures of arterial structure and function (cIMT and cfPWV) after 5 years in the HIV-infected and uninfected groups.

Hypotheses

- Measures of arterial structure (cIMT) and function (cfPWV) will be higher in the HIV-infected individuals living with HIV infection for more than five years compared to age, sex and locality matched uninfected controls.
- Baseline markers of endothelial activation namely, ICAM-1 and VCAM-1 will associate positively with measures of arterial structure and function (cIMT and cfPWV) at follow-up in both the HIV-infected and uninfected groups.
- Baseline cardiovascular risk factors (CRP, GGT, HIV status, ART use, age, sex, BMI, mean arterial pressure, HbA1c, TG: HDL-c ratio and tobacco use) will correlate positively with measures of arterial structure and function (cIMT and cfPWV) at follow-up in both the HIV-infected and uninfected groups.

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Chapter 2

Study Design and Research Methodology

1. COMPONENTS OF THIS THESIS

The data that was used for this study was mainly from the multi-national Prospective Urban and Rural Epidemiology (PURE) study and the narrative review of 19 studies done in Sub Saharan Africa (SSA) (**Figure 1**). The main objective of the multinational PURE study is to examine the changes in lifestyle, risk factors and causes of chronic diseases, through periodic standardised data collection [1]. In line with the objectives of the PURE study, a narrative review was conducted to clarify whether blood pressure (BP) and hypertension estimates are higher in the SSA population living with human immunodeficiency virus (HIV) compared to their uninfected counterparts.

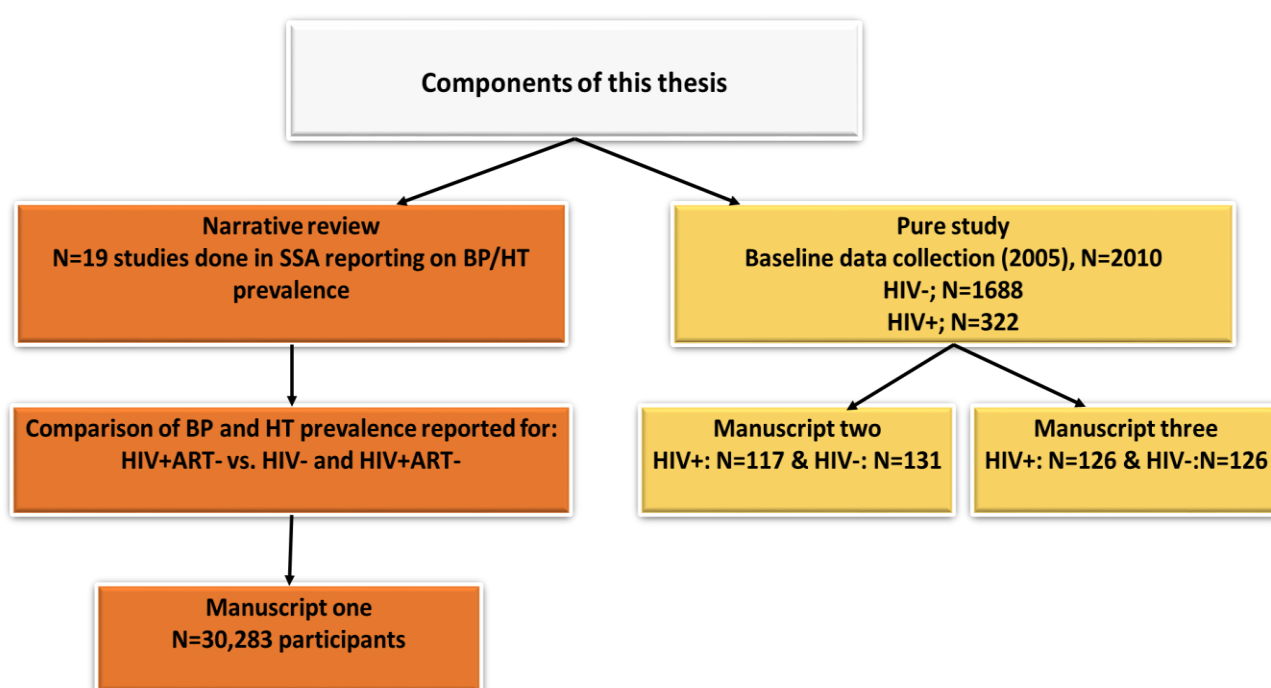


Figure 1. Components of this study

N, number; BP, blood pressure; HT, hypertension; HIV-, HIV-uninfected individuals; HIV+, HIV-infected individuals.

This study was compiled in the article format and manuscript one (Chapter 3) was a narrative review of 19 studies reporting on BP and prevalence of hypertension among HIV-infected people on ART versus untreated HIV-infected and uninfected counterparts residing in SSA countries, while manuscript two and three (Chapters 4 and 5, respectively) made use of existing data from the South African leg of the PURE study conducted in the North West Province.

2. NARRATIVE REVIEW METHOD

2.1 Literature search for the narrative review

The data search was done using PubMed and EBSCO Discovery Service (EDS). The following search terms were used in these search engines: HIV or human immunodeficiency virus, hypertension or high BP, Sub Saharan Africa or Sub-Saharan Africa. The same search keywords were repeated but SSA was replaced with the specific countries found in this region such as South Africa, Nigeria, Zimbabwe, Lesotho, Cameroon, Rwanda, Ghana, Congo and Ethiopia. Based on the reviews done for SSA, countries that were commonly stated in the articles that were reviewed were targeted. This study pooled studies that reported actual BP readings, raised/higher BP, diagnosed and prevalence of hypertension, isolated systolic and diastolic hypertension and use of hypertension medication. Two searches were done for this study, with the first, performed from 30 October 2018 to 31 July 2019 and followed by a second search on 09 May 2020.

2.2 Inclusion and exclusion criteria of the narrative review

Table 1. The inclusion and exclusion criteria as well as the justification used in the article selection of the narrative review

Review study	
Inclusion for the review study	Justification
Studies reporting on the estimates of BP and/ or hypertension prevalence in SSA countries including Africans	Previous studies conducted in the SSA region and reporting on BP and hypertension for the HIV-infected individuals compared to their uninfected controls had contradictory findings. Hence, there is a need to clarify the BP estimates in this population. The BP estimates differ by regions and ethnicity.
Original articles	The main and fundamental purpose of writing a review is to create a readable synthesis of the

	best resources available in the literature for an important research question or a current area of research. These resources include original articles with clearly defined study designs and results as well as drawn conclusions.
Articles published between 2005 - 2020	Research topics change significantly over time and it is important to keep information that is current and informed. Moreover, a narrative review used current research to synthesise information on a particular topic. The roll-out of ART in most African countries started in the year 2004 and beyond.
Exclusion for the review study	Justification
Pregnant women and children	Due to the known influences of hormones on blood pressure pregnant women are excluded. It is known that arterial structure for children and adults differs and BP is diagnosed differently for children and adults.
No access to full-text	The abstract does not deliberate on the full methods, results and conclusion of the articles – aspects that are critical for a good quality review.

2.5 Data extraction for the narrative review

Two reviewers independently assessed the articles for eligibility. Any discrepancies in eligible studies listed were resolved by consensus discussion. Based on the inclusion criteria, 779 records were retrieved and 760 excluded. Nineteen eligible articles were included from eight countries in the SSA

region (**Figure 2**). The following information was extracted: authors, year published, the origin of the study population, study design, population size, age, use and duration of ART, BP measurement/hypertension prevalence, use of antihypertensive medication, comorbidities and indicated significance value.

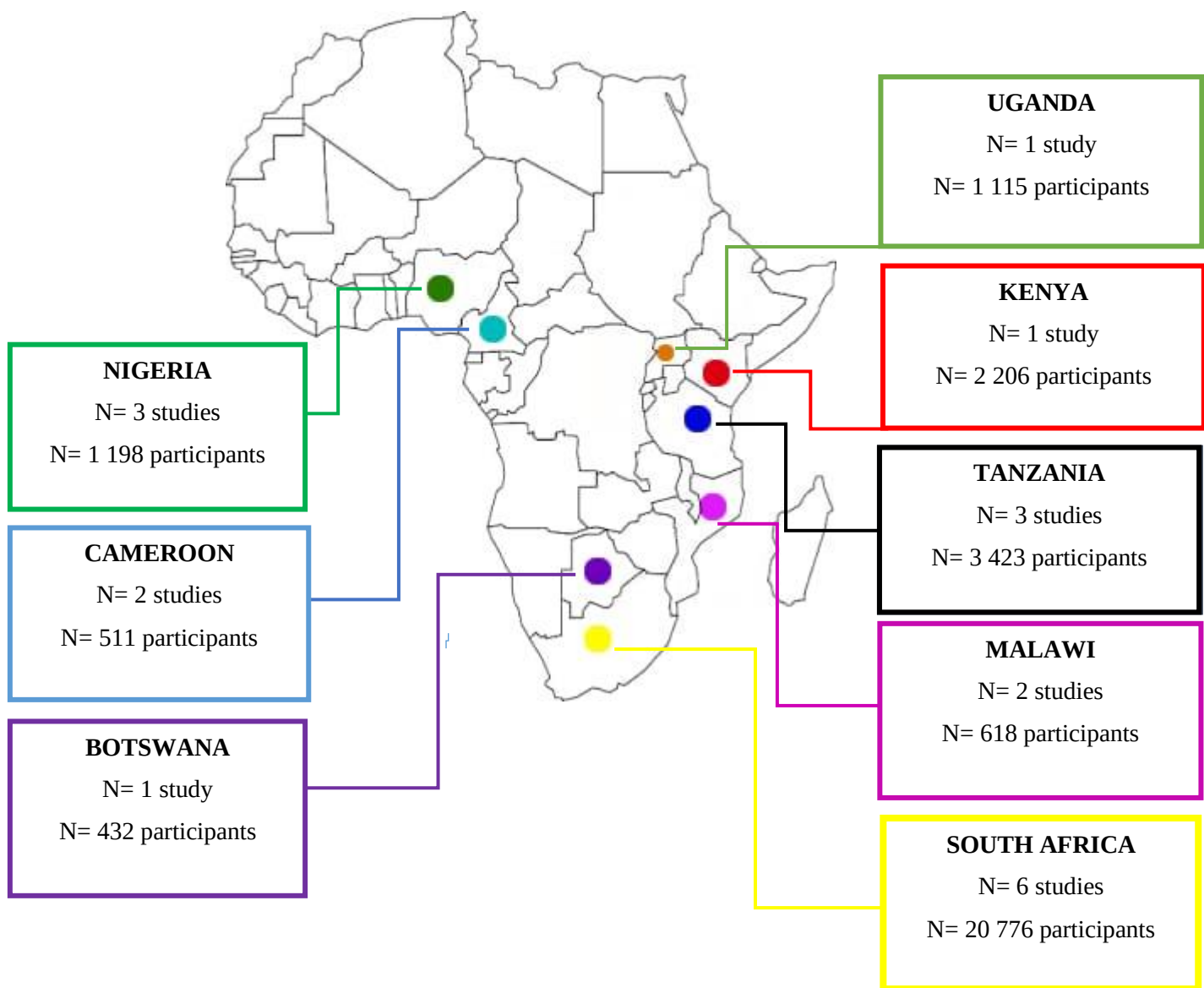


Figure 2. Sub Saharan Africa countries contributing to the data on blood pressure and hypertension prevalence in the narrative review.

3. METHODOLOGY FOR THE PROSPECTIVE URBAN AND RURAL EPIDEMIOLOGY STUDY

3.1 Study design and participants recruitment

This study is embedded within the Prospective Urban and Rural Epidemiology (PURE) study, which is a multinational longitudinal study examining changes in lifestyle and focusing on low-, middle- and high-income countries [1]. The international PURE study was designed to gather information on the social, behavioural, environmental and biological factors that impact on CVD development in 17 countries [1]. For the SA leg of the PURE done in the NWP, the Potchefstroom and Ikageng were selected as the urban areas, while in Ganyesa and Tlaskgameng rural participants were recruited (Figure 3). The urban research settings comprised the Metabolic Clinic and the HealthCare Centre on the North-West University's Potchefstroom campus.

In the rural areas, the research set-up at baseline study (2005) included tents and gazebos on the outdoor at Setlhare Guest Lodge while at the five-year and 10-year follow-up data collections were done inside the buildings of the Setlhare Guest Lodge. In the recruiting phase of the PURE-SA-NWP study, sampling at either the urban or rural settings focused on the issues of both “representativeness” and feasibility of long-term follow-up. In urban areas, it was attempted to enrol all eligible households from three sources: a) households of industrial workers from specified factories, b) geographically defined middle-class housing areas and c) defined slum or shanty town dwellers. A household census documenting the number of people, their ages and health profile was done in 6 000 houses. Census questionnaire was used to collect information on physical and psychological health, socio-economic status, lifestyle habits and available support systems.

The household census was conducted by rigorously trained fieldworkers from the different communities. The fieldworkers maintained communication with the participants by contacting the participants every three months to keep track of any changes in contact information and to capture event information. In the South African leg of the PURE study, performed in the North West Province, 2 010 African men and women, older than 35 years participated at baseline study in 2005 from a sample of 6000 randomly selected households in both rural and urban areas of the North West Province. Of the 2 010 baseline participants, 322 were newly diagnosed with HIV. In 2010, 1 288 participants were successfully followed. Of the 923, who took part in the 10-year follow-up study

(2015), 161 were infected with HIV. Participants were continuously followed over this period by fieldworkers and information on cardiovascular risk markers was assessed and captured.

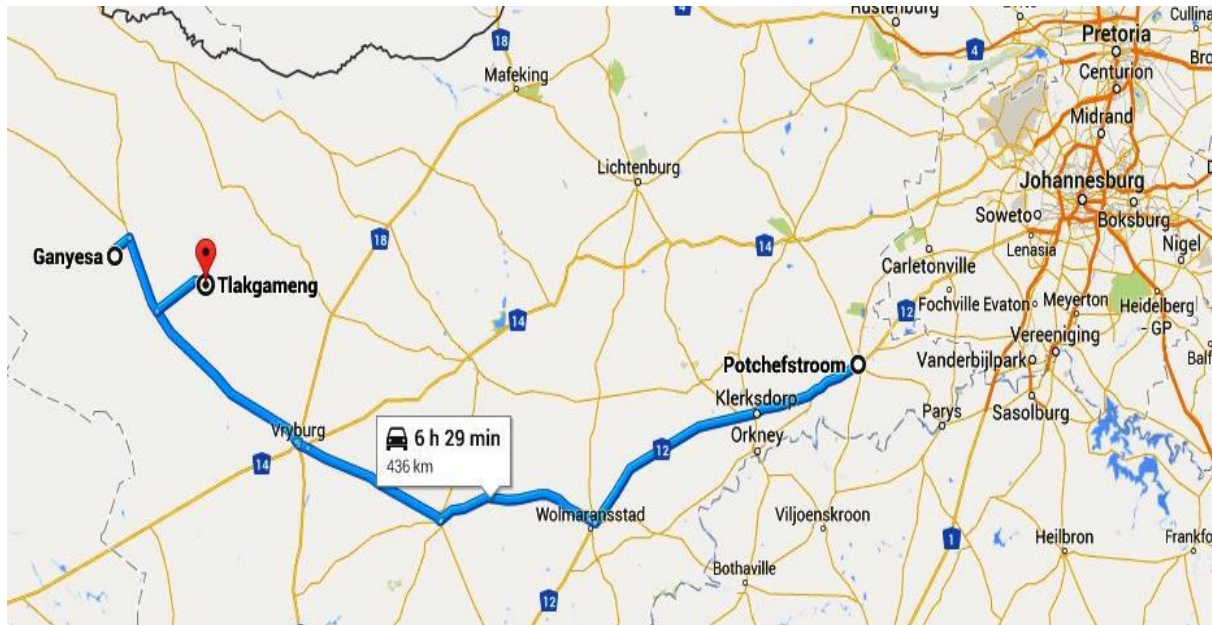


Figure 3. Areas used in the North West Province, leg of the South African of the PURE study

3.2 Ethical considerations

Permission to conduct the PURE study was obtained from the Health Research Ethics Committee of the North-West University (approval number: 2005: 04M10, 2010: NWU-00016-10-A1 and 2015: 00016-10-A1), Department of Health (North West Province) and from the Tribal Chief from each specified area. Likewise, this study was approved by the Health Research Ethics Committee of the North-West University with approval number: NWU-00074-17-S1 (Appendix B). The PURE study complied with all the applicable requirements of the Helsinki Declaration of 1975 and subsequent revisions for the investigation of human participants. Before participation in the PURE study, the participants were fully informed about the research objectives and procedures. In cases where the participants needed clarity, they were given a chance to ask questions. Where necessary, the information was communicated in the participant's home language by trained field workers eloquent in English and Setswana. All participants voluntarily participated in the study and gave written informed consent.

3.3 Inclusion and exclusion criteria of the PURE study

Table 2. The inclusion and exclusion criteria as well as the justification used in the screening phase of the PURE study.

PURE study	
Inclusion criteria for the PURE study	Justification
Black men and women older than 35 years	The PURE study focuses on tracking changes in lifestyle, risk factors and chronic diseases using periodic standardised data collection in urban and rural areas. In the literature cardiovascular diseases (CVD) such as hypertension are growing rapidly in the black population. Moreover, ageing is associated with a higher risk of developing CVD. Men and women were used to determining whether differences occur according to sex.
Participants without self-reported use of any medication for chronic disease and/or conditions.	Individuals with any known diseases or risk factors may influence cardiovascular health.
Eligible participants must reside in either urban or rural households.	Due to the longitudinal nature of the PURE study researchers had to make sure that participants could be followed over the required period.
Exclusion criteria for the PURE study	Justification
Pregnant and lactating women	Due to the known influences of hormones on cardiovascular health pregnant and lactating

	women are excluded.
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4. RESEARCH METHODOLOGY

During the three data collection periods, an appointment was made with each participant that was included in the PURE study. A dedicated driver from the research team was assigned to pick the participants from 08h00 from their home and transported them to the research sites (Potchefstroom, Ganyesa or Tlaskgameng) for their appointments. All the study procedures as well as the research setting were explained to the participants. Thereafter each participant gave written informed consent. The fieldworks assisted with translating the information to the participants in their preferred language or mother tongue.

4.1 Questionnaire

Validated PURE SA adult questionnaires were used to collect data in this study population with help from the trained field workers. The PURE SA adult questionnaire was used to collect data on socio-economic and demographic information, current health status, medical and family history, medication, tobacco and alcohol use. The use of any alcohol or tobacco product was recorded in the PURE Adult questionnaire in terms of the following codes: 0= not answered; 1= formerly used; 2= currently use; 3= never used. Demographic factors such as age and gender together with the use of alcohol and tobacco are known to play an important role in CVD development [2].

4.2 Anthropometric measurements

Standardised procedures for measuring weight, height, hip and waist circumference were used according to the International Society for the Advancement of Kinanthropometry (ISAK) [3] and the same procedures were used at baseline and follow-up. Participants were measured in an appropriate body posture (with the head, shoulders, buttocks and heels touching the flat surface and looking straight ahead), with minimal clothing and barefoot by two researchers in a private enclosure/room. Height was measured to the nearest 0.1 cm with a stadiometer (IP 1465, UK was used in 2005 and 2010 and Leicester height measure, Seca, Birmingham, UK was used in 2015). Weight was recorded

on a portable electronic scale to the nearest 0.01 kg (Precision Health Scale, A & D Company, Japan). Waist circumference was measured at the narrowest point between the lower rib border and the iliac crest and recorded to the nearest 0.1 cm with a steel tape (Holtain unstretchable metal tape was used in 2005 & 2010; Lufkin, Cooper Tools, Apex NC, USA was used in 2015). Body mass index (BMI) - an indicator of overall adiposity was calculated as weight (in kilograms) divided by the square of height (in meters).

4.3 Cardiovascular measurements

4.3.1 Blood pressure

The validated OMRON HEM-757 and OMRON M6 (Omron Healthcare, Kyoto, Japan) devices were used to measure blood pressure at baseline and follow-up study, respectively. Each participant could rest for 10 minutes before the blood pressure (BP) measurement can be taken. The measurement was done with the participants in a seated and rested state. It was necessary for the participant to be properly seated and rested, since talking, crossing legs and unsupported back and arm during BP measurement could raise the BP readings [4]. An appropriately sized cuff was placed the upper right arm, where after the brachial systolic (SBP) and diastolic BP (DBP) was measured and recorded. An appropriate sized BP cuff was used because an under or oversized cuff increases errors in measurement [4]. A second BP measurement was done by the researcher after five minutes resting period and this measurement was used for the statistical analysis. The same protocol was followed during the 2010 and 2015 follow-up studies. Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg and/or the use of antihypertensive medication [5].

4.3.2 Carotid intima-media thickness

Carotid intima-media thickness (cIMT) is a surrogate marker for atherosclerosis – a phenomenon associated with cardiovascular events and all-cause mortality [6, 7]. To assess the arterial structure in this cohort, the SonoSite Micromax ultrasound system (SonoSite, Inc., Bothel, WA, USA) was used, with a 6-13 MHz linear array transducer was used to measure cIMT at follow-up (2015). Images of the left and right common carotid artery were obtained from at least two optimal angles. The segments

were then imaged and measured by prescribed protocols and were digitised and imported into the Artery Measurement Systems (Gothenburg, Sweden) semi-automated software, where a dedicated analysis of the cIMT was performed [8, 9]. The analysis was done on a selected segment of a maximum of 10 mm with good image quality. The borders of the near and far wall of the intima-media, as well as the inner diameter of the vessel, are automatically identified by the program. This was followed by the calculation of the mean IMT and vessel diameter throughout the 10 mm segment, by using around 100 discrete measurements.

4.3.4 Pulse wave velocity

The measurement of cfPWV was done with the participant in a supine position using the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) at follow-up (2015). The use of cfPWV is considered a gold-standard for measuring arterial stiffness [10] and it is an independent predictor of all-cause CVD mortality [11]. With the participant in a supine position, PWV was measured along the descending thoracoabdominal aorta using the foot-to-foot velocity method. The participant's right carotid artery pulse wave was taken with a tonometer while the femoral pulse was evaluated with a femoral cuff placed around the participant's upper thigh. During the measurement, the 80% rule was applied for the calculation of the distance used (common carotid artery (measured with infantometer (SECA))- common femoral artery (done with a tape measure) x 0.8) [12]. The measurement was then, entered onto the SphygmoCor software on a laptop that is linked to a SphygmoCor. Of all currently used distances, 80% of the direct carotid to femoral distance appears to be the most accurate, only overestimating the real distance from the carotid to the femoral artery as measured by magnetic resonance imaging by 0.4% [12]. The result of the PWV calculated as distance/pulse transit time, was then automatically generated. Prior to this PWV measurement and for accurate measurements, the participants were not allowed to eat at least 3 hours before the measurement as well as not talk or sleep during the measurement. The measurement was taken in duplicate and the average of the two measurements was taken. A third measurement was recommended and taken, if the first two cfPWV measurements differed by more than 0.5 m/s and the median of the three cfPWV measurements was calculated and used.

4.4 Blood collection

Before taking part in the measurements, participants were asked to fast overnight for at least eight hours. Sterilised needles were used for each participant and blood was drawn by a qualified registered nurse, with a doctor on call, ensuring that all necessary precautions were taken. Venous blood samples were collected using a winged infusion set. The participants were aware of this procedure beforehand and gave consent. Serum and plasma were prepared according to standardised procedures and were stored at -80 degrees Celsius at the North-West University until analysis. Spot urine samples were also collected and stored in a bio-freezer. In the rural areas (Ganyesa) the samples were frozen at -20 degrees Celsius for no longer than five days before being transferred to -80 degrees Celsius bio-freezers. The storage materials were marked only with the participant's unique number to protect their identity. To optimise data integrity, frozen biological samples are stored in bio-freezers which is connected to an alarm system via cell phone. This alarm notifies the researcher of any malfunction regarding the freezer so that the samples are protected at all times. Only appropriate/eligible researchers have access to this bio-freezer where the samples are stored frozen.



Figure 4. Collection venous blood from the arm using a winged infusion set by a registered nurse

4.5 Biochemical analyses

At baseline (2005) the Konelab20i™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland) and follow (2010 and 2015) the Cobas Integra 400 plus auto-analysers (Roche, Basel, Switzerland) were used to analyse levels of serum high-sensitivity C-reactive protein (hsCRP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG) γ -glutamyltransferase (GGT), aspartate transaminase (AST), alanine transaminase (ALT) and creatinine. Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula [13].

Glucose levels from fluoride plasma samples were determined using the Vitros DT6011 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York, USA) in 2005 and Cobas Integra 400 plus (Roche, Indianapolis, IN) in 2010 and 2015 follow-up studies. Glycated haemoglobin (HbA1c) was determined (by Ion-exchange high-performance liquid chromatography) using D-10 Haemoglobin testing system from Bio-Rad (Hercules, California, United States) for all the data collections. The above-mentioned biochemical markers are risk factors for the development of CVD; hence they were included in the analysis of the study.

Intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were determined using human sICAM-1 and human sVCAM-1 enzyme-linked immunosorbent assays (IBL, Hamburg, Germany) at baseline (2010). Urinary creatinine and albumin were analysed using the Cobas Integra 400 plus (Roche, Basel, Switzerland) in 2005 and 2015. Urinary albumin-creatinine ratio (uACR) was calculated by the following formula: albumin/creatinine at both baseline (2005) and follow-up (2010 and 2015). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations were used to calculate the estimated glomerular filtration rate (eGFR) (ml/min/1.73 m²) [14] in 2010 and 2015.

4.6 Human immunodeficiency virus testing

The South African National Department of Health protocol was followed to perform the HIV testing. All HIV testing procedures took place in a private room, with the participant and the HIV counsellor. The participants gave written informed consent for HIV testing. They were given pre-counselling before they could be tested by a qualified HIV counsellor and they also received post-counselling

after the procedure. The participants were referred to the clinic/hospital if they tested positive. All the participants were treated the same to prevent stigmatisation such as undergoing the same procedure of pre-and post-test counselling.



Figure 5. Testing of human immunodeficiency virus by a counsellor using the rapid card test

The HIV status was determined from whole blood finger prick according to the protocol of the South African Department of Health, using the first response rapid HIV card test (Premier Medical Corporation Limited, Daman, India). In the case of a positive result, the test was confirmed with Pareeshak card test (BHAT Bio-tech, India), SD BIOLINE HIV 1/2 3.0 card test (Standard Diagnostics, INC, Korea) and Abon (Biopharm Corporation Limited Hanyzhou, China) rapid card in 2005, 2010 and 2015 respectively. According to standard procedures, the reliability of the rapid card tests is 96-98%, to prevent wrongly identifying a patient as being HIV infected every positive HIV rapid card test was repeated with a rapid card test from another manufacturer. During the follow-up in 2010 and 2015, the participants that were HIV positive were not tested for HIV again, since they

knew their status from 2005. The CD4 cell counts were determined by the National Health Laboratory services using Beckman COULTER® EPICS® XI™, Fullerton, USA in 2005 and 2010. In 2015 the CD4 cell count was measured at the research site using finger-prick blood and point-of-care device, PIMA™ CD4 (Alere, Jena, Germany).

4.7 The use of antiretroviral therapy

The participants were newly diagnosed with HIV in the baseline study (2005) and were ART-naïve. The HIV-infected ART-naïve participants were referred for follow-up and CD4 cell count determination to start ART according to the South Africa Department of Health guidelines. In follow-up studies (2010 and 2015), the HIV-infected individuals were on a fixed-dose combination comprising of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs).

5. STATISTICAL ANALYSES

The detailed statistics that were used in this study are described within the two manuscripts (Chapter 4 and 5).

6. CONTRIBUTIONS OF THE STUDENT TO RESEARCH MEASUREMENTS

As a post-graduate student, the researcher was diligently involved in the follow-up research measurements of various studies and performed the following activities within the Hypertension in Africa Research Team:

This study

The researcher compiled the original proposal for this study did the literature review, wrote all the three manuscripts and performed all statistical analysis, including cleaning of the data sets, checking for normality and all other advanced statistics which are already described in the three manuscripts. The researcher furthermore prepared all the documents and ethics application to request ethical

approval for this study. The thesis was written under the guidance of both my promoter (Prof. CMT Fourie) and co-promoters (Prof. AE Schutte and Prof CMC Mels).

The PURE, AfricanPredict and EndoAfrica studies

Retrieving medical records and participants information

- The researcher assisted with collecting missing information on the use of ART namely the type of ART regimen, duration on ART, switch in ART over time, an ART start date for HIV-infected participants that took part in the PURE study by retrieving their medical records at the local clinics where they were receiving treatment for HIV.
- Before accessing the medical records at the clinics, the study was presented to the Research Committee of the Potchefstroom Hospital and Department of Health at the district level, to get permission to access the files of the patients that participated in the PURE study and have missing data on ART use and duration in the records.
- PURE questionnaires were also retrieved to gather information on the socio-economic status and education of the participants.

Laboratory and biobank work

- The researcher was responsible for the preparation and processing of blood and urine samples. Samples were collected from the hypertension clinic, centrifuged the blood samples, aliquoted samples into appropriate cryovials and stored in bio-freezers in the African Predict study. Arranging urine samples from the bio-freezer in preparation of biochemical analysis in the PURE study.

Data collection in the hypertension clinic

- The researcher was involved in obtaining pulse wave analysis and PWV using the Sphygmorcor XCEL device. This was done while she was assisting in the Hypertension Clinic, where interaction with participants took place.
- Measurements for electrocardiogram (ECG) were also performed by registering a standard 12 lead ECG (PC-ECG 1200, Norav® Medical Ltd, Kiryat Bialik, Israel), with the participant in a supine position.

- Furthermore, the researcher was responsible for doing continuous arterial blood pressure measurements using the Finometer device (Finapres Medical Systems, Amsterdam, Netherlands).
- The researcher also assisted in the EndoAfrica study with measurements of endothelial function, namely flow-mediated dilation by preparing the participant, explaining the research procedure, calibration, entering participant's information onto the CardioVascular Suite software (CardioVascular Suite™ version 2.9, Quipu, Pisa, Italy, as well as data analysis and export.

Data capturing

- The researcher was responsible for downloading and capturing the data of the flow-mediated dilation.
- The information on ART use, employment history and education in the RedCap programme were also captured by the researcher.

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Chapter 3, Manuscript one

A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls residing in Sub Saharan Africa: a narrative review



A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls residing in Sub-Saharan Africa: a narrative review

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Abstract

Elevated blood pressure (BP) is an important risk factor for cardiovascular disease and mortality in Sub-Saharan Africa (SSA). We reviewed the literature comparing BP in treated HIV-infected populations against untreated and/or uninfected controls from SSA. We conducted a narrative review through PubMed and EBSCO Discovery Service to determine estimates of raised BP and hypertension in HIV-infected patients versus untreated/uninfected controls (1 January 2005 to 31 July 2019 and 9 May 2020). We included 19 eligible studies that compared treated HIV-infected with untreated and/or uninfected controls. In studies comparing treated HIV-infected patients to uninfected controls, studies including 6882 (56.30%) and 21,819 (79.2%) participants reported lower BP and hypertension prevalence, respectively in HIV-infected patients; whereas studies including 753 (6.16%) and 3553 (12.9%) participants showed a higher BP and hypertension prevalence, respectively. In studies comparing treated HIV-infected patients to untreated HIV-infected patients, studies including 1,111 (56.30%) and 2,181 (79.2%) participants reported lower BP and hypertension prevalence, respectively in HIV-infected patients; whereas studies including 753 (6.16%) and 3553 (12.9%) participants showed a higher BP and hypertension prevalence, respectively. In studies comparing treated HIV-infected patients to untreated HIV-infected patients, studies including 1,111 (56.30%) and 2,181 (79.2%) participants reported lower BP and hypertension prevalence, respectively in HIV-infected patients; whereas studies including 753 (6.16%) and 3553 (12.9%) participants showed a higher BP and hypertension prevalence, respectively.

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Summary of instructions to authors used for the preparation of manuscript one

Details of the Journal	
Name of Journal	Journal of Human Hypertension
Impact factor	2.433
Aim and scope	<i>Journal of Human Hypertension</i> is published monthly and is of interest to health care professionals who deal with hypertension (specialists, internists, primary care physicians) and public health workers.
Publisher	Springer Nature
Guidelines for Authors	
Review article	The text should be double spaced with a wide margin. The main body of text (excluding abstract, tables/figures and references) should not exceed 4,000 words.
Preparation of the article	The following instructions were followed: Title page: Title should be brief, informative, of 150 characters or less. Running title should convey the essential message of the paper in no more than 50 characters. Full names of all the authors and their affiliations, as well as the e-mail address of the corresponding author. Abstract: Unstructured abstract with a maximum of 250 words Text: should be divided into the following headings: introduction, materials/subjects and methods, results, discussion (the last paragraph should indicate conclusion), acknowledgements, conflict of interest and funding. References: A maximum of 35 references can be cited using the Vancouver format. Figure Legends: Liberal use of tables and figures is encouraged.
Website for more information	Complete details of the instruction to the author can be viewed using the following web address: https://www.nature.com/jhh/authors-and-referees/gta

(Please note that some of the formats were changed to ensure uniformity throughout the thesis).

**A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls
residing in Sub-Saharan Africa: a narrative review**

Running title: Blood pressure analysis in HIV-infected versus uninfected individuals

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ABSTRACT

Elevated blood pressure (BP) is an important risk factor for cardiovascular disease and mortality in Sub-Saharan Africa (SSA). The literature was reviewed comparing BP in treated HIV-infected populations against untreated and/or uninfected controls from SSA. A narrative review was conducted through PubMed and EBSCO Discovery Service to determine estimates of raised BP and hypertension in HIV-infected patients versus untreated/uninfected controls (01 January 2005 to 31 July 2019 and 09 May 2020). Nineteen eligible studies were included that compared treated HIV-infected with untreated and/or uninfected controls. In studies comparing treated HIV-infected patients to uninfected controls, studies including 6,882 (56.30%) and 21,819 (79.2%) participants reported lower BP and hypertension prevalence, respectively in HIV-infected patients, whereas studies including 753 (6.16%) and 3,553 (12.9%) participants showed a higher BP and hypertension prevalence. Lastly, 4,588 (37.54%) and 2,180 (7.91%) participants showed no difference in BP and the prevalence of hypertension. When comparing BP of treated versus untreated HIV-infected patients, studies including 5,757 (44.2%) patients reported lower BP in treated patients; while studies with 200 (1.53%) patients showed higher BP and 7,073 (54.28%) showed no difference in BP. For hypertension status, studies with 4,547 (74.5%) patients reported a lower prevalence of hypertension in treated patients; whereas studies with 598 (9.80%) patients showed higher prevalence; and 959 (15.7%) no difference in prevalence between treated versus untreated HIV-infected patients. In studies conducted in Sub-Saharan Africa, most of the findings indicate lower BP and/or prevalence of hypertension in treated HIV-infected individuals compared to untreated and uninfected controls.

Keywords: Human immunodeficiency virus, raised blood pressure, hypertension, Sub-Saharan Africa.

INTRODUCTION

According to the World Health Organization Progress Monitoring Report on Non-communicable Diseases of 2017, approximately one billion people globally are affected by raised blood pressure (BP) [1], making it a major public health concern. Elevated BP is well documented as a modifiable risk factor for the development of cardiovascular disease (CVD) and cardiovascular mortality [2]. The CVD burden is rising disproportionately among low- and middle-income countries within Sub-Saharan Africa (SSA), where almost half of the premature deaths are due to CVD [3].

In addition to raised BP, the SSA region is also faced with higher rates of infectious diseases, particularly infection with the human immunodeficiency virus (HIV) [4]. SSA contributed about 66% of the new HIV infections globally in 2017 [5]. This colliding burden of communicable and non-communicable diseases poses a health threat in a region with restricted financial support and qualified trained personnel to deal with the impact effectively [6].

After the initiation of the antiretroviral treatment (ART) roll-out programme in SSA, the use of treatment has increased extensively with an estimation of more than 12.9 million people on ART [7]. The era of ART significantly increased the life expectancy of the HIV-infected population [8] and as a result, people living with HIV are now exposed to non-communicable diseases typically associated with ageing [2]. It has been suggested that HIV-infected patients may suffer early-onset CVD such as hypertension due to premature vascular ageing associated with HIV and immune activation, as well as chronic inflammation coupled with the use of ART [9]. As such, previous studies including HIV-infected populations have reported elevated BP in HIV-infected patients when compared to uninfected controls [10-12]. Conversely, other studies found no increased risk for hypertension [13-15] and similar BP measurements [16-18] in HIV-infected patients compared to their uninfected counterparts.

The association between raised BP and ART-use is also inconclusive, with studies showing raised BP with ART use [13, 19, 20] and while other studies indicate lower BP readings with ART [21-23]. These inconsistencies may be because of different ART treatment regimens across studies and

infrequent use of protease inhibitors (PIs) in comparison to nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) [24]. Since there are opposing findings, it is vital to clarify the prevalence of raised BP and hypertension in HIV-infected populations, particularly when considering the total combined weight of HIV and hypertension on the disease burden in SSA.

Critically, many studies reporting hypertension prevalence or BP in HIV patients did not include an uninfected control group to compare BP estimates [13]. Often studies also focused more on reporting hypertension prevalence rather than mean BP [11, 25]. Without a control group, one cannot effectively determine whether BP is different in HIV-infected patients due to HIV-related aspects or due to traditional cardiovascular risk factors associated with ageing [26]. Before the introduction of ART, HIV-infection was shown to be associated with immune suppression and wasting, resulting in BP lowering [21]. On the other hand, the use of ART is linked to immune reconstitution [27] and increased body weight [24] which tend to be proportional to BP [28].

When comparing studies done in Africa to those of Western origin, research suggests variances in cardio-metabolic risk profiles (dyslipidemia, different fat distribution and insulin resistance) among populations of African descent versus those of European descent [29, 30]. Also, the main virus strain responsible for HIV infection in SSA is HIV-1 subtype C, while HIV-1 subtype B is more predominant in North America and Europe [31]. Besides, the ART regimens used in Africa differ from those in high-income countries such as Europe. For instance, in Africa, the first-line regimen consists of tenofovir+lamivudine/emtricitabine with either efavirenz/nevirapine or dolutegravir [32] while in Europe the preferred regimen consists of abacavir/lamivudine+dolutegravir or tenofovir/alefenamide+emtricitabine+dolutegravir [33]. This is important considering the role of ART on cardio-metabolic disease risk in HIV-infected patients.

Some of these reported inconsistencies or controversies concerning BP in HIV patients may be a result of small population sizes and methodological variance [34]. Whether BP estimates are indeed

higher in the HIV-infected SSA patients compared to their uninfected counterparts remains unclear. We, therefore, reviewed the literature focusing on SSA populations reporting on BP estimates in treated HIV-infected patients against untreated and HIV-uninfected controls.

LITERATURE SEARCH

We searched PubMed and EBSCO Discovery Service (EDS) and the summary of the search terms used in these search engines are included in Table 1. Based on the reviews done for SSA, countries that were frequently mentioned in the articles that were reviewed were targeted. Since this study focused on BP estimates, information was pooled that reported actual BP readings, raised/higher BP, diagnosed and prevalence of hypertension, isolated systolic and diastolic hypertension and use of hypertension medication. The first search was performed from 30 October 2018 to 31 July 2019 and followed by a second search on 09 May 2020. As part of good practice, a protocol for this narrative review was written for guiding the synthesis of this review (Supplementary File).

Table 1. List of the search items

HIV or human immunodeficiency virus, hypertension or high blood pressure, Sub Saharan Africa or Sub-Saharan Africa.
HIV or human immunodeficiency virus, hypertension or high blood pressure, Africa
HIV or human immunodeficiency virus, hypertension or high blood pressure, South Africa
HIV or human immunodeficiency virus, hypertension or high blood pressure, Nigeria
HIV or human immunodeficiency virus, hypertension or high blood pressure, Congo
HIV or human immunodeficiency virus, hypertension or high blood pressure, Ethiopia
HIV or human immunodeficiency virus, hypertension or high blood pressure, Lesotho
HIV or human immunodeficiency virus, hypertension or high blood pressure, Botswana
HIV or human immunodeficiency virus, hypertension or high blood pressure, Cameroon
HIV or human immunodeficiency virus, hypertension or high blood pressure, Zimbabwe
HIV or human immunodeficiency virus, hypertension or high blood pressure, Rwanda
HIV or human immunodeficiency virus, hypertension or high blood pressure, Ghana
HIV or human immunodeficiency virus, hypertension or high blood pressure, Uganda
HIV or human immunodeficiency virus, hypertension or high blood pressure, Kenya

HIV, human immunodeficiency virus.

The studies that were included in the final review were those comparing BP and/or hypertension status that achieved the following eligibility criteria: original research articles, published between 1 January 2005 to 09 May 2020, only black African descent, done in SSA, the inclusion of either uninfected control group (or compared to the general population) and/or untreated HIV-infected group as well as ART-use mentioned. Articles were excluded based on the following listed criteria: non-English articles, no access to full text, non-HIV focus, non-African focus, no quantitative data, pregnant women, children, editorials, letters to the editor, commentary articles, review articles and case reports (Fig 1).

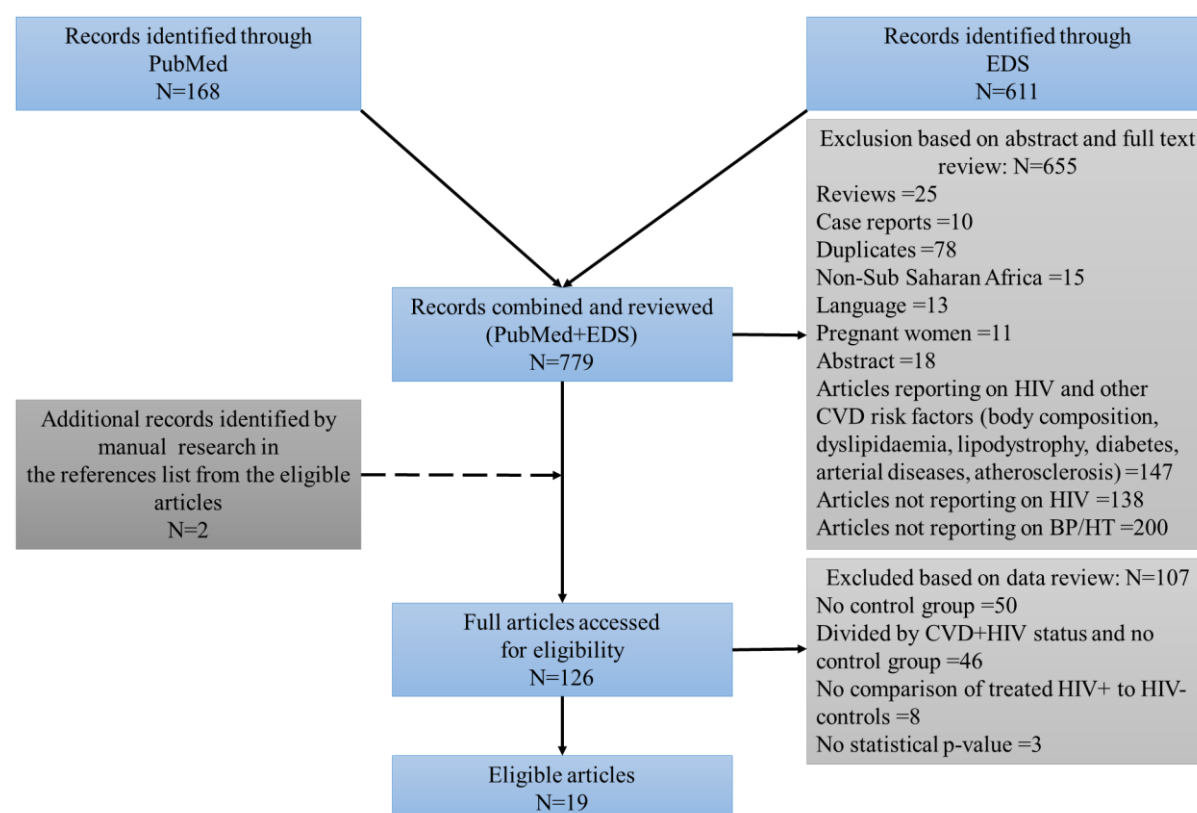


Fig 1. Flow chart of the literature selection process.

N, number; EDS, EBSCO Discovery Service; HIV, human immunodeficiency virus; HIV+, HIV-infected individuals; HIV-, HIV-uninfected individuals; ART, antiretroviral therapy; BP, blood pressure; CVD, cardiovascular diseases.

We reviewed the reference lists of the articles that met the inclusion criteria for additional sources and two articles were added. Two reviewers independently assessed the articles for eligibility. Any

discrepancies in eligible studies listed were resolved by consensus discussion. A total of 779 records were retrieved and 761 were excluded based on the inclusion criteria. From the 19 eligible articles, the following information was extracted: authors, year published, the origin of the study population, study design, population size, age, use and duration of ART, BP measurement/hypertension prevalence, use of antihypertensive medication, comorbidities and indicated significance value (Tables 2, 3 and 4).

Table 2. Summary of the reviewed articles that report elevated blood pressure in HIV-infected patients using ART compared to uninfected and HIV-infected without ART

Articles that report elevated BP in HIV infected patients using ART compared to uninfected and HIV infected without ART ⁻							
Author (s) and country	Study design, N	Age	ART use and duration	BP (mmHg) or HT (%): HIV ⁺ ART ⁺ vs. HIV ⁻	BP (mmHg) or HT (%): HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻	The anti-hypertensive medication	Co-morbidities
1. Pangmekeh et al. 2019 [1] Bamenda Regional Hospital, North West region of Cameroon.	Cross-sectional Sample size N=315 HIV⁺ ART⁺: N=228 HIV⁺ ART⁻: N=87	Mean: HIV⁺ ART⁺: 45.1±10.7 yrs HIV⁺ ART⁻: 41.6±9.16 yrs	All the patients were on the backbone of 3TC. Patients were on: TDF/3TC/EFV (54.91%) AZT/3TC/NVP (19.20%) TDF/3TC/ATV/r (14.73%) AZT/3TC/ATV/r (6.70%)	N/A	36.44% vs. 13.33% p=0.001	Not reported/stated.	Overweight: 33.97% Grade 1 obesity: 8.25%; and Grade 2 obesity: 6.98%

			TDF/3TC/NVP (2.68%) Range: 2-17 years				
2. Mosepele et al. 2017 [2] Botswana, Gaborone	Cross-sectional Sample size N= 432 HIV⁺ : N=208 HIV⁻ : N=224 Enrolment was balanced (but not matched) by gender and age (within 10-year age groups).	Mean: HIV⁺ : 39±5 yrs HIV⁻ : 37±5 yrs	Second-line regimen: 75% on NNRTIS, 25% on PIs. Mean 8.6±2.7 yrs	17% vs. 8% p=0.009	N/A	HIV ⁺ vs. HIV ⁻ Thiazide diuretic 15% vs. 5% Calcium channel blockers 8% vs. 2% Beta-blockers 3% vs. 1% ACE inhibitors 2% vs. 2% HMG Co-A inhibitor 5% vs. 0% Fibrates 0% vs. 0%	Did not report comorbidities.
3. Van Heerden et al. 2017 [3]	Cross-sectional Sample size	Categories: 18 – 25 yrs 26 – 35 yrs	ART use not specified.	39% vs. 34% p<0.001	N/A	Not reported/stated.	Overweight (27% vs. 22%) and obesity (50% vs.

South Africa, rural KwaZulu- Natal	N=570 HIV⁺ : N=189 HIV⁻ : N=381	36 – 45 yrs 46 – 65 yrs >66 yrs	However, the ART that was recommended by the South African DoH was [4]: First line regimen TDF/d4T/ABC + FTC (or 3TC) +EFV/NVP Second-line regimen AZT+3TC+ LPV/r TDF+3TC (or FTC) and LPV/r Third line regimen Raltegravir/Darunavir/ Etravirine Not mentioned.				46%) were higher in the HIV ⁺ than in the HIV ⁻ , respectively. Hyperglycaemia was higher in the HIV ⁻ compared to HIV ⁺ While hyperlipidaemia was similar in both groups (20% vs 20%).
4. Dimala et al. 2016 [5]	Cross-sectional Sample size N=200	Mean: HIV⁺ ART⁺ : 40.2±8.0 yrs	The patients were using NRTIs (d4T, 3TC, FTC, NVP) and NNRTIs (ZDV,	38% vs. 19% p=0.003	N/A	Not reported/stated.	BMI-defined overweight was higher in the HIV ⁺ ART ⁺ (50) than in

Limbe, Cameroon	HIV⁺ ART⁺: N=100 HIV⁺ ART⁻: N=100	HIV⁺ ART⁻: 38.0±10.6 yrs	TDF, EFV). Duration on ART not specified.				the HIV ⁺ ART ⁻ (31%)
5. Edwards et al. 2015 [6] Kenya, Kibera, Nairobi	Retrospective Sample size N=2206 adults HIV⁻: N=1996 HIV⁺: N=210	Median: HIV⁺: 43 [39-50] yrs HIV⁻: 49 [40-56] yrs	First-line regimen TDF/3TC/EFV Second-line regimen LPV/RTV Median years on ART Men: 4 [3-6] yrs Women: 4 [2-6] yrs	HT frequency for 1697 HIV ⁻ and 200 HIV ⁺ : Stage 1 HT: 44.0% vs. 30.9% p<0.0003	N/A	Not reported/stated.	Type 1 diabetes was 13.0% in HIV ⁺ and 0% in HIV ⁻ Chronic Kidney disease (CKD) was 12% overall. HIV⁺ vs HIV⁻ Stage 1 CKD 29.7% vs. 36.2% Stage 2 CKD 58.9% vs 50.5% Stage 3 CKD 10.4% vs. 10.6% Stage 4 CKD 1.0% vs 2.7%
6. Osegbe et al.	Cross-sectional	Mean: HIV⁺ ART⁺:	2NRTIs+1PI N=85 on NRTIs	124.9±20.7 mmHg vs. 114.8±11.7	124.9±20.7 mmHg vs. 121.5±20.7 mmHg	Not reported/stated.	HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻

2015 [7] Nigeria, Lagos	Sample size N=283 HIV⁺ART⁺: N=100 HIV⁺ART⁻: N=100 HIV⁻: N=83 Groups matched for age and sex.	37.5±7.8 yrs HIV⁺ART⁻: 35.6±8.1 yrs HIV⁻: 36.6±7.2 yrs	N=15 on PIs (15%) Participants on ART for more than a year.	mmHg p=0.014	p=0.001 Systolic HT (%): 23% vs. 12% p=0.04 Diastolic HT (%): 20% vs. 11% p=0.08		Obesity: 17% vs. 13% Diabetes: 9% vs. 2% Dyslipidaemia defined by: TC ($\geq$ 5mmol/l): 47% vs. 34% TG ($\geq$ 7 mmol/l): 24% vs. 21% LDL-c ($\geq$ 3.3 mmol/l): 37% vs. 30% HDL-c ($<$ 1.0 mmol/l): 11% vs. 42% Inflammation: 36% vs. 51% Atherosclerosis: 39% vs. 35%
7.	Cross-sectional	Median:	First-line regimen	28.7% vs. 16.3%	N/A	Not reported/stated.	Overweight

Peck et al. 2014 [8] Tanzania, Mwanza	Sample size N=454 HIV⁺ART⁺: N=150 HIV⁺ART⁻: N=151 HIV⁻: N=153	HIV⁺ART⁺: 40 [38-47] yrs HIV⁺ART⁻: 37 [32-44] yrs HIV⁻: 38 [32-46] yrs	TDF+FTC or AZT/3TC and/or EFV or NVP Second-line regimen PI (12.0%) Median 56 [31 to 68] months.	p=0.01 Stage 1 HT (%): 22.0% vs. 13.1% p=0.01 Stage 2 HT (%): 6.7% vs. 3.3%: p=0.01			HIV ⁺ ART ⁺ : 30.0% HIV ⁺ ART ⁻ : 6.0% HIV ⁻ : 21.6% Obesity HIV ⁺ ART ⁺ : 21% HIV ⁺ ART ⁻ : 7.3% HIV ⁻ : 7.2% Diabetes HIV ⁺ ART ⁺ : 0.7% HIV ⁺ ART ⁻ : 0.7% HIV ⁻ : 0% Kidney diseases HIV ⁺ ART ⁺ : 41.3% HIV ⁺ ART ⁻ : 34.4% HIV ⁻ : 25.6%
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N, number; N/A, not applicable; NS, not significant; yrs, years; PA, physical activity; DoH, Department of Health; HIV, human immunodeficiency virus; ART, antiretroviral therapy; HIV⁻, HIV-uninfected; HIV⁺, HIV-infected; HIV⁺ART⁻, HIV-infected ART-naive; HIV⁺ART⁺, HIV-infected taking ART; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood

pressure; HT, hypertension; BMI, body mass index; MetS, metabolic syndrome; CVD, cardiovascular disease, NCD, non-communicable disease; CKD, chronic kidney disease; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; FDC, fixed-dose combination; 3TC, lamivudine; EFV, efavirenz; TDF, tenofovir; NVP, nevirapine; FTC, emtricitabine; AZT, zidovudine; LPV, lopinavir; RTV, ritonavir.

N/A: The blood pressure estimates or hypertension prevalence was not elevated in the HIV+ ART+ compared to HIV- and/or HIV+ ART-.

Table 3. Summary of reviewed articles that reported lower BP in HIV infected patients using ART compared to uninfected and HIV infected without ART.

Articles that reported lower BP in HIV infected patients using ART compared to uninfected and HIV infected without ART ⁻							
Author (s) and country	Study design, N	Age	ART use and Duration	BP (mmHg) and/or HT (%): HIV ⁺ ART ⁺ vs. HIV ⁻	BP (mmHg) and/or HT (%): HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻	The anti-hypertensive medication	Co-morbidities
1. Benzekri et al 2019 [9] Senegal	Retrospective Sample size N=2848 HIV⁺: N=1687 HIV⁻: N=1161	Range 18-84 yrs HIV⁺ (median): 37 [18-72] yrs HIV⁻ (median): 33 [18-84] yrs	Did not specify the type of ART regimen used. N=161 (11.0%) Did not specify the duration	11.9% vs. 17.6% p<0.001	N/A	Not reported/stated.	HIV ⁺ vs. HIV ⁻ Overweight 11.2% vs. 19.2% Obesity 5.3% vs. 10.4%
2. Kelly et al	Prospective	>18 yrs HIV⁺:	Triple-drug ART (did not specify	120 [108-128] mmHg vs. 128 [114-	N/A	Not reported/stated.	HIV ⁺ vs. HIV ⁻ Pre-existing CVD

2019 [10] Blantyre, Malawi	Sample size N=389 HIV⁺ : N=279 HIV⁻ : N=110	36.6 [31.1-43.37] yrs HIV⁻ : 30.8 [30.8-41.2] yrs	individual regimen) Supplied adjunctive Raltegravir (12 weeks) 44 weeks	134] mmHg p=0.001			1% vs. 0.4% Pre-existing HT 2.0% vs. 3.0% Pre-existing diabetes 0.4% vs. 1%
3. Kamtchum-Tatuene et al 2019 [11] Malawi	Cross-sectional Compared HIV-infected and uninfected with ischemic stroke and those without ischemic stroke (IS) Sample size N=61 HIV⁺ with IS: N=19 HIV⁻ with IS: N=42	HIV⁺ with IS: 42 [30.0-52.0] yrs HIV⁻ with IS: 63 [44.0-77.0] yrs HIV⁺ without IS: 47.5 [36.5-60.0] yrs HIV⁻ without IS: 58 [43.0-65.0] yrs	Stavudine (d4T), lamivudine (3TC) and nevirapine (NVP) and alternative first line was zidovudine (AZT), 3TC and NVP With IS 9 (47.4%) Without IS 17 (53.1)	With IS HIV⁺ vs. HIV⁻ 63.2% vs. 90.5% p<0.001 Without IS HIV⁺ vs. HIV⁻ 34.4% vs. 56.6% p<0.001	N/A	Not reported/stated.	The study included HIV-infected patients with stroke (N=19). Endothelial activation was common in HIV-infected patients with stroke. Diabetes with IS HIV⁺ vs. HIV⁻ 3.8% vs. 0.0% Without IS HIV⁺ vs. HIV⁻ 3.1% vs. 1.5%

	N=168 HIV⁺ without IS: N=32 HIV⁻ without IS: N=136						
4. Geldsetzer et al. 2018 [12] South Africa, Kwa-Zulu Natal	2 x Cross-sectional (2004 & 2010) 2004- Before the introduction of ART 2010- After the introduction of ART Results of the 2x cross-sectional were compared. Sample size Men 2004: N=761	Men: 25-54 yrs Women: 25-49 yrs	In 2010, 25% of the HIV-infected participants were on ART. Duration on ART not mentioned.	N/A	Adjusted for age: Blood pressure comparison was done for: Women, N=706 and men, N=239 Women 78.1 [76.7-79.5] mmHg vs. 80.4 [79.4-81.5] mmHg p=0.010 Men 117.3 [114.2-120.4]	Not reported/stated.	Female HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻ Overweight 44.8% vs. 63.7% Obesity 16.2% 31.6% Male HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻ Overweight 9.7% vs. 19.7% Obesity 1.6% vs. 6.1%

	2010: N=759 HIV⁺ 2004: N=157 2010: N=239 ART ⁺ : N=81 ART ⁻ : N=158 HIV⁻ 2004: N=332 2010: N=336 Women 2004: N=1557 2010: N=1825 HIV⁺ 2004: N=335 2010: N=706 ART ⁺ : N=256 ART ⁻ : N=450 HIV⁻ 2004: N=580 2010: N=778				mmHg vs. 121.90 [119.7-124.1] mmHg p=0.020 76.1 [73.7-78.5] mmHg vs. 80.0 [78.3-81.7] mmHg p=0.010		
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6. Van Heerden et al. 2017 [3] South Africa, rural KwaZulu- Natal	Cross-sectional Sample size N=570 HIV⁺ : N=189 HIV⁻ : N=381	Categories: 18 – 25 yrs 26 – 35 yrs 36 – 45 yrs 46 – 65 yrs >66 yrs	ART use not specified. However, the ART that was recommended by the South African DoH was [4]: First line regimen TDF/d4T/ABC + FTC (or 3TC) +EFV/NVP Second-line regimen AZT+3TC+ LPV/r TDF+3TC (or FTC) and LPV/r Third line regimen Raltegravir/Daruna vir/ /Etravirine Duration on ART	Stage 1 HT (%): 16% vs. 25% p<0.001 Stage 2 HT (%): 8% vs. 13% p<0.001	N/A	Not reported/stated.	Overweight (27% vs. 22%) and obesity (50% vs. 46%) were higher in the HIV ⁺ than in the HIV ⁻ , respectively. Hyperglycaemia was higher in the HIV ⁻ compared to HIV ⁺ While hyperlipidaemia was similar in both groups (20% vs 20%).

			not mentioned.				
7. Manne-Goehler et al. 2017 [13] South Africa Agincourt, sub-district	Longitudinal Sample size N= 4547 HIV⁺ ART⁺: N=662 HIV⁺ ART⁻: N=373 HIV⁻: N=3512	Mean: HIV⁺ ART⁺: 55.9 yrs HIV⁺ ART⁻: 54.5 yrs HIV⁻: 63.6 yrs	ART use not specified. However, the ART that was recommended by the South African DoH was [4]: First line regimen TDF/d4T/ABC + FTC (or 3TC) +EFV/NVP Second-line regimen AZT+3TC+ LPV/r TDF+3TC (or FTC) and LPV/r Third line regimen Raltegravir/Darunavir/ Etravirine	38.7% vs. 63.7% p<0.001 129 mmHg vs. 140 mmHg p<0.001	38.7% vs. 43.5% p<0.001 129 mmHg vs. 132 mmHg p<0.001	Treated HT HIV ⁺ ART ⁺ : 57.8% HIV ⁺ ART ⁻ : 49.1% HIV ⁻ : 56.5%	Diabetes HIV ⁺ ART ⁺ : 7.8% HIV ⁺ ART ⁻ : 6.3% HIV ⁻ : 12%

8. Okello et al. 2017 [14] Uganda, Mbarara Municipality	Cross-sectional Sample size N= 1115 HIV⁺ : N=577 HIV⁻ : N=538 Groups were matched for age, gender and neighbourhood.	Median: HIV⁺ : 45 [42-49] yrs HIV⁻ : 46 [43-50] yrs	91.5% were on ART and 3% of them using PIs, 9% using d4T First-line regimen FTC+3TC 64% on ART.	123±18.3 mmHg vs. 127±17.8 mmHg p<0.001 76±11.5 mmHg vs. 78±12.3 mmHg p<0.027	N/A	Not reported/stated.	HIV ⁺ vs HIV ⁻ Overweight 22.7% vs. 24.7% Obese 15.4% vs. 24.7%
9. Msoka et al. 2016 [15] Tanzania, Moshi	Cross-sectional Sample size N=121 HIV⁺ART⁺ : N=34 HIV⁺ART⁻ : N=47 HIV⁻ : N=40	Mean: HIV⁺ ART⁺ : 45±4 yrs HIV⁺ART⁻ : 45±6 yrs HIV⁻ : 45±5 yrs	First-line regimen 3TC+AZT+NVP Median 32.4 months	N/A	122±11 mmHg vs. 128±10 mmHg p<0.05	Not reported/stated.	No mention of comorbidities.

10. Edwards et al. 2015 [6] Kenya, Kiberia, Nairobi	Retrospective Sample size N=2206 adults HIV⁻ : N=1996 HIV⁺ : N=210	Median: HIV⁺ : 43 [39-50] yrs HIV⁻ : 49 [40-56] yrs	First-line regimen TDF/3TC/EFV Second line regimen LPV/RTV Median years on ART Men: 4 [3-6] yrs Women: 4 [2-6] yrs	HT frequency for 1697 HIV ⁻ and 200 HIV ⁺ : Stage 3 HT: 14.5% vs. 32.9% p<0.001	N/A	Not reported/stated.	Type 1 diabetes was 13.0% in HIV ⁺ and 0% in HIV ⁻ CKD was 12% overall. HIV⁺ vs HIV⁻ Stage 1 CKD 29.7% vs. 36.2% Stage 2 CKD 58.9% vs 50.5% Stage 3 CKD 10.4% vs. 10.6% Stage 4 CKD 1.0% vs 2.7%
11. Fourie et al. 2015 [16] South Africa, North West Province	Cross-sectional study Sample size N=309 HIV⁺ART⁺ : N=66 HIV⁺ART⁻ :	Mean HIV⁺ART⁺ : 49±0.96 yrs HIV⁺ART⁻ : 48±0.89 yrs HIV⁻ : 50.3± 0.61yrs	2 NRTIs (D4T, 3TC) and 1 NNRTIs (EFV, NVP) Mean 2.86 ±0.13 years	124±2.54 mmHg vs. 132±1.59 mmHg p<0.05	124±2.54 mmHg vs. 122±2.28 mmHg p<0.05	HIV ⁺ ART ⁺ : 17.6% HIV ⁺ ART ⁻ : 14.0% HIV ⁻ : 9.09%	No mention of comorbidities.

	N=78 HIV⁻ : N=165						
13. Malaza et al. 2012 [17] South Africa, KwaZulu-Natal Province	Population-based survey embedded within a longitudinal study Sample size Whole study population N=14918 Out of the entire study population, only 79% (N=11789) measured their BP and Only 69.9% did an HIV test	Range 15 and older than 70 yrs	ART regimen included: d4T+3TC+EFV d4T+3TC+NVP N=6909 were on ART and N=4688 (67.9%) were females The duration is not specified.	19.5% vs. 27.9% p<0.001	N/A	Not reported/stated.	HIV ⁺ women vs. men Overweight 29.3% vs. 11.4% Obesity 23.8% vs. 4.1% HIV ⁻ women and men Overweight 26.4% vs. 10.4% Obesity 34.1% vs. 5.1%

N, number; N/A, not applicable; NS, not significant; yrs, years; PA, physical activity; DoH, Department of Health; HIV, human immunodeficiency virus; ART, antiretroviral therapy; HIV⁻,

HIV-uninfected; HIV⁺, HIV-infected; HIV⁺ ART⁻, HIV-infected ART-naive; HIV⁺ ART⁺, HIV-infected taking ART; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood

pressure; HT, hypertension; BMI, body mass index; MetS, metabolic syndrome; CVD, cardiovascular disease, NCD, non-communicable disease; CKD, chronic kidney disease; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; FDC, fixed-dose combination; 3TC, lamivudine; EFV, efavirenz; TDF, tenofovir; NVP, nevirapine; FTC, emtricitabine; AZT, zidovudine; LPV, lopinavir; RTV, ritonavir.

N/A: The blood pressure estimates or hypertension prevalence was not lower HIV+ ART+ compared to HIV- and/or HIV+ ART-.

Table 4. Summary of reviewed articles that reported no differences BP in HIV infected patients using ART compared to uninfected and HIV infected without ART

Articles that reported no differences BP in HIV infected patients using ART compared to uninfected and HIV infected without ART							
Author (s) and country	Study design, N	Age	ART use and duration	BP (mmHg) and HT (%): HIV ⁺ ART ⁺ vs. HIV ⁻	BP (mmHg) and HT (%): HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻	The anti-hypertensive medication	Co-morbidities
1. Kelly et al 2019 [10] Blantyre, Malawi	Prospective Sample size N=389 HIV⁺: N=279 HIV⁻: N=110	>18 yrs HIV⁺: 36.6 [31.1-43.37] yrs HIV⁻: 30.8 [30.8-41.2] yrs	Triple-drug ART (did not specify individual regimen) Supplied adjunctive Raltegravir (12 weeks) The patients were 44 weeks on ART	73 [68-80] mmHg vs. 75 [68-82] mmHg p=0.27	N/A	Not reported/stated.	HIV ⁺ vs. HIV ⁻ Pre-existing CVD 1% vs. 0.4% Pre-existing HT 2.0% vs. 3.0% Pre-existing diabetes 0.4% vs. 1%
2. Geldsetzer et	2 x Cross-sectional (2004 &	Men: 25-54 yrs	In 2010, 25% of the HIV-infected	N/A	Adjusted for age: Women	Not reported/stated.	HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻ (female)

al. 2018 [12] South Africa, Kwa-Zulu Natal	2010) 2004- Before the introduction of ART 2010- After the introduction of ART Results of the 2x cross-sectional were compared. Sample size Men 2004: N=761 2010: N=759 HIV⁺ 2004: N=157 2010: N=239 ART ⁺ : N=81 ART ⁻ : N=158 HIV⁻ 2004: N=332 2010: N=336	Women: 25-49 yrs	participants were on ART. Duration on ART not mentioned.		113.7 [111.8-115.7] mmHg vs. 115.9 [114.4-117.3] mmHg p=0.086		Overweight 44.8% vs. 63.7% Obesity 16.2% 31.6% HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻ (male) Overweight 9.7% vs. 19.7% Obesity 1.6% vs. 6.1%
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	Women 2004: N=1557 2010: N=1825 HIV⁺ 2004: N=335 2010: N=706 ART⁺: N=256 ART⁻: N=450 HIV⁻ 2004: N=580 2010: N=778						
3. Mosepele et al. 2017 [2] Botswana, Gaborone	Cross-sectional Sample size N= 432 HIV⁺: N=208 HIV⁻: N=224 Enrolment was	Mean: HIV⁺: 39±5 yrs HIV⁻: 37±5 yrs	Second-line regimen: 75% on NNRTIS, 25% on PIs. Mean 8.6±2.7 yrs	130.3±15.7 mmHg vs. 131.5±14.4 mmHg p=0.40 85.1±12.4 mmHg vs. 84.8±11.8 mmHg p=0.78	N/A	HIV⁺ vs. HIV⁻ Thiazide diuretic 15% vs. 5% Calcium channel blockers 8% vs. 2% Beta-blockers 3% vs. 1% ACE inhibitors	HIV⁺ vs. HIV⁻ Personal history: Diabetes mellitus 0% vs. 1% HT 17% vs. 8% CKD 3% vs. 0% Dyslipidaemia

	balanced (but not matched) by gender and age (within 10-year age groups).					2% vs. 2% HMG Co-A inhibitor 5% vs. 0% Fibrates 0% vs. 0%	8% vs. 4% Family history: Myocardial infarction 1% vs. 1% Stroke 12% vs. 16%
4. Nduka et al. 2016 [18] Nigeria, Makurdi	Prospective Unmatched study sample N=512 adults HIV⁺ART⁺: N=306 HIV⁺ART⁻: N=100 HIV⁻: N=106 Initial and final propensity score-matched sample	Mean: HIV⁺ART⁺: 38.56±38.56 yrs HIV⁺ART⁻: 35.63±12.48 yrs HIV⁻: 36.67±12.77 yrs	First-line regimen 2NRTIs+ 1NNRTIs Second-line regimen 2NRTIs+1PI Mean 45.5±28.2 months	N/A	16.3% vs. 9.0% p=0.071 111.8±15.6 mmHg vs. 112.7±18.5 mmHg p=0.376 73.6±11.6 mmHg vs. 74.1±12.3 mmHg p=0.800 Final propensity score after controlling all baseline cofounders: 113.96 mmHg vs.	Unmatched study sample HIV ⁺ ART ⁺ : 7.8% HIV ⁺ ART ⁻ : 4.0% HIV ⁻ : 31.1% Initial propensity-matched sample HIV ⁺ ART ⁺ : 1.52% HIV ⁺ ART ⁻ : 0% Final propensity-matched sample HIV ⁺ ART ⁺ : 1.75%	Central obesity HIV ⁺ ART ⁺ : 45.8% HIV ⁺ ART ⁻ : 42.0% HIV ⁻ : 34.9% Hyperglycaemia HIV ⁺ ART ⁺ : 3.4% HIV ⁺ ART ⁻ : 4.2% HIV ⁻ : 7.8%

	size N=303 HIV⁺ART⁺: N=229 HIV⁺ART⁻: N=74				114.21 mmHg p=0.944 76.07 mmHg vs. 76.14 mmHg p=0.976	HIV ⁺ ART ⁻ : 1.75%	
5. Feigl et al. 2016 [19] South Africa, Kwa-Zulu Natal	Longitudinal Sample size N=2616 adults Baseline (2003): N=2111 Follow-up (2010): N=505 Did not specify sample size according to HIV status	Mean: Baseline (2003) HIV⁺: 36.0±7.2 yrs HIV⁻: 39.0±7.2 yrs Follow-up (2010) HIV⁺: 38.0±7.6 yrs HIV⁻:	29% on ART for 0-1.9 years ART regimens not specified. Note: The study was done in South Africa, between 2003 and 2010. The ART which was recommended by the DoH [63] in South Africa during	Unadjusted for age, sex, weight, height, BMI, SBP, DBP, HIV status, educational status, self-reported health status and assets index. 122.3±17.4 mmHg vs. 127.1±20.7 mmHg p=0.270	Unadjusted for age, sex, weight, height, BMI, SBP, DBP, HIV status, educational status, self-reported health status and assets index. 122.3±17.4 mmHg vs. 129.3±22.2 mmHg p=0.270 78.0±10.7 mmHg vs. 81.0±14.0 mmHg	Not reported/stated.	No mention of comorbidities.

		40.8±7.0 yrs	this period was 2NRTIs+ 1NNRTIs as the first-line regimen while the second-line regimen was 2 NNRTIs+1PI Mean 2.5 (1.4) yrs	78.0±10.7 mmHg vs. 80.9±13.5 mmHg p=0.430	p=0.430		
6. Dimala et al. 2016 [5] Limbe, Cameroon	Cross-sectional Sample size N=200 HIV⁺ ART⁺: N=100 HIV⁺ ART⁻: N=100	Mean: HIV⁺ ART⁺: 40.2±8.0 yrs HIV⁺ ART⁻: 38.0±10.6 yrs	The patients were using NRTIs (d4T, 3TC, FTC, NVP) and NNRTIs (ZDV, TDF, EFV). Duration on ART not specified.	N/A	131±31 mmHg vs. 125±19 mmHg p=0.060 80±13 mmHg vs. 77±12 mmHg p=0.118	Not reported/stated.	BMI-defined overweight was higher in the HIV ⁺ ART ⁺ (50%) than in the HIV ⁺ ART ⁻ (31%)
7. Edwards et al. 2015 [6]	Retrospective Sample size	Median: HIV⁺: 43 [39-50] yrs	First-line regimen TDF/3TC/EFV Second-line	HT frequency for 1697 HIV ⁻ and 200 HIV ⁺ :		Not reported/stated.	Type 1 diabetes was 13.0% in HIV ⁺ and 0% in HIV ⁻

Kenya, Kibera, Nairobi	N=2206 adults HIV⁻ : N=1996 HIV⁺ : N=210	HIV⁻ : 49 [40-56] yrs	regimen LPV/RTV Median years on ART Men: 4 [3-6] yrs Women: 4 [2-6] yrs	Stage 2 HT: 39.0% vs. 33.2% p=NS Isolated systolic HT: 2.5% vs. 2.9% p= NS			CKD was 12% overall. HIV ⁺ vs HIV ⁻ Stage 1 CKD 29.7% vs. 36.2% Stage 2 CKD 58.9% vs 50.5% Stage 3 CKD 10.4% vs. 10.6% Stage 4 CKD 1.0% vs 2.7%
8. Osegbe et al. 2015 [7] Nigeria, Lagos	Cross-sectional Sample size N=283 HIV⁺ART⁺ : N=100 HIV⁺ART⁻ : N=100 HIV⁻ : N=83	Mean: HIV⁺ART⁺ : 37.5±7.8 yrs HIV⁺ART⁻ : 35.6±8.1 yrs HIV⁻ : 36.6±7.2 yrs	2NRTIs+1PI N=85 on NRTIs N=15 on PIs (15%) Participants on ART for more than a year.	75.4±13.9 mmHg vs. 72.9±10.7 mmHg p=0.38	75.4±13.9 mmHg vs. 73.8±13.9 mmHg p for trend=0.40	Not reported/stated.	HIV ⁺ ART ⁺ vs. HIV ⁺ ART ⁻ Obesity: 17% vs. 13% Diabetes: 9% vs. 2% Dyslipidaemia defined by: TC (≥ 5mmol/l): 47% vs. 34%

	Groups matched for age and sex.						TG (≥ 7 mmol/l): 24% vs. 21% LDL-c (≥ 3.3 mmol/l): 37% vs. 30% HDL-c (< 1.0 mmol/l): 11% vs. 42% Inflammation: 36% vs. 51% Atherosclerosis: 39% vs. 35%
9. Fourie et al. 2015 [16] South Africa, North West Province	Cross-sectional study Sample size N=309 HIV⁺ART⁺: N=66 HIV⁺ART⁻: N=78 HIV⁻: N=165	Mean: HIV⁺ART⁺: 49±0.96 yrs HIV⁺ART⁻: 48±0.89 yrs HIV⁻: 50.3±0.61yrs	2 NRTIs (D4T, 3TC) and 1 NNRTIs (EFV, NVP) Mean 2.86 ±0.13 years	83±1.49 mmHg vs. 84±1.34 mmHg p: NS	83±1.49 mmHg vs. 84±1.34 mmHg p: NS	HIV ⁺ ART ⁺ : 17.6% HIV ⁺ ART ⁻ : 14.0% HIV ⁻ : 9.09%	No mention of comorbidities.

10. Peck et al. 2014 [8] Tanzania, Mwanza	Cross-sectional Sample size N=454 HIV⁺ART⁺: N=150 HIV⁺ART⁻: N=151 HIV⁻: N=153	Median: HIV⁺ART⁺: 40 [38-47] yrs HIV⁺ART⁻: 37 [32-44] yrs HIV⁻: 38 [32-46] yrs	First-line regimen TDF+FTC or AZT/3TC and/or EFV or NVP Second-line regimen PI (12.0%) Median 56 [31 to 68] months.	120 [110-137.5] mmHg vs. 120[110- 130] mmHg p=0.16 80 [70-90] mmHg vs. 75 [70-80] mmHg p=0.11	N/A	Not reported/stated.	Overweight HIV ⁺ ART ⁺ : 30.0% HIV ⁺ ART ⁻ : 6.0% HIV ⁻ : 21.6% Obesity HIV ⁺ ART ⁺ : 21% HIV ⁺ ART ⁻ : 7.3% HIV ⁻ : 7.2% Diabetes HIV ⁺ ART ⁺ : 0.7% HIV ⁺ ART ⁻ : 0.7% HIV ⁻ : 0% Kidney diseases HIV ⁺ ART ⁺ : 41.3% HIV ⁺ ART ⁻ : 34.4% HIV ⁻ : 25.6%

11. Ogunmola et al. 2014 [20] Nigeria, Ido Ekiti, Ekiti State, Southwest Nigeria. .	Cross-sectional Sample size N=403 adults HIV⁺ART⁺: N=130 HIV⁺ART⁻: N=120 HIV⁻: N=153 Groups were matched for age and gender	Mean: HIV⁺ART⁺: 38.6±8.0 yrs HIV⁺ART⁻: 36.5±9.1 yrs HIV⁻: 35.5±7.6 yrs	TDF+2NRTIs 2NRTIs+PI; 1NRTI+1NNRTIs+ PI 98% were on AZT+3TC+NVP 2% were on TDF+3TC+LPV/r Mean: 34.1±29.6 months	12.3% vs. 13.7% p=0.712 118.6±14.3 mmHg vs. 118.3±14.1 mmHg p=0.232 73.5±10.3 mmHg vs. 75.7±9.2 mmHg p=0.373	12.3% vs. 19.0% p=0.712 122.6±16.5 mmHg vs. 118.6±14.3 mmHg p=0.232 73.5±10.3 mmHg vs. 74.6±15.2 mmHg	Not reported/stated.	Obesity HIV ⁺ ART ⁺ : 8% HIV ⁺ ART ⁻ : 3% HIV ⁻ : 15.99%
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N, number; N/A, not applicable; NS, not significant; yrs, years; PA, physical activity; DoH, Department of Health; HIV, human immunodeficiency virus; ART, antiretroviral therapy; HIV-, HIV-uninfected; HIV+, HIV-infected; HIV+ ART-, HIV-infected ART-naive; HIV+ ART+, HIV-infected taking ART; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HT, hypertension; BMI, body mass index; MetS, metabolic syndrome; CVD, cardiovascular disease; NCD, non-communicable disease; CKD, chronic kidney disease; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; FDC, fixed-dose combination; 3TC, lamivudine; EFV, efavirenz; TDF, tenofovir; NVP, nevirapine; FTC, emtricitabine; AZT, zidovudine; LPV, lopinavir; RTV, ritonavir.

N/A: The blood pressure estimates or hypertension prevalence was not similar in HIV+ ART+ compared to HIV- and/or HIV+ ART-.

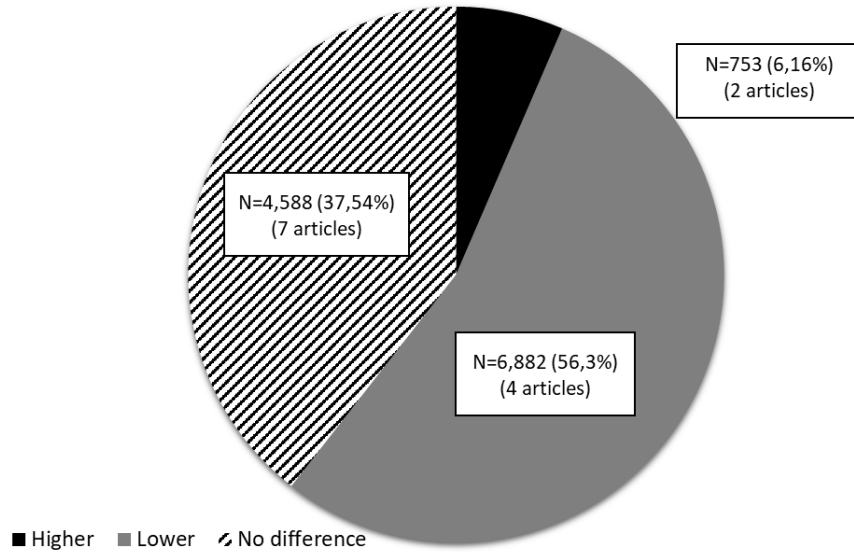
FINDINGS

We included 19 studies across eight countries in the SSA region. The BP of a total of 30,283 participants was compared between treated HIV-infected, untreated and HIV-uninfected controls. The characteristics of the 19 studies (cross-sectional (12 articles), retrospective cohort (2 articles), longitudinal cohort (5 articles)) included in this review are summarised in Tables 2, 3 & 4. In the final exclusion, three articles that reported on BP and hypertension prevalence and did not indicate statistical p-values were excluded. To determine the potential influence of these articles based on the lack of statistical p-value, the comparative analyses for the three groups were repeated. The results remained the same. In Fig 2 the number and percentage of participants and articles indicating BP and the prevalence of hypertension to be higher, lower or comparable in treated HIV-infected versus untreated and HIV-uninfected controls are shown. The sample size of the studies ranged from N=121 to N=14,918 participants. The age of the study participants ranged from 15 to 84 years. Overall, the studies included more women than men, ranging from N=41-1825 and N=44-761, respectively.

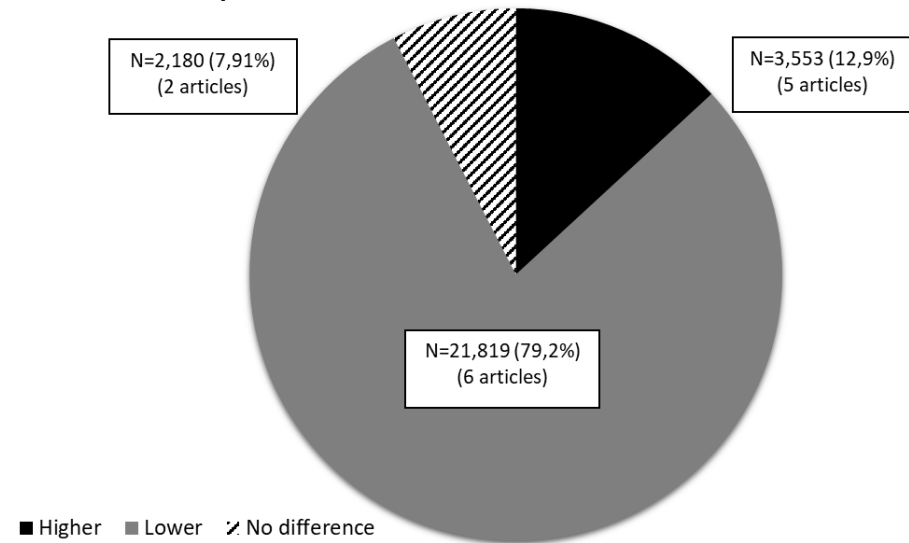
BP estimates in HIV-treated versus uninfected populations

As shown in Fig 2 (Panel A, left), 13 studies compared BP readings of treated HIV-infected individuals versus those uninfected. Two studies (combined number of participants, N=753 (6.16 %)) reported higher BP in the treated HIV-infected individuals when compared to uninfected counterparts. Contrarily, four studies (combined number of participants, N=6,882 (56.3%)) reported lower BP in the treated HIV-infected individuals compared to those uninfected, while seven studies found no difference (combined number of participants, N=4,588 (37.54%)) in BP between the treated HIV-infected and uninfected groups

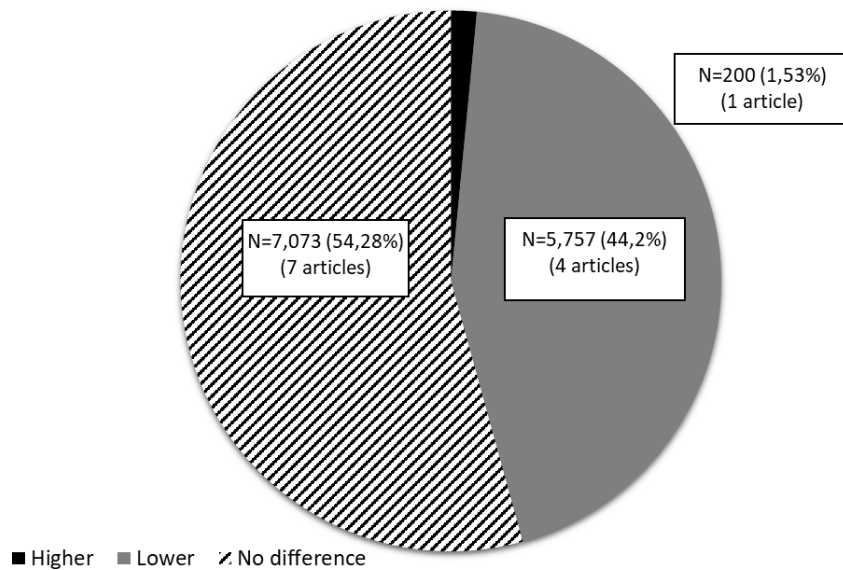
BP: HIV+ART+ vs. uninfected individuals



HT prevalence: HIV+ART+ vs. uninfected individuals



BP: HIV+ART+ vs. HIV+ART- individuals



HT prevalence: HIV+ART+ vs. HIV+ART- individuals

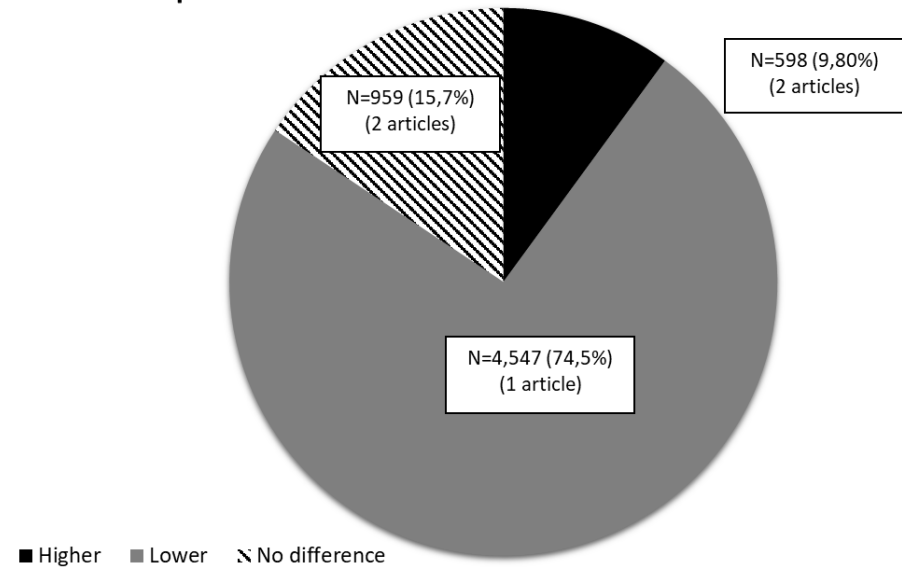


Fig 2. Do HIV-infected patients using ART have higher blood pressure (or prevalence of hypertension) than uninfected controls and untreated HIV-infected patients? Panel A: Comparison of treated HIV-infected vs. uninfected patients; Panel B: Comparison of treated HIV infected vs. untreated HIV-infected patients.

The number of eligible patients (and total number [N] of participants in these studies) indicating higher blood pressures/prevalence of hypertension (yes), lower blood pressure/ prevalence of hypertension (no) or no difference in blood pressure/ prevalence of hypertension between HIV infected patients using ART compared uninfected controls and untreated HIV-infected individuals.

N, number; BP, blood pressure; HT (%), hypertension prevalence; HIV, human immunodeficiency virus; ART, antiretroviral therapy; HIV-, HIV-uninfected; HIV+ART+, HIV-infected individuals taking antiretroviral therapy, HIV+ART-, HIV-infected individuals not taking antiretroviral therapy.

Since individuals may present with hypertensive status and yet have controlled BP, a separate comparison was performed for studies that reported on the prevalence of hypertension (Fig 2, Panel A, right). Five articles (combined number of participants, N=3,553 (12.9%)) reported a higher prevalence of hypertension in the treated HIV-infected group than uninfected participants. On the other hand, six studies (combined number of participants, N=21,819 (79.2%)) indicated a lower prevalence of hypertension in the treated HIV-infected group compared to the uninfected. The results also show two articles (combined number of participants, N=2,180 (7.91%)) that reported no difference in the prevalence of hypertension.

BP estimates in HIV-treated versus untreated HIV populations

Out of the 19 articles that were reviewed, 12 articles compared BP readings of treated and untreated HIV-infected patients (Fig 2, Panel B, left). One study (number of participants, N=200 (1.53%)) indicated raised BP in the treated versus untreated groups while four studies (combined number of participants, N=5,757 (44.2%)) reported lower BP in the treated versus untreated HIV-infected patients. Seven studies (combined number of participants, N=7,073 (54.28%)), showed no difference in BP between the HIV-infected treated compared to untreated HIV-infected groups.

We compared the prevalence of hypertension for treated and untreated HIV-infected individuals (Fig 2, Panel B, right) and a higher prevalence of hypertension was reported in two studies among treated HIV-infected patients (N=598 (9.80%)). One study reported a lower prevalence of hypertension (N=4,547 (74.5%)), while two studies showed no difference in the prevalence of hypertension (combined number of participants, N=959 (15.7%)).

Medication use and comorbidities

Lastly, the use of anti-hypertensive medication and comorbidities in the three groups were reported, as well as the use of ART in treated HIV-infected patients. The commonly used ART regimens were NRTIs and NNRTIs compared to PI in the treated HIV-infected patients. For example, in studies reporting higher, lower and similar BP or hypertension prevalence, the use of combined

NRTIs/NNRTIs ranged between 6.7% - 54.9%; 3% - 67.9% and 2% - 85%, respectively, while the use of PIs ranged between 6.7% - 25%; 3% - 15.8% and 2% - 25%, respectively. Out of the 19 studies, only N=3 studies specified the use of antihypertensive medication while N=16 studies did not specify. Concerning the comorbidities, N=16 indicated and N=3 did not indicate other comorbidities apart from raised BP or hypertension. The common comorbidities were overweight, obesity, kidney diseases, hyperglycemia and hyperlipidemia (Tables 1, 2 & 3).

DISCUSSION

Raised BP is an important risk factor for CVD development and mortality in SSA and a growing concern in adults living with HIV [3]. Understanding the reflection of hypertension estimates in HIV-infected patients is therefore vital for proper management, particularly since studies performed in Western countries suggest an increased risk for hypertension in HIV-infected patients [35]. Within this narrative review, 19 studies performed in SSA reporting on BP and hypertension status among treated HIV-infected patients and uninfected controls were included, as well as untreated HIV-infected patients.

Our main finding is that studies with larger cohort sizes showed lower BP and prevalence of hypertension in treated HIV-infected patients compared to uninfected [25, 36-43] and untreated HIV-infected controls [40, 41, 44, 45]. The findings thus oppose the common notion that raised BP is more prevalent in treated HIV-infected individuals [16, 42]. Only a few studies from SSA [11, 12, 37, 38, 46-48] reported higher BP estimates in treated HIV-infected patients, while several studies reported comparable BP estimates [11, 12, 16-18, 36, 37, 41, 44, 46, 47].

Indeed, the findings of lower BP in the treated HIV-infected patients compared to the uninfected are in contrast with previous studies [10, 46] which reported raised BP in HIV-infected vs uninfected people. These discrepancies may be because the populations from the latter studies were from Western countries and ethnic-specific differences in body composition and cardio-metabolic disease risk may exist [49, 50]. The findings agree with the findings Dillon et al [21] that included HIV-

infected Africans focusing on their cardio-metabolic profile and reporting lower BP in treated HIV-infected patients.

Apart from the lower BP estimates in HIV-infected patients, 44% of the studies showed lower BP measurements for HIV patients with ART versus those without ART [40, 41, 44, 45]. This finding is in contrast with a previous meta-analysis by Nduka et al [51] that reported higher BP in patients using ART than the HIV-infected ART-naïve. As opposed to the study, the former review included BP and hypertension comparison including population from Africa and Western countries. In this study, the patients were mainly using reverse transcriptase inhibitors [40, 41, 44, 45]. A study by Thiebaut et al [52] has shown that the use of NNRTIs along with the cumulative duration of use is associated with a lower risk for hypertension [52]. Other studies have linked PIs (e.g., lopinavir and indinavir) to elevated BP [53]; but some of these reported effects may be due to concurrent increases in weight gain after the introduction of this ART regimen [20]. In SSA the PIs are infrequently used compared to NRTIs and NNRTIs [54]. For example, in South Africa, the PI regimen is used as a second and third-line therapy when suppression of viral load failed on the first regimen line [55].

Interestingly, treated HIV-infected hypertensives were more aware of their hypertension status and with a possibility that more used anti-hypertensive medication compared to uninfected hypertensives which may explain the lower BP in the HIV-infected patients [11, 25, 34]. However, in this study, only three studies out of the N=19 studies reported the use of anti-hypertensive medication. Effective use of anti-hypertensive medication helps to lower BP; hence some individuals may be hypertensive and yet have a controlled BP [34]. With fewer studies that reported on the use of anti-hypertensive medication in this study, it is difficult to ascertain whether the estimates of the lower BP observed in HIV-infected patients as a result of using medication or not. For these reasons, in addition to reporting the BP estimates, the hypertension prevalence was also reported.

Other possible reason for the lower BP could be that more frequent visits to healthcare facilities by HIV-infected patients on ART offer more opportunities to be diagnosed with hypertension and subsequently treated and controlled [11]. Previous findings have shown that HIV-infected patients

attending integrated healthcare for HIV and hypertension had controlled BP compared to those seeking medical assistance for hypertension alone [56]. Furthermore, patients with controlled hypertension and had a regularly scheduled visit that was more than the clinical indication for patients was associated with uncontrolled BP [56]. With proper health care infrastructure, integration of the management of HIV and hypertension in the health care system may help to effectively manage future CVD events in HIV-infected patients. The studies also included more women than men, in general women, are known to have better health-seeking behaviour than men which increases their opportunity to access health care further supporting the lower BP.

Studies including 5,104 patients (N=10 articles) reported higher BP and hypertension prevalence in treated HIV-infected patients compared to uninfected and untreated HIV-infected controls. This agrees with findings by Masenga et al [57] and Xu et al [58] that reported a higher prevalence of hypertension in treated HIV-infected patients than uninfected and untreated HIV-infected controls. The pathophysiology of hypertension in HIV infection is not fully understood, but several factors such as chronic inflammation, immune reconstitution, lipodystrophy and HIV-related renal diseases have been proposed [59]. For instance, increased circulating inflammatory markers as a result of HIV-related chronic immune activation, such as interleukin-6 was shown to precede and predict hypertension development in HIV-infected patients [60]. Additionally, sCD14 plays a role in the underlying mechanism of hypertension development [61]. When comparing these findings to the majority of studies indicating lower BP in the treated HIV-infected patients, the collective findings suggest that raised blood pressure is less likely observed in SSA patients living with HIV on ART.

There are other factors such as the study designs and inconsistency in defining raised BP or hypertension that are inherent to the study and are worthy to be mentioned. The definition of raised BP and hypertension was not uniform in all studies or not specified (N=6) and this can have a consequent effect in terms of underestimating or overestimating BP or hypertension outcome. It is important to clarify the definition of raised BP and hypertension to effectively identify HIV-infected patients at risk of having raised BP or hypertension and consequently eligible for treatment. For

instance, in a study by Sarfo et al [62] the prevalence of hypertension among treated HIV-infected patients and untreated HIV-infected patients was 36.9% and 23.4%, using the 2017 American College of Cardiology and the American Heart Association (ACA/AHA) guidelines (BP $\geq$ 140/90 mm Hg) and 57.2% and 42.3%, respectively, using the updated ACA/AHA (BP $\geq$ 130/80 mm Hg). The above report further emphasises the need to have a consensus on proper diagnoses of raised BP in HIV-infected patients for integrated treatment care to effectively control BP [56]. Elevated blood pressure is an important risk factor for CVD development, misdiagnosis of hypertension mirrors the true burden of HIV-infected patients at risk of having raised BP or hypertension and consequently future risk for CVD development [3].

Estimates of BP and hypertension prevalence were mainly derived from cross-sectional studies (N=12) followed by longitudinal and retrospective cohort studies. Cross-sectional studies provide analyses of multiple variables at a given time-point but restrict influence about causality [63]. The use of specified groups in cross-sectional studies allows the selection of specific individuals who might have a predisposed risk. In this narrative review, the high number of cross-sectional studies used may have resulted in the inclusion of selected individuals at higher or lower risk for developing hypertension. While with prospective studies (N=5), confounding bias is frequent and can cause over- or under-estimation of the true relationship and may even change the direction of the observed effect [64]. The lost-to-follow-up of participants in longitudinal studies may reflect those with elevated BP or vice versa influencing the reported estimates of BP and prevalence of hypertension. Lastly, in retrospective studies (N=2) the major source of bias is recall bias as people with the outcome are more likely to remember certain experiences or exaggerate or minimise what they consider to be risk factors [64]. The design of the studies used in this narrative review may affect the outcomes of the BP and hypertension estimates in the HIV-infected patients against the untreated and uninfected controls.

However, this study has some limitations in that the researchers were only able to include data from eight SSA countries, thus limiting generalisability. This highlights a limitation in the number of studies that report on raised BP in treated HIV-infected patients compared to their untreated and

uninfected counterparts residing in the SSA region. A strength of the study is the inclusion of control groups which enabled comparison of BP estimates between treated HIV-infected patients and uninfected patients or untreated patients to clarify the controversies in reports on BP.

In summary, in studies conducted in Sub-Saharan Africa, it was found that most studies reported treated HIV-infected patients to present with lower BP and hypertension estimates compared to untreated patients and uninfected controls. Considering that raised BP is known to be predictive of future CVD, the lower blood pressure reported in these treated HIV-infected patients could play a role in reducing the CVD burden.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article (and its supplementary information files).

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Chapter 4, Manuscript two

A 10-year follow-up study of demographic and cardiometabolic factors in HIV-infected South Africans.

Cardiovascular Topics

A 10-year follow-up study of demographic and cardiometabolic factors in HIV-infected South Africans

Edith Phalane, Carla Maria Theresia Fourie, Catharina Martha Cornelia Mels, Aletta Elisabeth Schutte

Abstract

Objectives: Understanding of the interplay between human immunodeficiency virus (HIV) and cardiovascular disease,

Since the discovery of the human immunodeficiency virus (HIV) in the 1980s,¹ the global prevalence of HIV infection has increased from 7.6 million patients in 1990 to 36.7 million in 2017.² Of global infections, eastern and southern Africa

This manuscript was published in the Cardiovascular Journal of Africa (DOI: 10.5830/CVJA-2019-034). An abstract is attached (Appendix D).

Summary of instructions to authors used for the preparation of manuscript two

Details of the Journal	
Name of Journal	Cardiovascular Journal of Africa (CVJA)
Impact factor	1.400
Aim and scope	The CVJA is an international peer-reviewed journal that keeps cardiologists up to date with advances in the diagnosis and treatment of cardiovascular disease.
Publisher	Clinics Cardive Publishing
Guidelines for Authors	
Original article	This is an original article; therefore, the following guidelines were followed: Maximum of six keywords can be used for indexing.
Preparation of the article	The following outlines were followed: Title page: descriptive title; authors' surname and forename, address of each author and full address, telephone, fax and e-mail contacts for the corresponding author. Abstract: should not exceed 150 words and should include the purpose of the study, key methods, the main results and the conclusion. The body should include an introduction, methods, results, discussion (with the conclusion), acknowledgement, references, figures and tables. References: should be numbered in the order of appearance in the text, according to Vancouver style.
Website for more information	Complete details of the instruction to the author can be viewed using the following web address: http://www.cvja.co.za/authors.php

(Please note that some of the formats were changed to ensure uniformity throughout the thesis).

A 10-year follow-up study of demographic and cardio-metabolic factors in HIV-infected South Africans

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ABSTRACT

Objectives: The understanding of the interplay between human immunodeficiency virus (HIV) and cardiovascular disease is limited by evidence from longitudinal studies, especially in Africa. Therefore, the demographic, cardio-metabolic, renal and liver function of an HIV-infected South African population were profiled from 2005 to 2015.

Methods: The study included 117 HIV-infected and 131 HIV-uninfected controls examined at baseline over a follow-up period of 5 and 10 years.

Results: Mortality declined from 24% (2005-2010) to 0% (2010-2015) after introduction of ART. Longitudinal increases in C-reactive protein ($p=0.002$), alanine transaminase ($p=0.006$), gamma-glutamyl transferase ($p=0.046$) and estimated glomerular filtration rate ($p<0.001$) were seen only in the HIV-infected group. The HIV-infected group showed increased high-density lipoprotein-cholesterol (HDL-c) ($p<0.001$), total cholesterol ($p<0.001$) and decreased triglycerides: HDL-c ($p=0.011$). Low-density lipoprotein-cholesterol decreased in both groups ($p<0.001$).

Conclusion: Despite the trajectories of deranged lipid and inflammatory profiles, the cardio-metabolic disease risk seems stable in HIV-infected South Africans. Inflammation, renal and liver function warrant regular monitoring.

Keywords: Human immunodeficiency virus, antiretroviral therapy, cardio-metabolic factors, renal function, liver enzymes and South Africa.

INTRODUCTION

Since its discovery in the 1980s [1], the global prevalence of the human immunodeficiency virus (HIV) infection has drastically escalated from 7.6 million in 1990 to 36.7 million in 2017 [2]. On a global scale, Eastern and Southern Africa constitute 53% of new infections with 19.6 million people living with HIV in these regions [2]. South Africa became the focus of global attention in the 1990s following the rapid spread of HIV and the attendant pervasiveness of acquired immunodeficiency syndrome (AIDS) related deaths in the country [3]. To date, the HIV epidemic remains a cause of global and national concern. To address the increasing burden of HIV/AIDS, in April 2004 [4] the South African government implemented the antiretroviral therapy (ART) roll-out programme which is currently the world's largest ART roll-out programme for HIV-infected South Africans [5]. The use of potent ART has significantly improved the life expectancy of people infected with HIV in a country that has experienced an alarming increase in the number of HIV-infected patients from 11.5% (5.35 million) in 2005 to 12.5% (6.19 million) in 2015 (**Figure 1**) and a further increase to 12.6% (7.1 million) in 2017 [6]. Despite various campaigns designed to curb the HIV infection rates in South Africa, the country continues to bear a disproportionate share of the global burden of HIV as it still has one of the highest global records of new HIV infections [2].

The invaluable role of ART in the battle against HIV/AIDS notwithstanding, its introduction has led to new health concerns such as treatment-related metabolic complications and cardiovascular diseases (CVD). These include cardio-metabolic changes such as elevated blood pressure (BP) [7], dyslipidaemia [7], lipodystrophy [8] and chronic inflammation [9] – all of which are associated with increased development of cardiovascular disease (CVD). The use of ART is further associated with hepatotoxicity which often manifests as liver disease in HIV-infected patients [10]. In addition, a renal disease characterised by higher urinary protein excretion and elevated serum creatinine has been observed in HIV-infected individuals [11]. With 56% of the HIV- infected patients in South Africa on ART [5], an increasing prevalence of cardio-metabolic and renal diseases in the HIV-infected patients has emerged [12].

Recent research suggests that the HIV-infected population bears a higher risk of developing CVD due to the complex interconnections between HIV, ART and cardio-metabolic disease [13]. However, despite the prevalence of the co-epidemics of HIV infection and CVD in Sub-Saharan Africa [14], there is a paucity of research on the nexus between these conditions and even scarcer evidence from longitudinal studies, especially in South Africa. For example, in cross-sectional analyses of 300 newly diagnosed HIV-infected, ART-naive individuals who were individually matched with uninfected controls by age, gender, body mass index (BMI) and locality, the revelation of dyslipidaemia and inflammation in the HIV-infected group suggested an increased risk for the development of CVD [15]. Therefore, in this study, HIV-positive South Africans and controls were closely monitored over 10 years to profile the demographic factors and to investigate the impact of long-term HIV infection and ART use on their cardio-metabolic factors, liver and renal functions.

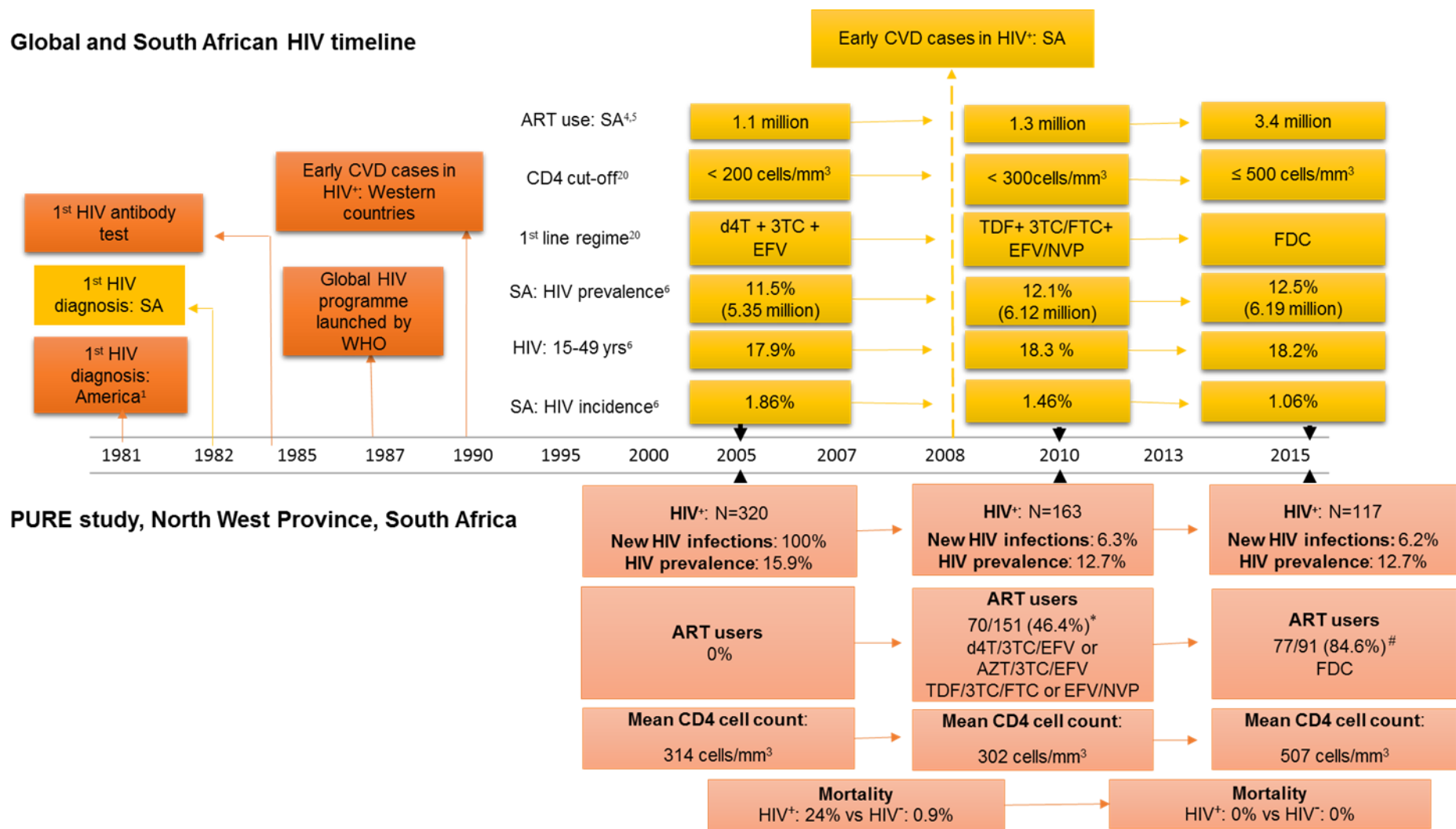


Figure 1. Selected events in tracking the human immunodeficiency virus, antiretroviral therapy and cardiovascular disease.

HIV, human immunodeficiency virus; ART, antiretroviral therapy; FDC, Fixed-dose combination; d4T, Stavudine; TDF, Tenofovir; 3TC, Emtricitabine; EFV, Efavirenz; FTC, Lamivudine; NVP, Nevirapine; AZT, Zidovudine; SA, South Africa; WHO, World Health Organisation; CVD, cardiovascular disease; PURE, Prospective Urban and Rural Epidemiology study; HIV+, HIV infected; HIV-, HIV uninfected; VS, versus.

* Out of the 163 HIV-infected individuals that were followed in 2010, information on the use of ART was available for 151 participants.

Out of the 117 HIV-infected individuals that were followed in 2015 information on ART use was available for 91 participants.

METHODS

The PURE study

This study falls within the ambits of the Prospective Urban and Rural Epidemiology (PURE) study, which is a multinational longitudinal study that examines lifestyle changes in low, middle and high-income countries [16]. In the South African constituent of the PURE study, performed in the North-West Province, N=2010 participants were randomly recruited from Potchefstroom (urban N=1004) and Ganyesa (rural N=1006). Data were collected thrice with the baseline in 2005 and the two follow-ups in 2010 and 2015 respectively. Black individuals above the age of 35 years were invited to participate in the study, pregnant and lactating women were excluded, the participants were fully informed about the objectives of the study, the procedures were explained and written informed consent were duly obtained.

During the first follow-up in 2010, N=1288 participated in the study, N=221 died, while N=501 were lost to a lapse in the follow-up. In the second follow-up (2015), N=926 committed to the follow-up, N=127 deaths were recorded, while N=307 did not come back for follow-up (**Figure 1**). The attrition rate of participants from baseline (2005: N=2005) to follow-up (2015: N=926) resonates with similar challenges encountered in previous longitudinal studies either because of participants' refusal participate, relocation to other parts of the country, ill health of the older individuals or mortality.

The current study

At baseline (2005), N=320 out of the total study population of N=2010 were newly-identified with HIV, 10 years later the number decreased to N=117. The then 320 newly-identified HIV-infected participants were matched with uninfected controls according to age, gender, BMI and locality at baseline. For this longitudinal study, the 117 HIV-positive and 131 uninfected participants who partook in the 10-year follow-up data collection were assessed. **Figure 2** presents a graphical overview of the study population.

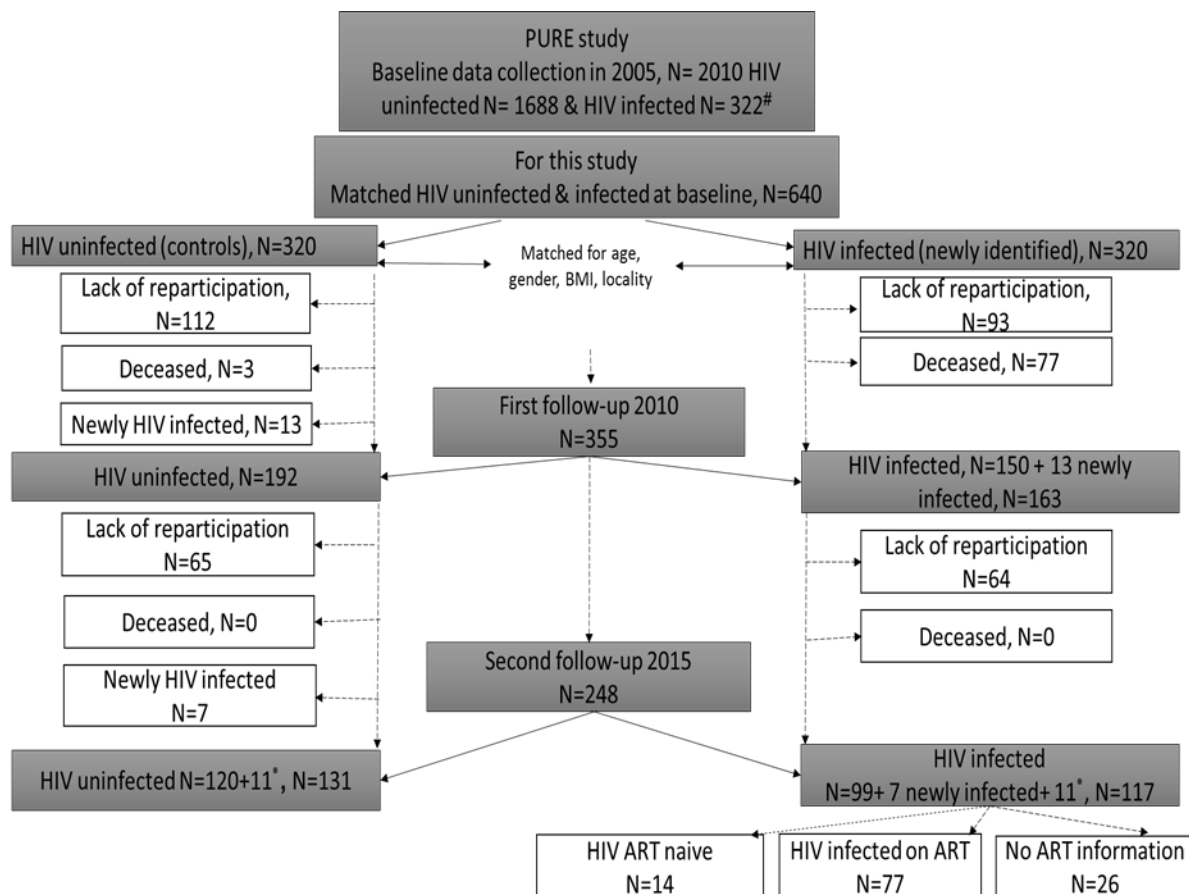


Figure 2. Outline of the study population.

ART, antiretroviral therapy; BMI, Body mass index; HIV, human immunodeficiency virus; N, number of participants;

PURE, Prospective Urban and Rural Epidemiology study.

Two of the participants were excluded due to incomplete data

* Participants that were not followed in 2010, but in 2015: 11 HIV infected and 11 HIV uninfected participants.

In 2005, the newly-HIV-diagnosed and ART-naïve participants were referred for follow-up and CD4 cell count determination to place them on ART following the guidelines of the South African Department of Health. Five years later (2010), N=70 was on ART, this number increased to N=77 in 2015. The ART comprised of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTIs) (Figure 1). It is crucial to note that there were modifications to the ART guidelines in South Africa during the 10 years of follow-up. For example, in 2004 when ART was introduced, Stavudine was the backbone of the ART regimen. However, due to its association with lipodystrophy, it was phased out and replaced with Tenofovir in 2010 [17].

Similarly, the fixed-dose combination was introduced in 2012 [17] and a “test-and-treat” programme was implemented in September 2016 [5].

Ethical considerations

The PURE study was approved by the Health Research Ethics Committee of the North-West University, South Africa. The study protocol and procedures were explicitly explained to the participants in their home language (Setswana) and they duly gave written informed consent. Questionnaires were used to collect data on demographic information, current health status, medical and family history, medication use, tobacco and alcohol use.

Anthropometry

The study employed standardised procedures for anthropometric measurements including height, weight, waist and hip circumferences [18].

Blood pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were taken in duplicate at an interval of five minutes on the right arm, whilst in a sitting position. The validated OMRON HEM-757 device was utilised during the baseline and 2010 follow-up while the OMRON M6 device (Omron Healthcare, Kyoto, Japan) was used during the 2015 follow-up.

Biological sampling and biochemical analyses

Venous blood samples were collected from the participants after fasting for at least eight hours. Serum and plasma were prepared and stored at -80 degrees Celsius along with spot urine samples. Glucose levels from fluoride plasma samples were determined using the Vitros DT6011 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York, USA) in 2005 and the Cobas Integra 400 Plus (Roche, Indianapolis, IN) during the follow-up. Furthermore, glycated haemoglobin (HbA1c) was determined using the D-10 Haemoglobin testing system from Bio-Rad (Hercules,

California, United States). Serum samples were used to analyse high sensitivity C-reactive protein (CRP), total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-c), γ -glutamyltransferase (GGT), aspartate transaminase (AST), alanine transaminase (ALT) and creatinine using a Konelab20i™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland) in 2005 and a Cobas Integra 400 Plus Auto-analyser (Roche, Indianapolis, IN) in 2010 and 2015 respectively. Low-density lipoprotein-cholesterol (LDL-c) levels were calculated [19] and the estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in ml/min/1.73m² [20]. Urinary creatinine and albumin were analysed using the Cobas Integra 400 Plus (Roche, Basel, Switzerland) in 2005 and 2015 and the urinary albumin to creatinine ratio (uACR) was calculated.

HIV testing

The HIV status of all the participants was determined through a whole blood finger-prick test using the First Response Rapid HIV card test (Premier Medical Corporation Limited, Daman, India). In the case of positive results, the tests were confirmed using the Pareeshak card test (BHAT Bio-tech, India), SD BIOLINE HIV 1/2 3.0 card test (Standard Diagnostics, INC, Korea) and Abon (Biopharm Corporation Limited, Hanyzhou, China) rapid card tests in 2005, 2010 and 2015 respectively. The CD4 cell counts were determined by the National Health Laboratory Services (Beckman Coulter® EPICS® X1™, Fullerton, USA) in 2005 and 2010. In 2015, the CD4 cell count was measured on-site using finger-prick blood and a point-of-care device, PIMA™ CD4 (Alere, Jena, Germany).

STATISTICAL ANALYSES

Statistical analyses were performed using Statistica version 13.3 (TIBCO Software Inc, Palo Alto, United State of America, 2017). Normally distributed variables were presented as means with standard deviation, while the logarithmically transformed variables were presented as geometric means with 5th and 95th percentiles. The variables that remained skewed after log-transformation was presented as median with 25th and 75th percentiles. Independent t-tests were used to compare the

means of continuous variables and Chi-square tests to compare frequencies in these groups. Percentage change was calculated and comparison was done using an independent T-test between the HIV-infected and HIV-uninfected groups. Mann-Whitney U tests were used to compare the percentage change of skewed variables between these groups. Repeated measure analysis of variance was used to determine the change in continuous variables in these groups over time.

RESULTS

The baseline and follow-up characteristics of the HIV-infected and uninfected groups were compared over 10 years (Table 1). The HIV-infected participants included fewer men (24 versus 38%) and were younger than the HIV-uninfected participants (baseline mean ages 43 versus 45 years). The measurements of body composition and blood pressure did not differ between the two groups. Contrary to the uninfected population, the HIV-infected participants presented with lower TC ($p<0.001$) and HDL-c ($p<0.001$) and higher TC: HDL-c ($p=0.003$) and TG: HDL-c ($p<0.001$) at baseline. At follow-up, the HIV-infected participants displayed higher LDL-c ($p=0.038$) and TC: HDL-c ($p=0.016$). Also, a lower concentration of HbA1c ($p=0.013$) was recorded after 10 years (2015) among the HIV-infected than the uninfected participants. In comparison to their counterparts, at follow-up (2015) higher CRP ($p=0.022$) and liver enzymes, ALT ($p<0.001$), AST ($p=0.011$) and GTT ($p=0.006$) levels were observed in the HIV-infected group, despite comparable levels at baseline. At baseline, the HIV-infected displayed higher serum creatinine ($p=0.003$) and uACR ($p=0.003$) and lower eGFR ($p=0.001$), unlike the HIV-uninfected.

Interestingly, the mortality rate among the HIV-infected group (Table 1) declined from 24% (2005-2010) to 0% (2010-2015).

To ascertain whether certain attributes can be ascribed to the loss of participants or the deceased during the 10-year follow-up, the baseline characteristics of the HIV-infected assessed, lost to lapses in the follow-up and the deceased were compared (see Table 2). The findings revealed that unlike the aforementioned categories, the deceased HIV-infected participants were older ($p=0.002$), had a higher

heart rate ($p=0.001$), lower HDL-c ($p=0.024$), higher lipid ratios (all $p=0.033$), HbA1c ($p=0.044$), CRP ($p<0.001$) and GGT ($p=0.046$).

In Table 3, the 10-year % change between the HIV-infected and uninfected groups was compared. No difference was recorded in the % change in BP and body composition between the groups, however, TC and HDL-c increased in HIV-infected participants as opposed to the obvious decrease in the uninfected (all $p<0.001$). Although LDL-c (-1.9% vs. -16%, $p<0.001$) decreased in both groups, a lesser decrease was observed in the HIV-infected population. The change in TG: HDL-c differed between the HIV-infected and uninfected (-8.1% vs. 21%, $p=0.011$). Likewise, glucose increased significantly (8.5% vs. 4.7%, $p=0.046$), while HbA1c remained the same in the HIV-infected group, unlike the HIV-uninfected group that experienced an increase (0% vs. 2.1%, $p=0.009$). The HIV infected group displayed a significant increase in CRP (76% vs. 0.1%, $p=0.047$), ALT (25% vs. -19%, $p<0.001$) and GGT (12% vs. -34%, $p<0.001$) compared to the uninfected group. The HIV-infected group demonstrated an increase in eGFR (4.8% vs. -2.1%, $p=0.010$) while a decline in eGFR was recorded in the HIV-uninfected group.

The changes in the cardio-metabolic characteristics of both the HIV-infected and uninfected participants was determined over the three data collection time points (Table 4). For this analysis, fewer participants were available due to some missing data concerning some variables for the 2010 follow-up. Nevertheless, the TC, LDL-c and TG: HDL-c (all $p\leq 0.88$) in the HIV-infected group remained the same, but HDL-c increased ($p=0.017$), whereas in the HIV-uninfected group all the above lipids decreased over time (all $p\leq 0.023$). The HIV-infected group showed no changes for AST ($p=0.11$) and an increase in ALT ($p=0.006$) and GGT ($p=0.046$), conversely, these markers all decreased (all $p\leq 0.002$) in the HIV-uninfected participants. Relatively, the HIV-infected group CRP increased ($p=0.002$) and remained stable ($p=0.45$) in the uninfected group. An increase in uACR was noticed in the HIV-infected ($p<0.001$) and HIV-uninfected participants ($p<0.001$) and eGFR ($p<0.001$) increased over time in the HIV infected group whereas in the uninfected counterparts no change was recorded ($p=0.53$).

Table 5 clearly shows the proportion and percentage of cardiometabolic risk factors of the HIV-infected and HIV-uninfected individuals over the 10-year period. Few of the HIV-infected participants had elevated blood pressure than HIV-uninfected controls at both baseline ($p=0.011$) and 5 years later ($p=0.043$). At the baseline study, more of the HIV-infected participants had elevated HDL-c in comparison to the uninfected men ($p=0.016$). Five years later, the HIV-infected men ($p=0.046$) and women ($p=0.002$) presented with a lesser number of individuals having elevated HDL-c. Also, there was a higher prevalence of central obesity at the 2010 follow-up ($p=0.003$) among the HIV-uninfected women than the HIV-infected women.

Table 1. Characteristics of the HIV infected and uninfected individuals successfully followed over 10 years: baseline data (2005) and follow-up (2015) data.

	Followed in 2015 using baseline data (2005)		P	10-year follow-up, (2015)		P
	HIV infected N=117	HIV uninfected N=131		HIV infected N=117	HIV uninfected N=131	
<i>Socio-demographic factors</i>						
Male, N (%)	28 (23.9)	50 (38.2)	0.016	28 (23.9)	50 (38.2)	0.016
Urban, N (%)	51 (43.6)	52 (36.7)	0.53	51 (43.6)	52 (36.7)	0.53
Age, years	42.9 ± 5.64	44.7 ± 7.63	0.037	52.4 ± 5.66	54.2 ± 7.59	0.036
<i>Anthropometry</i>						
WC, cm	74.2 [61.5; 98.0]	75.8 [62.8; 100]	0.22	81.8 [64.1; 114]	82.8 [65.3; 105]	0.57
BMI, kg/m ²	22.5 [16.4; 35.6]	22.4 [16.9; 32.9]	0.85	23.2 [16.1; 37.5]	23.4 [16.1; 35.7]	0.79
<i>Cardiovascular measurements</i>						
SBP, mmHg	122 [97.0; 155]	126 [95.0; 172]	0.11	123 [94.0; 177]	128 [99.0; 177]	0.13
DBP, mmHg	84.7 ± 13.2	85.6 ± 14.4	0.59	82.3 ± 14.3	85.4 ± 13.8	0.22
PP, mmHg	37.1 [25.0; 59.0]	40.3 [25.0; 67.0]	0.029	40.2 [24.0; 75.0]	42.6 [28.0; 73.0]	0.15
MAP, mmHg	97.5 ± 14.5	99.6 ± 16.4	0.29	97.4 ± 17.1	100 ± 16.4	0.18
HR, beats/min	76.2 ± 13.1	73.5 ± 15.5	0.14	75.3 ± 15.0	73.1 ± 13.9	0.23
<i>Biochemical variables</i>						
TC, mmol/l	4.46 ± 1.27	5.05 ± 1.26	<0.001	4.54 ± 1.01	4.32 ± 1.12	0.13
HDL-c, mmol/l	1.19 [0.56; 2.43]	1.58 [0.72; 3.19]	<0.001	1.29 [0.76; 2.48]	1.32 [0.77; 2.53]	0.74
LDL-c, mmol/l	2.56 ± 1.04	2.80 ± 1.12	0.085	2.56 ± 0.23	2.25 ± 1.31	0.038
TG, mmol/l	1.11 [0.52; 2.42]	1.01 [0.57; 2.46]	0.11	1.14 [0.51; 2.79]	1.01 [0.55; 2.52]	0.099
TC:HDL-c ratio	3.59 [1.84; 7.21]	3.10 [1.71; 5.88]	0.003	3.43 [1.86; 16.0]	3.01 [1.56; 6.18]	0.016
TG:HDL-c ratio	0.93 [0.30; 3.17]	0.64 [0.22; 1.79]	<0.001	0.89 [0.35; 2.39]	0.76 [0.26; 2.53]	0.11
Glucose, mmol/l	4.59 [3.30; 5.70]	4.79 [3.50; 6.30]	0.078	5.13 [4.14; 6.48]	5.09 [3.85; 7.22]	0.74
HbA1c,%	5.46 [4.80; 6.20]	5.49 [4.90; 6.20]	0.52	5.46 [4.80; 6.30]	5.70 [4.90; 6.70]	0.013
CD4 cell count, cell/mm ³	314 [126; 702]	-	-	429 [145; 1035]	-	-
CRP, mg/l	2.31 [0.25; 40.9]	2.25 [0.23; 28.8]	0.88	4.06 [0.38; 56.9]	2.61 [0.91; 16.1]	0.022
ALT, U/l	17.5 [7.81; 46.4]	18.8 [8.00; 59.0]	0.34	20.7 [10.1; 49.3]	15.0 [8.00; 36.8]	<0.001
AST, U/l	31.5 [14.2; 96.5]	29.5 [13.4; 108]	0.41	28.2 [15.3; 88.5]	23.5 [13.5; 62.1]	0.011
GGT, U/l	48.2 [17.2; 2228]	57.5 [18.8; 442]	0.128	53.3 [13.9; 342]	36.9 [10.2; 226]	0.006
<i>Renal function</i>						
Serum creatinine, µmol/l	69.2 [47.8; 101]	63.5 [47.0; 88.8]	0.003	55.7 [37.7; 79.8]	57.8 [37.6; 78.4]	0.42

uACR, mg/mmol	0.92 [0.15; 16.3]	0.57 [0.12; 2.63]	0.003	1.89 [0.48; 10.3]	1.48 [0.38; 9.41]	0.095
eGFR, ml/min/1.73m ²	107 [67.8; 144]	118 [85.1; 149]	0.001	118 [95.6; 139]	116 [97.9; 140]	0.64
<i>Lifestyle factors</i>						
Tobacco use, N (%)	71 (60.7)	82 (62.6)	0.76	44/114 (38.6)	61/129 (47.3)	0.17
Alcohol use, N (%)	53 (45.3)	58 (44.3)	0.87	38/114 (33.3)	49/129 (37.9)	0.45
<i>Medication use</i>						
ART use, N (%)	0 (0%)	-	-	77/91 (84.6%) [#]	-	-
< 5years on ART, N (%)	0 (0%)	-	-	24/77 (31.2%)	-	-
≥ 5years on ART, N (%)	0 (0.0)	-	-	53/77 (68.8%)	-	-
Anti-hypertensive med, N (%)	12 (10.3)	14 (10.7)	0.91	25/112 (22.3)	39/129 (30.2)	0.17
Statins, N (%)	0 (0.00)	0 (0.00)	1.00	2/112 (1.79)	1/129 (0.78)	0.48
Anti-inflammatory med, N (%)	15 (12.8)	22 (16.8)	0.38	7/112 (6.25)	11/129 (8.53)	0.50
Anti-diabetic med, N (%)	0 (0.00)	1 (0.76)	0.34	0 (0.00)	3/129 (2.33)	0.10

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. SD, standard deviation; HIV, human immunodeficiency virus; N, number of participants; WC, waist circumference; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; TC, Total cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol; HDL-cholesterol, high-density lipoprotein-cholesterol; HbA1c%, glycated haemoglobin, GGT, γ -glutamyltransferase; CRP, C-reactive protein; AST, aspartate transaminase; ALT, alanine transaminase; uACR, urinary albumin creatinine ratio; eGFR, estimated glomerular filtration rate; med, medication.

[#] Out of the 117 HIV-infected individual information on ART use was available for 91 participants.

Table 2. Baseline characteristics of HIV infected individuals followed, lost to follow-up and deceased

	Followed N=117	Lost to follow N=144	Deceased N=77	<i>P for trend</i>
<i>Socio-demographic factors</i>				
Male, N (%)	28 (23.9)	64 (44.4)	29 (37.7)	0.003
Urban, N (%)	51 (43.6)	75 (54.2)	33 (42.9)	0.14
Age, years	42.9 ± 5.64 ^a	43.5 ± 7.86 ^b	46.9 ± 10.3 ^{ab}	0.002
<i>Anthropometry</i>				
WC, cm	74.2 [16.5; 98.0]	75.7 [64.2; 98.0]	74.6 [59.5; 99.8]	0.47
BMI, kg/m ²	22.5 [16.4; 35.6]	22.6 [17.3; 34.8]	21.0 [15.1; 32.5]	0.052
<i>Cardiovascular measurements</i>				
SBP, mmHg	122 [97.0; 155]	125 [98.0; 167]	119 [85.0; 166]	0.090
DBP, mmHg	84.6 ± 13.2	84.7 ± 15.3	81.8 ± 16.0	0.34
PP, mmHg	37.1 [25.0; 59.0] ^a	40.6 [27.0; 62.0] ^a	37.3 [25.0; 66.0]	0.015
MAP, mmHg	97.5 ± 14.5	98.7 ± 17.1	97.8 ± 18.3	0.25
HR, beats/min	76.2 ± 13.1 ^a	74.2 ± 15.0 ^b	82.4 ± 18.9 ^{ab}	0.001
<i>Biochemical variables</i>				
TC, mmol/l	4.46 ± 1.27	4.46 ± 1.25	4.30 ± 1.31	0.62
HDL-c, mmol/l	1.19 [0.56; 2.43] ^a	1.12 [0.56; 2.26]	0.97 [0.26; 2.61] ^a	0.024
LDL-c, mmol/l	2.56 ± 1.04	2.65 ± 1.02	2.52 ± 10.4	0.63
TG, mmol/l	1.11 [0.52; 2.42]	1.13 [0.56; 3.03]	1.22 [0.51; 3.10]	0.45
TC/HDL-c ratio	3.59 [1.84; 7.21] ^a	3.85 [2.21; 6.51]	4.22 [1.97; 13.4] ^a	0.033
TG/HDL-c ratio	0.93 [0.30; 3.17] ^a	0.99 [0.38; 4.23]	1.25 [0.51; 3.10] ^a	0.033
Glucose, mmol/l	4.59 [3.30; 5.70]	4.67 [3.40; 5.90]	4.51 [3.50; 5.70]	0.38
HbA1c,%	5.46 [4.80; 6.20]	5.47 [4.70; 6.10]	5.62 [4.80; 6.40]	0.044
CD4 cell count, cells/mm ³	314 [243; 407]	306 [240; 389]	260 [178; 379]	0.70
CRP, mg/l	2.31 [0.25; 40.9] ^a	2.79 [0.31; 35.98] ^b	6.48 [0.46; 53.8] ^{ab}	<0.001
ALT, U/l	17.5 [15.7; 19.6]	18.1 [16.3; 19.9]	18.9 [16.5; 21.8]	0.68
AST, U/l	31.5 [28.0; 35.4]	32.3 [29.1; 35.9]	36.6 [31.7; 42.2]	0.23
GGT, U/l	48.2 [17.2; 228] ^a	54.6 [18.3; 320]	67.0 [20.3; 489] ^a	0.046
<i>Renal function</i>				
Serum creatinine, , µmol/l	69.2 [47.8; 101]	63.5 [44.7; 94.4]	67.9 [45.5; 114]	0.17
uACR, mg/mmol	0.92 [0.15; 16.3]	0.67 [0.14; 6.83] ^a	1.19 [0.20; 32.7] ^a	0.012

eGFR, ml/min/1.73m ²	107 [67.8; 144] ^a	118 [79.8; 156] ^a	108 [58.5; 143]	0.008
<i>Lifestyle factors</i>				
Tobacco use, N (%)	71 (60.7)	86 (59.7)	43 (55.8)	0.79
Alcohol use, N (%)	53 (45.3)	69 (47.9)	40 (60.0)	0.66
<i>Medication use</i>				
ART use, N (%)	-	-	-	-
Anti-hypertensive med, N (%)	12 (10.3)	11 (7.64)	9 (11.7)	0.58
Anti-inflammatory med, N (%)	15 (12.8)	11 (7.64)	12 (15.6)	0.16

Data are presented as mean and standard deviation for normally distributed variables and logarithmically transformed variables are presented as geographic mean and 5th and 95th percentile.

HIV, human immunodeficiency virus; N, number of participants; WC, waist circumference; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; TC, Total cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol; HDL-cholesterol, high-density lipoprotein-cholesterol; HbA1c%, glycated haemoglobin, GGT, γ -glutamyltransferase; CRP, C-reactive protein; AST, aspartate transaminase; ALT, alanine transaminase; uACR, urinary albumin creatinine ratio; eGFR, estimated glomerular filtration rate; med, medication.

Table 3. Percentage change in the cardio-metabolic characteristics of HIV infected and uninfected individuals (2005-2015).

Percentage change between groups			
	HIV infected N=117	HIV uninfected N=131	P
Age, %	22.4 ± 3.04	21.7 ± 3.75	0.15
<i>Anthropometry</i>			
WC, %	9.12 [1.09; 18.7]	8.62 [1.73; 16.5]	0.80
BMI, %	3.70 ± 17.2	4.87 ± 11.2	0.53
<i>Cardiovascular measurements</i>			
SBP, %	0.00 [-8.01; 12.2]	0.83 [-9.82; 14.6]	0.84
DBP, %	-0.17 ± 19.1	1.54 ± 18.5	0.48
PP, %	4.82 [-12.5; 33.3]	4.76 [-13.9; 30.8]	0.65
MAP, %	0.84 ± 18.0	2.17 ± 18.0	0.56
HR, %	-1.30 [-13.9; 11.0]	0.00 [-12.6; 13.1]	0.60
<i>Biochemical variables</i>			
TC, %	6.48 [-12.2; 24.3]	-14.5 [-28.8; 2.90]	<0.001
HDL-c, %	12.7 [-20.0; 46.0]	-15.1 [-31.8; 10.9]	<0.001
LDL-c, %	-1.92 [-21.5; 33.3]	-16.4 [-38.8; 2.22]	<0.001
TG, %	2.13 [-28.6; 46.3]	-1.86 [-22.0; 37.5]	0.83
TC:HDL-c ratio, %	-1.63 [-25.5; 22.1]	0.53 [-17.0; 23.1]	0.48
TG:HDL-c ratio, %	-8.10 [-40.5; 54.3]	21.4 [-17.1; 86.6]	0.011
Glucose, %	8.46 [-5.85; 21.6]	4.72 [-9.56; 16.3]	0.046
HbA1c, %	0.01 [-5.71; 5.31]	2.12 [-1.77; 7.34]	0.009
CRP, %	75.8 [-38.1; 353]	0.08 [-52.1; 178]	0.047
ALT, %	25.4 [-15.9; 78.8]	-19.3 [-42.9; 15.1]	<0.001
AST, %	-13.6 [19.7; 139]	-20.5 [-47.7; 13.4]	0.15
GGT, %	11.7 [-24.3; 68.7]	-33.9 [-55.5; -3.52]	<0.001
<i>Renal function</i>			
Serum creatinine, %	-20.4 [-34.2; -2.28]	-10.8 [-26.9; 6.60]	0.033
uACR, %	106 [-36.3; 465]	132 [9.77; 365]	0.52
eGFR, %	4.83 [-5.90; 34.7]	-2.09 [-9.17; 10.5]	0.010

Data are presented as mean and SD for normally distributed variables and the variables that remained skew after log-transformation was presented as median with 25th and 75th percentiles. SD, standard deviation; HIV, human immunodeficiency virus; N, number of participants; WC, waist circumference; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure, MAP, mean arterial pressure; HR, heart rate; TC, Total cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol; HDL-cholesterol, high-density lipoprotein-cholesterol; HbA1c%, glycated haemoglobin; GGT, γ -glutamyltransferase; CRP, C-reactive protein; AST, aspartate transaminase; ALT, alanine transaminase; uACR, urinary albumin creatinine ratio; eGFR, estimated glomerular filtration rate.

Table 4. Change in the cardio-metabolic characteristics of the HIV infected and uninfected individuals over 10 years

	HIV infected			<i>P</i>	
	Year:	2005	2010		2015
<i>Biochemical variables(N=93 to 101)*</i>					
TC, mmol/l		4.41 ±1.24	4.57 ±0.16	4.57 ±1.03	0.31
LDL-c, mmol/l		2.58 ±0.99	2.68±1.01	2.59 ±0.90	0.45
HDL-c, mmol/l		1.18 [1.09; 1.29]	1.18 [1.10; 1.27]	1.30 [1.22; 1.39]	0.017
TG, mmol/l		1.08 [0.98; 1.19]	1.07 [0.91; 1.27]	1.15 [0.21; 1.28]	0.63
TC:HDL-c ratio		3.58 [3.32; 3.85]	3.72 [3.45; 4.02]	3.41 [3.18; 3.65]	0.003
TG:HDL-c ratio		0.91 [0.80; 1.04]	0.91 [0.76; 1.09]	0.88 [0.77; 1.01]	0.88
Glucose, mmol/l		4.61 [4.44; 4.79] ^a	4.70 [4.45; 4.96] ^b	5.15 [5.01; 5.28] ^{ab}	<0.001
HbA1c,%		5.48 [5.40; 5.57] ^a	5.67 [5.58; 5.77] ^{ab}	5.46 [5.37; 5.55] ^b	<0.001
CRP, mg/l		2.35 [1.69; 3.25] ^{ab}	3.47 [2.60; 4.63] ^a	4.16 [3.11; 5.58] ^b	0.002
ALT, U/l		17.0 [15.3; 18.9]	19.6 [17.5; 22.0]	21.1 [18.9; 23.7]	0.006
AST, U/l		31.9 [28.5; 35.7]	28.1 [25.5; 31.0]	28.8 [26.0; 31.9]	0.11
GGT, U/l		45.9 [39.2; 53.6]	44.4 [36.8; 53.6]	53.3 [43.7; 65.0]	0.046
<i>Renal function</i>					
Serum creatinine, µmol/l		69.4 [65.9; 73.1] ^{ab}	56.3 [53.8; 58.9] ^a	56.1 [53.2; 59.1] ^b	<0.001
uACR, mg/mmol		0.93 [0.71; 1.23] ^a	-	1.92 [1.54; 2.39] ^a	<0.001
eGFR, ml/min/1.73m ²		106 [100; 112] ^b	122 [118; 127] ^a	118 [113; 122] ^{ab}	<0.001
	HIV uninfected			<i>P</i>	
	Year:	2005	2010		2015
<i>Biochemical variables(N=102 to 113)*</i>					
TC, mmol/l		4.99 ±1.19 ^a	4.86 ±1.13 ^b	4.27 ±1.08 ^{ab}	<0.001
LDL-c, mmol/l		2.74 ±1.09 ^a	2.78 ±1.10 ^b	2.20 ±1.33 ^{ab}	<0.001
HDL-c, mmol/l		1.59 [1.48; 1.71] ^a	1.47 [1.38; 1.57]	1.31 [1.14; 1.51] ^a	0.006
TG, mmol/l		1.01 [0.94; 1.10]	1.05 [0.97; 1.13]	0.99 [0.88; 1.11]	0.58
TC:HDL-c ratio, %		3.04 [2.84; 3.26]	3.21 [2.99; 3.44]	2.97 [2.72; 3.24]	0.007
TG:HDL-c ratio, %		0.63 [0.57; 0.72] ^a	0.71 [0.63; 0.80]	0.75 [0.66; 0.86] ^a	0.023
Glucose, mmol/l		4.81 [4.65; 4.96]	4.94 [4.76; 5.12]	5.04 [4.84; 5.25]	<0.001
HbA1c,%		5.48 [5.40; 5.57] ^{abc}	5.92 [5.79; 6.06] ^{abc}	5.65 [5.52; 5.79] ^{abc}	<0.001
CRP, mg/l		2.35 [1.78; 3.10]	2.72 [2.087; 3.55]	2.75 [2.10; 3.59]	0.45
ALT, U/l		18.6 [16.7; 20.9]	16.8 [15.2; 18.5]	15.0 [13.4; 16.9]	0.001
AST, U/l		29.0 [25.5; 33.1] ^a	26.1 [23.5; 28.9]	23.6 [21.1; 26.4] ^a	0.002
GGT, U/l		58.8 [49.3; 70.1] ^{abc}	47.0 [38.6; 57.2] ^{abc}	37.9 [31.1; 46.0] ^{abc}	<0.001
<i>Renal function</i>					

Serum creatinine, $\mu\text{mol/l}$	63.9 [61.7; 66.2]	59.3 [57.1; 61.5] ^a	57.7 [52.9; 62.9] ^a	0.027
uACR, mg/mmol	0.60 [0.48; 0.74] ^a	-	1.44 [1.17; 1.77] ^a	<0.001
eGFR, ml/min/1.73m ²	118 [114; 122]	121 [118; 124]	116 [106; 127]	0.53

Data are presented as mean and SD for normally distributed variables and for logarithmically transformed variables are presented as geographic mean and 5th and 95th percentile. *Data available for all three data collection time points were less than those for 2005 to 2015. SD, standard deviation; HIV, human immunodeficiency virus; N, number of participants; TC, Total cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol; HDL-cholesterol, high-density lipoprotein-cholesterol; HbA1c%, glycated haemoglobin; GGT, γ -glutamyltransferase; CRP, C-reactive protein; AST, aspartate transaminase; ALT, alanine transaminase; uACR, urinary albumin creatinine ratio; eGFR, estimated glomerular filtration rate.

Table 5. Percentage of cardio-metabolic risk factors exceeding specific cut-offs of the HIV infected and uninfected individuals over 10 years

Cardio-metabolic factors			
	HIV infected	HIV uninfected	P
	N=320	N=320	
2005 baseline study			
WC, N (%)			
Women ≥ 94 cm	16/198 (8.0)	19/194 (10)	0.55
Men ≥ 80 cm	17/117 (15)	17/116 (15)	0.98
Blood pressure $\geq 130/85$ mmHg, N (%)	105/320 (32.8)	136/320 (42.5)	0.011
Glucose ≥ 5.6 mmol/l, N (%)	35/301 (12)	47/307 (15)	0.18
HDL-C, N (%)			
Women ≤ 1.29 mmol/l	34/124 (27.4)	50/161 (31)	0.504
Men ≤ 1.03 mmol/l	48/125 (38)	21/108 (19)	0.016
TG ≥ 1.7 mmol/l, N (%)	55/313 (18)	48/318 (15)	0.40
2010 follow-up study			
	HIV infected	HIV uninfected	P
	N=163	N=192	
WC, N (%)			
Women ≥ 94 cm	6/114 (5)	21/117 (18)	0.003
Men ≥ 80 cm	9/47 (19)	12/71 (17)	0.75
Blood pressure $\geq 130/85$ mmHg, N (%)	58/160 (36)	87/185 (47)	0.043
Glucose ≥ 5.6 mmol/l, N (%)	14/161 (9)	27/184 (15)	0.087
HDL-C, N (%)			
Women ≤ 1.29 mmol/l	44/115 (38)	66/113 (58)	0.002
Men ≤ 1.03 mmol/l	33/44 (75)	59/66 (89)	0.046
TG ≥ 1.7 mmol/l, N (%)	42/162 (26)	38/192 (20)	0.18
2015 follow-up study			
	HIV infected	HIV uninfected	P
	N=117	N=131	
WC, N (%)			
Women ≥ 94 cm	18/73 (25)	36/95 (38)	0.504
Men ≥ 80 cm	11/25 (44)	17/53 (32)	0.31
Blood pressure $\geq 130/85$ mmHg, N (%)	42/117 (36)	61/131 (47)	0.088
Glucose ≥ 5.6 mmol/l, N (%)	23/104 (22)	32/120 (27)	0.43
HDL-C, N (%)			
Women ≤ 1.29 mmol/l	39/83 (47)	30/77 (39)	0.31
Men ≤ 1.03 mmol/l	17/23 (75)	41/49 (84)	0.33

TG ≥ 1.7 mmol/l, N (%)	20/92 (22)	19/141 (13)	0.098
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Data are presented as proportion and percentage. HIV, human immunodeficiency virus; N, number of participants; WC, waist circumference; HDL-cholesterol, high-density lipoprotein-cholesterol; TG, triglycerides.

The cardio-metabolic factors were defined using the International Diabetes Federation (IDF) as follows: Central obesity: men ≥ 94 cm and women ≥ 80 cm; hyperglycaemia: fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed with type 2 diabetes; dyslipidaemia ≥ 1.7 mmol/l or specific treatment; Dyslipidaemia (second criteria), HDL cholesterol men < 1.03 mmol/l and women < 1.29 mmol/l or specific treatment; and raised blood pressure ≥ 130 mmHg systolic or ≥ 85 mmHg diastolic blood pressure or on treatment.

DISCUSSION

With 12.7% of the PURE study participants living with HIV after 10 years, the prevalence rate of HIV infections corresponds with the South African national rate (12.5%) [6]. One of the primary aims of the Joint United Nations Programme on HIV/AIDS (UNAIDS) is to increase the number of HIV-infected patients on ART [21], this objective resonates with the outcomes of this study cohort — with nearly a two-fold increase in ART from 46% in 2010 to 85% in 2015. This is however not reflective of the total South African population, as the use of ART is estimated at 56% [22]. Even though 46% of the HIV-infected cohort was on ART in 2010, a marked decline in mortality was observed in apt consistency with the global decline in mortality due to ART use [2]. The HIV-infected participants in this study were relatively younger than their counterparts and showed no differences in chronic medication use. As such, the observed decline in mortality could be attributed to those factors together with the beneficial effects of ART.

The assessment of the HIV-infected participants and their controls over the 10 years indicates similar changes in BP and body composition, remarkable differences concerning lipid profile and a trajectory of elevated CRP, liver enzymes and eGFR in the HIV-infected. Contrary to the expectations of eGFR decline due to ageing [23], the HIV-infected participants displayed an increase in eGFR. This suggests the probability of improved renal function with ART use. However, if the data is appraised as a trajectory towards likely future outcomes, then continuous increases in eGFR might reach the hyperfiltration range that typically precedes the development of the renal disease. Additionally, this population is predisposed to several risk factors such as ageing, the use of tobacco, alcohol consumption and anti-hypertensive medication that may increase their CVD risk in the future [24].

A previous study using the PURE study population reported a prevalence of dyslipidaemia among newly-diagnosed, HIV-infected, ART-naïve Africans [15]. Now, based on the 10-year follow-up data with 85% of the HIV-infected participants on ART, the present study reported an increase in HDL-c and TC and a decrease in TG: HDL-c in the HIV-infected compared to the uninfected controls. In

support of the increase in HDL-c, a larger proportion of the HIV-infected men had an elevated HDL-c than the HIV-uninfected men at baseline. However, after the introduction of ART in 2010, a lesser proportion of the HIV-infected participants presented with lower HDL-c, whereas no difference was observed after 10 years. The finding that Higher HDL-c is cardio-protective is consistent with a recent study which reported an increase in HDL-c over 96 weeks in HIV-infected patients on the first-line regimen, especially Nevirapine (NVP) [25]. The participants in this study were using NVP (**Figure 1**) which is associated with favourable lipid changes [26]. The decrease noticed in TG: HDL-c in the HIV-infected participants over time may be attributed to the effects of the increase in HDL-c levels [27]. These findings suggest that increased longevity on ART provides benefits and the low-to-moderate effect of the ART on the CVD risk factors might be modulated by the mean age of the [28] participants being 52.4 ± 5.66 years at follow-up. It has been established that old age is associated with higher CVD risks [29] and the study participants were mostly mid-aged and younger than their uninfected counterparts.

The 10-year continued elevation in circulating CRP in HIV-infected South Africans confirm the established inflammatory response to HIV infection, which is marked by excess leukocytes and cytokines [9]. Early and effective ART initiation plays an invaluable role in the reduction of immune activation and inflammation in HIV-infected individuals [30]. However, findings from randomised controlled trials reported persistent elevated CRP levels in HIV infected patients before and after ART use and CRP elevation was associated with HIV-disease progression [31]. The findings of this study suggest persistent low-grade inflammation in HIV-infected patients even after long-term ART, this is attributable to on-going viral replication or microbial translocation [32]. Moreover, CRP is associated with CVD risk and all-cause mortality not only in the general population [33] but also in HIV-infected patients [9]. Inflammation may be further aggravated by the presence of oxidative stress, which might in turn be a consequence of both HIV infection and the use of ART [34].

In addition, higher levels of GGT were recorded, a liver enzyme known to play an important role in maintaining glutathione homeostasis and normal redox status [35], along with a greater increase in

GGT over 10-year period in the HIV-infected group. Although other studies have reported that ART lowers serum GGT in HIV-infected patients [10], the findings of this study contradict previous reports, this can be attributed to the effects of the nucleosides [10]. The use of GGT applies beyond oxidative stress as it is also a marker of non-fatty and alcohol-related liver disease [36]. Even though self-reported alcohol use was significantly high in the study, it did not differ between these groups. Additionally, GGT, higher ALT and AST were reported at follow-up and there was an increase in ALT over time in the HIV-infected participants unlike the decrease recorded in uninfected counterparts. These results are in concordance with previous findings [10]. It is crucial to note that ALT and GGT levels were not above the cut-off values of 40 U/l and 50 U/l respectively for the liver disease [37, 38]. The administration of NRTIs is associated with mitochondrial toxicity, while NNRTIs is metabolised by the cytochrome P450 that is known to increase activities of co-administered ART, hence elevating the toxic effect on the hepatocytes [39]. Since the HIV-infected participants will continue ART, it is expected that these liver enzymes will increase over time; this necessitates the regular monitoring and management of liver function among HIV-infected individuals.

In participants with HIV, higher uACR and lower eGFR was indicated at baseline, with an increased eGFR over the 10-year gap. This increase in glomerular filtrate rate contradicts the normal expectations of a decrease in eGFR with ageing [23] but concurs with the findings of the Multicenter AIDS Cohort Study where an increase in eGFR in HIV-patients was defined as hyperfiltration (eGFR ≥ 140 ml/min/1.73m²) [40]. This finding might be an indication of renal deterioration and may lead to a higher prevalence of renal failure in HIV-infected populations in the future [11]. However, in this study, the eGFR was not above the cut-off value proposed by the Multicenter AIDS Cohort Study. This increase of eGFR may also suggest a catch-up effect in renal function over time due to ART. As renal impairment occurs dependent or independent of HIV infection. In the former pattern, HIV alters renal function because of immune suppression and when ART is introduced renal function improves by exerting its antiviral effects [41]. In the latter pattern, patients have improved immune function due to long-term ART use which later results in nephrotoxicity or as a result of pre-existing renal

impairment and ageing. Although the renal function markers did not indicate renal disease over time, the importance of regular renal screening cannot be overemphasised as this HIV-infected cohort ages and continue using life-long ART [42].

The findings of this study must be interpreted within the ambits of the strengths and limitations of the study design. Demographic and cardio-metabolic profiling of HIV-positive and control participants were carefully performed over 10 years to contribute to the existing paucity of longitudinal data on South Africans living with HIV. The newly diagnosed HIV-positive participants (N=20) during the duration of the follow-up studies were included in the study population. Regarding ART information, ART use was not available for twenty-six (N=26) HIV-infected participants. The relatively small sample size of the study notwithstanding, its utilisation of a longitudinal design validates the findings even though it may arguably not be exhaustively representative of the entire HIV-positive population in South Africa.

CONCLUSION

In conclusion, the findings of this study demonstrated that in comparison to their uninfected counterparts, South Africans living with HIV over the 10 years presented with similar changes in BP and body composition. However, HIV infection triggered longitudinal changes in the lipid profile, which indicates the possibility for the development of dyslipidaemia in the future. In addition to these changes, the prevalence of low-grade inflammation and oxidative stress among HIV infected individuals using ART, together with the lipid changes reflect the development of a pro-atherogenic profile — a profile associated with increased risk of CVD. Based on the foregoing, the recommendation made herein is that it is pertinent to monitor the trajectories of increased CRP, elevated liver enzymes and increased eGFR concerning HIV infection and ART use in South Africa.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Chapter 5, Manuscript three

**Arterial structure and function in Africans infected with HIV for >5
years:**

**longitudinal relationship with endothelial activation and
cardiovascular risk markers**

**Arterial structure and function in Africans infected with HIV for
>5 years: longitudinal relationship with endothelial activation and
cardiovascular risk markers**

Manuscript three is under review in the journal of HIV Medicine

Summary of instructions to authors used for the preparation of manuscript one

Details of the Journal	
Name of Journal	HIV Medicine
Impact factor	3.556
Aim and scope	<i>HIV Medicine</i> covers aims to provide an alternative outlet for the publication of international research papers in the field of HIV Medicine, embracing clinical, pharmacological, epidemiological, ethical, preclinical and in vitro studies.
Publisher	Wiley
Guidelines for Authors	
Original article	The main text should be up to 3,500 words
Preparation of the article	Title page: The title should be informative and conscience. Give full names, highest academic degrees and institutional affiliations of all authors. Include a complete mailing address and contact details of the corresponding author. Abstract: Should not exceed 250 words and divided into objectives, method, results and conclusion. Text: The manuscript main text must include the following sections in this order – introduction, methods, results, discussion and conclusion References: Should be between 20-50 and follow Vancouver style. Figures and tables: a maximum of 5 five figures and/or tables
Website for more information	Complete details of the instruction to the author can be viewed using the following web address: https://www.atherosclerosis-journal.com/content/authorinfo

(Please note that some of the formats were changed to ensure uniformity throughout the thesis)

**Arterial structure and function in Africans infected with HIV for >5 years:
longitudinal relationship with endothelial activation and cardiovascular risk markers**

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ABSTRACT

Objective: We determined whether individuals living with human immunodeficiency virus (HIV) for over five years have increased measures of arterial injury (carotid intima-media thickness (cIMT)) and large artery stiffness (carotid-femoral pulse wave velocity (cfPWV)) compared to HIV-uninfected counterparts and whether baseline markers of endothelial activation and cardiovascular risk are associated with cIMT and cfPWV after five years.

Methods: We matched 126 HIV-infected and 126 uninfected controls according to age, sex and locality. Cardiovascular risk and endothelial function markers were measured at baseline and cIMT and cfPWV at follow-up.

Results: At follow-up cIMT ($p=0.90$) and cfPWV ($p=0.35$) were similar between the groups. Despite elevated levels of soluble intracellular adhesion molecule and soluble vascular cell adhesion molecule in the HIV-infected group (all $p<0.001$) at baseline, these markers did not associate with cIMT and cfPWV over time. Age ($\beta=0.31$, $p=0.002$) and triglyceride: high-density lipoprotein-cholesterol ($\beta=0.23$; $p=0.016$) associated positively with cIMT in the HIV-infected group. Mean arterial pressure (MAP) ($\beta=0.28$; $p=0.010$) associated positively with cfPWV in the HIV-infected group. In the uninfected group, sex ($\beta=0.31$; $p=0.004$) and glycated hemoglobin (HbA1c) ($\beta=0.24$; $p=0.026$) associated positively with cIMT while age ($\beta=0.17$; $p=0.049$), sex ($\beta=0.29$; $p=0.003$), MAP ($\beta=0.31$; $p=0.001$) and HbA1c ($\beta=0.21$; $p=0.041$) associated positively with cfPWV.

Conclusion: Measures of arterial structure and function were similar in HIV-infected Africans and their age, sex and locality matched uninfected controls. Traditional cardiovascular risk markers rather than elevated endothelial activation at baseline were independently associated with cIMT and cfPWV over five years.

Keywords: Human immunodeficiency virus, atherosclerosis, arterial stiffness and South Africa.

INTRODUCTION

Sub-Saharan Africa (SSA) bears the greatest burden of human immunodeficiency virus (HIV) infections, with an estimated 25.6 million people living with HIV in this region [1]. Alongside the increasing HIV infection rates, cardiovascular disease (CVD) in HIV-infected individuals is also eminent. With the use of antiretroviral therapy (ART), HIV-infected individuals are living longer and as such, these individuals are now exposed to cardiovascular risk factors typically associated with ageing [2, 3]. Previous studies have shown higher estimates of atherosclerosis (reflected by increased carotid-IMT (cIMT)) [4-6] and arterial stiffness [7, 8] in HIV-infected people compared to HIV-uninfected controls. Moreover, the HIV-infected individuals are suggested to have a two-fold greater risk for myocardial infarction and coronary artery disease relative to their age-matched HIV-uninfected counterparts [9-11].

The pathophysiology underlying the development of CVD in the HIV-infected population is not fully understood. However, several factors such as socio-demographic factors [12], effects of the HIV infection (inflammation and endothelial activation) [13] and adverse metabolic complications associated with ART (namely hypertension, dyslipidaemia and diabetes mellitus) [14, 15] have been implicated to play a major role in CVD development in the HIV-infected individuals. The CVD risk profile may, however, differ between the SSA and Western populations as a result of disparities in socio-demographic profiles and the prevalence of CVD risk factors [16, 17]. Moreover, HIV subtype C is commonly found in the African setting, while subtype B is most common in Western countries [18] and the effects of the different viral subtypes on the cardiovascular system are not clear.

HIV infection is also associated with endothelial activation as part of its inflammatory response, characterised by higher expression of vascular adhesion molecules such as soluble intracellular adhesion molecule (ICAM-1) and soluble vascular cell adhesion molecule (VCAM-1) [19]. Endothelial activation markers are suggested to be diagnostic indicators of atherosclerotic disease burden and/or progression [20, 21].

We have previously reported in a cross-sectional study that HIV-infected individuals have elevated ICAM-1 and VCAM-1 levels compared to their HIV-uninfected counterparts [22]. However, it is not clear if endothelial activation coupled with traditional cardiovascular risk factors (age, sex and cardio-metabolic factors) in HIV-infected individuals is associated with accelerated arterial and arterial stiffness. It was firstly aimed to determine whether HIV-infected individuals living with HIV for more than five years have higher measures of arterial injury (cIMT) and arterial stiffness (carotid-femoral pulse wave velocity (cfPWV)) when compared to their age, sex and locality matched uninfected counterparts. Secondly, it was aimed to determine whether baseline markers of endothelial activation and traditional cardiovascular risk factors are associated with arterial injury (cIMT) and stiffness (PWV) after five years.

MATERIALS AND METHODS

Study design and population

This current study forms part of the Prospective Urban and Rural Epidemiology (PURE) study. The PURE study was designed to examine and compare changes in lifestyle factors focusing on low, middle and high-income countries. The participants for this study were recruited in the North West Province, South Africa, to take part in the study. Data collection was done on more than one occasion, at baseline (2005) and two follow-up phases in 2010 and 2015, respectively.

This current study followed African men and women who participated in the PURE study living in rural and urban areas from 2010 to 2015. One-hundred-and-twenty-six HIV-infected participants were matched for age, sex and locality to 126 HIV-uninfected controls taking part in both the 2010 and 2015 data collections (**Figure 1**). The study fulfilled all the applicable requirements of the Declaration of Helsinki and was approved by the Health Research Ethics Committee of the North-West University, South Africa (Ethics number: NWU-00074-17-S1). All the study participants provided written, informed consent and participation in the study were voluntary.

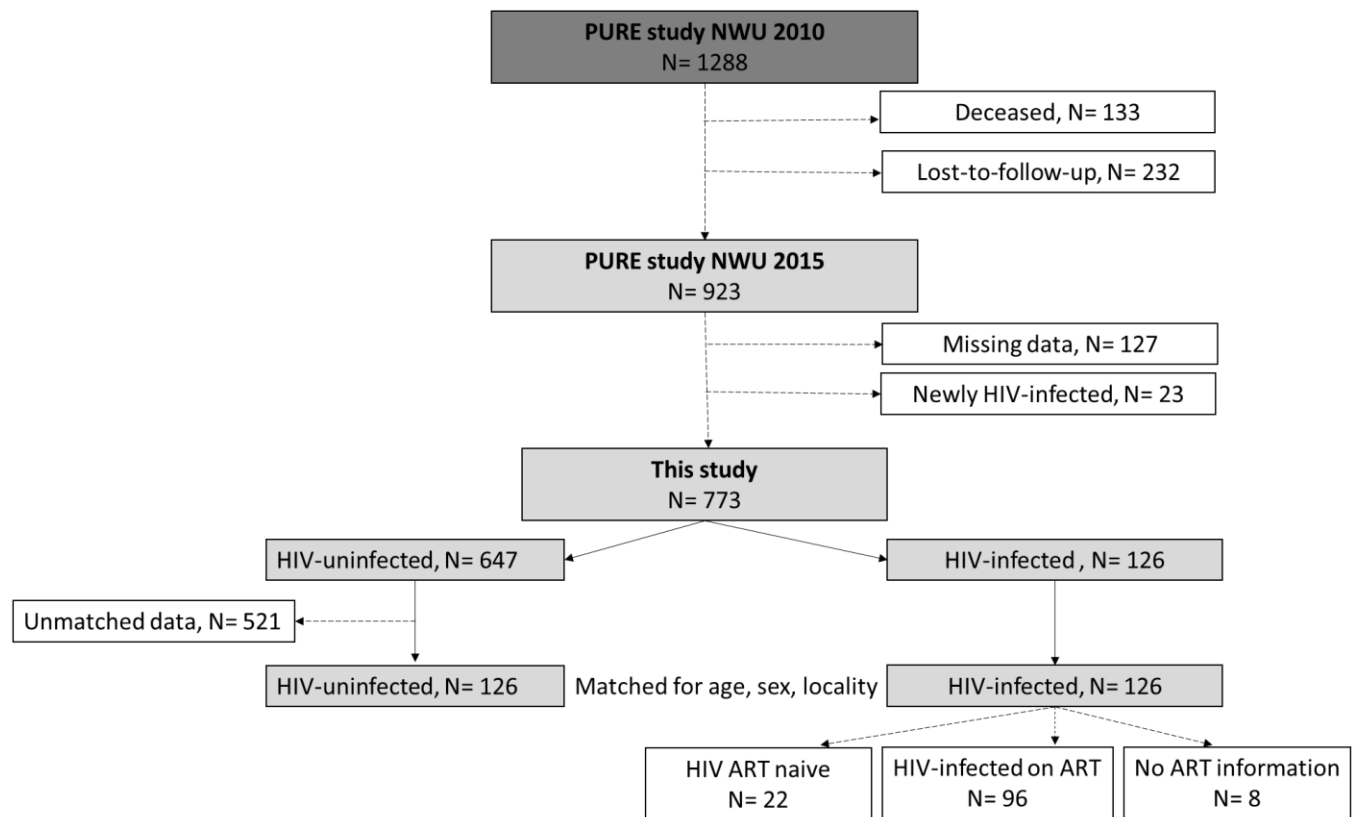


Figure 1. Outline of the study population.

PURE, Prospective Urban and Rural Epidemiology Study; N, number of participants; HIV, human immunodeficiency virus; ART, antiretroviral therapy

Questionnaire data

Demographic and lifestyle factors, as well as information on medical history, were collected using questionnaires.

Anthropometry

Following standardised procedures, height, weight, waist and hip circumferences were measured [16]. Body mass index was computed as weight (in kilograms) divided by the square of height (in meters).

Cardiovascular measurements

Blood pressure

After five minutes of rest, brachial blood pressure measurements were taken in a sitting position on the right arm in duplicate at an interval of five minutes. The validated OMRON HEM-757 (Omron Healthcare, Kyoto, Japan) device was used in 2010, while the OMRON M6 device (Omron Healthcare, Kyoto, Japan) was used during the 2015 follow-up study. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of anti-hypertensive medication [23].

Carotid intima-media thickness

Measurement of cIMT was done using a Sonosite Micromax ultrasound system (SonoSite, Inc., Bothel, WA, USA), with a 6-13 MHz linear array transducer at follow-up. Images of the left and right common carotid artery were obtained from at least two optimal angles. The segments were then imaged and measured by prescribed protocols and were digitised and imported into the Artery Measurement System (Gothenburg, Sweden) semi-automated software and analysis of IMT measurements was performed [24, 25]. The analysis was done on a selected segment of a maximum 10 mm with high image quality. The borders of the near and far wall of the intima-media, as well as the inner diameter of the vessel, were automatically identified by the program. This was followed by the calculation of the mean cIMT and vessel diameter throughout the 10 mm segment, by using around 100 discrete measurements.

Pulse wave velocity

The measurement of cfPWV was done with the participant in a supine position using the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia). The cfPWV measurements were done in duplicate and the average of the two measurements was used.

Biological sampling and biochemical analyses

The participants were asked to fast overnight for 8-10 hours and blood samples were taken by a registered nurse using a sterile winged infusion set. Serum samples were used to analyse high sensitivity C-reactive protein (CRP), total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-c) and γ -glutamyltransferase (GGT) with a Cobas Integra 400 plus (Roche, Basel, Switzerland). Low-density lipoprotein-cholesterol (LDL-c) levels were calculated [26]. The endothelial activation markers, VCAM-1 and ICAM-1 were determined with human sICAM-1 and sVCAM-1 enzyme-linked immunosorbent assays (IBI Hamburg, Germany). Glucose levels from fluoride plasma samples were determined with the Cobas Integra 400 plus (Roche, Basel, Switzerland). Glycated haemoglobin (HbA1c) was determined using the D-10 Hemoglobin testing system from Bio-Rad (Hercules, California, United States).

Human immunodeficiency virus

The First Response rapid HIV card test (Premier Medical Corporation Limited, Daman, India) was used to determine the HIV status of the participants from whole blood finger-prick. The SD BIOLINE HIV 1/2 3.0 card test (Standard Diagnostics, INC, Korea) and Abon (Biopharm Corporation Limited Hanyzhou, China) rapid card test in 2010 and 2015, respectively were used to repeat and confirm HIV positive results. In 2010, the CD4 cell counts were determined by the National Health Laboratory Services (Beckman Coulter® EPICS® XI™, Fullerton, USA) while in 2015 finger-prick blood was collected and CD4 cell count determined using a point-of-care device, PIMA™ CD4 (Alere, Jena, Germany) at the research site. The HIV-infected individuals were using ART composed of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitors (NNRTIs).

STATISTICAL ANALYSES

Statistical analyses were performed using Statistica version 13.3 (TIBCO Software Inc, Palo Alto, United States of America, 2017). The normality of the data was tested using histograms and q-q plots. Normally distributed variables (age, systolic and diastolic blood pressure and heart rate) were presented as means with standard deviation, while the logarithmically transformed variables (waist circumference, body mass index, mean arterial pressure, ICAM-1, VCAM-1, TC, HDL-c, LDL-c, TG, total cholesterol: high-density lipoprotein-cholesterol (TC: HDL-c) ratio, triglycerides: high-density lipoprotein-cholesterol (TG: HDL-c) ratio, HbA1c, CRP and GGT) were presented as geometric means with 5th and 95th percentiles. Independent t-tests were used to compare the means of continuous variables while dependent t-tests were used to determine changes over time. Chi-square and McNemar tests were done to compare frequencies in HIV-infected and uninfected groups. Partial correlations were used to assess the associations for cIMT and cfPWV at follow-up with baseline markers of endothelial activation (ICAM-1 and VCAM-1) and cardiovascular risk factors (CRP, GGT, HIV status, ART use, age, sex, body mass index, mean arterial pressure, HbA1c, TG: HDL-c ratio and tobacco use) while adjusting for age, sex, body mass index and mean arterial pressure.

Multivariable-adjusted linear regression analyses were conducted to determine prospective associations for cIMT and cfPWV with baseline markers of endothelial activation (ICAM-1 and VCAM-1) and cardiovascular risk factors (CRP, GGT, HIV status, ART use, age, sex, body mass index, mean arterial pressure, HbA1c, TG: HDL-c ratio and tobacco use). These analyses were performed in the entire group and separately in the HIV-infected and uninfected groups. Regression models included ICAM-1 and VCAM-1 separately and the following cardiovascular risk factors at baseline as the main independent variables: CRP, GGT, age, sex, body mass index, mean arterial pressure, HbA1c, TG: HDL-c ratio and tobacco use. The use of ART was included in the total group and HIV-infected group, while HIV status was included only in the total group.

RESULTS

Characteristics of the study population

The baseline and follow-up characteristics of the HIV-infected and matched uninfected participants are presented in **Table 1**. The HIV-infected patients had lower waist circumference (all $p<0.001$) and body mass index (all $p<0.001$) compared to the uninfected controls. Lower systolic (all $p\leq 0.015$) and diastolic blood pressure (all $p\leq 0.012$) were found as well as mean arterial pressure (all $p\leq 0.006$) in the HIV-infected group at baseline and follow-up.

Baseline ICAM-1 and VCAM-1 (all $p<0.001$) levels were higher in the HIV-infected group than the uninfected group. The HIV-infected participants presented with lower TC and HDL-c (both $p\leq 0.010$) at baseline and, lower glycated haemoglobin at baseline and follow-up (all $p\leq 0.001$). At follow-up, GGT ($p=0.002$) was higher in the HIV-infected participants. The measures of arterial structure and function in the HIV-infected versus uninfected controls are compared in **Figure 2**, where cIMT (0.60 ± 0.01 vs. 0.60 ± 0.01 mm; $p=0.90$) and cfPWV (7.98 ± 1.02 vs. 8.15 ± 1.02 m/s; $p=0.35$) were similar between the groups. We repeated the analysis by further adjusting for MAP and estimated glomerular filtration rate and the results remained unchanged, cfPWV (7.97 ± 1.02 vs. 8.16 ± 1.02 , $p=0.32$).

Changes over five years in the characteristics of the study population (**Supplementary Table 1**) indicated an increase in HDL-c ($p<0.001$), LDL-c ($p=0.003$) and GGT ($p=0.022$) and a decrease for TC: HDL-c ratio ($p<0.001$) and HbA1c ($p<0.001$) in the HIV-infected group. In the HIV-uninfected individuals, TC ($p<0.001$), TC: HDL-c ($p<0.001$) and HbA1c ($p=0.046$) decreased over time. The use of ART in the HIV-infected participants increased from 41.6% to 82.3% ($p=0.001$). The management of hypertension within the HIV-infected group improved slightly over this period, i.e. a small increase in the number of participants using anti-hypertensive medication (11.6% vs 14.6%, $p<0.001$), as well as a decrease in the frequency of hypertension, after five years (38.0% vs 28.1%, $p<0.001$). The use of anti-hypertensive and anti-diabetic medication within the uninfected group increased (all $p\leq 0.001$) from 21.6% to 28.8% and 4.80% to 11.2% respectively over the same period.

Table 1. Characteristics of HIV-infected and uninfected groups at baseline and five-year follow-up

	Baseline (2010)			Follow-up (2015)		
	HIV-infected N=126	Uninfected N=126	<i>P</i>	HIV-infected N=126	Uninfected N=126	<i>P</i>
<i>Socio-demographic factors</i>						
Age, years	47.8 ± 5.70	47.7 ± 5.63	0.99	52.7 ± 5.68	52.7 ± 5.65	0.95
Men, N (%)	27.0 (21.4)	27.0 (21.4)	1.00	27.0 (21.4)	27.0 (21.4)	1.00
Urban, N (%)	56.0 (44.4)	56.0 (44.4)	1.00	56.0 (44.4)	56.0 (44.4)	1.00
<i>Anthropometry</i>						
Waist circumference, cm	75.4 [62.0; 94.8]	82.3 [63.5; 108]	< 0.001	81.3 [64.6; 103]	89.2 [66.6; 114]	< 0.001
Body mass index, kg/m ²	22.9 [16.9; 34.9]	26.3 [17.2; 42.0]	< 0.001	22.9 [16.6; 35.5]	26.0 [16.9; 41.0]	< 0.001
<i>Cardiovascular measurements</i>						
Systolic blood pressure, mmHg	122 ± 19.5	133 ± 22.8	< 0.001	125 ± 24.7	133 ± 23.8	0.015
Diastolic blood pressure, mmHg	83.7 ± 12.1	89.2 ± 13.2	0.001	82.6 ± 13.8	86.9 ± 13.2	0.012
Mean arterial pressure, mmHg	95.6 [75.0; 118]	103 [83.0; 131]	0.001	95.3 [73.0; 128]	101 [79.0; 127]	0.006
Heart rate, beats/min	66.4 ± 16.6	65.1 ± 17.4	0.57	75.3 ± 14.5	74.5 ± 14.7	0.66
<i>Endothelial activation</i>						
ICAM-1, ng/ml	340 [155; 682]	275 [145; 508]	< 0.001	-	-	-
VCAM-1, ng/ml	976 [528; 1935]	708 [447; 1412]	< 0.001	-	-	-
<i>Biochemical variables</i>						
TC, mmol/l	4.39 [2.93; 6.55]	4.46 [3.30; 7.04]	0.010	4.43 [2.93; 6.16]	4.41 [3.00; 6.36]	0.93
HDL-c, mmol/l	1.17 [0.71; 2.04]	1.38 [0.80; 2.50]	0.001	1.31 [0.82; 2.45]	1.33 [0.71; 2.35]	0.81
LDL-c, mmol/l	2.47 [1.03; 4.22]	2.63 [1.32; 4.70]	0.23	2.73 [1.65; 4.45]	2.62 [1.14; 4.79]	0.45

TG, mmol/l	1.12 [0.50; 2.90]	1.13 [0.58; 2.36]	0.97	1.17 [0.51; 3.36]	1.13 [0.56; 2.72]	0.62
TC:HDL-c ratio	3.76 [2.27; 6.63]	3.45 [1.91; 6.34]	0.063	3.02 [1.82; 6.92]	3.24 [2.04; 9.33]	0.24
TG:HDL-c ratio	0.96 [0.30; 3.61]	0.82 [0.30; 2.43]	0.094	0.89 [0.35; 2.66]	0.85 [0.35; 3.19]	0.62
Glycated hemoglobin, %	5.70 [4.90; 6.40]	6.13 [5.30; 9.50]	<0.001	5.46 [4.90; 6.20]	5.96 [5.00; 11.7]	<0.001
C-reactive protein, mg/l	3.04 [0.38; 39.6]	3.29 [0.24; 23.8]	0.65	3.70 [0.28; 50.8]	2.92 [0.26; 18.1]	0.18
γ -glutamyltransferase, U/l	46.0 [12.4; 261]	39.5 [11.0; 374]	0.22	53.2 [14.9; 351]	36.3 [11.5; 196]	0.002
<i>Lifestyle factors</i>						
Tobacco use, n/N (%)	71/123 (57.7)	61/120 (50.8)	0.28	51/123 (41.5)	50/126 (39.7)	0.78
Alcohol use, n/N (%)	43/118 (36.4)	36/119 (30.3)	0.31	42/123 (34.1)	46/126 (36.5)	0.70
<i>Medication use</i>						
Antiretroviral therapy, n/N (%)	52/121 (43.0)	-	-	96/118 (81.4)	-	-
Anti-hypertensive therapy, n/N (%)	14/126 (11.1)	27/126 (21.4)	0.027	18/121 (14.9)	36/125 (28.8)	0.008
Hypertension frequency, n/N (%)	46/121 (38.0)	55/120 (45.8)	0.22	34/125 (27.2)	56/126 (44.4)	0.004
Statins, n/N (%)	0/126 (0.00)	0/126 (0.00)	1.00	2/121 (1.65)	6/125 (4.80)	0.16
Anti-inflammatory therapy, n/N (%)	7/126 (5.56)	10/126 (7.94)	0.45	8/121 (6.61)	13/125 (10.4)	0.29
Anti-diabetic therapy, n/N (%)	1/126 (0.79)	6/126 (4.76)	0.055	0/121 (0.00)	14/125 (11.2)	<0.001
<i>HIV-related factors</i>						
CD4 cell count, cell/mm ³	311 [106; 773]	-	-	453 [143; 927]	-	-

Data are arithmetic mean $\pm$ SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. SD, standard deviation; HIV, human immunodeficiency virus; n, number of participants; N, the total number of participants; TC, total cholesterol; LDL-c, low-density lipoprotein-cholesterol; HDL-c, high-density lipoprotein-cholesterol; TC: HDL-c ratio, total cholesterol: high-density lipoprotein-cholesterol ratio; TG: HDL-c ratio, triglycerides: high-density lipoprotein-cholesterol ratio; ICAM-1, intracellular adhesion molecule; VACM-1, vascular cell adhesion molecule; cf-pulse wave velocity, carotid-femoral pulse wave velocity; c intima-media thickness mean, carotid intima-media thickness mean.

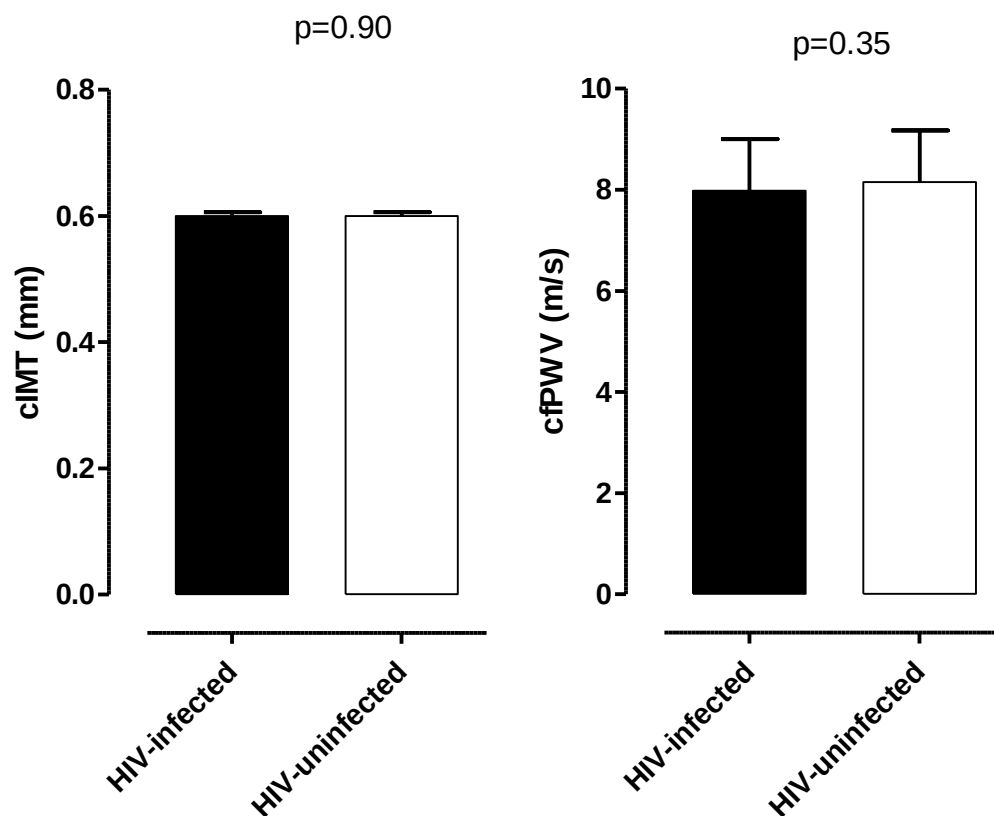


Figure 2. Carotid intima-media thickness and carotid-femoral pulse wave velocity in HIV-infected individuals (N=120) versus age, sex and locality matched HIV-uninfected controls (N=125).

cIMT and cfPWV were adjusted for mean arterial pressure. N, number of participants; HIV, human immunodeficiency virus; cIMT, carotid intima-media thickness; cfPWV, carotid-femoral pulse wave velocity.

Partial correlation analyses

In partial regression analyses (adjusted for age, sex, body mass index and mean arterial pressure), a positive correlation of cIMT with TG: HDL-c ratio ($r=0.24$; $p=0.012$) and negative association with the use of ART ($r=-0.21$; $p=0.040$) was found only in the HIV-infected group (**Table 2**). In the HIV-uninfected individuals, cIMT was positively associated with only HbA1C ($r=0.20$; $p=0.030$). With regard to cfPWV, it was negatively associated with GGT ($r=-0.19$; $p=0.049$) and VCAM-1 ($r=-0.27$; $p=0.005$) and positively correlated with HbA1c ($r=0.22$; $p=0.022$) only in the HIV-uninfected group.

Table 2. Regression analyses of follow-up markers of arterial structure and function with baseline markers of endothelial activation and cardiovascular disease risk

HIV-infected[#], N=116		
	cIMT, mm	cfPWV, m/s
ICAM-1, ng/ml	r=-0.06; p=0.55	r=0.10; p=0.32
VCAM-1, ng/ml	r=-0.12; p=0.22	r=0.05; p=0.63
C-reactive protein, mg/l	r=0.07; p=0.44	r=0.02; p=0.83
γ-glutamyltransferase, U/l	r=0.04; p=0.67	r=0.02; p=0.84
Antiretroviral therapy	r=-0.21; p=0.040	r=0.07; p=0.45
Glycated hemoglobin, %	r=-0.11; p=0.25	r=0.18; p=0.07
TG:HDL-c ratio	r=0.24; p=0.012	r=0.05; p=0.61
Tobacco use, %	r=-0.08; p=0.42	r=0.14; p=0.16

HIV-uninfected[#], N=121		
	cIMT, mm	cfPWV, m/s
ICAM-1, ng/ml	r=-0.02; p=0.87	r=0.06; p=0.55
VCAM-1, ng/ml	r=-0.05; p=0.60	r=-0.27; p=0.005
C-reactive protein, mg/l	r=0.04; p=0.65	r=0.02; p=0.86
γ-glutamyltransferase, U/l	r=-0.11; p=0.25	r=-0.19; p=0.049
Glycated hemoglobin, %	r=0.20; p=0.030	r=0.22; p=0.022
TG:HDL-c ratio	r=-0.04; p=0.71	r=0.14; p=0.17
Tobacco use, %	r=-0.13; p=0.18	r=-0.11; p=0.26

HIV, Human Immunodeficiency Virus; cIMT, carotid intima-media thickness; cfPWV, carotid-femoral pulse wave velocity; ICAM-1, intercellular adhesion molecule; VCAM-1, vascular cell adhesion molecule; TG: HDL-c ratio, triglycerides: high-density lipoprotein-cholesterol ratio.

[#] Adjusted for age, sex, body mass index and mean arterial pressure.

Multivariable adjusted regression analyses

Table 3 presents the results of the multivariable regression analysis of cIMT or cfPWV as dependent variables with markers of endothelial activation (ICAM-1, model 1 or VCAM-1, model 2) and cardiovascular risk factors (CRP, GGT, HIV status, ART use, age, sex, body mass index, mean

arterial pressure, HbA1c, TG: HDL-c ratio and tobacco use) as independent variables in the HIV-infected, uninfected and total group.

In the total group, age (all $\beta \leq 0.16$; $p \leq 0.024$) and mean arterial pressure (model 1: $\beta \leq 0.17$; $p \leq 0.023$) were positively associated with cIMT. The cfPWV was negatively associated with the use of ART (model 1: $\beta = -0.17$; $p = 0.001$ and model 2: $\beta = -0.20$; $p = 0.010$) and positively with female sex (all: $\beta = 0.14$; $p \leq 0.046$), mean arterial pressure (all $\beta = 0.30$; $p < 0.001$) and HbA1c (all $\beta = 0.16$; $p \leq 0.027$).

In the HIV-infected group, cIMT was positively associated with age (model 1: $\beta = 0.31$; $p = 0.002$ and model 2: $\beta = 0.29$; $p = 0.003$) and TG: HDL-c ratio (all $\beta \leq 0.23$; $p \leq 0.017$). A positive association for cfPWV with the mean arterial pressure (all $\beta = 0.28$; $p \leq 0.010$) was observed in the HIV-infected group. With regard to the HIV-uninfected group, cIMT was positively associated with female sex (all $\beta = 0.31$; $p = 0.004$) and HbA1c (all $\beta = 0.24$; $p = 0.026$). Age ($\beta = 0.17$; $p = 0.049$), female sex ($\beta = 0.29$; $p = 0.003$), mean arterial pressure ($\beta = 0.31$; $p = 0.001$) and HbA1C ($\beta = 0.21$; $p = 0.034$) were positively associated with cfPWV in model 1 while in model 2 cfPWV was negatively associated with VCAM-1 ($\beta = -0.21$; $p = 0.010$) and positively associated with female sex ($\beta = 0.30$; $p < 0.001$), mean arterial pressure ($\beta = 0.31$; $p < 0.001$) and HbA1c ($\beta = 0.19$; $p = 0.041$).

Table 3. Multivariable-adjusted regression analyses with cIMT and cfPWV as dependent variables

	Total group (N=229)		HIV-infected (N=109)		Uninfected (N=115)	
	cIMT (mm)	cfPWV (m/s)	cIMT (mm)	cfPWV (m/s)	cIMT (mm)	cfPWV (m/s)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Model 1						
Adjusted R^2	0.07	0.20	0.16	0.09	0.07	0.30
ICAM-1, ng/ml	-0.04 [-0.17; 0.10]	0.04 [-0.24; 0.02]	-0.04 [-0.23; 0.14]	0.08 [-0.12; 0.28]	0.01 [-0.18; 0.21]	0.02 [-0.15; 0.20]
C-reactive protein, mg/l	0.07 [-0.07; 0.21]	-0.03 [-0.14; 0.12]	0.08 [-0.11; 0.27]	0.01 [-0.19; 0.21]	0.04 [-0.16; 0.24]	-0.03 [-0.21; 0.34]
GGT, U/l	-0.07 [-0.25; 0.07]	0.11 [-0.02; 0.24]	-0.02 [-0.23; 0.19]	0.06 [-0.17; 0.28]	-0.13 [-0.33; 0.07]	0.16 [-0.01; 0.07]
HIV status, neg/pos	0.03 [-0.13; 0.19]	-0.02 [-0.11; 0.20]	-	-	-	-
ART use, no/yes	0.08 [-0.07; 0.24]	-0.17 [-0.35; -0.05][†]	-0.01 [-0.21; 0.21]	-0.21 [-0.44; 0.02]	-	-
Age, years	0.16 [0.02; 0.29]*	0.08 [-0.05; 0.20]	0.31 [0.12; 0.50][†]	0.04 [-0.16; 0.24]	-0.01 [-0.20; 0.19]	0.17 [0.03; 0.35]*
Sex, women/men	0.10 [-0.04; 0.24]	0.14 [0.01; 0.27]*	-0.04 [-0.23; 0.16]	0.03 [-0.19; 0.24]	0.31 [0.10; 0.52][†]	0.29 [0.11; 0.47][†]
Body mass index, kg/m ²	0.04 [-0.12; 0.20]	-0.13 [-0.230; 0.01]	0.12 [-0.08; 0.33]	-0.09 [-0.31; 0.12]	0.07 [-0.16; 0.30]	-0.14 [0.14; 0.49]
Mean arterial pressure, mmHg	0.17 [0.63; 0.31]*	0.30 [0.17; 0.43][‡]	0.15 [-0.04; 0.34]	0.28 [0.07; 0.48]*	0.15 [0.05; 0.35]	0.31 [0.14; 0.49][‡]
HbA1c, %	0.06 [-0.08; 0.21]	0.16 [0.02; 0.30]*	-0.10 [-0.31; 0.10]	0.09 [-0.13; 0.31]	0.24 [0.03; 0.44]*	0.21 [0.02; 0.40]*
TG:HDL-c ratio	0.13 [0.01; 0.26]	0.06 [-0.07; 0.19]	0.23 [0.05; 0.41]*	0.05 [-0.14; 0.25]	-0.10 [-0.31; 0.10]	0.04 [-0.14; 0.22]
Tobacco use, %	-0.12 [-0.25; 0.01]	0.03 [-0.09; 0.16]	-0.09 [-0.27; 0.10]	0.14 [-0.06; 0.33]	-0.12 [-0.30; 0.07]	-0.10 [-0.27; 0.07]
Model 2						
Adjusted R^2	0.07	0.21	0.18	0.08	0.07	0.35
VCAM-1, ng/ml	-0.06 [-0.19; 0.08]	-0.11 [-0.09; 0.18]	-0.14 [-0.32; 0.05]	0.02 [-0.18; 0.23]	-0.04 [-0.22; 0.14]	-0.21 [-0.37; -0.05]*
C-reactive protein, mg/l	0.07 [-0.06; 0.21]	-0.01 [-0.16; 0.09]	0.10 [-0.09; 0.30]	0.01 [-0.19; 0.22]	0.05 [-0.15; 0.24]	0.04 [-0.16; 0.17]
GGT, U/l	-0.08 [-0.22; 0.06]	0.11 [-0.03; 0.24]	-0.05 [-0.26; 0.15]	0.08 [-0.15; 0.30]	-0.13 [-0.32; 0.07]	0.16 [-0.01; 0.32]
HIV status, neg/pos	0.04 [-0.12; 0.20]	0.04 [-0.17; 0.13]	-	-	-	-
ART use, no/yes	0.07 [-0.08; 0.23]	-0.20 [-0.32; -0.02]*	-0.03 [-0.24; 0.18]	-0.22 [-0.44; 0.01]	-	-
Age, years	0.15 [0.02; 0.29]*	0.07 [-0.05; 0.21]	0.29 [0.10; 0.48][†]	0.04 [-0.16; 0.24]	-0.01 [-0.20; 0.19]	0.17 [0.01; 0.33] [‡]
Sex, women/ men	0.11 [-0.03; 0.25]	0.14 [0.01; 0.27]*	-0.04 [-0.24; 0.15]	0.02 [-0.19; 0.24]	0.31 [0.10; 0.52]*	0.30 [0.13; 0.48]
Body mass index, kg/m ²	0.04 [-0.12; 0.20]	-0.15 [-0.28; 0.02]	0.09 [-0.11; 0.30]	-0.10 [-0.32; 0.12]	0.15 [-0.04; 0.34]	-0.14 [0.15; 0.48]
Mean arterial pressure, mmHg	0.16 [0.02; 0.30]*	0.30 [0.16; 0.43][‡]	0.15 [-0.04; 0.34]	0.28 [0.07; 0.49]*	0.07 [-0.17; 0.30]	0.31 [0.15; 0.48][‡]

HbA1c, %,	0.06 [-0.08; 0.21]	0.16 [0.02; 0.30]*	-0.09 [-0.30; 0.11]	0.09 [-0.13; 0.31]	0.24 [0.03; 0.44][‡]	0.19 [0.01; 0.38]*
TG:HDL-c ratio	0.13 [-0.01; 0.26]	0.06 [-0.07; 0.19]	0.22 [0.04; 0.41]*	0.06 [-0.14; 0.25]	-0.11 [-0.31; 0.10]	0.02 [-0.16; 0.20]
Tobacco use, %	-0.12 [-0.25; 0.01]	0.03 [-0.09; 0.16]	-0.11 [-0.30; 0.01]	0.14 [-0.06; 0.35]	-0.11 [-0.30; 0.07]	-0.08 [-0.24; 0.08]

β, partial regression coefficient; 95% CI, 95% confidence interval of β; HIV, human immunodeficiency syndrome; cfPWV, pulse wave velocity; cIMT, carotid intima-media thickness; ICAM-1, intracellular adhesion molecule; VCAM-1, vascular cell adhesion molecule; GGT, γ-glutamyltransferase; pos, positive; neg, negative; ART, antiretroviral therapy; HbA1C, glycated haemoglobin; TG: HDL-c ratio, triglycerides: high-density lipoprotein cholesterol ratio; Independent variables included in the model for the total group: ICAM-1 or VCAM-1, C-reactive protein, GGT, HIV status, ART use, age, sex, body mass index, HbA1C, TG: HDL-c ratio and tobacco use; HIV-infected group: ICAM-1, VCAM-1, C-reactive protein, GGT, ART use, age, sex, body mass index, mean arterial pressure, HbA1C, TG: HDL-c ratio and tobacco use; HIV-uninfected group: ICAM-1, VCAM-1, C-reactive protein, GGT, age, sex, body mass index, mean arterial pressure, HbA1C, TG: HDL-c ratio and tobacco use. All independent variables were added at the same time. ¥, p<0.001; ‡, p<0.01; *, p ≤ 0.05; – Variable was not included in the model.

DISCUSSION

Measures of arterial structure (cIMT) and function (cfPWV) were determined in an HIV-infected cohort living with HIV for more than five years and compared to age, sex and locality matched HIV-uninfected controls. There was no difference in the cIMT or cfPWV in the HIV-infected individuals compared to their uninfected counterparts. It was also determined whether baseline markers of endothelial activation and cardiovascular risk factors are prospectively associated with cIMT and cfPWV. Although endothelial activation markers (ICAM-1 and VCAM-1) were markedly higher at baseline in HIV-infected individuals, these markers did not contribute to higher IMT measures or arterial stiffness after five years. Rather, traditional cardiovascular risk factors such as age and TG:HDL-c ratio associated positively with cIMT in the HIV-infected group, with female sex and HbA1c associating positively with cIMT in the uninfected group. In the HIV-infected group, the mean arterial pressure at baseline was positively associated with cfPWV. Lastly, a positive association for cfPWV with age, sex, mean arterial pressure and HbA1c were observed in the HIV-uninfected individuals.

Our finding of similar cIMT measures between the HIV-infected individuals and age, sex and locality matched uninfected controls is consistent with the previous study that included HIV-infected Africans from Botswana [27]. In line with the previously mentioned study, the HIV-infected individuals were on ART (mainly reverse transcriptase inhibitors) and had viral suppression. It has been shown that cIMT tends to decrease with the use of ART, particularly NNRTIs and viral suppression [28]. Moreover, in this study, 84% of the HIV-infected individuals were taking ART comprising of a combination of NNRTIs and NRTIs at follow-up when the IMT measurements were taken. Contrary to the finding, other studies have reported a higher IMT in the HIV-infected individuals compared to their uninfected controls and showed that IMT was strongly associated with increasing age, altered lipid metabolism and inflammation [4, 5]. This study cohort had similar age, CRP levels, as well as favourable lipid profile and this, may in part, help to explain the similar cIMT in the two groups.

Previous studies that have reported on cfPWV in HIV-infected individuals compared to uninfected controls have produced inconsistent findings [7, 8, 29, 30]. As such, some of the studies reported

higher cfPWV in HIV-infected individuals than uninfected counterparts. But, consistent, with the finding of the study, Monteiro et al. [29] and Ferraioli et al. [30] reported no difference in cfPWV for the HIV-infected individuals and uninfected controls. These inconsistencies may be due to different methods used to measure PWV, study designs and characteristics of the participants. HIV infection *per se* is known to have deleterious effects on the arterial wall properties either via immunosuppression and inflammation [31]. However, with the use of ART, the direct effects of HIV on the arterial wall tend to be lessened due to immune reconstitution [32] and in this study, the mean CD4 cell count was 453 cells/mm³ and this is above the cut-off of ≤ 200 cells/mm³ to define immunosuppression. Moreover, the effects of ART on arterial stiffness vary between different ART regimen classes. Hence, the use of a protease inhibitors (PI) regimen is associated with an increased risk of arterial stiffness compared to the use of NRTIs [33]. In this study, HIV-infected individuals were using a combination of NRTIs. The balance between the beneficial effects of diminishing active HIV infection and the adverse effects of ART may be essential to determine the net effect in an individual and the different arterial segments.

Regardless of the expectation that endothelial activation is associated with measures of arterial structure and function [21, 34, 35], cellular adhesion molecules did not associate with cIMT and cfPWV over time in the HIV-infected individuals. In large cohort studies, it has been shown that the endothelial activation marker, ICAM-1 is independently associated with the presence of carotid plaque [20] and/or the progression of carotid plaque [34], despite no significant association for ICAM-1 with cIMT. Based on these abovementioned studies, it is suggested that endothelial activation contributes to atherogenesis in the presence of advanced carotid plaque, while in the study, the mean cIMT and cfPWV were within normal ranges. A cross-sectional study done by Ross et al. [35] assessed a relationship between ICAM-1 and VCAM-1 with IMT in HIV-infected individuals taking ART for more than 24 weeks and the endothelial activation markers did not correlate with cIMT. However, a positive association was shown for VCAM-1 with internal carotid artery IMT. Endothelial activation may, thus, affect carotid thickness in a way that depends on the arterial region being assessed. More so, atherosclerosis in different arterial beds such as the carotid and coronary

arteries may have differential expression of cell adhesion molecules. In the study of Ross et al [35], the HIV-infected individuals had higher IMT compared to the uninfected controls, as opposed to the study.

It is also important to highlight that the previous findings were mostly reported in Western populations and ethnic-specific variances in atherosclerotic and arterial stiffening may exist. Also, in the previous studies [20, 21, 34], the study population had overt dyslipidaemia and obesity – factors known to contribute to increased CVD risk.

Independent traditional CVD risk factors that were associated with cIMT and cfPWV differed for HIV-infected versus HIV-uninfected participants. Mean arterial pressure was positively associated with cfPWV in both groups and this association is consistent with previously reported studies [7, 29, 36]. The segments of the arterial wall are highly sensitive to an increase in blood pressure and raised blood pressure is an independent predictor of arterial stiffness [36]. The positive association for age and sex with cfPWV in the uninfected group is consistent with previous findings [29, 37]. Only in the HIV-infected group, cIMT was positively associated with TG: HDL-c ratio, despite similar ratio's in both groups. The presence of this association in the HIV-infected group may reflect the role of HIV infection and its treatment in altering lipid metabolism [38]. Regardless of the two groups having similar age as a result of matching, age contributed to cIMT over time only in the HIV-infected individuals. It is suggested that CVD occurs earlier in HIV-infected individuals than uninfected counterparts [39] and early accelerated vascular ageing that is seen in the HIV-infected population may explain the association of age with cIMT only in the HIV-infected group [40].

When compared to the HIV-infected individuals, the uninfected controls, presented with higher concentrations of HbA1c and revealed a positive association between HbA1c and measures of atherosclerosis and arterial stiffness. This finding is aligned with a cross-sectional study that was done in Botswana that showed HbA1c as a significant contributor to cIMT in HIV-uninfected controls but not in HIV-infected individuals [27].

The association of these traditional CVD risk factors with markers of atherosclerosis and arterial stiffness are important for HIV care in Sub-Saharan African settings where CVD is increasing as a result of epidemiological transition and urbanisation. Therefore, it is critical to screen for these CVD risk factors in HIV-infected individuals to reduce future CVD burden.

The findings of this study should be interpreted in the context of the strengths and limitations of the study design. This small sample population may not be representative of the HIV-infected population of South Africa, however the study benefits by including a population with a detailed longitudinal phenotype and a comparable age, sex and locality matched HIV-uninfected control group – an aspect that many similar studies on HIV and CVD lack. The study was done in controlled conditions and gold standard techniques were used. The participants were taken from a longitudinal cohort residing in South Africa – a country with the highest HIV epidemic in the world and thus provide important evidence addressing the impact of HIV on cardiovascular risk in follow-up studies.

To conclude, after five years of HIV infection, measures of arterial structure and function were similar in the HIV-infected and uninfected controls, suggesting that HIV infection does not pose an increased risk for arterial injury and stiffness. Despite elevated endothelial activation in the HIV-infected group at baseline, endothelial activation did not contribute to increased arterial injury or stiffness after five years. Consistent with previous studies, traditional CVD risk factors contributed to cIMT and cfPWV over time in both HIV-infected and uninfected groups.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Table 1. Changes in cardio-metabolic characteristics within the HIV-infected and uninfected groups

	HIV-infected			Uninfected		
	Baseline	Follow-up		Baseline	Follow-up	
	N= 126	N= 126	<i>P</i>	N= 126	N= 126	<i>P</i>
Age, years	47.8 ± 5.70	52.7 ± 5.68	< 0.001	47.7 ± 5.63	52.7 ± 5.65	< 0.001
<i>Anthropometry</i>						
Waist circumference, cm	75.4 [62.0; 94.8]	81.3 [64.6; 103]	< 0.001	82.3 [63.5; 108]	89.2 [66.6; 114]	< 0.001
Body mass index, kg/m ²	22.9 [16.9; 34.9]	22.9 [16.6; 35.5]	0.88	26.3 [17.2; 42.0]	26.0 [16.9; 41.0]	0.24
<i>Cardiovascular measurements</i>						
Systolic blood pressure, mmHg	122 ± 19.5	125 ± 24.7	0.12	133 ± 22.8	133 ± 23.9	0.99
Diastolic blood pressure, mmHg	83.7 ± 12.1	82.6 ± 13.6	0.59	89.2 ± 13.2	86.8 ± 13.2	0.049
Mean arterial pressure, mmHg	95.6 [75.0; 118]	95.3 [73.0; 128]	0.86	103 [83.0; 131]	101 [79.0; 127]	0.19
Heart rate, beats/min	66.4 ± 16.6	75.6 ± 14.3	< 0.001	65.1 ± 17.5	73.5 ± 13.4	< 0.001
<i>Biochemical variables</i>						
TC, mmol/l	4.39 [2.93; 6.55]	4.43 [2.93; 6.16]	0.53	4.46 [3.30; 7.04]	4.41 [3.00; 6.36]	< 0.001
HDL-c, mmol/l	1.17 [0.71; 2.04]	1.31 [0.82; 2.45]	< 0.001	1.38 [0.80; 2.50]	1.33 [0.71; 2.35]	0.13
LDL-c, mmol/l	2.47 [1.03; 4.22]	2.73 [1.65; 4.45]	0.003	2.63 [1.32; 4.70]	2.62 [1.14; 4.79]	0.78
TG, mmol/l	1.12 [0.50; 2.90]	1.17 [0.51; 3.36]	0.34	1.13 [0.58; 2.36]	1.13 [0.56; 2.72]	0.84
TC:HDL-c ratio	3.76 [2.27; 6.63]	3.02[1.82; 6.92]	< 0.001	3.45 [1.91; 6.34]	3.24 [2.04; 9.33]	< 0.001
TG:HDL-c ratio	0.96 [0.30; 3.61]	0.89 [0.35; 2.66]	0.45	0.82 [0.30; 2.43]	0.85 [0.35; 3.19]	0.34
Glycated hemoglobin, %	5.70 [4.90; 6.40]	5.46 [4.90; 6.20]	< 0.001	6.13 [5.30; 9.50]	5.96 [5.00; 11.7]	0.046
C-reactive protein, mg/l	3.04 [0.38; 39.6]	3.70 [0.28; 50.8]	0.18	3.29 [0.24; 23.8]	2.92 [0.26; 18.1]	0.25
γ-glutamyltransferase, U/l	46.0 [12.4; 261]	53.2 [14.9; 351]	0.022	39.4 [11.0; 374]	36.3 [11.5; 196]	0.10

<i>Lifestyle factors</i>						
Tobacco use, n/N (%)	70/120 (58.3)	50/120 (41.7)	0.91	61/120 (50.8)	49/120 (40.8)	0.31
Alcohol use, n/N (%)	41/115 (35.7)	39/115 (33.9)	<0.001	36/119 (30.3)	44/119 (37.0)	<0.001
<i>Medication use</i>						
Antiretroviral therapy, n/N (%)	47/113 (41.6)	93/113 (82.3)	0.001	-	-	-
Anti-hypertensive therapy, n/N (%)	14/121 (11.6)	18/121 (14.9)	<0.001	27/125 (21.6)	36/125 (28.8)	<0.001
Hypertension frequency, n/N (%)	46/121 (38.0)	34/121 (28.1)	<0.001	55/120 (45.8)	55/120 (45.8)	0.30
Anti-inflammatory therapy, n/N (%)	7/121 (5.79)	8/121 (6.61)	<0.001	10/125 (8.00)	13/125 (10.4)	<0.001
Anti-diabetic therapy, n/N (%)	-	-	-	6/125 (4.80)	14/125 (11.2)	<0.001
<i>HIV-related factors</i>						
CD4 cell count, cell/mm ³	311 [106; 773]	453 [143; 927]	<0.001	-	-	-

Data are arithmetic mean $\pm$ SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. SD, standard deviation; HIV, human immunodeficiency virus; n, number of participants; N, the total number of participants; TC, total cholesterol; LDL-c, low-density lipoprotein-cholesterol; HDL-c, high-density lipoprotein-cholesterol; TG, triglycerides; TC: HDL-c ratio, total cholesterol: high-density lipoprotein-cholesterol ratio; TG: HDL-c ratio, triglycerides: high-density lipoprotein-cholesterol ratio.

Chapter 6

Summary of Main Findings and Conclusions

1. INTRODUCTION

This is a summative chapter that outlines the main findings of this study and refers to the original hypotheses. This is then followed by a summary model proposing the longitudinal cardiovascular health profile of human immunodeficiency virus (HIV)-infected Africans based on the findings of this study. Lastly, recommendations and the overall conclusion of the study are presented.

2. AIM OF THIS STUDY

The central aim of this study was to investigate the longitudinal cardiovascular health of a black South African cohort infected with HIV and review the literature based on studies conducted in the Sub Saharan African (SSA) region.

3. SUMMARY OF MAIN FINDINGS

The findings from this study reveal lower estimates of blood pressure (BP) and the prevalence of hypertension in HIV-infected patients on antiretroviral therapy (ART) compared to their untreated HIV-infected and uninfected counterparts. A South African cohort including of HIV-infected population living with HIV for years, presented with lipid derangement, inflammatory status and oxidative stress coupled with elevated levels of liver enzymes and increased eGFR. In addition, traditional cardiovascular risk factors (but not elevated baseline endothelial activation and HIV status), associated with the measures of atherosclerosis and arterial stiffness over time in the HIV-infected population. **Figure 1** below presents an overview of the main findings from three manuscripts that form part of this study.

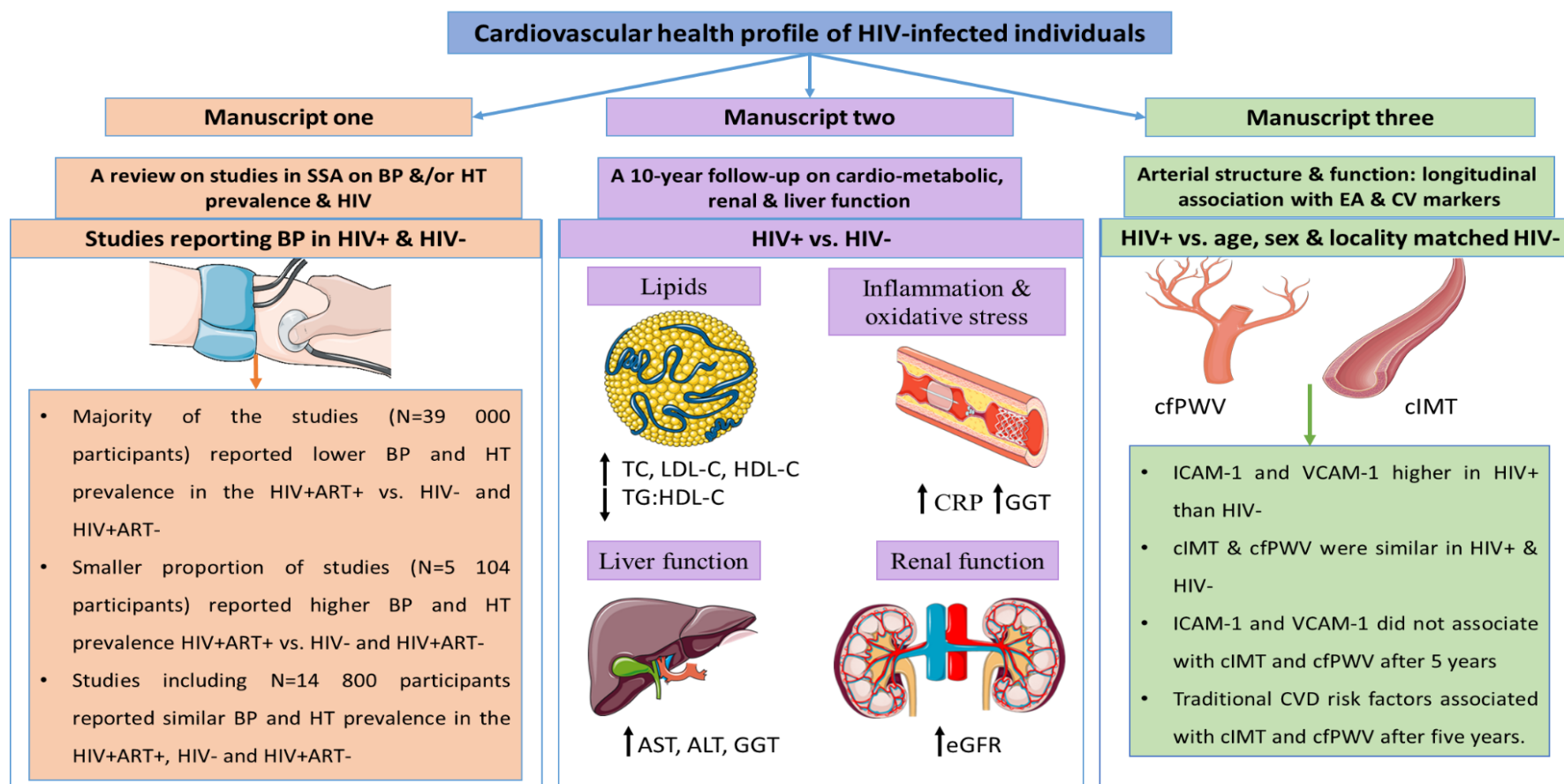


Figure 1. Presentation of the main findings of the study (manuscripts 1, 2 and 3).

HIV, human immunodeficiency virus; BP, blood pressure; HT, hypertension; HIV+ART+, treated HIV-infected; HIV+ART-, untreated HIV-infected; HIV-, HIV-uninfected; HIV+, HIV-infected; EA, endothelial activation; CV, cardiovascular; TC, total cholesterol; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; TG: HDL-c, triglycerides: high-density lipoprotein-cholesterol ratio; CRP, C-reactive protein; AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; cIMT,

carotid intima-media thickness; cfPWV, carotid-femoral pulse wave velocity; ICAM-1, intracellular adhesion molecule; VACM-1, vascular cell adhesion molecule. *Images obtained from Servier Medical art.*

4. REFERENCE TO THE HYPOTHESES AND COMPARISON WITH RELEVANT LITERATURE

Manuscript 1: A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls residing in Sub-Saharan Africa: a narrative review

- Blood pressure estimates and hypertension prevalence will be higher in the treated HIV-infected patients compared to uninfected controls
- Blood pressure estimates and hypertension prevalence will be higher in the treated HIV-infected patients compared to untreated HIV-infected controls.

In the narrative review, a large proportion of the studies indicated lower estimates of blood pressure (BP) and the prevalence of hypertension that the treated HIV-infected population compared to untreated HIV-infected and uninfected controls. The finding of lower blood pressure is consistent with finding by Malaza et al [1], Geldsetzer et al [2] and Okello et al [3] who reported lower BP and prevalence of hypertension in the HIV-infected population living in Sub-Saharan Africa (SSA). Lower BP in this population could be explained by the phenomenon of cardiovascular autonomic dysfunction related to HIV and this autonomic dysfunction is associated with lower BP in HIV-infected people regardless of immunosuppression [4]. Another possible reason could be attributed to the frequent use of non-nucleoside reverse transcriptase inhibitors (NRTIs) and nucleoside reverse transcriptase inhibitors (NRTIs), which is associated with lower BP compared to protease inhibitors (PIs) [5]. In other studies that report elevated BP with ART, it is due to concurrent weight gain as a result of immune reconstitution with the use of ART [6, 7]. However, in this review the majority of the studies that reported lower BP the HIV-infected individuals had a lower prevalence of overweight and obesity compared to the uninfected individuals [2, 3, 8].

These hypotheses are therefore rejected specifying higher BP and hypertension prevalence in the HIV-infected individuals compared to uninfected controls since it was showed that treated HIV-infected patients have lower BP and prevalence of hypertension compared to their untreated HIV-infected and uninfected counterparts.

Manuscript 2: A 10-year follow-up study of demographic and cardio-metabolic factors in HIV-infected South Africans

- HIV-infected individuals will have higher systolic and diastolic BP readings than the HIV-uninfected controls from baseline to follow-up.

However, using the PURE population, we reported similar BP in the HIV-infected individuals and uninfected controls. Similarly, Ogunmola et al [9] and Feigl et al [10] reported a similar BP for HIV-infected people and uninfected controls living in Sub-Saharan Africa. The PURE population also presented with similar waist circumference (WC), body mass index (BMI) and use of tobacco – these factors that are strongly associated with increased risk of raised BP or hypertension. This may help to explain in part this finding.

This hypothesis is rejected because the HIV-infected individuals and uninfected individuals presented with similar BP.

- HIV-infected individuals will have a lower BMI and WC when compared to the uninfected controls as well as a decrease in BMI and WC over time.

We reported similar measures of BMI and WC in the HIV-infected individuals compared to uninfected controls at both baseline and follow-up as well as no changes over time. This finding is consistent with those reported by Arbeitman et al [11] that reported similar WC and BMI in African Americans. Contrary to this study, the former study was a national wide study including children and youth which may not be comparable to our study population.

I, therefore, reject this hypothesis specifying that HIV-infected people will have lower WC and BMI since similar WC and BMI were reported at baseline and follow-up. These markers remained unchanged over time.

- HIV-infected individuals will have higher TG, TC, LDL-c and HDL-c than HIV-uninfected controls at baseline (2005) and follow-up (2015) as well as greater increases in TG, TC, LDL-c and HDL-c over time.

Regarding the lipid profile, the HIV-infected people had higher TC and HDL-c levels while TG and LDL-c were similar in the two groups at baseline. Additionally, TC and HDL-c increased over time in this group. At follow-up, the HIV-infected group had elevated levels of LDL-c while TG, HDL-c and TC concentration remained the same in the two groups. Lipid metabolism HIV-infected people are altered by HIV and ART either synergistically or independently of each other via mitochondrial toxicity, inhabitation of lipogenesis and adipocyte differentiation [12, 13]. Hence, NRTIs and NNRTIs are associated with hypercholesterolemia and lower TC and HDL-c which may help to explain the findings [14]. The increase HDL-c, as well as the decrease in LDL-c over time, could be attributed to improvement in immune function (high CD4 cell count) due to the efficacy of ART, which is known to be associated with a favorable lipid profile. increase in CD4 cell count, indicating improved immunity [15].

The HIV-infected group at baseline had higher levels of TC and HDL-c than the uninfected group, while TG and LDL-c were similar, hence the hypothesis is partially accepted. Because LDL-c was higher in the HIV-infected group and TG, TC and HDL-C did not differ between the two groups, the hypothesis is partially accepted. The last part of the hypothesis is partially accepted since the 10-year percentage change between the HIV-infected and uninfected group showed an increase in TC and HDL-c levels in the HIV-infected group as opposed to a decrease in the uninfected group. A lesser decrease in LDL-c levels was evident in the HIV-infected than the uninfected group.

- Plasma glucose and HbA1c will be similar in the HIV-infected group and uninfected controls at baseline and follow-up.

The HIV-infected individuals had similar glucose levels at both studies and lower HbA1c at follow-up. An increase in glucose levels over time were observed. These findings of a similar level of glucose in the HIV-infected and uninfected group is consistent with previous findings [16, 17]. The increase in glucose levels over time might be attributed to decreased expression of glucose transporter (GLUT 4) and this decreased expression impairs insulin-mediated intake of glucose into the adipocytes [18].

I, therefore, the first part hypothesis is rejected because glucose levels were similar at both baseline and follow-up while HbA1c was lower only at follow-up. The second part is partially accepted because glucose increased while HbA1c decreased over time in the HIV-infected group than the uninfected group.

- HIV-infected patients will have higher CRP levels than uninfected controls from baseline to follow-up studies as well as a greater increase in CRP over time.

The HIV-infected individuals had higher CRP levels only at follow-up and the CRP levels increased greatly over the 10 years indicating an inflammatory response due to HIV infection, which is marked by excess leukocytes and cytokines. Inflammation in HIV-infected people is induced as a result of compromised immune function coupled with an ongoing immune activation with a resultant increase in inflammatory markers [19].

The first part of hypothesis 4 is therefore partially accepted since CRP levels were higher in the HIV-infected at follow-up and similar in the two groups at baseline. The second part of the hypothesis is fully accepted because CRP levels increased over time in HIV-infected individuals.

- HIV-infected patients will have higher levels of GGT (as a marker of oxidative stress), than uninfected controls at baseline and follow-up as well as a greater increase in GGT over time

We reported higher concentrations of GGT, a marker of oxidative stress in the HIV-infected group than uninfected controls only at follow-up. In support of the high levels of GGT, the HIV-infected group had greater increases for GGT over time. The findings are consistent with a previous study that reported higher levels of GGT in HIV-infected individuals compared to uninfected individuals [20]. Oxidative stress is associated with HIV disease progression [21] and HIV induces oxidative stress through deregulation of oxidative stress pathways with an increase of reactive oxygen species production and by stimulating mitochondrial dysfunction [22].

This hypothesis is partially accepted, for the reason being that the HIV-infected individuals had elevated levels of GGT at follow-up, but at follow up the GGT levels were similar for the two groups. The second part of this hypothesis is fully accepted because GGT increased over time in the HIV-infected individuals than uninfected controls.

- Markers of liver function namely, γ -glutamyltransferase (GGT), aspartate transaminase (AST) and aspartate transaminase (ALT) will be higher and will have a greater increase over time in the HIV-infected individuals than in uninfected controls.

Liver enzymes namely, GGT, AST and ALT were elevated in the HIV-infected population compared to their uninfected controls at follow-up but not at baseline. Over the 10 years, both GGT and ALT but not AST increased over time in the HIV-infected population. The use of ART, especially NRTIs and an increase in CD4 cell count are associated with liver injury characterised by elevated levels of liver enzymes [23, 24] and in this study, the mean CD4 cell count of the HIV-infected increased from 314 cells/mm³ at baseline (2005) to 507 cells/mm³ at follow-up (2010).

The two groups had similar GGT, ALT and AST at baseline while these markers being higher in the HIV-infected population at follow-up, hence the hypothesis is partially accepted. The second part of the hypothesis is partially accepted because only GGT and ALT increased over time in the HIV-infected population.

- Renal function markers such as serum creatinine and urinary albumin creatinine ratio (uACR) will be higher and estimated glomerular filtration rate (eGFR) lower in the HIV-infected individuals than in uninfected controls from baseline to follow-up studies. Serum creatinine and uACR will increase and eGFR decrease over 10 years in the HIV-infected group than in the uninfected controls.

Consistent with previous studies [25, 26], the HIV-infected population presented with high creatinine and uACR as well as lower eGFR compared to their uninfected counterparts at baseline. However, at follow-up, these measures of renal function were similar in the two groups. The use of ART, especially tenofovir is associated with nephrotoxicity as a result of mitochondrial toxicity leading to renal dysfunction [25, 27]. Moreover, the HIV proteins exert their effect on the kidney by infecting the epithelial cells and podocytes, causing renal alteration [28]. Over the 10-year follow-up, serum creatinine levels reduced while eGFR increased in the HIV-infected group. An increase in eGFR above physiological normal is an indicator of glomerular hyperfiltration and it occurs due to compensatory hemodynamic alterations within the kidney [29]. However, it is important to note that

the eGFR in this study did not increase to a level of $\text{eGFR} \geq 140 \text{ ml/min/1.73 m}^2$ which indicate hyperfiltration [29]. This increase in eGFR may also suggest an improvement in renal function over time due to ART [30].

This hypothesis is therefore partially accepted because the HIV-infected population had higher levels of serum creatinine, uACR and presented with a decline eGFR at baseline study while at follow-up these measures of renal function did not differ for the two groups. The second part of this hypothesis is also partially accepted since only serum creatinine decreased, eGFR increased and uACR being similar in the two groups over time.

Manuscript 3: Arterial structure and function in Africans infected with HIV for >5 years: longitudinal relationship with endothelial activation and cardiovascular risk markers

- Measures of arterial structure (cIMT) and function (cfPWV) will be higher in the HIV-infected individuals living with HIV infection for more than five years compared to age, sex and locality matched uninfected controls.

In an HIV-infected cohort living with HIV for 5 years, similar cIMT and cfPWV were reported when compared to uninfected individuals. This is consistent with a study done by Mosepele et al [31] who reported no difference in cIMT for the HIV-infected people on ART and uninfected controls. In agreement, with the finding of this study, Monteiro et al. [32] and Ferraioli et al. [33] reported similar cfPWV for the HIV-infected individuals and uninfected controls. It is suggested that HIV infection exert deleterious effects on the arterial wall inducing alteration in arterial structure and function [34, 35]. However, with the use of ART, especially transcriptase inhibitors, the effects of HIV on the arterial wall is mitigated to a certain extent [36].

The hypothesis is rejected because cIMT and cfPWV did not differ between the HIV-infected individuals and the uninfected controls.

- Baseline markers of endothelial activation namely, soluble intracellular adhesion molecule (ICAM-1) and soluble vascular cell adhesion molecule (VCAM-1) will associate positively with

measures of arterial structure and function (cIMT and cfPWV) at follow-up in both the HIV-infected and uninfected groups.

Despite endothelial activation being higher at baseline, it was not associated with measures of arterial structure and function after 5 years in the HIV-infected group. In a study by Ross et al [37] ICAM and VCAM were not associated with cIMT in HIV-infected on ART. The association between endothelial activation and arterial injury and stiffness has been investigated majorly in the general population and individuals with established comorbidities such as hypertension, diabetes and obesity [38, 39].

The hypothesis is rejected because in the HIV-infected group ICAM-1 and VCAM-1 did not associate with cIMT and cfPWV while only in the HIV-uninfected VCAM-1 was negatively associated with cfPWV.

- Baseline cardiovascular risk factors (CRP, GGT, HIV status, ART use, age, sex, BMI, mean arterial pressure, HbA1c, TG: HDL-c ratio and tobacco use) will correlate positively with measures of arterial structure and function (cIMT and cfPWV) at follow-up in both the HIV-infected and uninfected groups.

There is controversy as to whether traditional CV risk factors or HIV-related contribute mostly to changes in arterial structure and function [32, 35, 40, 41]. In this study, a positive association for cIMT with TG: HDL-c ratio was showed as well as cfPWV with mean arterial pressure in the HIV-infected group. In the uninfected individuals, cIMT was positively associated with female sex and HbA1c levels. A positive association for cfPWV with age, female sex, MAP and HbA1c overtime was reported in the uninfected individuals. These findings are supported by other previous studies that reported an independent association between cIMT and cfPWV with traditional CV risk factors such as age [32, 42], sex [32, 43], lipid perturbation [44], raised BP [32, 41] and HbA1c [31].

The hypothesis is partially accepted, since the HIV-infected group, the results showed a positive association for cIMT with TG: HDL-c ratio and cfPWV associated only with mean arterial pressure over time. In the uninfected controls, cIMT positively associated with female sex and HbA1c levels. Regarding cfPWV, a positive association was found for cfPWV with age, female sex, MAP and HbA1c over time.

5. THE CONCEPTUALISATION OF THE LONG-TERM CARDIOVASCULAR HEALTH PROFILE OF PEOPLE LIVING WITH HIV AND THE CONTRIBUTION OF THIS STUDY

5.1 The longitudinal cardiovascular health risk profile of people living with HIV

This study adds to the body of knowledge on the longitudinal cardiovascular health profile of Africans living with HIV, which is concurrently limited by evidence from longitudinal studies. In this black South African cohort, the HIV-infected cohort is suggested to have a steady-to-moderately higher cardiovascular health risk profile. The conceptualisation of the long-term cardiovascular health risk profile of the HIV-infected cohort is illustrated below in **Figure 2**.

5.2 Contributions of this study

- The findings of the narrative review indicate lower estimates of BP and hypertension prevalence in treated HIV-infected compared to uninfected controls, residing in the SSA region (**Figure 2**). This finding is contrary, to previous findings from Western populations that reported elevated BP in the HIV-infected patients than uninfected controls [6, 45]. This finding suggests ethnic-specific differences in factors such as cardio-metabolic factors and body fat distribution that contribute to BP [46-48]. People living with HIV tend to visit health care facilities more often than uninfected counterparts as part of their monitoring progress for ART treatment and this offer more opportunities to be diagnosed with hypertension and subsequently treated and controlled [31]. Moreover, it has been shown that HIV-infected patients attending integrated health care for HIV and hypertension had controlled BP compared to those seeking medical assistance for hypertension alone [49].
- The lower BP and/or hypertension estimates in the treated patients compared to the untreated counterparts could be explained in part by the varying effects of different ART regimens on BP. The use of PI rather than NNRTIs and NRTIs frequently contribute to elevated BP in HIV-infected individuals [50]. Moreover, in the SSA region, PIs are used as second and third-line regimens after the patients have immunologically failed on the first-line regimen, making it the

less frequently used ART regimen as compared to NRTIs and NNRTIs [51]. Hypertension in HIV-infected people may also develop indirectly as an effect of weight gain with the use of ART – however in this review occurrence of obesity or overweight was not frequent in the HIV-infected individuals [10, 45] Since raised BP is predictive of future CVD, this study suggests that the lower BP reported in these treated HIV-infected patients do not contribute to the CVD burden.

- Similar BP was reported between the HIV-infected individuals and uninfected controls. This finding is contrary, to those of the recent review study which showed lower BP in HIV-infected individuals than uninfected control [52]. One of the possible reasons for the similar BP could be that the two groups did not differ in either WC or BMI as well as lifestyle factors - which are known to contribute significantly to hypertension. Although the PURE study benefit from its longitudinal design, the relatively smaller size compared to that of the review study, might have affected the BP outcome.
- Other inherent factors such as the type of study designs and research quality of the included articles in the review might have influenced the outcome of the review. A comprehensive search strategy was used to search for manuscripts/articles relevant to the topic and limit publication bias [53]. Two reviewers independently assessed the articles for eligibility for inclusion in the review. Any discrepancies in eligible studies listed were resolved by consensus discussion ensuring that relevant and quality articles are included in the review. Estimates of BP and hypertension prevalence were mainly derived from cross-sectional studies (N = 12) followed by longitudinal and retrospective cohort studies. Cross-sectional studies provide analyses of multiple variables at a given time-point but the results reported are only associations from which no conclusions regarding causality can be drawn [54]. The advantage of cross-sectional studies is that it allows selection of specific individuals which might have a predisposed risk. In this narrative review, the high number of cross-sectional studies used may have resulted in the inclusion of selected individuals at higher or lower risk for developing hypertension. While with prospective studies (N = 5), confounding bias is frequent and can cause over-or under-estimation of the true relationship and may even change the direction of the observed effect [55]. In retrospective studies (N = 2) the major source of bias is recall bias as people with the outcome are more likely to remember certain

experiences, or exaggerate or minimize what they consider to be risk factors [55]. The design of the studies used in this narrative review might have affected the outcomes of the BP and hypertension estimates in the HIV-infected patients against the untreated and uninfected controls.

- The HIV-infected South Africans living with HIV for 10 years, presented with higher HDL-c and lower TG: HDL-c ratio than uninfected counterparts, known to be cardiovascular protective [56] (**Figure 2**). Nonetheless, the HIV-infected group also seem to be at a risk for cardio-metabolic disease as they presented with higher concentrations of TC and LDL-c.

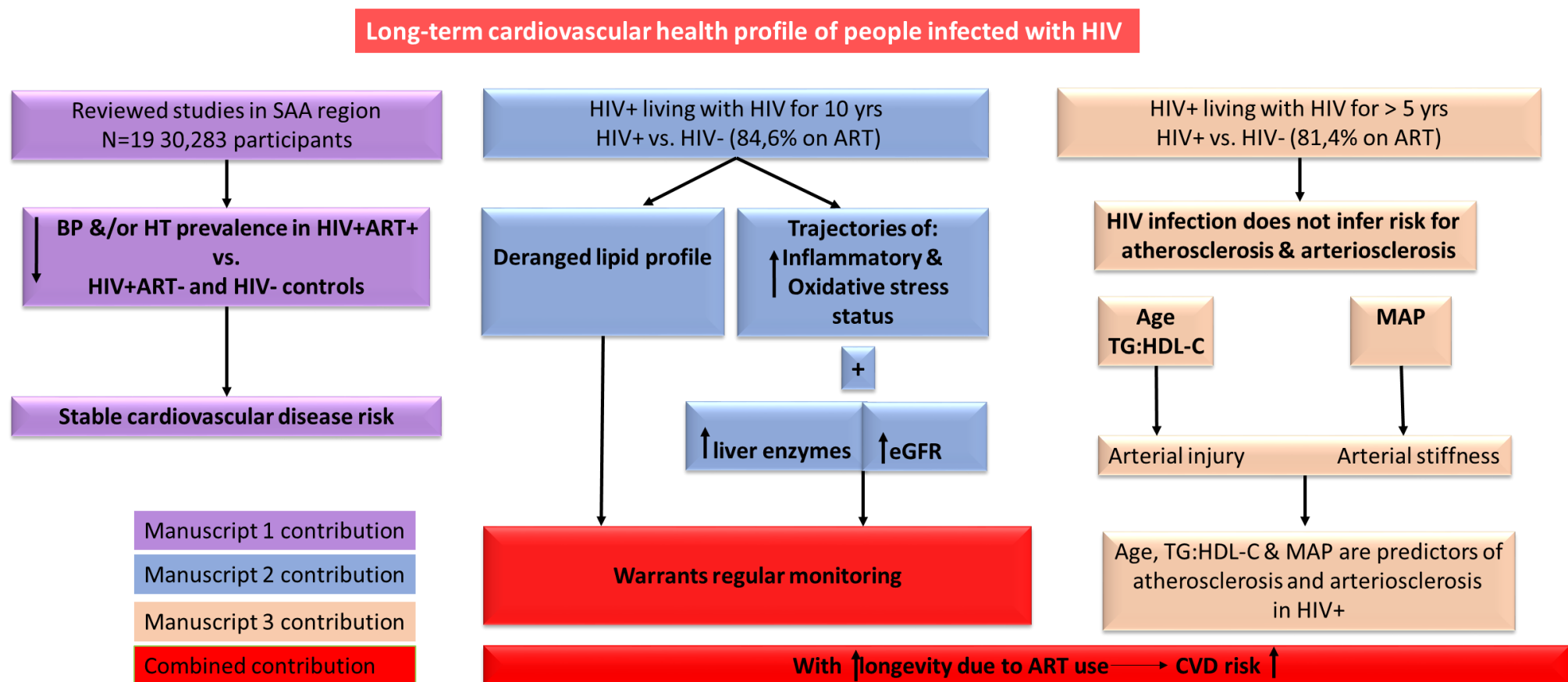


Figure 2. A proposed model highlighting the contributions of this study on the longitudinal cardiovascular health risk profile of Africans living with HIV

HIV, human immunodeficiency virus; ART, antiretroviral therapy; CVD, cardiovascular disease; CV, cardiovascular; BP, blood pressure; MAP, mean arterial pressure; HT, hypertension; HIV+ART+, treated HIV-infected; HIV+ART-, untreated HIV-infected; HIV-, HIV-uninfected; HIV+, HIV-infected; TG: HDL-c, triglycerides: high-density lipoprotein-cholesterol ratio; AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; cIMT, carotid intima-media thickness; cfPWV, carotid-femoral pulse wave velocity; ICAM-1, intracellular adhesion molecule; VACM-1, vascular cell adhesion molecule. *Images obtained from Servier Medical art.*

- The 10-year increase in CRP in the HIV-infected individuals, support a persistent inflammatory status with HIV infection and even in the presence of ART use since the HIV-infected individuals in this study were on ART. Guimaraes et al [57] have shown that CRP is an important contributor to CVD development in HIV-infected individuals and this finding of elevated CRP levels may pose an increased CVD risk in people infected with HIV.
- In addition to the inflammation, oxidative stress as highlighted by elevated levels of GGT was evident in the HIV-infected individuals compared to uninfected controls. The combined presence of inflammation, oxidative stress and deranged lipid profile in HIV might promote a pro-atherogenic risk [58].
- The higher levels of liver enzymes in people living with HIV for 10 years suggest a possible alteration in liver function with HIV and ART use. In this study, the HIV-infected individuals were using a combination of NRTIs and NNRTIS, regimens known to exacerbate the activities of co-administered ART and hence increasing the toxic effect on the liver cells [59]. Since the HIV-infected participants will continue taking ART, it is expected that these liver enzymes will increase further over time, which warrants regular monitoring of liver function in people living with HIV.
- The HIV-infected individuals demonstrated an increase in eGFR over 10 years and this is in line with the findings of the Multicenter AIDS Cohort Study where an increase in eGFR in the hyperfiltration range ($\text{eGFR} \geq 140 \text{ ml/min/1.73 m}^2$) were noted in HIV-infected patients [29]. Although, in the study, the eGFR increase did not reach the hyperfiltration range, with continued exposure to HIV and ART toxicities as well as ageing the eGFR may reach the hyperfiltration range. Moreover, hyperfiltration precedes renal diseases and renal diseases are an important cause of CVD development and mortality in the HIV-infected population [25]. Therefore, this advocates for regular screening and monitoring of renal function in HIV-infected Africans.
- The measures of arterial injury (cIMT) and stiffness (cfPWV) were similar in the HIV-infected living with HIV for more than five years compared to age, sex and locality matched uninfected counterparts, irrespective of the HIV status and the exposure to ART (**Figure 2**). Atherosclerosis

and arterial stiffness tend to reduce with the use of ART (such as NRTIs and NNRTIs) and viral suppression, as a result of mitigating the viral effects of HIV on the arterial system [36].

- The lack of prospective associations between endothelial activation with measures of atherosclerosis and arterial stiffness in HIV-infected individuals suggests there are other factors beyond endothelial activation in HIV infection that contribute to CVD. Contrarily, other studies [37-39, 60] showed an association for endothelial activation with atherosclerotic plaque burden in a Western population with existing CVD risk factors such as obesity and dyslipidaemia. This may become evident in the HIV-infected Africans as they live longer with the use of ART and develop associated metabolic disorders. Also, the relatively small sample of the study cohort could have influenced the association. However, Ross et al [37] included a smaller study population (73 HIV-infected individuals and 21 uninfected controls) than this current study and reported an association for internal carotid artery IMT with VCAM in HIV-infected individuals.
- The association of TG: HDL-c ratio with cIMT found only in the HIV-infected group may reflect the potential role of HIV infection and ART treatment in altering lipid metabolism and mediating higher IMT measures [61]. The positive contribution of age to cIMT in HIV-infected individuals may elute to accelerated vascular ageing in the HIV-infected population [62]. It is suggested that CVD occurs earlier in HIV-infected individuals than uninfected counterparts [63], emphasising the need to screen CVD at an earlier age in HIV-infected individuals.

6. STRENGTHS OF THE STUDY

- The participants were taken from a longitudinal cohort (Prospective Urban and Rural Epidemiology study) residing in South Africa – a country with the highest HIV epidemic in the world and thus provide important evidence addressing the impact of HIV on cardiovascular risk in follow-up studies.
- The study participants were carefully matched for age, sex and locality for the third manuscript. A matched control group is a unique feature of this study as most studies including HIV-infected individuals lack an HIV-uninfected control group. Furthermore, the matching of the classical CVD risk factors in the two groups may help to minimise the confounding effects of these factors

on the results of the study. Regarding the narrative review paper (manuscript one), the inclusion of control groups enabled a comparison of BP estimates between treated HIV-infected patients and uninfected patients or untreated patients to clarify the controversies in reports on BP.

- The study included the assessment of the cardiovascular variables of interest, including measures of blood pressure, arterial stiffness (cfPWV) and atherosclerosis (cIMT). The study was done in controlled conditions and gold-standard techniques performed by trained and experienced researchers were used.

7. LIMITATION OF THE STUDY

- With the review article (Chapter 3), the data was generated using 19 studies from eight countries in SSA regions (namely South Africa, Botswana, Cameroon, Kenya, Tanzania, Malawi, Uganda and Nigeria), thus minimising generalisability for the broader SSA population. This highlights a limitation in the number of studies that report on raised BP in treated HIV-infected patients compared to their untreated and uninfected counterparts residing in the SSA region.
- The present study in the South African leg was done in two regions of the North West Province, Potchefstroom-area and Ganyesa-area and was not randomised. As such the study population is not a representative sample of the HIV-infected population of South Africa and/or other SSA Africans (Chapters 4 and 5).
- In Chapter 4 (Manuscript two) the cross-sectional examinations that were done do not infer any causal associations between CVD risk factors and applicable measures of arterial structure and function.
- The studies (Chapters 4 and 5) were limited by a relatively small sample size. But the study benefits from the longitudinal design of the study and the inclusion of an uninfected companion group.
- Information on the use of ART was not complete for 26 HIV-infected participants in manuscript two and eight participants in manuscript three. Additionally, a comparison for HIV-infected individuals with and without ART could not be made. However, the study participants were all on

a fixed-dose combination, consisting of NRTIs and NRTIs. The duration of ART was not known for all the participants on ART.

8. POTENTIAL CONFOUNDERS

It is important to consider factors that may have influenced the results of this study. These include the methodology, statistical analyses and interpretation of the results.

- During the profiling of the demographic and cardio-metabolic risk factors in the HIV-infected population in Chapter 4, we included N=11 participants that were diagnosed with HIV at follow-up as opposed to those N=106 who were diagnosed at baseline (2005). However, to check the robustness we excluded the N=11 and repeated the comparative analysis for the HIV-infected individuals (N=106) and uninfected controls (N=131). The age was similar between the two groups. There was no difference in their anthropometric and cardiovascular measurements (except for pulse pressure).
- During the investigation of the association between cardiovascular risk markers with cIMT and cfPWV in Chapter 5, several covariates were adjusted. The covariates were chosen among many others using suitable selection criteria and were based on their interrelationships with cfPWV and cIMT.
- Testing of tuberculosis, hepatitis C and other infectious diseases was not done in all three studies, however, information on the use of chronic medication was collected. These non-measured factors could have confounded the findings of this present study, but the longitudinal aspect of this study could remedy the effect in this regard.
- We could not do a separate analysis for the HIV-infected individuals with and without ART because the majority (85% in manuscript two and 81% in manuscript three) of the HIV-infected were on ART leaving few HIV-infected without ART to do effective comparisons.
- Furthermore, only patients who voluntarily accepted to participate in the study were examined; thus, a selection bias resulting from higher compliance with treatment in the patient population could have affected the results of this study. It could be speculated that these patients were also better motivated to comply with treatment.

- The PURE study participants were given referral letter in case of CVD parameters that were above recommended range to consult a doctor or visit health services to receive further medical assistance and this could have affected the results at follow-up study.

9. RECOMMENDATIONS

According to the findings of this study, the following recommendations are made:

- Education on CVD and the risk involved, as well as the promotion of healthier lifestyle choices, is needed amongst South Africans infected with HIV.
- Screening of the CVD risk markers such as renal function, liver function and traditional cardiovascular risk markers in the HIV-infected individuals should be done regularly to allow for early detection of CVD.
- To further the understanding of the mechanisms involved in the pathophysiology of CVD in the HIV-infected population, future studies (which include uninfected controls) are recommended with randomised and larger sample size as well as assessing the role of the different ART regimens on the CVD development to better inform the physiological mechanisms. Investigations should also focus on the role of different ART regimens in the development of CVD in the HIV-infected population.

10. CONCLUSIONS

This study investigated the longitudinal cardiovascular health of a black South African cohort infected with HIV. In a review of studies done in the SSA region, the majority of studies in treated HIV-infected individuals presented with lower BP and/or hypertension estimates compared to their uninfected and untreated HIV-infected controls, thus not suggesting an increased cardiovascular disease risk. In the PURE-study, we, however, report deranged lipid profiles accompanied by increased trajectories of inflammatory status, oxidative stress, liver enzymes and eGFR in HIV-infected individuals living with HIV for 10 years, proposing increased long-term risk for CVD, beyond blood pressure. Finally, traditional cardiovascular risk factors (but not elevated baseline endothelial activation and HIV status), contributed to measures of arterial injury and stiffness over

time in the HIV-infected people. The findings of this study emphasise the need for HIV care in SSA settings where CVD is increasing due to the epidemiological transition. Therefore, it is critical to screen for CVD risk factors in HIV-infected individuals to reduce future CVD burden. More large-scale studies in SSA are required to corroborate these findings.

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APPENDICES

Appendix A

Turnitin Report

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Appendix B

Ethics Approval



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24 July 2017

Prof CMT Fourie
Physiology-HART

Dear Prof Fourie

**APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH
ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES**

Ethics number: NWU-00074-17-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: A longitudinal investigation of the cardiovascular health of a black South African cohort infected with the human immunodeficiency virus

Study leader/supervisor: Prof CMT Fourie

Student: E Phalane-28149866

Application type: Single study

Risk level: Minimal

You are kindly informed that your application was reviewed at the meeting held on 14/06/2017 of the HREC, Faculty of Health Sciences, and was approved on 24/07/2017.

The commencement date for this study is 24/07/2017 dependent on fulfilling the conditions indicated below. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

Appendix C

Manuscript one: Abstract



A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls residing in Sub-Saharan Africa: a narrative review

Edith Phabane¹ · Carla M. T. Fourie^{1,2} · Catharina M. C. Mels^{1,2} · Netta E. Schutte^{1,3,4}

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Abstract

Elevated blood pressure (BP) is an important risk factor for cardiovascular disease and mortality in Sub-Saharan Africa (SSA). We reviewed the literature comparing BP in treated HIV-infected populations against untreated and/or uninfected controls from SSA. We conducted a narrative review through PubMed and EBSCO Discovery Service to determine estimates of raised BP and hypertension in HIV-infected patients versus untreated/uninfected controls (1 January 2005 to 31 July 2019 and 9 May 2020). We included 19 eligible studies that compared treated HIV-infected with untreated and/or uninfected controls. In studies comparing treated HIV-infected patients to uninfected controls, studies including 6882 (56.30%) and 21,819 (79.2%) participants reported lower BP and hypertension prevalence, respectively in HIV-infected patients; whereas studies including 753 (6.16%) and 3553 (12.9%) participants showed a higher BP and hypertension prevalence. Lastly, 4588 (37.54%) and 2180 (7.91%) participants showed no difference in BP and the prevalence of hypertension. When comparing BP of treated versus untreated HIV-infected patients, studies including 5757 (44.2%) patients reported lower BP in treated patients; while studies with 200 (1.53%) patients showed higher BP and 7073 (54.28%) showed no difference in BP. For hypertension status, studies with 4547 (74.5%) patients reported a lower prevalence of hypertension in treated patients; whereas studies with 598 (9.80%) patients showed higher prevalence; and 959 (15.7%) no difference in prevalence between treated versus untreated HIV-infected patients. In studies conducted in Sub-Saharan Africa, the majority of the findings indicate lower blood pressure and/or prevalence of hypertension in treated HIV-infected individuals compared to untreated and uninfected controls.

Supplementary Information The online version of this article (<https://doi.org/10.1038/s41371-020-0385-6>) contains supplementary material, which is available to authorized users.

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Introduction

According to the World Health Organization Progress Monitoring Report on Non-communicable Diseases of 2017, ~1 billion people globally are affected by raised blood pressure (BP) [1], making it a major public health concern. Elevated BP is well documented as a modifiable risk factor for the development of cardiovascular disease (CVD) and cardiovascular mortality [2]. The CVD burden is rising disproportionately among low- and middle-income countries within Sub-Saharan Africa (SSA), where almost half of the premature deaths are due to CVD [3].

In addition to raised BP, the SSA region is also faced with higher rates of infectious diseases, particularly infection with the human immunodeficiency virus (HIV) [4]. SSA contributed about 66% of the new HIV infections globally in 2017 [5]. This colliding burden of communicable and non-communicable diseases poses a health threat in a region with restricted financial support

Appendix D

Manuscript two: Abstract

Cardiovascular Topics

A 10-year follow-up study of demographic and cardiometabolic factors in HIV-infected South Africans

Edith Phalane, Carla Maria Theresia Fourie, Catharina Martha Cornelia Mels, Aletta Elisabeth Schutte

Abstract

Objectives: Understanding of the interplay between human immunodeficiency virus (HIV) and cardiovascular disease, especially in Africa, is limited to evidence from longitudinal studies. Therefore the demographic profile and cardiometabolic, renal and liver function of an HIV-infected South African population were profiled from 2005 to 2015.

Methods: The study included 117 HIV-infected and 131 uninfected controls that were examined at baseline, five and 10 years.

Results: Mortality rate declined from 24% (2005–2010) to 0% (2010–2015) after the introduction of ART. Longitudinal increases in C-reactive protein ($p = 0.002$), alanine transaminase ($p = 0.006$) and gamma-glutamyl transferase ($p = 0.046$) levels and estimated glomerular filtration rate ($p < 0.001$) were seen only in the HIV-infected group. This group also showed increased high-density lipoprotein cholesterol (HDL-C) ($p < 0.001$) and total cholesterol ($p < 0.001$) levels and decreased triglyceride:HDL-C ($p = 0.011$) levels. Low-density lipoprotein cholesterol decreased in both groups ($p < 0.001$).

Conclusion: Despite trajectories of deranged lipid and inflammatory profiles, the cardiometabolic disease risk seems stable in HIV-infected South Africans. Inflammation and renal and liver function warrant regular monitoring.

Keywords: human immunodeficiency virus, antiretroviral therapy, cardiometabolic factors, renal function, liver enzymes, South Africa

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Since the discovery of the human immunodeficiency virus (HIV) in the 1980s,¹ the global prevalence of HIV infection has increased from 7.6 million patients in 1990 to 36.7 million in 2017.² Of global infections, eastern and southern Africa contributed 53% of new infections and include 19.6 million people living with HIV in these regions.³

The HIV epidemic in South Africa received significant attention during the 1990s, due to the growing incidence of HIV infection and deaths related to acquired immunodeficiency syndrome (AIDS).⁴ In order to address the growing burden of HIV/AIDS, the South African government implemented the antiretroviral therapy (ART) roll-out programme for HIV-infected patients in April 2004,⁵ which is now the world's largest ART roll-out programme.⁶ The use of ART has improved the life expectancy of people infected with HIV in a country that has seen a dramatic increase in the number of HIV-infected patients, namely 11.5% (5.35 million) in 2005 to 12.5% (6.19 million) in 2015 (Fig. 1) and 12.6% (7.1 million) in 2017.⁴ Despite various campaigns to reduce HIV infection rates, South Africa continues to bear a disproportionate share of the global burden of HIV, as the highest rates of new HIV infection are still reported in South Africa.⁷

The era of ART has exposed new challenges, including cardiometabolic changes such as elevated blood pressure,⁸ dyslipidaemia,⁹ lipodystrophy¹⁰ and chronic inflammation,¹¹ which are all associated with increased development of cardiovascular disease (CVD). The use of ART is associated with hepatotoxicity, which often manifests as liver disease in HIV-infected patients.¹² In addition, renal disease, characterised by higher urinary protein excretion and elevated serum creatinine, was observed in HIV-infected individuals.¹³ With 56% of the HIV-infected patients on ART in South Africa,⁴ the prevalence of cardiometabolic and renal diseases in HIV-infected patients has increased.¹⁴

The HIV-infected population is at a higher risk of developing CVD due to the complex interlinkage between HIV, ART and cardiometabolic disease.¹⁵ Furthermore, sub-Saharan Africa is faced with co-epidemics of HIV infection and CVD.¹⁶ Further research is required to understand the nexus between these conditions, and limited evidence is available from longitudinal studies in Africa.

In a cross-sectional analysis of 300 newly diagnosed HIV-infected, ART-naïve individuals individually matched with uninfected controls by age, gender, body mass index (BMI) and locality, we previously reported dyslipidaemia and inflammation in the HIV-infected group, which suggested increased risk for the development of CVD.¹⁷ In this study, HIV-infected black Africans and controls were followed over 10 years to profile the

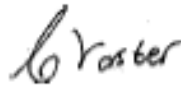
Appendix E

Proof of Language Editing

CERTIFICATE OF EDITING

I, C Vorster (ID: 710924 0034 084), Language editor and Translator and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of a thesis written by Ms E Phalane from the North-West University (student number 28149866).

Title of the thesis: A longitudinal investigation of the cardiovascular health of a black South African cohort infected with the human immunodeficiency virus



13 December 2020

C Vorster

Date

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