Cardiovascular disease risk assessment in HIV-infected black South Africans: A longitudinal study

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Affirmation by authors

The following researchers contributed to this project:

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Responsible for literature research, statistical analyses, interpretation of results and writing of the manuscript and dissertation.

Prof. Carla MT Fourie *(Supervisor)*

Responsible for this study design and planning of the study, took part in PURE study data collection, supervised statistical analysis of data, as well as giving recommendations regarding the writing and construction of the script.

Prof. Johannes M van Rooyen *(Co-supervisor)*

Took part in the PURE study data collection and planning of this study and gave recommendations regarding interpretation of data, writing and construction of the manuscript and dissertation.

This is a statement from the co-authors confirming their individual role in the study and giving their permission that the manuscript may form part of this dissertation.

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Summary

Motivation

A double burden of non-communicable -and communicable diseases exists in South Africa which include high prevalence of cardiovascular disease (CVD) and human immunodeficiency virus (HIV) infection. Coronary heart disease (CHD), a sub-category of CVD, affects more African-American individuals than white individuals. The high prevalence of CVD and HIV decreases the quality of life for those living with HIV. However, being treated with antiretroviral drugs are known to increase the life expectancy for the HIV-infected population. Those living with HIV presented with higher prevalence of arterial stiffness, atherosclerosis, kidney disease and left ventricular hypertrophy (LVH). Despite the advantages of antiretroviral therapy (ART) use, it was shown to have worsen CVD risk for the HIV-infected population. The need for risk assessment in the African HIV-infected population burdened by both coronary heart disease (CHD) and CVD are important.

Aim

The aim of this study was to determine the risk scores of 10-year CHD and CVD by making use of the Framingham -and Reynolds risk score models, respectively, and to determine associations of the risk score models with measures of end-organ damage.

Methodology

This study is embedded in the South African arm of the Prospective Urban and Rural Epidemiological (PURE) study and included African participants from the North-West Province of South Africa who were infected with HIV for at least 10-years. The PURE-South African study consisted of baseline (2005) and follow-up (2015) data. A number of 2010 individuals participated in the PURE study during baseline, where 322 participants were newly identified as being infected with HIV. Ten years later 100 of the 322 HIV-infected participants took part in the follow-up study of which 29 participants were excluded due to incomplete data sets. A number of 71 HIV-infected participants remained and they were matched to 71 HIV-free controls according to age, sex and locality.

Anthropometric measurements included height, weight and waist circumference (WC), followed by the calculation of body mass index (BMI). Regarding cardiovascular measurements, systolic and diastolic blood pressure (SBP and DBP), carotid-femoral

pulse wave velocity (cfPWV), only for follow-up, and intima-media thickness (IMT) were determined. Biochemical variables included total cholesterol (TC), high and low-density lipoprotein cholesterol (HDL-C and LDL-C), triglycerides (TG), glycosylated haemoglobin A1c (HbA1c), C-reactive protein (CRP) and HIV status. Creatinine clearance (CrCl) was calculated with the Cockcroft-Gault formula. The CD4 counts were determined, in whole blood, at baseline by the National Health Laboratory using flow cytometric analysis and at follow-up with finger-prick blood and a point-of-care device PIMA^{TM} CD4 (Alere, Jena, Germany).

The statistical analyses were performed by using Statistica® 13 (StatSoft, Inc., Tulsa, OK, USA). The Framingham and Reynolds risk score were determined with excel spreadsheets, separately, during baseline and follow-up. Basic descriptive statistics were used to determine normal distribution of the data and logarithmic transformation was applied and presented as geometric mean with 5th and 95th percentiles if skewed. Groups were compared using independent t-tests and Chi-square tests as appropriate. Co-morbidity prevalence was defined by using cut-off values. Associations of measures of end-organ damage with the risk scores were determined by making use of Pearson and partial correlations. Odds ratios with 95% confidence intervals (CI) were calculated. Median values of risk scores and measures of end-organ damage were used as cut-off values.

Results and conclusion

The HIV-infected group presented with lower HDL-C ($p<0.01$) and CrCl levels ($p=0.02$) at baseline, compared to the HIV-free group. At follow-up the HIV-infected group had significant lower BMI ($p<0.01$), WC ($p<0.01$) and HbA1c ($p=0.01$) compared to the HIV-free control group.

The CD4 counts of the HIV-infected group was higher ($p=0.03$) at follow-up compared to baseline.

More HIV-free participants were overweight ($p=0.02$), had diabetes ($p=0.04$), had lower HDL-C (men) ($p=0.013$) and had a higher prevalence of microalbuminuria ($p=0.04$), compared to the HIV-infected group at follow-up.

No differences were seen between the HIV-infected and HIV-free group with either the Framingham or Reynolds risk score at baseline or follow-up. The Framingham risk score was higher in the HIV-infected group at follow-up when compared to the HIV-free controls, however both the HIV-free controls and HIV-infected group had a higher

Reynolds risk score at follow-up than at baseline. No differences were seen between the ART and no-ART group.

A borderline negative correlation (p=0.053) was seen between CHD and CrCl, while CVD risk correlated negatively with cfPWV in the HIV-infected group at follow-up, however after adjusting for age, sex, WC, SBP, CRP, CD4 and ART use no correlations were seen. The CVD risk correlated negatively with Cornell product in the HIV-free group, however after adjusting for age, sex, WC, SBP, CRP, tobacco use and alcohol use no correlations were seen. No significant odds ratios were found for having a higher than median CHD or CVD risk with higher than median PWV, IMT and Cornell product or lower than median CrCl.

To conclude, despite their HIV-status for at least 10-years and 80% of participants receiving ART, we found that the HIV-infected participants did not have higher CHD or CVD risk when compared to the HIV-free participants. Those infected with HIV did also not show associations of risk scores with measures of end-organ damage.

Keywords: Cardiovascular disease, Human immunodeficiency virus, Risk assessment, Africans, End-organ damage

Preface

The article format was used for the completion of this dissertation. The chosen journal for publication of the manuscript is *Heart, Lung and Circulation*. This dissertation is written in English.

The structure of this dissertation is as follows:

- **Chapter 1:** The introductory chapter consists of a background, motivation, aim, objectives and hypotheses of the study.
- **Chapter 2:** A complete literature study of the relevant topics.
- **Chapter 3:** A complete methods section.
- **Chapter 4:** The research manuscript which includes instructions for authors of the journal *Heart, Lung and Circulation*, an introduction, the materials and methods, results, discussion, conclusion and acknowledgements.
- **Chapter 5:** Concluding remarks, a critical discussion of the findings and recommendations.

A reference list is provided at the end of each chapter, according to the Vancouver referencing style, as prescribed by the journal *Heart, Lung and Circulation* and was used throughout the dissertation.

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List of abbreviations

AIDS	-	Acquired Immunodeficiency Syndrome
ART	-	Antiretroviral therapy
BMI	-	Body mass index
cfPWV	-	Carotid-femoral pulse wave velocity
CDC	-	Centers for Disease Control and prevention
CHD	-	Coronary heart disease
CI	-	Confidence interval
CKD	-	Chronic kidney disease
CP	-	Cornell product
CrCl	-	Creatinine clearance
CVD	-	Cardiovascular disease
DBP	-	Diastolic blood pressure
ECG	-	Electrocardiography
FSGS	-	Focal Segmental Glomerulosclerosis
GFR	-	Glomerular filtration rate
GGT	-	Gamma-glutamyltransferase
HbA1c	-	Glycosylated haemoglobin A1c
HDL-C	-	High-density lipoprotein cholesterol
HIV	-	Human immunodeficiency virus
hsCRP	-	High-sensitivity C-reactive protein
IL-6	-	Interleukin 6
IMT	-	Intima-media thickness
LDL-C	-	Low-density lipoprotein cholesterol
LVH	-	Left ventricular hypertrophy

NCEP	-	National Cholesterol Education Program
NRTI	-	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	-	Non-nucleoside Reverse Transcriptase Inhibitor
PI	-	Protease inhibitor
PURE	-	Prospective Urban and Rural Epidemiological
SBP	-	Systolic blood pressure
TC	-	Total cholesterol
TG	-	Triglyceride
uACR	-	Urinary albumin to creatinine ratio
WC	-	Waist circumference
WHO	-	World Health Organization

Chapter 1: Introduction

Background

Cardiovascular disease (CVD) is considered a leading cause of morbidity and mortality in the African population [1, 2]. Nearly 38.3% of non-communicable disease (NCD) deaths were due to CVD in sub-Saharan Africa in 2013 [3]. During 2015, statistics showed that the Eastern and Southern parts of Africa had the highest number of human immunodeficiency virus (HIV) infections, compared to other African regions, with the number being 19 million individuals [4]. It has been concluded by Triant et al. (2007) that HIV-infected individuals will most likely develop an increasing burden of CVD as they age and live longer [5]. Aging also leads to chronic inflammation development and this process may be described as “inflammaging” [6]. In addition to inflammaging, HIV infection as well as the aging process itself share cellular immunologic similarities such as reduced T-cell function [7].

Lipid profile disturbances, or better known as dyslipidaemia, contribute to CVD development in those infected with HIV and may be due to the virus itself, ART or both [8]. In 2009, Duprez et al. stated that, regardless of other CVD risk factors present in HIV-infected individuals, lower levels of high-density lipoprotein cholesterol (HDL-C) were highly associated with developing CVD [9]. Black HIV-infected individuals usually have lower levels of total cholesterol (TC), triglycerides (TG) [8] and higher levels of HDL-C which is seen to be a “protective” pattern against the development of ischaemic heart disease and atherosclerosis in this population [10]. However, being treated with ART may contribute to developing hyperlipidaemia [11, 12].

Hypertension is the most significant CVD risk factor and accounts for more than half of CVD morbidity and mortality [13]. It is also the main cause of 7.6 million deaths worldwide every year [14]. In a study conducted by Lloyd-Sherlock et al. (2014) it has been found that South Africa is the country with the highest prevalence of hypertension among the elderly [15]. Black individuals are also more prone to develop hypertension [16, 17].

Studies have indicated that the risk of developing CVD is even more likely to occur in individuals who are classified as being diabetic and as much as 80% of mortality in individuals with diabetes occur through CVD [18, 19]. Diabetes, a state of chronic inflammation [20], is higher amongst HIV-infected individuals compared to their HIV-free counterparts [21].

Smoking is, according to literature, known as the most established modifiable CVD risk factor and the relationship thereof with CVD development is well-described [22]. HIV-infected individuals are more likely to be smokers, when compared to their HIV-free counterparts [23, 24]. Up to 40%, if not more, of myocardial infarction risk may be prevented by the cessation of smoking among those infected with HIV [25]. In a study conducted by Ohsawa et al. (2005), it has been found that smokers have higher levels of C-reactive protein (CRP) [26]. Those living with HIV show elevated levels of CRP [27, 28] and it has also been suggested that CRP may be used as a marker for the atherosclerotic burden in the African-American population [29]. The use of ART in previously untreated HIV-infected female individuals is associated with an increase in CRP levels [30].

Microalbuminuria may be seen as an early marker of renal damage and it is known to be associated with CVD risk [31-34]. HIV-infected Africans have a higher prevalence of chronic kidney disease (CKD) [35, 36]. Low-grade albuminuria is associated with the progression of arterial stiffness [37]. It has been found that HIV-infected individuals without ART show early onset of aortic stiffness which correlates with atherosclerosis and is also associated with CVD [38]. Van Vonderen et al. (2009) concluded that HIV-infected individuals show lower compliance and distensibility components in the carotid, femoral and brachial arteries [39]. A recent study has found that an increase in the pulse wave velocity (PWV) is associated with the use of ART, especially efavirenz [40]. Schoffelen et al. (2015) concluded that intima-media thickness (IMT) of the HIV-infected South African women is increased when compared to their male counterparts and has a stronger association with cardiovascular risk factors [41].

Cardiovascular risk assessment is seen to be beneficial, and Blom (2011) concluded that it is a science in “rapid evolution” [42]. Many cardiovascular risk assessment methods exist of which the Framingham risk score is probably the most popular. The risk factors currently included in the Framingham risk score models (one for men and women) [43] are: age, TC, smoking, HDL-C and SBP. The Framingham risk model is used to calculate the 10-year coronary heart disease (CHD) score [42]. Cardiovascular risk also relates to family history, inflammation (CRP) and glycosylated haemoglobin A1c (HbA1c) (among diabetics) [44, 45]. The Reynolds risk score includes the same risk variables as those of the Framingham risk score, but CRP and HbA1c are also incorporated [44]. These risk factors are included in two separate models for men [45]

and women [44]. The Reynolds risk score is part of the risk scores which calculate the 10-year cardiovascular risk.

Both the Reynolds and Framingham risk models received class one recommendations from the American Heart Association as well as the American College of Cardiology [46] and both risk models were endorsed as part of the national guidelines for the CVD prevention programme in Canada [47]. Both these risk models were also mainly developed for Caucasians [43, 44, 48], however, in a recent study it was found that the application of the Framingham risk model to a large black study population was applicable to the black participants [49]. The Reynolds risk score on the other hand showed improved discrimination overall and in black and white women in particular [50]. Therefore this risk model was also used in the present study.

End-organ damage has an independent prognostic significance, irrespective of whether it involves the function and/or structure of the blood vessels, kidney, brain or heart [51]. According to Mancia et al. (2007), once organ damage has been detected, the patients usually already have a high cardiovascular risk [52].

Motivation

A dual burden of non-communicable (CVD) and communicable (HIV) diseases exist in South Africa and the literature suggests that HIV-infected individuals have higher prevalence of CVD. Literature regarding cardiovascular risk in HIV-infected Africans are sparse. Risk monitoring is also known to be a considerable component to achieve risk management, and therefore we thought it well to implement these risk scores in our study (longitudinal regard) in order to identify risk in the African HIV-infected population.

This data may be beneficial to all healthcare systems in order to make important decisions concerning the cardiovascular risk among HIV-infected black South Africans and to develop the necessary treatment.

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Chapter 2: Literature review

1. Cardiovascular disease in Africa

Non-communicable diseases are predicted to become the leading cause of mortality in Africa by 2030 [1]. The World Health Organization (WHO) has identified cardiovascular disease (CVD) as being one of the main categories of NCD [2]. In a study conducted in an urban East African setting, the researchers found that the participants showed a high prevalence of cardiovascular risk, especially among the women [3]. Coronary heart disease (CHD) may be classified as a sub-category of CVD, mainly affecting the coronary arteries, and the mortality thereof is known to be higher in African-Americans than in white Americans [4].

Besides being a region with a high prevalence of CVD, sub-Saharan Africa is also known as a region with a high prevalence of human immunodeficiency virus (HIV) infection [5].

2. Cardiovascular disease and Human Immunodeficiency Virus in Africa

According to the WHO, HIV continues to be a crucial global public health concern and has claimed roughly 35 million lives up until 2016 [6]. In 2015 there were approximately 19 million HIV-infected individuals in Eastern and Southern Africa [7] and it is predicted that over 10 million individuals aged older than 50 years will be living with HIV in sub-Saharan Africa by 2030 [8, 9].

Human immunodeficiency virus is described as a lentivirus (member of the *Retroviridae* family) that progresses to acquired immunodeficiency syndrome (AIDS) [10]. The immune system is demolished by retroviruses and the enzyme called reverse transcriptase is produced, which plays a role in the demolition of the human immune system [10].

Human immunodeficiency virus-1 is characterised by genetic heterogeneity, which is driven by several factors, including a lack of proofreading ability of the reverse transcriptase [11, 12], rapid in vivo HIV-1 turnover [13], host selective immune pressures [14] and recombination of events during the viral replication [15]. Due to the variability of HIV-1 variants, it may be divided into three main phylogenetic groups: group M (main), group O (outlier) and group N (non-M/non-O) [16-18]. Different subtypes of HIV-1 exist, whereas the main virus which prevails in South Africa is HIV-1 subtype C [19, 20]. Human immunodeficiency virus-1 subtype B is responsible for

the infections in North America, Europe and Australia and its genome differs from HIV-1 subtype C by as much as 30% [19, 21, 22].

HIV demolishes the T-helper cells, which play an essential role in immunity, and infects any cell that expresses CD4 proteins [10]. The CD4 T-helper cell count indicates the immune status and is associated with clinical manifestations of the HIV infection [23]. Numerous mechanisms are related to viral replication during HIV progression. During the primary infection, HIV-1 leads to strong T-cell responses which may persist during the phase of chronic infection and this may be due to the replication of the virus [24, 25]. Several studies suggest that HIV gene products may directly induce the activation of macrophages, lymphocytes and proinflammatory chemokine and cytokine production [14-16]. The protein gp120 activates cells or enhances their responsiveness to activation through binding to CD4 or other co-receptors [26-28].

The use of antiretroviral therapy (ART) is needed to decelerate the progression of HIV replication. ART may be associated with higher levels of inflammation, lower levels of high-density lipoprotein cholesterol (HDL-C) [29] and higher levels of triglycerides (TG) which may aggravate CVD risk in HIV-infected individuals and may be directly related to viral infection, ART use or both [30]. In contrast with the latter, Rajasuriar et al. (2015) concluded that early ART use may reduce inflammation, however, the timing and duration of ART use are important factors [31]. High-density lipoprotein cholesterol has anti-inflammatory characteristics through the inhibition of the expression of endothelial adhesion molecules [32-34]. Therefore lower levels of HDL-C may reduce the anti-inflammatory function of the molecule and lead to further aggravation of inflammation. Participants exhibiting high levels of TG frequently have additional risk factors, such as insulin resistance which may affect their sensitivity to atherosclerosis development [35].

Untreated HIV infection induces pro-atherogenic mechanisms in the body and speeds up the progression of atherosclerosis leading to the development of CVD [29]. According to Baker et al. (2015) accelerated cardiovascular risk is becoming more prevalent in the HIV-infected population [29] due to prolonged life expectancy and a higher prevalence of traditional lifestyle cardiovascular risk factors in the HIV-infected population, such as smoking [36, 37].

3. Cardiovascular risk factors

3.1 Non-modifiable cardiovascular risk factors

3.1.1 Age

Age is known to be non-modifiable cardiovascular risk factor and Petoumenos et al. (2014) have found that CVD events in men increase with an increase in age [38]. A general "hallmark" seen in aging tissues is chronic inflammation [39]. Low-grade inflammation together with chronic –and systemic inflammation during the aging process can be described as a process called inflammaging [39].

Petoumenos et al. (2014) stated that the risk for developing CVD, myocardial infarction and CHD increase with twofold as the individual grows older [38]. As a person ages, arterial stiffness, measured by pulse wave velocity (PWV), increases with approximately 0.1 m/s per year [40]. As demonstrated by Lee et al. (2010), endothelial dysfunction, lower elastin levels, higher collagen levels, increased deposition of calcium and the growth of smooth muscle cells lead to vascular wall thickening and lower compliance of blood vessels [41] which may increase the risk for developing CVD.

In addition to inflammaging, HIV infection and the aging process share cellular immunological similarities [42]. It includes reduced naïve T-cell generation, T-cell receptor diversity, more memory T-cells and reduced function and shortened telomeres [42]. Early immune aging (continuous processes of antigen burden and immune activation) has been seen in the HIV-infected population [42].

Those infected with HIV are at increased risk of age-related non-AIDS mortality and morbidity compared to HIV-free controls [43]. It is hypothesised that HIV-infected individuals not only undergo chronological aging, but also biological aging which is mediated by increased cellular deterioration [44]. Chronological age is an indefinite measure of biological aging.

Triant et al. (2007) have shown diverging rates of myocardial infarction between HIV-infected and HIV-free participants with increasing age [45]. They also concluded that CVD will most likely become an increasing burden in HIV-infected individuals as they age and live longer [45].

3.1.2 Sex and Ethnicity

Studies found that ethnicity is highly associated with the development of CVD [45]. Black individuals living in South Africa are more likely to develop hypertension, heart failure and atherosclerosis [46, 47] and it has been suggested that black Africans are less susceptible to angiotensin converting enzyme inhibitors, mainly due to the frequency of low renin hypertension, compared to white individuals [48]. The above mentioned may be the reason for black Africans developing hypertension and being more prevalent to develop CVD.

According to the Centers for Disease control and Prevention (CDC), black African American men accounted for one third of all HIV diagnoses in 2015 [49]. They also reported that African American women accounted for 11% of all HIV diagnoses in 2015 and black women were 16 times more likely to be diagnosed with HIV when compared to white women [49].

Njelekela et al. (2009) have found strong evidence of high cardiovascular risk in Tanzania, particularly among the women in their study group [3]. Receiving ART along with Framingham risk factors and other cardiovascular risk factors such as hypertension and renal disease may contribute to CVD development in both men and women [50].

3.2 Modifiable cardiovascular disease risk factors

3.2.1 Lipids

Dyslipidaemia is known to be a predominant CVD risk factor, especially in the HIV-infected population [51]. Lipid profile alterations, indicating lower HDL-C and higher triglycerides levels, form part of the risk to develop CVD in HIV-infected individuals and may be due to the virus itself, ART or both [30]. Black individuals have lower levels of total cholesterol (TC), TG [30] and higher levels of HDL-C compared to other populations such as white individuals [52] and it may indicate a “protective” pattern against the development of atherosclerosis and ischaemic heart disease in black individuals [52]. However, in a study conducted by Fourie et al. (2010), the HDL-C levels of the black HIV-infected African participants were lower than those normally associated with increased cardiovascular risk [53]. Triglyceride and HDL-C levels are evaluated together and inversely correlated [54-57].

Total cholesterol includes low-density lipoprotein cholesterol (LDL-C), HDL-C and TG. Elevated serum TC levels are associated with the development of atherosclerosis and correlate with CHD [58]. Levels of TC above 11.60 mmol/L have been directly associated with a two-fold higher risk of developing hypertension in the general population [59] and may increase the risk for developing CVD.

Atherosclerosis is an inflammatory disease. Studies have shown that HDL-C has important anti-inflammatory effects and promotes these anti-inflammatory effects by inhibiting the expression of endothelial adhesion molecules [32-34]. The latter therefore explains why an increase in HDL-C levels with 0.06 mmol/L may reduce the risk of developing CVD by 2% [60].

Low-density lipoprotein cholesterol is seen as the main source of cholesterol build-up in the arteries and arterial blockage [61]. Three clinical trials showed that lower levels of LDL-C in diabetics reduced the incidence of CVD development [62-64]. Howard et al. (2000) have found that LDL-C is a powerful predictor of the development of CHD and have suggested that a 0.60 mmol/L increase in LDL-C would lead to a 12% increase in CVD [65].

Triglycerides are main constituents of natural oils and fats in the human body. A triglyceride molecule consists of three fatty acid groups and glycerol. Elevated TG levels are predictors of CVD development [66, 67] as studies been suggested that increased TG levels are major independent risk factor for the development of CHD [68-70]. Studies concluded that TG may contribute to the development of CVD since an association of TG with atherogenic lipoproteins exists [71].

Several studies have found that ART may contribute to developing hyperlipidaemia and/or lipodystrophy (abnormal conditions of the body's adipose tissue) [72, 73]. Nucleoside reverse transcriptase inhibitors (NRTI), most likely stavudine (which form part of the first line ART in South Africa), may lead to increased levels of TG, LDL-C and TC [74].

3.2.2 Obesity

Obesity has been acknowledged for decades as a significant contributing factor to develop various chronic diseases such as hypertension and CVD [75-77]. An increased prevalence of obesity is also seen in low-and-middle income countries, and people classified as being middle-aged (± 45 – 64 years of age) have the highest

prevalence of obesity [78]. African-American women are more likely to be obese [79] and it has been suggested that black South African women are also more likely to have a high prevalence of abdominal obesity [80]. McCormick et al. (2014) have also found in their study that HIV-infected women from sub-Saharan Africa are more prone to present with obesity than their male counterparts [81].

By 2008, an estimated 502 million adults were classified worldwide as being obese (BMI >30 kg/m^2) and 14.6 million adults as being overweight [82]. Body mass index (BMI) has been used for the identification of individuals at risk of developing obesity and related conditions such as CVD [83]. Each five-unit increase in BMI was associated with an increase in CVD mortality of 29% in women and 34% in men [84]. Another measuring method is the waist circumference (WC) which is one of the most common proxy measures of the presence of visceral fat accumulation [85]. Central obesity, as indicated by increased WC measurements, is an important cardiovascular risk factor in populations globally, including those living in sub-Saharan Africa [86, 87].

In a study conducted by Ogunmola et al. (2014), the HIV-free individuals were more likely to be obese, compared to HIV-infected individuals [88]. A recent study found that the women attributed their weight gain to ART and it was suggested that weight gain in the HIV-infected women occurred as a result of successful ART [81]. An increase in BMI with an average of 2.4 kg/m^2 is seen during a six-month ART initiation period and 3.5 kg/m^2 after a year of ART use among HIV-infected individuals living in SA [89].

3.2.3 Hypertension

Hypertension is the most significant cardiovascular risk factor accounting for more than half of cardiovascular morbidity and mortality [90] and is the main cause of 7.6 million deaths every year, around the globe [91]. An increase in arterial blood pressure may lead to organ damage via hemodynamic load. Thus hypertension and the resulting hemodynamic load may lead to functional and structural cardiac changes [92], such as left ventricular hypertrophy (LVH) which is associated with hypertension. Hypertension may be characterised by the remodelling of small and large arteries as well as endothelial dysfunction [93]. This may lead to reduced dilation capability of high resistance vessels and the development of plaque formation, reduced coronary reserve and stenosis [93].

A study conducted by Yang et al. (2016) found that hypertension prevalence was 53.2% in the elderly (65 years and older), of which only 55% were aware of their hypertension status [94]. The prevalence of hypertension is higher in developing countries, as these countries have higher hypertension risk factors such as urbanisation and lifestyle changes [94]. Lloyd-Sherlock et al. (2014) found that South Africa is the country with the highest prevalence of hypertension among the elderly [95]. Although studies found that hypertension is more prevalent in sub-Saharan African countries, a meta-analysis conducted by Dillon et al. (2013) showed that HIV-infected sub-Saharan Africans had lower systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels than the HIV-free control group [96].

The Heart of Soweto Study found that the prevalence of hypertension was 56% for urban South African study participants [47].

3.2.4 Hyperglycaemia

Diabetes mellitus is a significant contributor to the overall CVD burden [97]. It has been suggested by the International Diabetes Foundation that the global population of individuals classified as being diabetic will increase from 382 million in 2013 to 592 million in 2035 with the highest increase of 109% in sub-Saharan Africa where an estimated number of 19.8 million individuals with diabetes will double to 41.5 million [98].

A recent study conducted showed that diabetes accounted for nearly 8.6% of the total mortality rate in sub-Saharan Africa in 2013 [99]. Several studies have indicated that the risk of developing CVD is twice more likely to occur in individuals with diabetes and 80% of mortality in individuals with diabetes occur as a result of CVD [100, 101]. Diabetes has been considered a state of chronic inflammation [102] and there is evidence to suggest that immune activation may precede insulin resistance in both diabetic and pre-diabetic conditions and may be seen as the factor which initially increases the CVD risk during the process of the disease [103].

The prevalence of diabetes is higher in HIV-infected individuals, compared to HIV-free individuals [104]. It has also been found that HIV-infected individuals receiving ART have an increased incidence of high fasting glucose levels (hyperglycaemia) [104].

The formation of glycosylated haemoglobin A1c (HbA1c) occurs by glycation of lysine and valine residues within haemoglobin [105]. The value of HbA1c is the bound fraction

of haemoglobin molecules to glucose molecules [106]. Glycosylated haemoglobin A1c is used to monitor diabetes as it is an index of glycaemia [107, 108].

Glycosylated haemoglobin A1c levels are associated with a higher risk of CVD [109]. Adams et al. (2009) have concluded that the risk to develop CHD is higher in women compared to their male counterparts when HbA1c was less than 6% [109]. Hypertriglyceridemic HIV-infected African-Americans making use of highly active antiretroviral treatment have a higher risk to develop diabetes [110].

3.2.5 Smoking

Smoking is one of the most important established modifiable CVD risk factors and smoking may lead to CVD development [111]. Smoking is also related to lower levels of HDL-C [112] and to the progression of atherosclerosis [113]. Smoking could promote atherosclerosis by leading to alterations in lipid profiles of smokers, such as higher levels of serum cholesterol, triglycerides and LDL-C levels and lower HDL-C levels [114]. Cigarette smoking may also lead to decreased amounts of paraoxonase which is known as an enzyme that protects against the oxidation of LDL-C [115].

Black South Africans are more likely to be smokers compared to other races [116], and individuals infected with HIV are two to three times more likely to be smokers compared to HIV-free controls [117, 118]. Cardiovascular risk and mortality, due to smoking, have been found to be substantial in HIV-infected participants [119, 120]. Smoking and myocardial infarction development are directly related among the HIV-infected population [119]. Three out of four myocardial infarction events are associated with smoking in the HIV-infected population, whereas only one out of four myocardial infarction events are attributable to smoking among the general population [119]. The cessation of smoking could prevent up to 40% and more of myocardial infarction risk among those infected with HIV [119].

Those living with HIV who are smokers lose more “life-years” to smoking than to the virus itself [120].

3.2.6 Alcohol

Alcohol use is seen as a lifestyle cardiovascular risk factor and is also known to be a coping mechanism, especially among the HIV-infected African population [121]. Alcohol consumption is a key risk factor for developing hypertension and mortality in the HIV-infected population [122, 123].

A positive relationship between alcohol consumption and lipids, such as HDL-C, LDL-C and TG together with blood pressure exists [124]. The above mentioned risk variables are elevated in heavy drinking, increasing the risk for these people to suffer from myocardial infarction [124]. Alcohol consumption may also have harmful effects on the kidneys, as it may lead to higher blood pressure due to increased pressure on the walls of arteries by injurious kidneys [125]. High levels of gamma-glutamyltransferase were found in smokers with chronic kidney disease (CKD) and this finding was associated with alcohol consumption [126]. Gamma-glutamyltransferase (GGT) is an enzyme found in the liver and is used as a marker of high levels of alcohol intake.

Africans show higher levels of GGT when being compared to other ethnic groups [127]. According to Lee et al. (2007) GGT correlates positively with cardiovascular risk markers such as, blood pressure, diabetes mellitus, dyslipidaemia and obesity [128]. It was reported that HIV infection was associated with higher GGT in Africans [129].

In terms of atherosclerosis [130], GGT was considered to be proatherogenic in atherosclerotic plaques [131] and alcohol consumption is significantly associated with arterial stiffness [132].

4. Inflammation, cardiovascular disease and human immunodeficiency virus

C-reactive protein (CRP) is one of the most common markers used to assess and diagnose inflammation [133]. C-reactive protein belongs to the pentraxin family of proteins and levels increase 1000-fold or more during the occurrence of injury, inflammation or tissue death [134].

Literature suggests that CRP is considered a biomarker for the progression of CVD [135-137] and elevated CRP levels have been linked to future CVD events [138]. Atherosclerosis is regarded as an inflammatory disease [139] and it has been suggested that CRP may be used as a marker of atherosclerotic burden in African-Americans [140].

HIV-infection is associated with chronic activation of the immune system and a pro-inflammatory states [141]. CRP is known to be an indicator of immune activation in response to damage due to inflammation or infection [134, 142-144] and it binds to pathogens which then leads to the activation of the complement system for the enhancement of opsonisation and clearance [145]. Studies found higher levels of CRP

in those living with HIV [146, 147]. Elevated levels of CRP, in those infected with HIV, are associated with the progression of HIV and with mortality in women [146, 148]. IL-6 is a is an immune protein (marker of inflammation) and is released in response to inflammation and/or infection [149].

Interleukin 6 (IL-6) levels are higher in the HIV-infected population (due to the presence of inflammation and infection) and may lead to CVD development [150]. It has also been found that black individuals living in sub-Saharan Africa have higher levels of CRP and IL-6 compared to white individuals [151].

Cigarette smoke, LDL-C and HDL-C were predictive in the development of elevated CRP levels [152]. Sex differences in CRP levels have been reported in the general population whereas women that complied with National Cholesterol Education Program (NCEP) metabolic syndrome criteria, have been reported to have higher CRP levels compared to their male counterparts [153].

5. End-organ damage and cardiovascular disease

5.1 Chronic kidney disease

The prevalence of kidney insufficiency and CVD rises with age [154] and even in the absence of kidney disease it is thought that human kidney function declines by 10 ml/min/1.73m^2 per decade [155]. Chronic kidney disease seen in the HIV-infected population develops as a result of viral-related risk factors as well as risk factors for developing kidney disease. HIV associated nephropathy develops in individuals with high viremia, typically seen during the advanced HIV disease stage or during acute HIV infection [156, 157].

According to the literature, HIV is associated with focal segmental glomerulosclerosis (FSGS) which is mainly seen in individuals of African descent [158]. Ethnic disparities in the rate of kidney failure progression to end-stage kidney disease exist between the African-American population and white individuals [159]. Focal segmental glomerulosclerosis associated with HIV, may present with low levels of protein excretion in urine to severe proteinuria [158]. According to the literature HIV-associated FSGS rapidly progresses to end-stage renal disease [158].

The determination of renal function includes isotopic determination of the glomerular filtration rate (GFR) [160] and creatinine clearance (CrCl) with the Cockcroft-Gault

equation [161]. A decrease in GFR was previously associated with an increased risk of CVD development together with higher morbidity and mortality [162]. Elevated levels of serum creatinine were associated with cardiovascular events and mortality [154]. In the HIV-infected population, lower GFR and higher serum creatinine occur [163].

The introduction of ART dramatically changed the clinical picture of HIV progression and HIV associated CKD [158]. With the effective use of ART, renal disease may stabilise, the disease process may be reversed and even disappear [164]. However, receiving tenofovir (a nucleoside transcriptase inhibitor commonly used as a first-line ART agent) is characterized by a significant loss of kidney function in those living with HIV [165].

5.2 Arterial stiffness

Stiffness of large elastic blood vessels is mainly determined by the extracellular matrix components situated in the arterial wall. Smooth muscle cells contribute minimally to mechanical behaviour of large elastic arteries [166]. The most important extracellular matrix components seen in large elastic arteries are elastin and collagen [167]. Elastin is a protein which provides reversible extensibility during cyclic loading of the cardiac cycle [167], while strength and prevention of failure at high pressure are provided by collagen [168]. During the aging process and disease progression, the elastic fibres are degraded and fragmented, which may lead to stiffening of the arterial wall [169].

An increase in arterial stiffness, as assessed by the pulse wave velocity measurement, is directly associated with the progression of atherosclerosis [170]. Schutte et al. (2011) reported that black individuals living in South Africa have higher PWV values, compared to their white counterparts [171]. Ngatchou et al. (2013) reported that HIV-infected individuals not receiving ART show a higher prevalence of aortic stiffness which may be associated with CVD [172].

Van Vonderen et al. (2009) have found that HIV-infected individuals have lower compliance and distensibility components in the carotid, femoral and brachial arteries [173]. Moreover, in a recent study it has been found that an increase in the PWV is associated with the use of efavirenz, traditional cardiovascular risk factors such as smoking, dyslipidaemia and systemic inflammation (CRP). These researchers have also suggested that ART may play a role in arterial stiffening in those living with HIV [174].

5.3 Left ventricular hypertrophy

Left ventricular hypertrophy may reflect increased work load to physiological adaption of the heart [175]. An increase in left ventricular mass is the principal factor in developing LVH and hypertension a powerful determinant of LVH [175]. Hypertension may be directly associated with deficient levels of nitric oxide [176], leading to the inability of the endothelium to function normally in maintaining normal vascular tone in preventing onset of vascular damage. Furthermore, dysfunctional endothelium leads to functional changes in vasculature [177] and alterations in the function of the heart. According to literature, LVH is an independent predictor of cardiovascular events [178]. It has been concluded that LVH is more prevalent in black Africans compared to their white counterparts [179]. Havranek et al. (2008) indicated that LVH contributes to the risk of cardiovascular mortality in African individuals compared to their white counterparts [180].

Mansoor et al. (2009) found a positive association between LVH and HIV [181]. Several autopsy studies have reported that HIV infection directly affects myocardial cells and is associated with local cytokine release and other factors leading to inflammation in those living with HIV [182]. It has also been found that individuals exposed to protease inhibitors show higher intraventricular septal -and posterior wall thickness compared to those not receiving protease inhibitor [183] which may lead to a higher prevalence of LVH.

5.4 Atherosclerosis

Atherosclerosis may be described as a chronic immune-inflammatory fibroproliferative (proliferation of fibroblasts) disease which occurs in blood vessels [184-186]. Endothelial cells, leukocytes and intimal smooth muscle cells are few of the major determinants of the development of atherosclerosis. Atherosclerotic lesions initially develop with leaky, activated and dysfunctional endothelium [187]. Recruitment of circulating monocytes and T- lymphocytes are of the earliest cellular responses in atherogenesis [185, 186]. The perseverance of these cellular responses seems to underlie disease progression [185, 186]. During disease progression, the immune-inflammatory response is accompanied by the fibro proliferative response which is mediated by intimal smooth muscle cells and they are responsible for the healing and repair process after arterial injury [188]. Smooth muscle cells produce collagen-rich

matrix compartments which offer stability to plaques with the aim of protecting them against plaque rupture and thrombosis [189].

Structural changes in blood vessels could be identified by conducting a measurement of the arterial intima-media thickness of the common carotid, femoral and brachial arteries by making use of the non-invasive B-mode ultrasonography [190, 191]. From the literature, it is evident that the intima-media thickness (IMT) is strongly associated with future CVD events especially stroke and myocardial infarction [192] and IMT is also seen as a marker for atherosclerosis. In a study by Okeahialam et al. (2011), Nigerian participants, who were apparently non-diabetic and non-hypertensive, showed a high CVD with regard to their IMT values [193]. Schoffelen et al. (2015) concluded that IMT was thicker in HIV-infected South African women compared to men and IMT associated better with CVD risk factors [194]. According to Mangili et al. (2011) IMT is associated with HIV mortality [195].

In a cross-sectional study conducted by Pen et al. (2013), it was found that the Framingham risk score displayed a significant correlation with coronary atherosclerotic burden [196]. However, Parra et al. (2010) did not find any correlation between the Framingham risk score and sub-clinical atherosclerosis in HIV-infected individuals [197].

6. Cardiovascular risk assessment

Cardiovascular risk assessment may be used to select participants for intervention [198]. Cardiovascular risk assessment involves “trying to predict the future adverse outcomes” by the implementation of risk score models [198]. All risk scores are designed in the same manner, where multiple clinical variables are documented in a population of individuals who are free of clinical CVD [198]. These individuals are then followed up over many years and the cardiovascular events well-documented [198]. Finally, the dataset is analysed to identify clinical variables which may be associated with cardiovascular outcomes [198]. The final product consists of a mathematical risk model and equation [198]. Even though risk models may be seen as reliable tools for clinicians to assess cardiovascular risk, algorithms generally do not account for risk variables which change over time. For instance, smoking is a categorical variable classified as “yes” or “no” with little regard to the duration and dose of smoking. A person classified as being a non-smoker may have been a chain smoker for 30 years before quitting or exposed to second-hand smoking.

According to Blom (2011), models used to analyse the data generated have been fairly simplistic [198] and more sophisticated techniques may improve the performance of the various algorithms [198]. Blom has also concluded that cardiovascular risk assessment is a science in “rapid evolution” [198].

7. The Framingham risk score

The Framingham study was the first study of its kind to investigate risk factors in a well-constructed longitudinal cohort. It consisted of 5209 men and women who were aged between 30 and 62, free of CVD and recruited in 1948 from Framingham, Massachusetts [199].

In 1961, a paper published by Kannel and colleagues on “Factors of Risk” enabled physicians as well as scientists to be more assured that blood pressure and other risk factors lead to CHD development [200].

The Framingham study reported other risk equations forming a link of common risk factors with CHD, stroke, fatal and non-fatal CVD [201, 202]. Moreover, these publications led to more refined screening techniques and served as the “primary” of many clinical guidelines existing worldwide [203].

After the identification of individual risk factors for CHD development, the Framingham study enhances the methods which assess one’s overall risk established from numerous risk factors [204]. The risk of CVD development is strongly influenced by a cluster of risk factors present, notably CVD history, age, sex, diabetes, smoking, blood pressure and the concentrations of blood lipids [205].

In 1967, an analysis of the Framingham cohort was published and this analysis included seven risk factors to create a risk function for assessment in men and women aged between 30 to 62 years, namely age, cholesterol, systolic blood pressure, weight, haemoglobin, cigarette smoking and electrocardiography (ECG) (as evidence of LVH) [206].

In the 1990s improvements were made with the addition of HDL-C and extended duration for allowing risk assessment in an even older population (up to 74 years of age). A point scoring system [201] was added in particular for the clinicians who wanted to calculate the risk and in 1998, LVH was excluded [207]. Further changes included the impact of blood pressure treatment on CVD risk and tools for computing

risk scores were added on the web for the ease of use among clinicians. The additions made it possible for clinicians to determine CHD development without the use of a calculator. The risk factors currently included in the Framingham risk score [208] are: age, TC, smoking, HDL-C and SBP, which are included in two separate models, one for men and one for women.

Dada et al. (2016) found that the female participants of their study did not show a high Framingham risk [209]. Bergersen et al. (2004) demonstrated that twice as many HIV-infected individuals receiving ART, had a 10-year Framingham risk of more than 20%, compared to the HIV-free controls [210].

8. The Reynolds risk score

Cardiovascular risk also relates to family history, inflammation (CRP) and HbA1c (among diabetics) [211, 212]. These cardiovascular risk factors are included in the Reynolds risk score, which is an alternative global risk model developed in 2007 for men [211], and women [212]. This risk model also includes the same risk factors as proposed in the Framingham risk score [213].

The Reynolds risk score [212] was developed in the Women's Health Study Cohort. Recruitment of female participants took place in a nationwide cohort of the United States and these women were 45 years and older and did not have CVD. Cook et al. (2012) concluded that the Reynolds risk score was better calibrated than the Framingham risk score and showed improved discrimination overall and in black and white women [214]. The Reynolds risk score for men was developed in 2008 from a prospective cohort of 10000 American men where the risk score also included family history of myocardial infarction and high levels of CRP as risk factors [211].

Both the Reynolds and Framingham risk models received class one recommendations from the American Heart Association as well as the American College of Cardiology [215] and both these risk models were endorsed as part of the national guidelines for CVD prevention programme running in Canada [216].

Both these cardiovascular risk models were mainly developed for white men and women [208, 212, 217] however, in a recent study it has been found that the application of the Framingham risk model to a large black population is applicable to black individuals and not easily improved on, which suggests that no unique risk model needs to be developed for black individuals [218]. Mashinya et al. (2015) also

concluded that there is no need to develop a race/ethnicity specific risk model for HIV infected Africans [219].

To the best of my knowledge, there are no studies incorporating the Reynolds risk score to assess a 10-year risk in the HIV-infected population, especially in women [220].

9. Cardiovascular risk assessment and end-organ damage

The literature found that organ damage has an independent prognostic significance, irrespective of whether it involves the function and/or structure of the blood vessels, kidney, brain or heart [93]. It has also been shown that, once organ damage has been detected, the patients usually have a high cardiovascular risk [221], thus a chance of having either a fatal or a morbid cardiovascular event over a period of 10 years [222].

Table 1: Cardiovascular disease risk factors

Modifiable cardiovascular disease risk factors	Africans compared to white individuals	HIV-infected individuals compared to HIV-free individuals
Lipids	↑ HDL-C [52] ↓ TG [30] ↓ TC [52]	↓ HDL-C [30] ↑ TG [30]
Obesity	↑ Women [80]	↑ Women [81]
Hypertension	↑ Elderly [95]	↓ SBP and DBP [96]
Glucose	↑ Diabetes [98]	↑ Diabetes [104] Hyperglycaemia [104]
Smoking	↑ Smoking [116]	↑ Smoking [117, 118]
Alcohol	↑ GGT [127]	↑ GGT [129]
Inflammation	↑ CRP [151] ↑ IL-6 [151]	↑ CRP [146, 147] ↑ IL-6 [146, 147]
CKD	↑ CKD [223]	↓ GFR [163] ↑ Serum creatinine [163] Albuminuria [224]
LVH	LVH [179]	LVH [181]
Arterial stiffness	↑ PWV [171]	↑ PWV [174]
Atherosclerosis	↑ IMT [193]	↑ IMT in women [194]
Framingham risk score	↓ Women [209]	↑ FRS [210]
Reynolds Risk Score	No studies incorporating this risk score in Africans	No studies incorporating this risk score in HIV-infected populations [220]

HDL-C, High-density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; GGT, gamma-glutamyltransferase; CRP, c-reactive protein; IL-6, interleukin-6; CKD, chronic kidney disease; GFR, glomerular filtration rate; LVH, left ventricular hypertrophy; PWV, pulse wave velocity; IMT, intima-media thickness; FRS, Framingham risk score. ↑ indicates an increase and ↓ indicates decreased levels.

Summary

A dual burden of non-communicable (CVD) and communicable (HIV) diseases exist in South Africa and literature suggests that HIV-infected individuals have higher cardiovascular risk than HIV-free individuals. It is important to identify risk in this

population. This study will be conducted in longitudinal context and few studies in this regard exist.

Overall literature regarding CVD in the HIV-infected African population is sparse. Health care systems may benefit from this data in order to make important decisions concerning the CVD risk among HIV-infected black South Africans.

Aims

- To determine (1) the prevalence of cardiovascular disease risk markers and (2) cardiovascular disease risk with the Framingham and Reynolds risk score models in a cohort infected with HIV for at least ten years, compared to a HIV-free control group.
- To determine associations of the risk scores with measures of end-organ damage in this cohort.

Objectives

- To determine the levels of cardiovascular disease risk markers among HIV-infected participants and HIV-free controls at baseline (2005) and follow-up (2015) and to compare the 10-year cardiovascular disease risk score between HIV-infected and HIV-free participants using the Framingham and Reynolds risk score models;
- To determine the prevalence of co-morbidities (Table 4) among this cohort after ten years;
- To determine whether these risk scores associate with markers of end-organ damage [sub-clinical atherosclerosis (IMT), arterial stiffness (PWV), ECG derived left ventricular hypertrophy (Cornell product) and chronic kidney disease (CrCl)] in the HIV-infected and HIV-free group.

Hypotheses

- HIV-infected participants have a higher 10-year cardiovascular disease risk compared to HIV-free participants in both risk score models.
- A high cardiovascular disease risk score correlates positively with markers of end-organ damage [increased sub-clinical atherosclerosis (IMT), increased

arterial stiffness (PWV), left ventricular hypertrophy (LVH) and chronic kidney disease (CrCl)] in the HIV-infected group.

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Chapter 3: Methodology

Materials and methods

Study design and population

This study is embedded in the South African arm of a longitudinal multi-national study, the Prospective Urban and Rural Epidemiological (PURE) study conducted in the North-West province. This study is known for assessing lifestyle changes and causes of development of cardiovascular disease (CVD) [1]. The PURE-South African study of the North-West province started in 2005 (baseline) and consists of a follow-up period of 10 years (2015) which targeted urban and rural areas in low and middle-income countries such as South Africa [1]. The inclusion criteria consisted of volunteers older than 35 years of age who did not receive any chronic medication and did not have any self-reported diseases.

A number of 2010 individuals participated during baseline, of which 322 participants were newly identified as being infected with human immunodeficiency virus (HIV). During follow-up, 100 participants of the 322 HIV-infected participants took part in the 10-year follow-up study of which 29 participants were excluded due to incomplete data sets. The remaining 71 participants, who were infected with HIV for at least 10 years, were matched to 71 HIV-free controls according to age, sex and locality.

Ethical considerations

Taking part in this study was completely voluntary and the participants could withdraw at any time. The involved procedures were explained to each participant in their own language, followed by the signing of an informed consent form. Each participant was allowed to ask questions when needed. The Ethics Committee of the North-West University, Potchefstroom campus approved the protocol of the PURE-South African study in 2005 (04M10) and 2015 (0016-10-A1) which complies with the Declaration of Helsinki. This study protocol was also approved (NWU-00019-16-A1).

Experimental protocol

Permission for undertaking the PURE study was obtained from the provincial Department of Health, the local authorities and the tribal chief from the specific rural area. The experimental protocol for the data collection at follow-up was consistent with the protocol for the data collection during baseline. In short, lifestyle data (including self-reported tobacco use, alcohol intake and medical history) of the participants was obtained by trained field workers, in the participant's own language. During individual

post-counselling, each participant was informed about his/her HIV status, blood pressure levels and fasting glucose levels, followed by the referral of the infected participants to the local clinic or hospital for further follow-up and CD4 cell count determination. The fieldworkers signed a confidentiality agreement to protect the privacy of the participants.

Anthropometric measurements

Calibrated instruments were used to measure the participant's height to the nearest 0.1 cm (Invicta Stadiometer, IP 1465, London, UK) at baseline and follow-up (Leicester height measure, Seca, Birmingham, UK). Weight was measured to the nearest 0.01 kg (Precision Health scale; A & D Company, Tokyo, Japan) at baseline and follow-up. Waist circumference (WC) was measured between the lowest rib and the lateral iliac crest and recorded to the nearest 0.1 cm with a non-stretchable metal tape (Holtain, Crymych, UK) at baseline [2] and with a steel tape (Lufkin, Cooper Tools, Apex NC, USA) at follow-up. The body mass index (BMI) of each participant was calculated (at baseline and follow-up) with the formula: weight (kg)/ height (m^2). The above measurements were conducted by trained researchers in a private room.

Cardiovascular measurements

Systolic and diastolic blood pressure (SBP and DBP) as well as the heart rate of the participants were measured with the validated OMRON HEM-757 (Omron Healthcare, Kyoto, Japan) device during baseline and with a validated OMRON MI 6 (Omron Healthcare, Kyoto, Japan) device during follow-up, according to appropriate and standardised methods. Each participant was fitted with the correct cuff size. The participant had to be calm and rested for more than/at least five minutes before the measurement, should not have smoked, should not have conducted any form of exercise or eaten during the last 30 minutes and should not have climbed stairs during the last 15-30 minutes before completing this measurement. The participant should have been seated in a supine upright position with his/her arm supported at heart level. After the first measurement was conducted, the procedure was repeated five minutes apart and the last measurement was used (a total of two measurements were conducted).

The carotid-femoral pulse wave velocity (cfPWV) was measured non-invasively on the right side of each participant in a supine position. The carotid pulse wave velocity

(cfPWV), which is seen as the golden standard for the measurement of arterial stiffness [3], was measured. The validated SphygmoCor device (ATCor Medical Pty Ltd, Sydney, Australia) used superficial pulses to measure the PWV. The intima-media thickness (IMT) was measured non-invasively with the SonoSite Micromaxx ultrasound system (SonoSite, Inc., Bothel, WA, USA) with a 6-13 MHz linear array transducer on a selected segment of maximum ten millimeter with good image in each subject. The cardiovascular measurements were conducted by researchers in controlled and private conditions. A standard 12-lead electrocardiography (ECG) was recorded during resting conditions (PC 1200, v5.030, Norav Medical, Yokneam, Israel). Electrocardiography left ventricular hypertrophy (ECG-LVH) was determined using the Cornell product [4-6]. The golden standard for the measurement of left ventricular hypertrophy is echocardiography. However, literature suggests that both ECG-LVH and Echocardiography left ventricular hypertrophy (echo-LVH) are equally predictive of incident heart failure and can be used interchangeably in heart failure risk-prediction models [7]. In a middle-income country such as South Africa the measurement of echo-LVH is not always feasible; we therefore used the ECG-LVH in this study.

Plaque scores were not determined in this study. Although ECG LVH is not seen as the golden standard it remains an appropriate measurement.

Blood, serum and plasma samples

Blood samples from fasting participants were collected by a registered nurse with a sterile winged infusion set and syringes. The blood was drawn from the antebrachial vein and the preparation of both the serum and the plasma was carried out according to standardised methods, snap frozen on dry ice and stored in the laboratory at -80˚C. In the case of blood collection in rural areas, serum and plasma were snap frozen and stored at -20˚C for a maximum of five days. All the samples were transported to the laboratory and stored in a freezer at -80˚C which was/is connected to an alarm system via cell phone. This alarm notified the researcher on duty of any malfunction regarding the freezer, so that the samples were always protected. Mid-stream spot urine samples were collected and frozen at -80˚C.

Biochemical analyses

Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), gamma-glutamyltransferase (GGT) and C-reactive protein (CRP) levels were analysed at baseline (Konelab20i™ auto-analyser, Thermo Fisher Scientific Oy, Vantaa, Finland) and at follow-up by particle enhanced turbidimetric assay (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) from serum samples. The Friedewald formula [8] was used to calculate low-density lipoprotein cholesterol (LDL-C) levels. Albumin and creatinine levels were determined (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) and urinary albumin creatinine ratio (uACR) was calculated. Creatinine clearance (CrCl) was calculated with the Cockcroft-Gault formula: CrCl in ml/min= [(140-age) x (weight in kg) x (0.85 if female)]/(72 x creatinine in mmol/l) [9], at both baseline and follow-up. The glycosylated haemoglobin (HbA1c) levels were determined from blood samples collected in tubes with ethyl-enediamin-e-tetracetic acid. The D-10 haemoglobin testing system from Bio-Rad (#220-0101), which is based on ion-exchange high performance liquid chromatography, was used both at baseline and follow-up.

Glucose levels (blood collected in fluoride tubes) were determined at baseline (Vitros DT6011 Chemistry Analyser; Ortho-Clinical Diagnostics, Rochester, New York, USA) and at follow-up by an enzymatic reference method with hexokinase (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN).

The HIV status of the participants was determined after informed consent was given, in private, by a trained researcher/counsellor. At both baseline and follow-up, the First Response (PMC Medical, Daman, India) rapid HIV card test was used. If the participant tested positive at baseline, the test was repeated for confirmation by using the Pareekshak card test (BHAT Bio-tech, India) and at follow-up Abon card test (Biopharm Corporation Limited Hanyzhou, China). Feedback regarding the HIV status and post-counselling was given individually in a private room by trained counsellors. Participants who already tested positive at baseline were not tested again. For this study, only participants who were identified as being HIV-infected at baseline were included. The HIV-infected participants were treated with a combination pill containing efavirenz, tenofovir and emtricitabine at follow-up. The CD4 counts were determined (in whole blood) by the National Health Laboratory using flow cytometric analysis (Beckman COULTER® EPICS® XLTM, Fullerton, USA) at baseline and at follow-up

with finger-prick blood and a point-of-care device, PIMA^{TM} CD4 (Alere, Jena, Germany).

Risk analyses

The Framingham [10] and Reynolds [11] risk scores were calculated according to the risk score models in Excel spreadsheets at both baseline and follow-up. Both these risk models consisted of two separate models - one for men and one for women. The risk scores were determined as follows:

Table 2: The Framingham risk table implemented for South Africans [10]

Women

Age (years)	Points
20-34	-7
35-39	-3
40-44	0
45-49	3
50-54	6
55-59	8
60-64	10
65-69	12
70-74	14
75-79	16

	Age				
Total cholesterol (mmol/L)	20-39	40-49	50-59	60-69	70-79
≤4.09	0	0	0	0	0
4.10-5.10	4	3	2	1	1
5.11-6.20	8	6	4	2	1
6.21-7.20	11	8	5	3	2
≥7.21	13	10	7	4	2

High-density lipoprotein cholesterol (mmol/L)	Points
≥1.60	-1
1.30-1.59	0
1.00-1.29	1
<1	2

Systolic blood pressure (mmHg)	Untreated	Treated
<120	0	0
120-129	1	3
130-139	2	4
140-159	3	5
≥160	4	6

Smoking status	20-39	40-49	50-59	60-69	70-79
Non-smoker	0	0	0	0	0
Smoker	9	7	4	2	1

Total points	10-years cardiovascular disease risk (%)
<9	<1
9	1
10	1
11	1
12	1
13	2
14	2
15	3
16	4
17	5
18	6
19	8
20	11
21	14
22	17
23	22
24	27
>25	≥30

Men

Age (years)	Points
20-34	-9
35-39	-4
40-44	0
45-49	3
50-54	6
55-59	8
60-64	10
65-69	11
70-74	12
75-79	13

	Age				
Total cholesterol (mmol/L)	20-39	40-49	50-59	60-69	70-79
≤4.09	0	0	0	0	0
4.10-5.10	4	3	2	1	0
5.11-6.20	7	5	3	1	0
6.20-7.20	9	6	4	2	1
≥7.21	11	8	5	3	1

High-density lipoprotein cholesterol (mmol/L)	Points
≥1.60	-1
1.30-1.59	0
1.00-1.29	1
<1	2

Systolic blood pressure (mmHg)	Untreated	Treated
<120	0	0
120-129	0	1
130-139	1	2
140-159	1	2
≥160	2	3

Smoking status	20-39	40-49	50-59	60-69	70-79
Non-smoker	0	0	0	0	0
Smoker	8	5	3	1	1

Total points	10-years cardiovascular disease risk (%)
<0	<1
0	1
1	1
2	1
3	1
4	1
5	2
6	2
7	3
8	4
9	5
10	6
11	8
12	10
13	12
14	16
15	20
16	25
≥17	≥30

Modified from: *Blom (2011) [10]*

The Reynolds risk score models [11]

Women [11]

10-year CVD risk (%) = [1 – $0.98634^{(\exp [B-22.325])}$] x 100% where

B = 0.0799 x age + 3.137 x natural logarithm (SBP) + 0.180 x natural logarithm (hs-CRP) + 1.382 x natural logarithm (TC) – 1.172 x natural logarithm (HDL) + 0.134 x haemoglobin A_{1c} (%) (if diabetic) + 0.818 (if current smoker) + 0.438 (if family history of premature MI).

Men [11]

10-year CVD risk (%) = [1 – $0.908^{(\exp [B-30.651])}$] x 100% where

B = 4.034 x natural logarithm (age) + 2.562 natural logarithm (SBP) + 0.800 x natural logarithm (TC) – 0.760 x natural logarithm (HDL) + 0.352 (if current smoker) + 0.108 x natural logarithm (hs-CRP) + 0.487 (if family history of premature MI) + 0.098 x haemoglobin A_{1c} (%) (if diabetic).

Co-morbidity prevalence

The prevalence of co-morbidities was assessed as follows: Obesity, body mass index (BMI) >30 kg/m^2; Overweight, BMI >25 kg/m^2; Central obesity (WC: men ≥102 cm, women ≥88 cm); Hypertension [systolic blood pressure (SBP) ≥140 mmHg and diastolic blood pressure (DBP) ≥90 mmHg]; TC >5.1 mmol/L; LDL-C >3 mmol/L; HDL-C (men <1mmol/L, women <1.2 mmol/L); Microalbuminuria (ACR 3-30 mg/mmol); eGFR <60 ml/min; ECG derived LVH (Cornell product >244 mV/ms) [12] Diabetes (Glucose >7 mmol/L); cfPWV >10 m/s; sub-clinical atherosclerosis (IMT >0.9 mm) [13] TG >1.7 mmol/L; Atherogenic dyslipidaemia (TG ≥2.31 mmol/L and HDL-C ≤0.88 mmol/L) [14] TG: HDL-C ≥1.49 mmol/L [15] CRP >3 mg/L [16] GGT (men ≥80 U/L, women ≥50 U/L) [17] AIDS (CD4 <200 cells/mm^3) [18] CrCl <50 ml/min [19].

Statistical analysis

The statistical analysis was performed by using Statistica® 13 (StatSoft, Inc., Tulsa, OK, USA). Basic descriptive statistics were used to determine normal distribution of the data, logarithmic transformation was applied and presented as geometric mean with 5^{th} and 95^{th} percentiles if skewed. Groups were compared using independent t-tests and Chi-square tests as appropriate. Associations of measures of end-organ damage (PWV, IMT,

CrCl and Cornell product) with the risk scores were determined by making use of Pearson and partial correlations. Odds ratios with cut-off values and 95% confidence intervals (CI), were calculated. Since very few participants met the cut-off values for measures of end-organ damage and risk, the median values were used as cut-off values for the calculation of odds ratios. Median values were as follows: PWV median=8.15 m/s, IMT median=0.37 mm, CrCl median=103 ml/min, Cornell product median=64.6 mV/ms, CHD median=1% and CVD median=0.85%.

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Chapter 4: Manuscript for publication

INSTRUCTIONS TO AUTHORS; ***Journal:*** *Heart, Lung and Circulation*

- **Complete manuscript**. (1) Title page, (2) abstract and keywords if required, (3) text, (4) acknowledgments, (5) disclosures if required, (6) references, (7) tables (each complete with title) (8) figures and (9) figure legends.
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Cardiovascular disease risk assessment in HIV-infected black South Africans: A longitudinal study

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Abstract

Introduction:

Cardiovascular risk is increased in human immunodeficiency virus (HIV)-infected individuals and may be due to risk factors and/or antiretroviral treatment (ART). Africans may be burdened with both CVD and HIV. Those infected with HIV also show higher prevalence of measures of end-organ damage. We aimed to assess the 10-year coronary heart disease (CHD) and cardiovascular risk in a cohort infected with HIV. Furthermore, we determined whether high risk may be associated with measures of end-organ damage.

Methods:

In 71 HIV-infected participants and 71 controls (matched according to age, sex and locality) cardiovascular risk markers, co-morbidities, CHD and cardiovascular risk and associations of risk with measures of end-organ damage were determined.

Results:

The CHD and cardiovascular risk did not differ between the HIV-infected and their matched controls. Prevalence of obesity, diabetes and microalbuminuria were higher among the controls at follow-up. A borderline negative correlation (p=0.053) was seen between CrCl and CHD at follow-up among those infected.

Conclusion:

The HIV-infected participants did not have a higher CHD or CVD risk compared to their matched HIV-free controls, even though 80% of the HIV-infected participants were treated. No associations between risk scores and measures of end-organ damage were found.

Keywords: Cardiovascular disease, Human immunodeficiency virus, Risk assessment, Africans, End-organ damage

Introduction

Cardiovascular disease (CVD) is considered a leading cause of morbidity and mortality worldwide and especially among Africans [1]. Furthermore, African-Americans are known to have a higher risk of developing coronary heart disease (CHD) compared to other ethnicities [2]. Besides being burdened by non-communicable diseases (NCD), Southern and Eastern Africa are known as the regions that are home to the highest number of people living with HIV/AIDS [3]. The introduction of antiretroviral therapy (ART) has improved the prognosis for many HIV-infected individuals [4]. However, ART is associated with a disturbance in lipid metabolism which may lead to dyslipidaemia [5] and hypertension [6], significant CVD risk factors [7]. Human immunodeficiency virus-infected individuals are more likely to show elevated levels of inflammation [8] and the inflammatory marker C-reactive protein (CRP) is known to be a contributing risk marker to the progression of CVD in the general population [9] and in the HIV-infected population [10].

In a study conducted in Cameroon, treatment naïve HIV-infected individuals showed early onset of aortic stiffness [11] which may be directly associated with atherosclerosis and CVD [12]. Chronic kidney disease (CKD) is seen as a risk factor for CVD [13]. CKD may be promoted through various mechanisms; heart failure promoting decline in kidney function and atherosclerosis leading to renovascular disease [13]. Chronic kidney disease has long been recognised in the HIV-infected population especially those of African descent [14]. Microalbuminuria, indicating CKD, may be an early marker of CVD risk in the HIV-infected population [15]. Left ventricular hypertrophy (LVH), a predictor of CHD [16] and CVD mortality [17], are also more prevalent in the HIV-infected population [18].

A traditional risk factor contributing to the development of both CHD [19] and CVD [20] is smoking. This behaviour is aggravated in the HIV-infected population [21].

In light of the above the need for risk assessment in the African HIV-infected population burdened by both CHD and CVD is important. Cardiovascular risk assessment was described by Blom (2011) as “predicting the future” by implementing risk scores [22]. The Framingham Risk Score has been known as the “heart” of risk assessment for decades [9] and estimates the 10-year risk of coronary endpoints (myocardial infarction and coronary death) [22]. The Reynolds Risk Score, assessing 10-year CVD risk, incorporates CRP as a risk marker [23]. Therefore, we firstly aimed to assess the

10-year CHD (Framingham) and CVD (Reynolds) risk in an African HIV-infected cohort, and secondly to determine the associations of CHD and CVD risk with measures of end-organ damage.

Materials and methods

Study design and population

This study is embedded in the South African arm of the PURE Prospective Urban and Rural Epidemiological (PURE) study. Data were collected in the North-West Province during 2005 (baseline) when 2010 individuals participated, of which 322 were newly identified as being HIV-infected. During 2015 (follow-up) 100 of the 322 HIV-infected participants took part in the 10-year follow-up study of whom 29 participants were excluded due to incomplete data. The remaining 71 HIV-infected participants were matched to 71 HIV-free controls according to age, sex and locality.

Experimental protocol

Procedures regarding the experimental protocol were in accordance with those of Fourie et al. (2015) [24].

Ethical considerations

The Ethics Committee of the North-West University approved the protocol of the PURE-SA study in 2005 and 2015 which complies with the Declaration of Helsinki.

Anthropometric measurements

We used standardised procedures to measure height, weight and waist circumference (WC) during baseline and follow-up.

Cardiovascular measurements

Blood pressure measurements during baseline were in accordance with those of Fourie et al. (2015) [24] as it was calculated with the validated OMRON HEM-757 device and at follow-up the validated OMRON MI 6 device was used. The carotid-femoral pulse wave velocity (cfPWV) was measured with the validated SphygmoCor device (ATCor Medical Pty Ltd, Sydney, Australia) during follow-up. The intima-media thickness (IMT) measurements were in accordance with those of Schutte et al. (2012) where it was measured with the SonoSite Micromaxx ultrasound system (SonoSite, Inc., Bothel, WA, USA) with a 6-13 MHz linear array transducer on a selected segment

of maximum ten millimeter with good image in each subject during follow-up [25]. Electrocardiogram (ECG) measurements were performed [26] and ECG ventricular mass was determined with the Cornell product [27].

Biochemical analyses

Total cholesterol (TC), HDL-C (high-density lipoprotein cholesterol), triglycerides (TG), gamma-glutamyltransferase (GGT) and hs-CRP levels were analysed at baseline (Konelab20i™ auto-analyser, Thermo Fisher Scientific Oy, Vantaa, Finland) and at follow-up by particle enhanced turbidimetric assay (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) from serum samples. Low-density lipoprotein cholesterol (LDL-C) levels were determined with the Friedewald formula [28]. Urinary albumin and creatinine (uACR) levels were determined. Creatinine clearance (CrCl) was calculated with the Cockcroft-Gault formula [29]. The glycosylated haemoglobin A1c (HbA1c) levels were determined. The D-10 haemoglobin testing system was utilised at both baseline and follow-up. Glucose levels were determined at baseline and at follow-up.

At both baseline and follow-up, the First Response (PMC Medical, Daman, India) rapid HIV card test was used to determine the HIV-statuses. If the participant tested positive at baseline, the test was repeated for confirmation by using the Pareekshak card test (BHAT Bio-tech, India) and at follow-up Abon card test (Biopharm Corporation Limited Hanyzhou, China). The CD4 counts were determined (in whole blood) by the National Health Laboratory using flow cytometric analysis (Beckman COULTER® EPICS® XLTM, Fullerton, USA) at baseline and at follow-up with finger-prick blood and a point-of-care device, PIMA™ CD4 (Alere, Jena, Germany). Only participants who were identified as being HIV-infected at baseline were included in our study.

Risk analysis

The Framingham Risk Score [22] and the Reynolds Risk Score [23] were calculated according to the risk score models in Excel spreadsheets. Both these risk models consist of separate models for men and women.

Statistical analyses

The statistical analyses was performed by using Statistica® 13 (StatSoft, Inc., Tulsa, OK, USA). Basic descriptive statistics were used to determine normal distribution of the data, logarithmic transformation were applied and presented as geometric mean with 5th and

95th percentiles if skewed. Groups were compared using independent t-tests and Chi-square tests.

Associations of measures of end-organ damage (PWV, IMT, CrCl and Cornell product) with the risk scores were determined by making use of Pearson and partial correlations. Odds ratios with median values and 95% confidence intervals (CI) were calculated. Since very few participants met the cut-off values for measures of end-organ damage and risk, the median was used as cut-off value for the calculation of odds ratios. Median values were as follows: PWV median=8.15 m/s, IMT median=0.37 mm, CrCl median=103 ml/min, Cornell product median=64.6 mV/ms, CHD median=1% and CVD median=0.85%.

Results

From Table 3 the characteristics of the HIV-free control group and the HIV-infected group at baseline and 10-year follow-up are evident. The HIV-infected group had significantly lower HDL-C ($p<0.01$) and CrCl ($p=0.02$) levels at baseline, compared to the HIV-free group. At follow-up the HIV-infected group showed significantly lower BMI ($p<0.01$), WC ($p<0.01$) and HbA1c ($p=0.01$) compared to the HIV-free control group.

At follow-up the CD4 counts of those infected with HIV were higher compared to baseline ($p=0.03$).

The prevalence of co-morbidities among the HIV-free and HIV-infected groups at the 10-year follow-up study is reported in Table 4. This table shows that more HIV-free participants were overweight, had diabetes, had lower HDL-C (men) and had a higher prevalence of microalbuminuria compared to the HIV-infected group.

Table 3: Characteristics of HIV-free and HIV-infected participants at baseline and 10-year follow-up

	Baseline 2005			Follow-up 2015		
	HIV-free	**HIV-infected**	**p-value**	**HIV-free**	**HIV-infected**	**p**
	(*n=71*)	**(*n=71*)**		**(*n=71*)**	**(*n=71*)**	
Demographic variables						
Age (years)	43 ± 0.44	43 ± 5.85	0.95	53 ± 5.32	53 ± 5.43	0.80
Sex male, *n* (%)	15 (21)	15 (21)	-	15 (21)	15 (21)	-
Locality, *n* (%)*	34 (48)	34 (48)	-	34 (48)	34 (48)	-
Anthropometric variables						
BMI (kg/m^2)	24.2 (17.8-36.1)	22.9 (16.7-35.5)	0.16	25.9 (18.0-36.1)	23.1 (16.1-37.5)	<0.01
WC (cm)	79.8 ± 10.8	75.4 ± 10.1	0.54	89.4 ± 13.7	83.0 ± 14.5	<0.01
Cardiovascular variables						
SBP (mmHg)	124 ± 20.2	123 ± 20.8	0.80	129 ± 22.5	127 ± 24.3	0.73
DBP (mmHg)	84.1 ± 14.8	83.9 ± 13.6	0.49	85.5 ± 13.1	83.2 ± 13.4	0.30
Biochemical variables						
TC (mmol/L)	5.40 ± 1.44	4.36 ± 1.23	0.20	4.66 ± 1.22	4.48 ± 1.03	0.35
HDL-C (mmol/L)	1.59 (0.74-2.98)	1.19 (0.55-2.32)	<0.01	1.29 (0.74-2.27)	1.30 (0.74-2.48)	0.90
LDL-C (mmol/L)	3.09 ± 1.17	2.51 ± 1.00	0.20	2.83 ± 1.07	2.58 ± 0.86	0.13
TG (mmol/L)	1.12 (0.59-2.43)	1.05 (0.50-2.34)	0.45	1.14 (0.50-3.17)	1.10 (0.50-3.11)	0.73
HbA1c (mmol/L)	5.57 (5.00-6.30)	5.49 (4.90-6.20)	0.46	5.82 (5.00-8.80)	5.47 (4.80-6.20)	0.01
Glucose (mmol/L)	5.00 ± 1.80	4.70 ± 0.80	0.23	5.50 ± 1.80	5.12 ± 0.83	0.11
CRP (mg/L)	1.85 (0.10-15.3)	2.29 (0.26-47.7)	0.42	2.85 (0.44-33.9)	4.22 (0.54-44.7)	0.06
GGT (U/L)	53.9 (19.0-339)	44.3 (16.0-228)	0.16	39.1 (11.2-225)	52.7 (15.4-342)	0.07
Lifestyle variables						
Tobacco use, *n* (%)	25 (35)	28 (39)	0.60	25 (35)	28 (39)	0.60
Alcohol use, *n* (%)	22 (31)	25 (35)	0.59	22 (31)	25 (35)	0.59
HT med, *n* (%)	8 (11)	8 (11)	-	16 (23)	12 (17)	0.40
CHOL med, *n* (%)	-	-	-	2 (3)	1 (1)	0.56
CD4 (cells/mm^3)	-	362 ± 181	-	-	500 ± 258	-
ART, *n* (%)	-	-	-	-	57 (80)	-
ART duration, *n* (%)						
>5 years	-	-	-	-	31 (44)	-
<5 years	-	-	-	-	32 (45)	-
Fam hist. CHD, *n* (%)	23 (32)	32 (45)	0.12	-	-	-
Risk scores						
CHD, (%)	2.63 ± 3.30	1.80 ± 2.08	0.08	3.65 ± 4.39	3.22 ± 3.37	0.51
CVD, (%)	1.37 ± 1.78	1.88 ± 4.01	0.33	3.98 ± 3.82	4.27 ± 6.64	0.75
Measures of end-organ damage						
cfPWV (m/s)*	-	-	-	8.38 ± 1.77	8.27 ± 1.85	0.71
IMTf (mm)	-	-	-	0.39 ± 0.09	0.38 ± 0.12	0.53
uACR (mg/mmol)	1.13 ± 1.96	5.23 ± 20.7	0.10	11.43 ± 48.7	2.52 ± 3.30	0.14
CrCl (ml/min)	99.5 (61.7-193)	87.0 (51.1-156)	0.02	107 (71.5-194)	98.0 (55.8-167)	0.09
eGFR (ml/min)	122 ± 34.6	114 ± 37.0	0.20	132 (89.0-215)	136 (94.6-200)	0.62
Cornell product (mV/ms)	-	-	-	56.8 (12.7-179)	65.9 (19.4-247)	0.29

n indicates the number of participants; locality *(urban); BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; HbA1c, glycosylated haemoglobin; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; HT med, hypertension medication; CHOL med, cholesterol medication; ART, antiretroviral treatment; Fam hist. CHD, family history coronary heart disease; CHD, coronary heart disease (Framingham Risk Score); CVD, cardiovascular disease (Reynolds Risk Score); cfPWV, carotid-femoral pulse wave velocity *(adjusted for mean arterial pressure); IMTf, intima-media thickness-far wall mean; uACR, urinary albumin creatinine ratio; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate. Data are expressed as mean with standard deviation or geometric mean with 5th and 95th percentiles. P-values were obtained with independent t-tests, categorical variables with Chi-square tests and PWV p-value with ANCOVA. P-values ≤0.05 are regarded as significant.

Table 4: Prevalence of co-morbidities among HIV-free controls and HIV-infected participants at 10-year follow-up

	Follow-up 2015			
	Total Group	**HIV-free**	**HIV-infected**	**p**
	(n=142)	***(n=71)***	***(n=71)***	
Cardiovascular comorbidities				
Obesity, BMI >30 kg/m^2, *n* / total (%) [30]	34/141 (24)	20/70 (29)	14 (20)	0.22
Overweight, BMI >25 kg/m^2, *n* / total (%) [30]	63/141 (45)	38/70 (54)	25 (35)	0.02
Central obesity [30]				
Men ≥102 cm, *n* / total (%)	2/30 (7)	1/15 (7)	1/15 (7)	-
Women ≥88 cm, *n* / total (%)	52/112 (46)	31/56 (55)	21/56 (38)	0.06
Hypertension, *n* (%) [30]	28 (20)	16 (23)	12 (17)	0.40
Diabetes, *n* (%) [31]	4 (3)	4 (6)	0 (0)	0.04
TC >5.1 mmol/L, *n* (%) [30]	44 (31)	24 (34)	20 (28)	0.47
LDL-C >3 mmol/L, *n* (%) [30]	49 (35)	28 (40)	21 (30)	0.22
HDL-C [30]				
Men <1 mmol/L, *n* / total (%)	8/30 (27)	7/15 (47)	1/15 (7)	0.013
Women <1.2 mmol/L, *n* / total (%)	46/112 (41)	21/56 (38)	25/56 (45)	0.44
TG >1.7 mmol/L, *n* / total (%) [32]	26/141 (18)	13/70 (19)	13 (18)	0.97
TG: HDL-C ≥1.49 mmol/L, *n* / total (%) [33]	24/141 (17)	12/70 (17)	12/70 (17)	0.97
Atherogenic dyslipidaemia, *n* / total (%) [32]				
(TG ≥2.31 mmol/L and HDL-C ≤0.88 mmol/L)	5/141 (4)	4/70 (6)	1 (1)	0.17
CRP >3 mg/L, *n* (%) [34]	79 (56)	35 (49)	44 (62)	0.13
GGT [35]				
Men ≥80 U/L, *n* / total (%)	11/30 (37)	5/15 (33)	6/15 (40)	0.70
Women ≥50 U/L, *n* / total (%)	40/112 (36)	18/56 (32)	22/56 (39)	0.43
AIDS (CD4 <200 cells/mm^3), *n* / total (%) [36]	8/68 (12)	0 (0)	8/68 (12)	-
Risk assessment				
CHD (Framingham Risk Score)				
Low (<10%), *n* (%)	128 (90)	63 (89)	65 (92)	0.57
Medium (10-20%), *n* (%)	13 (9)	7 (10)	6 (8)	0.57
High (>20%), *n* (%)	1 (1)	1 (1)	0 (0)	-
CVD (Reynolds Risk Score)				
Low (<10%), *n* (%)	132 (93)	66 (93)	66 (93)	0.29
Medium (10-20%), *n* (%)	8 (6)	5 (7)	3 (4)	0.29
High (>20%), *n* (%)	2 (1)	0 (0)	2 (3)	0.29
Measures of end-organ damage				
Stiffness (cfPWV >10 m/s), *n* / total (%) [31]	15/126 (12)	8/65 (12)	7/61 (11)	0.89
SC atherosclerosis (IMTf >0.9 mm), *n* (%) [31]	0 (0)	0 (0)	0 (0)	-
Microalbuminuria, *n* / total (%) [30]	4/130 (3)	4/64 (6)	0 (0)	0.04
CrCl <50 ml/min, *n* / total (%) [37]	1/141 (1)	0 (0)	1(1)	0.32
eGFR <60 ml/min, *n* (%) [30]	0 (0)	0 (0)	0 (0)	-
ECG LVH (Cornell product >244 mV/ms), *n* (%) [30]	4/119 (3)	1/60 (2)	3/59 (5)	0.30

n indicates the number of participants; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; CHD, coronary heart disease; CVD, cardiovascular disease; cfPWV, carotid-femoral pulse wave velocity; SC atherosclerosis (IMTf), sub-clinical atherosclerosis intima-media thickness-far wall mean; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; ECG LVH, electrocardiographic derived left ventricular hypertrophy. P-values were obtained with Chi-square tests and p-values ≤0.05 are regarded as significant.

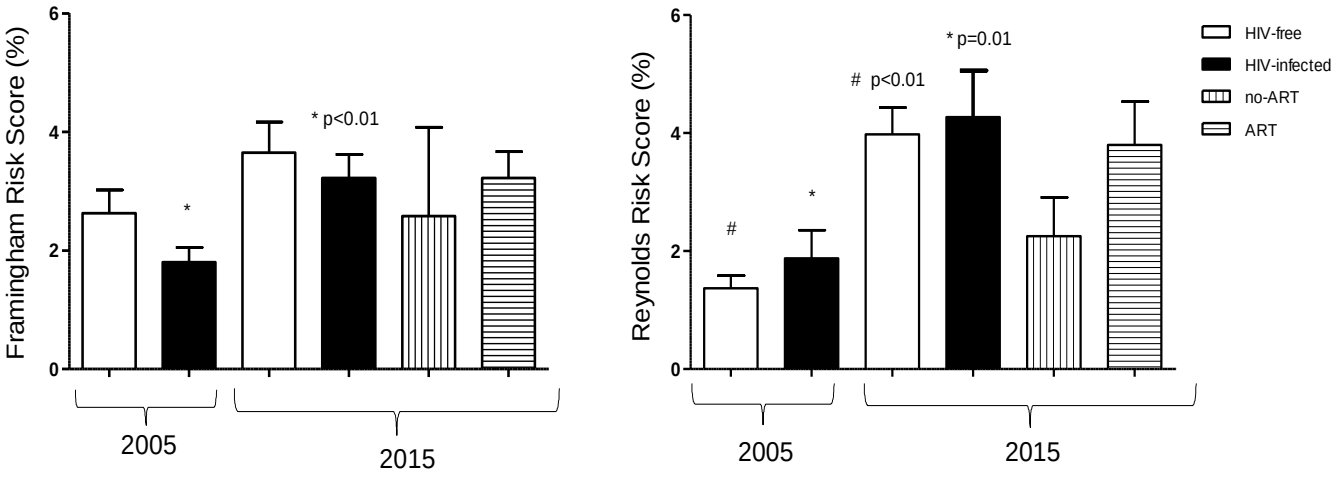

Figure 1: Baseline and follow-up Reynolds (CVD) and Framingham (CHD) risk scores in HIV-free and HIV-infected groups with the influence of treatment

Both the Framingham Risk Score (CHD risk) and Reynolds Risk Score (CVD risk) were determined for the HIV-free controls and HIV-infected group at baseline and follow-up. No differences were seen between the HIV-free and HIV-infected group with either the risk scores. Compared to baseline, the Framingham Risk Score was higher in the HIV-infected group at follow-up, however both the HIV-free controls and HIV-infected group had a higher Reynolds Risk Score at follow-up than at baseline. No differences were seen between the no-ART and ART groups (Figure 1).

Figure 2 reports the Pearson correlations between IMTf and CrCl with CHD risk and Cornell product with CVD risk in the HIV-free and HIV-infected group at 10-year follow-up. A borderline negative correlation (p=0.053) was seen between CrCl and CHD in the HIV-infected group at follow-up.

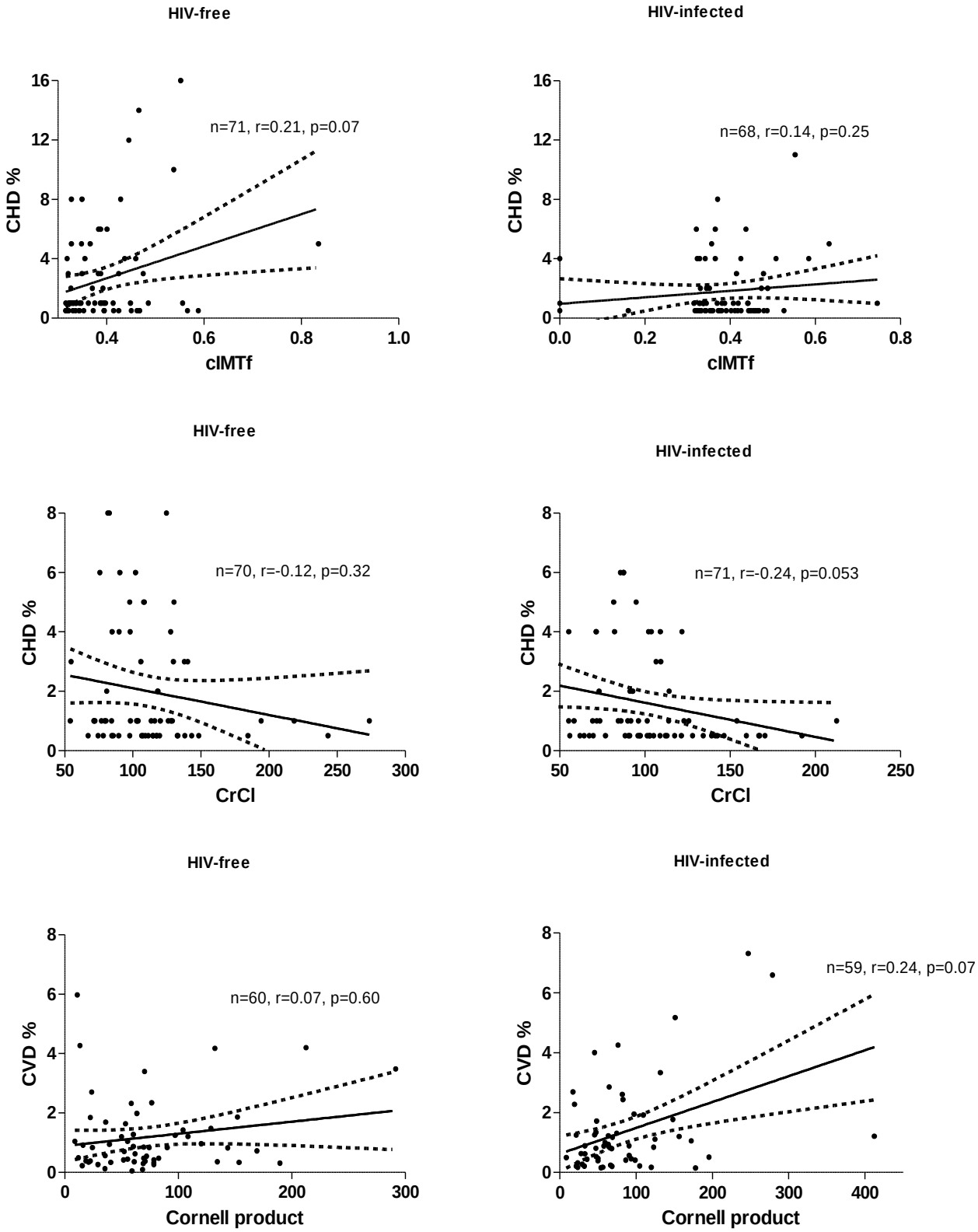

Figure 2: Scatterplots indicating the Pearson correlations between (intima-media thickness-far wall mean) IMTf and (creatinine clearance) CrCl with the Framingham Risk Score (CHD) and Cornell product with the Reynolds Risk Score (CVD) in the HIV-free and HIV-infected group at 10-year follow-up

Table 5: Partial correlations of 10-year Framingham (CHD) Risk Score and Reynolds (CVD) Risk Score with measures of end-organ damage at 10-year follow-up in the HIV-free and HIV-infected group

Coronary heart disease (Framingham Risk Score)				
	cfPWV (m/s)	**IMTf (mm)**	**CrCl (ml/min)**	**CP (mV/ms)**
Group				
HIV-free	r=-0.13, p=0.31	r=0.07; p=0.58	r=-0.07; p=0.58	r=-0.17; p=0.22
HIV-infected	r=-0.22; p=0.60	r=-0.08; p=0.82	r=0.16; p=0.67	r=-0.48; p=0.34
Cardiovascular disease (Reynolds Risk Score)				
	cfPWV (m/s)	**IMTf (mm)**	**CrCl (ml/min)**	**CP (mV/ms)**
Group				
HIV-free	r=0.06; p=0.65	r=0.03; p=0.82	r=-0.14; p=0.28	r=-0.30; p=0.03
HIV-infected	r=-0.82; p=0.01	r=-0.21; p=0.56	r=-0.25; p=0.49	r=-0.31; p=0.55

CHD, coronary heart disease; CVD, cardiovascular disease; cfPWV, carotid-femoral pulse wave velocity; IMTf, intima-media thickness (far wall mean); CrCl, creatinine clearance; CP, Cornell product. P-values ≤0.05 are regarded as significant

Table 5 reports the partial correlations of the risk scores (CHD -and CVD) respectively with measures of end-organ damage. In the HIV-infected group we adjusted for the risk variables: age, sex, WC, SBP, CRP, CD4 and ART use. Cardiovascular risk correlated negatively with cfPWV in the HIV-infected group. In sensitivity analyses we replaced ART with tobacco use or alcohol use, however the results did not differ. In the HIV-free group we adjusted for the risk variables: age, sex, WC, SBP, CRP, tobacco use and alcohol use. Cardiovascular risk correlated negatively with Cornell product in the HIV-free group.

Table 6: Odds Ratios of 10-year risk scores and measures of end-organ damage in the HIV-free and HIV-infected participants

Coronary heart disease (Framingham Risk Score)				
	cfPWV (95% CI)	**IMTf (95% CI)**	**CrCl (95% CI)**	**CP (95% CI)**
Group				
HIV-free	0.55 (0.19-1.57)	1.08 (0.40-2.93)	0.46 (0.16-1.31)	0.90 (0.31-2.65)
HIV-infected	1.23 (0.45-3.36)	0.60 (0.23-1.58)	0.39 (0.15-1.03)	2.17 (0.76-6.20)
Cardiovascular disease (Reynolds Risk Score)				
	cfPWV (95% CI)	**IMTf (95% CI)**	**CrCl (95% CI)**	**CP (95% CI)**
Group				
HIV-free	0.74 (0.28-1.96)	1.68 (0.65-4.30)	0.57 (0.22-1.47)	0.87 (0.31-2.39)
HIV-infected	1.23 (0.45-3.36)	0.61 (0.23-1.61)	0.55 (0.21-1.44)	1.42 (0.51-3.96)

OR, odds ratios; cfPWV, carotid-femoral pulse wave velocity; IMTf, intima-media thickness-far wall mean; CrCl, creatinine clearance; CP, Cornell product. Significance is indicated by *

The odds ratios of measures of end-organ damage and 10-year CHD -and CVD risk in the HIV-free and HIV-infected group are shown in Table 6. cfPWV was adjusted for mean arterial pressure (MAP). No significant odds were found for having a higher than median CHD or CVD risk with higher than median PWV, IMT and Cornell product or lower than median CrCl.

Discussion

To the best of our knowledge, we are the first to investigate CHD (Framingham Risk Score) and CVD (Reynolds Risk Score) risk and the association thereof with measures of end-organ damage in HIV-infected South Africans. The main finding of this study was that the HIV-infected participants did not have a higher CHD or CVD risk when compared to the age, sex and locality matched HIV-free control group; neither at baseline nor at 10-year follow-up. Furthermore, antiretroviral treatment did not seem to affect the CHD nor the CVD risk among those infected with HIV.

Although the Framingham risk model was mainly developed for risk prediction among white populations, it previously performed well to predict CHD risk in African American populations [38]. The Reynolds risk model also showed improved discrimination overall and especially in black and white women [39]. Mashinya et al. (2015) concluded that there is no need to develop a race/ethnicity specific risk model for HIV infected Africans [40].

In a cross-sectional study by Mashinya et al. (2015) the HIV-infected Africans in their study were classified as having a low 10-year Framingham Risk Score [40] which is in accordance with our findings.

ART may lead to lower immune activation [41] and this is seen in this study with the higher CD4 counts in the HIV-infected group (treated and not treated) at follow-up ($p=0.03$). Besides lowering the immune activation, ART may decrease the inflammatory response in those living with HIV [41]. However, in this study the CRP levels did not differ at baseline (all participants were ART naïve), while the CRP levels tended to be higher at follow-up ($p=0.06$) with 80% of the participants being on treatment.

Several cross-sectional studies reported that higher CRP levels were associated with an increase in IMT [42, 43] which correlated with arterial stiffness [11]. This correlation may be attributed to viral replication leading to an increase in inflammation and endothelial activation associated with atherosclerotic lesions and CVD [43]. We found no indication of atherosclerosis or arterial stiffness (PWV <10 m/s) in the HIV-infected group. Awotedu et al. (2015) found an increase in stiffness in their HIV-infected treatment-naïve group. They speculated that inflammation, induced by HIV, and increased collagen production, might have led to lower quantities of normal elastin,

and not receiving treatment may result in higher arterial stiffness especially in the elderly [44].

Left ventricular hypertrophy is a maladjusted response to chronic pressure overload and one of the most important risk factors in individuals with hypertension, especially high SBP [45]. Neither blood pressure nor prevalence of hypertension or ECG derived LVH differed between those infected with HIV and the HIV-free in this study. Our findings are in accordance with those of Ogunmola et al. (2014) where no differences in blood pressure or associations with hypertension were found between HIV-infected and HIV-free Nigerians [46].

Human immunodeficiency virus associated nephropathy describes glomerular and end stage renal disease in those infected with HIV-1 and may present with excretion of low levels of protein in urine (<2 g/day) or acute nephrotic proteinuria [47]. The HIV-infected group of this study were ART-naïve at baseline and started with ART during the course of the 10 years. Although the use of ART dramatically improved renal function, the participants were treated with a tenofovir-containing regimen which may result in declining kidney function with long-term use [48]. Although 44% of the HIV infected participants of this study received the tenofovir based regimen for more than five years, our results did not indicate kidney dysfunction or a prevalence of lower than normal CrCl at 10-year follow-up.

As ART lowers inflammation levels in those living with HIV [41] it will return the lipid profile towards normal, resulting in an increase in HDL-C levels [49]. The latter is seen in this study where lower levels of HDL-C were seen among those infected at baseline, but not at follow-up.

The HIV-infected group of this study were treated with efavirenz, emtricitabine and tenofovir containing regimen and may explain the resemblance of our results to those of Ogunmola et al. (2014) who have found a low prevalence of obesity in the HIV-infected group [46]. Metabolic complications usually arise with the use of protease inhibitors (PI) [50]. A lower prevalence of diabetes (also known as a metabolic complication), was reported among the HIV-infected individuals in our study.

Smoking [51] and alcohol consumption [52] are well-established traditional cardiovascular risk factors. Although it was found that HIV-infected individuals are

more likely to smoke [53] and drink excessive alcohol [54], tobacco and alcohol use did not differ between the HIV-infected and HIV-free control group of this study.

This study should be interpreted within the context of its strengths and limitations. Longitudinal studies assessing the 10-year CHD and cardiovascular risk among South Africans are sparse. To the best of our knowledge, the present study is the first to investigate the association between established cardiovascular risk scores and measures of end-organ damage in HIV-infected South Africans and matched HIV-free controls. This study is a well-controlled study and gold standard measurements were conducted with validated apparatus where possible. As it was not possible to measure left ventricular hypertrophy with echocardiography, the Cornell product was used to measure ECG-LVH. Limitations of this study is that this study group was relatively small, however the HIV-infected participants were matched to the HIV-free participants according to age, sex an locality and the HIV-infected participants were known to be infected for at least 10 years. Another limitation is that the plaque scores were not indicated with the measurement of the intima media thickness.

Conclusion

To conclude, despite their HIV-status of at least 10 years and 80% being treated, as well as an increase during the 10 years in the CHD risk only among those infected, the HIV-infected participants of our cohort did not have higher CHD or CVD risk when compared to the matched HIV-free participants. Furthermore, no indication of a higher prevalence of end-organ damage was detected among those infected with HIV, which correlates with the findings of the risk score models. Should our results be confirmed in larger studies, the studies would be of great interest to South Africa burdened by the high prevalence of both HIV and CVD.

Conflict of interest

Authors declared no conflict of interest.

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Chapter 5: General conclusions and recommendations

Introduction

In this chapter, the main findings are summarised, compared to the relevant literature, discussed and concluded in order to point out the differences in the coronary heart disease (CHD) risk score (Framingham) and the cardiovascular risk score (Reynolds) between human immunodeficiency virus (HIV)-infected and HIV-free Africans matched according to age, sex and locality. Associations of the risk scores with measures of end-organ damage are shown. Recommendations for future studies are also given.

Hypotheses and comparison to relevant literature

The risk scores did not differ between the HIV-infected and HIV-free participants, despite their HIV status of at least 10 years and 80% being treated with antiretroviral treatment (ART). No indication of a higher prevalence of end-organ damage was detected among the HIV-infected participants, which correlates with the findings of the risk score models. Therefore,

the first hypothesis namely: *HIV-infected participants have a higher 10-year cardiovascular disease risk compared to HIV-free participants in both risk score models* is rejected because the HIV-infected participants did not show higher risk when compared to the HIV-free controls.

Vos et al. (2017) have found in a study executed in a study group of black participants living in Limpopo, that the Framingham risk did not differ between the HIV-infected and HIV-free group and also did not differ between the treatment-naïve and treated participants [1]. This is in accordance to the findings of the present study, whereas the Framingham risk score between the HIV-infected and HIV-free group did not differ.

There are no studies making use of the Reynolds risk score to assess a 10-year CVD risk in HIV-infected individuals, especially in women [2]. However, according to the literature, the Reynolds risk model showed improved overall discrimination and especially in black and white women [3] and therefore there is no need to develop a race/ethnicity specific risk model for HIV infected Africans [4].

The second hypothesis, namely: *A high cardiovascular disease risk score correlates with markers of end-organ damage [increased sub-clinical atherosclerosis (IMT),*

increased arterial stiffness (PWV), left ventricular hypertrophy (LVH) and chronic kidney disease (CrCl)] is also rejected.

Parra et al. (2010) did not find any correlation between the Framingham risk score and sub-clinical atherosclerosis in the HIV-infected individuals [5]. This is in accordance with our results, as no significant associations of the risk scores to atherosclerosis were found. Also, no associations of risk scores with arterial stiffness were found. According to the literature, atherosclerosis correlates with arterial stiffness [6]. No associations of risk scores to left ventricular hypertrophy (LVH) or creatinine clearance (CrCl) were seen in both study groups.

Discussion of main findings

According to the literature, Africans have a high prevalence of cardiovascular risk [7]. During 2015, Eastern and Southern Africa had 19 million individuals infected with HIV [8]. Studies predicted that over 10 million individuals aged older than 50 years will be HIV-infected in sub-Saharan Africa by 2030 [9, 10]. Human immunodeficiency virus infected individuals may show higher cardiovascular risk due to infection, immune activation and the use of ART [11].

In a study conducted by Bergersen et al. (2004), twice as many HIV-infected individuals receiving ART, had a 10-year Framingham risk score of more than 20%, compared to their HIV-free counterparts [12]. However, our results differ from those of Bergersen et al. (2004), as the Framingham risk score of the HIV-infected participants and HIV-free participants did not differ. It was found in a recent study that the HIV-infected African individuals who were treated did not show a higher Framingham risk score [1, 4] which is more in line with our results.

The HIV-infected participants and the HIV-free participants had no difference in the Reynolds risk score in this study. As mentioned, there are no studies incorporating the Reynolds risk score to assess CVD risk in HIV-infected individuals [2], therefore this risk score was included in our study since it is the only risk score that includes C-reactive protein (CRP) as a CVD risk marker [13] and CRP is known to be higher in the HIV-infected population [14, 15].

No associations between the risk scores and measures of end-organ damage were found.

As mentioned, Parra et al. (2010) did not find any correlation between the Framingham risk score and sub-clinical atherosclerosis in the HIV-infected individuals [5]. No indication of atherosclerosis or increased arterial stiffness in the HIV-infected group of this study was found. Several studies reported that higher levels of CRP were associated with an increase in IMT [16, 17] which correlated with arterial stiffness [6]. Because the HIV-infected participants of this study did not show higher levels of CRP, this may be the reason why no prevalence of atherosclerosis or arterial stiffness in the HIV-infected participants was observed.

The prevalence of electrocardiography (ECG) derived LVH did not differ between the HIV-infected and HIV-free participants in this study. Left ventricular hypertrophy is a response to high systolic blood pressure (SBP) [18]. The HIV-infected participants did not have a higher prevalence of hypertension when compared to the HIV-free participants.

The results did not indicate kidney dysfunction or a prevalence of lower than normal CrCl at 10-year follow-up, although nearly half of our HIV-infected participants were treated with tenofovir and this ART is known to be associated with a decrease in kidney function.

Conclusion

The HIV-infected participants of this cohort of whom 80% were using ART and were infected for at least 10 years, did not have higher CHD or CVD risk scores when compared to the HIV-free participants. No indication of a higher prevalence of measures of end-organ damage was detected among those infected with HIV, which correlated with the findings of the risk score models.

Chance and confounding

It is of importance to critically reflect on some of the factors that may have confounded the results of this study. After 2005, participants commenced with treatment as their CD4 cell count declined below 200 cells/mm^3. Although the duration of treatment is shown in Table 1 (Chapter 4), the exact duration (months) of each participant's treatment could not be determined.

The study did not test for any opportunistic infections (this was only reported on questionnaires) and final mortality and events data were not available.

Statistical results were evaluated from a physiological perspective and statistical significance does not necessarily indicate physiological significance.

Recommendations to future research

- Studies should be conducted where all treated participants receive treatment for a longer period than 5 years.
- A larger experimental - and control group, matched according to age, sex and locality, should be employed. Due to incomplete data sets, 29 of the participants had to be excluded which might have reduced the power of the study sample.
- The influence of ART on vascular function needs to be determined to assess whether being treated is the reason that we found differences in risk scores between the HIV-infected and HIV-free participants and no association of risk scores with measures of end-organ damage in this population.
- Similar studies in other provinces of South Africa should be conducted.

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Appendices

Untitled

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DECLARATION

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 dissertation written by ms Marlene Duvenhage (student number 23440848).

Title of the article: Cardiovascular disease risk assessment in HIV-infected black South Africans: A longitudinal study

C Vorster

28 June 2017

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Date