Effect of HIV treatment and other risk factors on cardiovascular disease risk in rural South Africa

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DECLARATION

I Wisdom Forward Mudombi declare that this research report is my own work, compiled under the supervision of Professor Kerstin Klipstein-Grobusch and Dr Alinda G Vos. The report is being submitted to the University of the Witwatersrand in partial fulfillment of a degree of Master of Epidemiology in the field of Epidemiology and Biostatistics. The material contained in this research report has not been submitted for any other degree or examination in this university or any other university.

Signature 

Date: 27 September 2019

DEDICATION

To all people who have inspired me and given me tenacity to progress on. Thank you for your inspiration and may your heart desires be granted.

ABSTRACT

Background. Cardiovascular disease (CVD) is increasing in resource-limited settings. Few studies on the effect of combination antiretroviral treatment (cART) on CVD have been conducted in rural HIV-infected populations. There is need to further identify CVD risk burden and to estimate the influence of cART on CVD risk. This study assesses the CVD risk using the reduced Data Collection on Adverse Effects of Anti-HIV Drugs (D:A:D) CVD risk score and determines the effect of current and previous cART on the D:A:D risk score in HIV-positive participants in a rural African HIV-positive population. In addition the study identifies which determinants not accounted for in the original D:A:D risk score within a rural HIV-positive cohort are significantly associated with the D:A:D risk score.

Methods. A cross sectional analysis was performed including baseline data of all HIV-positive participants of the Ndlovu cohort study. This is a longitudinal study in rural South Africa including 1927 adults (46% HIV-positive) evaluating the influence of HIV on the development of CVD. Data were collected on demographics, education, cardiovascular risk factors, HIV and cART related characteristics. The reduced D:A:D score was calculated using the updated prediction model and multivariable regression was performed to determine associations with the D:A:D score.

Results. 885 participants (59.6% females) were included. The mean age was 42 years (standard deviation (SD) 10.3). 186(21%) were cART naïve and 699(79%) were already on cART. Median duration of cART use was 2.4 years (interquartile range (IQR) 0.0-6.8). The median D:A:D score was 2.2% (1.1-5.0); 2.4% (IQR 1.2-5.2) for participants on cART and 1.4% (IQR 0.7-3.5) for those not on cART ($p<0.001$). Factors associated with an increase in D:A:D score were male gender (0.987; 95% CI 0.851-1.122; $p<0.001$), no formal education (0.538; 95% CI 0.209-0.867; $p<0.001$), BMI (Overweight/obesity) (0.235; 95% CI 0.091-0.380; $p<0.001$) and Albumin creatinine ratio (ACR) (0.0015; 95% CI 0.00044-0.0025; $p=0.005$).

Conclusion. In this young HIV-positive rural African population 5-year CVD risk according to the D:A:D score is low. The risk is higher in participants on cART compared to that not on cART. Determinants of CVD risk are male gender, no formal schooling, and BMI and albumin/creatinine ratio. These factors have to be considered for preemptive interventions focused on the HIV infected population in order to reduce CVD morbidity and mortality.

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TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

ABC	Abacavir
ACE	Angiotensin Converting Enzyme
ACR	Albumin/Creatinine Ratio
AIDS	Acquired Immunodeficiency Syndrome
ARB	Angiotensin Receptor Blocker
ASCVD	Pooled Cohorts Equation for atherosclerotic CVD
BMI	Body Mass Index
cART	combination Antiretroviral Therapy
CI	Confidence Interval
CVD	Cardiovascular Disease
d4T	Stavudine
D:A:D	**D**ata Collection on **A**dverse Effects of Anti-HIV **D**rugs
ECG	Electrocardiogram
FRS	Framingham Risk Score
HDL	High density lipoprotein
HIC	High Income Countries
HIV	Human Immunodeficiency Virus
HIVAN	HIV associated nephropathy
LDL	Low density lipoprotein
LMIC	Low Medium Income Countries
MI	Myocardial Infarction
PI	Protease Inhibitor
PROCAM	Prospective Cardiovascular Munster Study
SSA	Sub-Saharan Africa
NCS	Ndlovu Cohort Study

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

1 CHAPTER 1 - INTRODUCTION

This chapter gives background detail to the global and national burden of Human Immunodeficiency virus (HIV) and cardiovascular disease (CVD). Thereafter the statement of the problem is given followed by the study justification. Relevant literature was searched and reviewed with respect to the effect of HIV treatment and other CVD risk factors in sub-Saharan Africa (SSA) and the gaps were described to give a justification of the study. The literature background and basis to utilize the Data Collection on Adverse Effects of Anti-HIV Drugs (D:A:D) risk score in this research is highlighted followed by the aim and objectives of the study being stated at the end of the chapter.

1.1 Background

Approximately more than 35 million individuals are infected and living with HIV of which 71% are from SSA (1). In SSA nearly 58% of individuals living with HIV are females (1). The morbidity and mortality in HIV populations has decreased as a result of combination antiretroviral therapy (cART) (2, 3) and mortality trends shifted from AIDS related infections and malignancies to conditions not related to AIDS including CVD (4). Non-communicable diseases such as cardio metabolic complications are increasingly occurring in HIV-positive people who now have an increased life expectancy due to treatment of HIV by use of cART as well as lifestyle changes (5). CVD manifests as (i) Coronary heart disease (CHD) which can be fatal myocardial infarction (MI), (ii) Cerebrovascular disease (nonfatal/fatal stroke and transient ischemic attack (TIA) (iii) Aortic atherosclerosis and thoracic/abdominal aortic aneurysm and (iv) Peripheral artery disease (PAD).

CVD is a major cause of mortality in the general population (6). Whether CVD burden differs for those infected with HIV from the general population it is under debate. This emphasizes the importance of a wider understanding of the risk of CVD and necessitates preemptive interventions focused on the HIV positive population.

Highly active antiretroviral therapy (HAART) also called cART has been introduced for a significant period of time with very good results in HIV-positive individuals. In the post cART introduction era there is now data showing that non-AIDS defining illnesses have been considered to attribute to approximately 50% of deaths in North American HIV-positive cohorts. In these observational studies 7% to 19% of all deaths were attributed to CVD (4).

The continuous and ongoing inflammation and extensive immune activation persisting in well-treated HIV infection, has been implicated for the rise of morbidity and mortality from cardio-metabolic conditions in the HIV population (7-10). This persistent inflammatory process has been found to have an association with a higher relative risk of CVD of close to 50% and a prior 'accelerated' onset of CVD in HIV-positive patients in comparison to HIV-negative persons (7-10). The D:A:D study was established to investigate emerging trends on major causes of death and the factors associated with each specific cause of death amongst HIV-positive populations (11). In an observational multinational prospective cohort, Smith et al studied an association between non HIV-specific risk factors, HIV-specific factors and death using multivariable Poisson regression (11). Of the 2482 deaths in 180176 person-years (PY) on 33308 participants, the deaths that were CVD related were 289 (11.6%) at 1.6 per 1000 person years. This is a high burden as CVD is ranked third with respect to mortality after HIV/AIDS (743; 29.9%) and liver related death (341; 13.7%) in the 212 clinics for the HIV positive D:A:D cohort study from 21 high-income countries (HIC) in Europe, United States and Australia (11). Liver related deaths in this cohort were mainly due to Hepatitis C. When comparison is made to the year of publication in 2010 and the recent developments in curative anti-Hepatitis C drugs the deaths due to Hepatitis C may decrease in the future. This may imply that death from CVD as the D:A:D study progresses may possibly increase. Smith et al concluded that CVD risk factors need to be appropriately addressed in order to maximally reduce mortality in the HIV-infected populations (11). Palella FJ Jr et al also observed that CVD contributed a significant percentage of deaths in a prospective observational and multicenter study from 1996 to 2004. From their analysis, non-AIDs defining illnesses which included CVD showed a rise from 13.1% in 1996 to 42.5% in 2004 ($P<0.001$ for trend) (12). These trends point to the need to closely follow up and monitor for CVD in HIV populations on cART.

In South Africa, CVD is in the top three leading causes of natural deaths. Non-communicable diseases form approximately 60% of the top ten natural causes of death. Together with diabetes mellitus, CVD is contributing significantly to the rise in non-communicable diseases in South Africa. The persistent rise in non-communicable diseases has been influenced by the ageing of both males and females (13).

It is important to note that increasing prevalence of CVD in SSA is related to changes in life-style factors like diet, physical inactivity even in the rural areas (14, 15). As the population advances in age it is vital to estimate the CVD risk into the future.

The utilization of cardiovascular risk prediction scores, adopted from the practice in the general population, is a useful tool to get insight into the future burden of CVD (16). These CVD prediction scores guide physicians and cardiologists as an adjunct for clinically correct interventions like lifestyle modification or whether to introduce medications to control hypertension or total cholesterol levels.

Currently, only one cardiovascular risk score specifically modeled from a large multicenter prospective observational HIV cohort is available for HIV-positive populations (17). The D:A:D risk score was modeled mainly with HIV patient data contributed from Australia, Europe and North America. The D:A:D risk score equation considers antiretroviral drug exposure to be a potential risk factor of cardiovascular outcomes. It is important to point out that the D:A:D study had limited data on women as only 26% were women and the CVD risk profile was found to be different for women compared to men. Only 9% of the D:A:D study population was none whites. Therefore, conclusions and interpretations should be done with caution given that the majority of the HIV infected population worldwide are Blacks, and in the SSA context 58% are female (16).

Few studies from low and medium income countries (LMIC) with a significant percentage of women have applied the D:A:D risk score for CVD risk estimation and in one study the D:A:D risk score showed that 31.1% of patients had a moderate to high 5-year risk in CVD (18). These studies did not however account for chronic inflammation markers such as C-reactive protein (CRP), which could be high in HIV-positive populations. The studies were generally of small sample size (18, 19) suggesting overestimation of the CVD risk score as a result of the studies' sample size not fulfilling the normal distribution population principles to correctly then estimate CVD risk for generalizability. Longitudinal studies are further required in LMIC with larger sample sizes assessing CVD endpoints to correctly estimate the CVD risk.

Another factor to take into account is that there is paucity of data in LMIC on the relation between frequently used cARTs like protease inhibitors (PI), previous or current use of stavudine (d4T) and CVD risk. d4T used to be part of the first line therapy and it contributed to lipodystrophy and metabolic disorders, and this has been linked to an increased CVD risk (20) Although d4T is hardly used anymore, HIV positive people who have previously used d4T might still be at a higher CVD risk compared to people who did not use d4T or HIV negative people due to the issue of residual lipodystrophy. The same applies to the current PIs

use, which may contribute to CVD risk as a synergistic effect with other traditional risk factors.

In addition urine micro albuminuria or albumin creatinine ratio (ACR), duration of cART and socioeconomic status were not factored for in most of the studies from LMIC. These CVD risk factors, which were not accounted for in the D:A:D prediction model could be prevalent in poor resource settings and there may be a need to consider them for estimating the CVD risk. Given that the D:A:D score is a fixed score and that it is not possible to add more CVD risk factors that are not yet part of the equation, it is important to investigate other biologically plausible CVD risk factors in the HIV-positive population in SSA.

1.2 Statement of the problem

CVD is increasing in resource-limited settings (13, 14). There are several risk factors that have been postulated to explain the CVD burden in the HIV populations. The traditional CVD risk factors still contribute to the pathogenesis of CVD in the HIV populations. In resource limited environments the effect of the added inflammatory component of HIV and co-infections from parasitic infections and other opportunistic infections may be contributing to the CVD burden. The adverse effects of cART may increase the CVD risk in HIV rural populations and this has not been evaluated in resource-limited settings especially with specific reference to previous and current cART.

1.3 Justification for the research

Few studies on CVD risk in SSA HIV-positive individuals have been conducted among rural people with a low socioeconomic status. The differences in HIC and LMIC with respect to ethnicity, genetics, environmental factors, and populations' uptake of stopping smoking campaigns can give rise to differences in the prevalence of CVD risk factors (19, 21, 22).

There is need to conduct a study in SSA factoring in previous, current cART use and other confounding CVD risk factors in the CVD causal pathway to further explain CVD in low and medium countries especially the rural HIV populations. This study will be useful to inform policy and guidelines on CVD risk assessment in HIV management. There is also need to improve CVD prevention utilizing and considering other CVD risk factors to establish whether observed associations in literature are possibly causal in SSA especially in resource limited settings. From that perspective it is important and justified to investigate the CVD risk

factors in HIV persons in resource-limited settings like the Moutse area in Limpopo province of South Africa.

1.4 LITERATURE REVIEW

1.4.1 HIV infection: burden of classic cardiovascular disease risk factors

The burden of CVDs in SSA is increasing and deaths from CVDs are projected to double to 2.4 million in 2030 compared to 2004 (23).This implies that cardiovascular and cardio-metabolic disease are a significant health burden in SSA competing with communicable diseases for scarce health resources (23-25).Traditional and HIV-related CVD risk factors together with cART-associated cardiovascular complications raise CVD risk 1.5- to 2-fold in HIV-positive persons in comparison to HIV-negative individuals (10, 26, 27). HIV-positive patients just like their HIV-negative counterparts are also burdened with classic CVD risk factors like hypertension, insulin resistance, increased body mass index (BMI), metabolic syndrome (MetS) and central obesity (28, 29).

CVD risk factors are also prevalent among young and stable outpatients from clinics in the low and medium countries according to Raal et al (30). Raal et al reported on the multicenter ACE study, an epidemiological study that involved 14 countries from SSA and Middle East recruiting both rural and urban populations. In an age range of participants of 18 – 65 years, prevalence of dyslipidemia was high at 70%, central obesity high at 68%, followed by hypertension 43% and diabetes 25% (30).

Some of the CVD risk factors are a result of aging in the general population whilst on the contrary in the HIV-infected population the aging is accompanied by long periods of exposure to cART and its potential side effects on lipid metabolism (29, 31). However, the exposure to cART contributing to CVD is still debatable from the evidence in literature. The benefits of cART in decreasing deaths clearly seem to outweigh the risks of CVDs as noted in the Strategies for Management of Antiretroviral Therapy (SMART) study group (32). In HIV-positive individuals the management should include reduction of the metabolic abnormalities and identification of subgroups with a high risk of cardiovascular diseases.

Guidelines on CVD management in the general population (33) and for HIV-positive populations (34, 35) suggest recognizing and starting therapy for patients categorized as high CVD risk. That can only be done once the CVD risk is categorized. Therefore the recognition of people with an increased CVD risk is important. The way CVD risk is classified in the

general HIV-positive population follows the same principles in the general population. In the same vein recognizing the CVD risk factors in rural SSA and calculating their CVD risk is important to meet these societal guideline recommendations. In South Africa, the National Department of Health also follows the standard treatment recommendations in the updated 2017 guidelines by Meintjes et al. Meintjes et al suggests that the CVD risk must be assessed using the Framingham heart disease risk score (FRS) and not D:A:D risk equation in the HIV populations who have other CVD risk factors such as hypertension and diabetes (36). The performance of the FRS in the Dutch HIV-positive population different from South African rural population has been investigated by Krikke et al (37).By comparing the predictions of five popular CVD risk scores Krikke et al concluded that the FRS attributed a higher overall CVD risk to the HIV-positive patients than when using the D:A:D, the American Heart association (AHA) atherosclerotic cardiovascular disease risk score (ASCVD) model ,and the Systemic Coronary risk Evaluation for the Netherlands (SCORE-NL) model (37). Even though the South African HIV clinicians' society has recommended the Framingham risk score in the 2017 HIV guidelines, close monitoring of CVD outcomes may need to be followed up using the D:A:D risk score as it has been modeled from HIV populations. For this reason selecting the D:A:D risk score as a CVD risk assessment tool in this research project has logical and better biological plausible explanation to justify its use rather than the FRS as an outcome variable.

Traditional CVD risk factors like ageing, metabolic syndrome, smoking status, gender, race, menopausal status and physical inactivity contribute to cardiovascular events in HIV positive people. Considering smoking for instance, a dose–effect association of tobacco and CVD outcomes is well reported in literature (38). Increased prevalence of smoking has been described in HIV-positive individuals (17, 39, 40). Cardiovascular risk factors that should be addressed in HIV care are hypertension, increased BMI, metabolic syndrome, central obesity (increased waist circumference), high CRP, urine microalbuminuria and ACR. CVD risk prediction scores tend to underestimate the risk because HIV-positive individuals are a specific subgroup of which the likelihood of a coronary outcome is dependent not only on traditional usual recognized risk factors (41, 42). Risk factors such as race/ethnicity may contribute to CVD risk estimation and have to be assessed in the HIV rural people in South Africa. It is important to develop a CVD risk estimation model specific for the HIV African population and this research assists in that direction with reference to addressing that gap whereby other risk factors need to be factored into CVD risk models.

1.4.2 HIV infection: chronic inflammation

Evidence shows that a low level of persistent inflammation continues despite being on cART (9, 41). This is thought to be related to acceleration in the ageing process, deterioration of multiple body functions as well as high incidence of atherosclerotic coronary disease (9, 43). CVD and progression of coronary atherosclerosis is also associated with persistent immune activation. Krikke et al and other authors have shown that using the common carotid intima media thickness (CIMT) atherosclerosis at subclinical levels and some CVD risk factors are prevalent in HIV positive individuals at a younger age and CVD accelerates faster as well (44-46) in comparison to the general population. Several factors have been related with higher CIMT in HIV positive individuals and these factors include the HIV viraemia, dyslipidemia, hypertension and smoking (44). There are other CVD factors that also need to be assessed especially in rural settings where demographic changes are taking place and socioeconomic factors interplay to determine the CVD risk.

Ethnic background appears to have some influence on baseline CRP levels. A systematic review and meta-analysis also showed CRP levels varied significantly depending on ethnic background (47). The authors concluded that the interpretation of CRP concentration should be in the context of the ethnicity. Given the finding of Vos et al (35) that increased levels of CRP have been reported to contribute to an increased CVD risk it is vital to also assess the role of CRP to predict CVD risk in HIV-positive people in rural South Africa.

Concomitant opportunistic infections like tuberculosis and chronic Hepatitis-C, which are indirectly associated with HIV, enhance persistent immune activation in the HIV positive population (48). Tuberculosis could be prevalent in the HIV-positive rural people of South Africa. There are suggestions that chronic persistent infections, especially, dysregulated immunological and metabolic processes observed in HIV infected individuals, could have a relationship to an acceleration in the aging process of these populations (49). Certainly, risk assessment in HIV infected population is to be done bearing these facts in mind.

To override the challenge of integrating the assessment of cardiovascular risk on a background of persistent immune activation, CRP will need to be factored in to determine whether it predicts an increased D:A:D CVD risk equation score in HIV positive populations.

HIV per se is considered to be a risk factor for CVD (50), but it is also relevant to recognize that differences in virus type (HIV-1 type C versus HIV-1 type B in developed countries),

with variations in genetic background exist. HIV subtype C is the predominant circulating subtype in African countries whilst in the Western countries it is subtype B (51). Between these strains there is a difference of 30% in their genomes. This means that there may be variability in the cardiovascular clinical consequences depending on geographical location.

The contribution and role of different HIV subtypes on the cardiovascular system is still uncertain. Therefore the contributions of HIV subtypes and that of cART on cardiovascular mortality and morbidity in LMIC may be different from the HIC HIV populations. Extensive studies have been carried out in Western HIV populations and there are significant differences in the traditional CVD risk factors as well as the profiles of their socio-demographics. In fact, the average age of HIV-positive individuals in SSA is less than the age distribution in the environments from where the D:A:D risk equation score has been modeled. There is evidence indicating that there are differences in CVD risk profiles in individuals from Europe compared to SSA (10, 52-54). Other differences to consider are lifestyles, alcohol consumption patterns, environmental and genetic differences. Given these differences in populations as the literature suggests, the researcher has sought to investigate the gap and determine whether other risk factors such as educational level status should be considered as CVD risk factors.

1.4.3 HIV infection: Microalbuminuria as a cardiovascular disease risk factor

The HIV population in SSA has been reported to have co-morbid conditions affecting renal function and CVD (55). There are several factors that contribute to the renal disease in these populations. These can be the use of tenofovir-based regimen, predisposition to recurrent urinary tract infections, direct kidney injury as a result of HIV associated nephropathy (HIVAN) and the deposition of immune complexes from the HIV chronic infection and susceptibility to opportunistic infections prevalent in these individuals (56). Therefore checking CVD risk considering the contribution of CRP and urine micro-albuminuria or ACR in the HIV population to CVD risk is important.

Renal diseases in the HIV population are further aggravated by the presence of traditional cardiovascular risk factors. (17). A more hostile course of renal disease is associated with the African ethnicity (57-59). Studies have shown that HIVAN, and hypertensive renal disease contribute to the renal disease in these populations.

From a pathophysiological basis a vicious cycle occurs whereby CVD results in renal damage, which then contributes to worsening of the further cardiovascular risk. A higher all cause mortality in HIV populations and an increased risk of CVD is associated with albuminuria (60). The higher risk for a cardiovascular outcome and cardiac failure have been also reported by Choi et al in HIV populations with albuminuria in contrast to those without albuminuria (61).

Wensink et al reported that in rural HIV positive patients, there is an association between albuminuria (ACR>30mg/g) and traditional CVD risk factors and HIV viraemia (62). Therefore because micro albuminuria characterizes both renal disease and CVD in the HIV population it is important to determine its contribution in CVD risk in SSA HIV rural populations. Wensink et al concluded that in order to improve the long-term prognosis of HIV-positive populations CVD risk prevention is vital. The monitoring for HIV virological suppression with a goal to reach undetectable levels is also very important in the ongoing management of HIV patients. This would be appropriate to avoid the vicious circle of CVD and chronic kidney disease in SSA (62). On the basis of this evidence and the fact that urine microalbuminuria is used interchangeably with ACR from different studies the researcher opted to check the effect of ACR on the D:A:D risk score.

1.4.4 HIV infection: Combination antiretroviral therapy as additional cardiovascular disease risk factor

Combination antiretroviral therapy is considered to contribute to the increase in CVD risk in HIV–positive patients (50). Previous exposure to medications such as stavudine plays a role in pathogenesis of cardiovascular complications in HIV infection (20). Despite d4T being removed from HIV management guidelines, some subgroups of HIV infected patients in SSA still have manifestations like lipodystrophy as a result of previous exposure to d4T. Lipodystrophy in that subgroup of HIV people may result in metabolic syndrome, which is a CVD risk factor and will need to be explored further. Dave et al reported in a mainly urban HIV positive population from Cape Town an increase in dyslipidemia prevalence as a result of cART which consisted of d4T (63).

Currently, there is literature to indicate that HIV medications like protease inhibitors (PIs), particularly indinavir and lopinavir/r as well as other medications like abacavir, have independent adverse effects related to coronary heart disease besides their potential metabolic effects (64-67). It is important to point out that indinavir was barely used in Africa, it has not

been used in a decade and may not be of much relevance in the SSA HIV-positive populations. With respect to PIs, the relationship of these drugs to CHD is to a certain degree explained via the effect on blood cholesterol levels. Consequently, the total effect of PIs on risk of CHD encompasses the changes on blood cholesterol levels plus the drugs independent adverse effects. For this reason in estimating the CVD risk score in HIV infected populations, previous or current exposure to these drugs and d4T has to be factored in. This cross sectional study has considered the previous use of d4T and other HIV medications. This consideration or gap was not accounted for in SSA studies assessing CVD risk (18, 68).

HIV-infected individuals on cART experience cardio-metabolic complications, and potentially have an increased risk of coronary events. Conclusions from previous studies indicate that medication-induced lipid alterations and other traditional CVD risk factors accelerate the risk of myocardial infarction (MI) (69). According to the D:A:D prospective study, cART is associated with a 26% increase in MI rate per year of cART use in the first 4-6 years of therapy (70). The risk may be higher immediately after commencement of cART, and there are differences to be considered such as the population from where the patient is from. Aboud M et al in a cross sectional study called the CREATE 1 study using the Framingham risk score with an adjustment on family history found that HIV patients on first cART had higher CVD risk compared to non-users of cART which was strongly associated with duration of cART (71). The CREATE 1 study aimed to establish the CVD risk profile of an HIV cohort and how use of cART affected it. From this United Kingdom HIV cohort, the CREATE 1 authors concluded that cART duration is key and has valuable implications for the management and CVD screening of HIV infected patients (71).

The various potential interactions and pathophysiological mechanisms hypothesized to bring about acceleration in the progression of CVD in HIV infected populations are summarized in Figure 1 (9, 72).

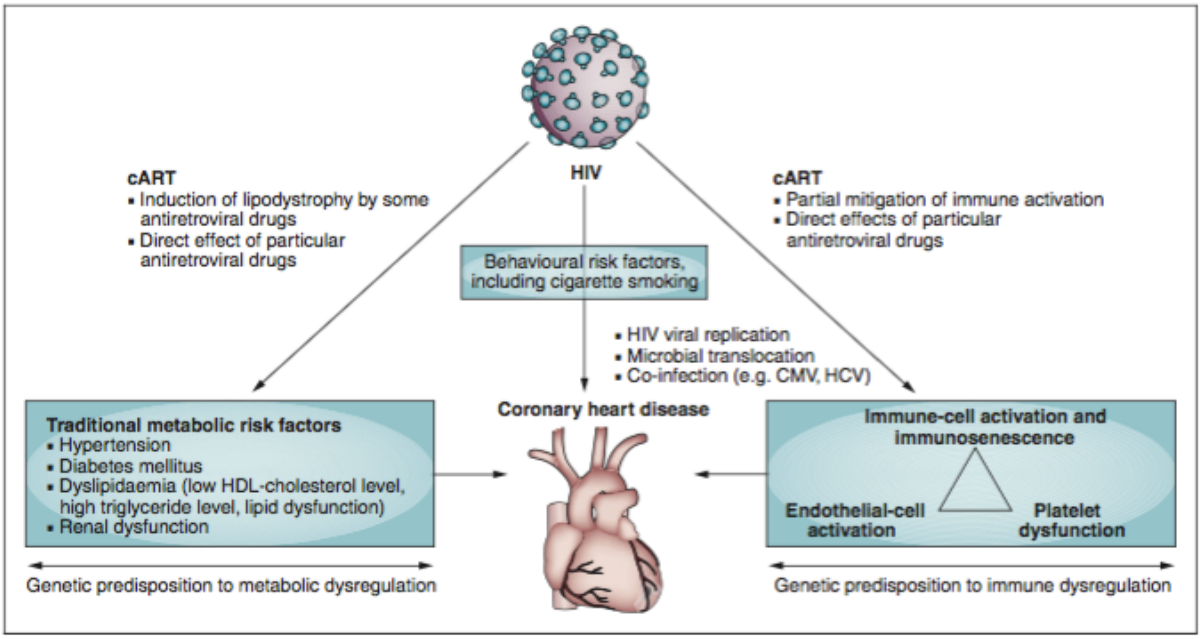

Figure 1: Mechanisms and risk factors postulated to be involved with an increased risk of coronary heart disease risk in patients with HIV. cART: combination antiretroviral therapy; CMV: cytomegalovirus; HCV: hepatitis C virus; HDL: high-density lipoprotein. (Reproduced with permission from Zanni MV, Schouten J, Grinspoon SK, et al. Risk of coronary heart disease in patients with HIV infection. Nat Rev Cardiol 2014; 11(12): 728–741. doi:10.1038/nrcardio.2014.167.

1.5 D:A:D and cardiovascular disease risk score

1.5.1 HIV infection: cardiovascular disease risk scores

Several CVD risk score equations have been studied in HIV-positive persons (19, 73-75). Most of the studies evaluated cardiovascular risk scores modeled for an HIV negative population, such as the Framingham risk scores (FRS) and the Prospective Cardiovascular Munster Study (PROCAM). However, the precision of these risk estimation tools for predicting cardiovascular outcomes in HIV-positive persons is not well established in low resource settings. The Framingham risk score has been used more frequently in studies estimating CVD risk in HIV positive patients unlike other cardiovascular risk equations, like PROCAM and Reynolds risk scores (73, 74, 76, 77). The predicted high-risk category for coronary outcomes in the next 10 years from most of the studies varies widely ranging from less than 1 percent up to 21 percent (74, 76).To date CVD risk scores have been modeled and cross-validated in developed and high resource nations. The D:A:D risk score equation is the one that predicts the 5-year CVD risk in HIV-positive populations.

1.5.2 D:A:D Risk score

The D:A:D risk score, gives an explanation of pathobiology of cardiac disease and mortality due to cART and the HIV infection (75). We introduce the D:A:D risk score as the choice for the current study stating its limitations and advantages.

Presently, HIV management guidelines reflect the difficulty of solutions to predict the accurate 5 or 10 year CHD risk. For instance, the European AIDS Clinical Society (EACS) Guidelines recommend that risk assessment be performed using FRS. In addition, they highlight the estimation must be done using the D:A:D equation (35). The D:A:D risk score takes into account collaboration of HIV infected patients in 11 cohorts from 212 centers in Europe, USA, Australia and Argentina to establish whether the use of cART is related to a higher risk of CVD (78). The authors acknowledge the controversy surrounding cART as a predictor of CVD and also the fact that overtime the HIV-patient's cART regimen may possibly be changed in follow up visits. For this reason the D:A:D score incorporated cART use and duration of cART use. However, as this information might not be generally available a reduced score has been developed as well. This reduced D:A:D model, excludes the cART covariates, was developed to circumvent the complexities that accompany switch of cART on subsequent review of HIV patients in clinics.

In the D:A:D study Cox regression was used to identify specific covariates that are associated with CVD (78). The main advantage of using Cox regression models in a study with the primary objective of determining whether cART increases CVD is that Cox model results in a risk equation that can simply be recalibrated to different cohorts. Friis-Moller et al (78) highlights that other HIV cohorts can recalibrate the full or reduced D:A:D risk score by calculating the mean values of predictors in the equation of their cohorts then replacing those in the D:A:D risk score equation with the ones from the specific HIV-cohorts (78).

The Cox regression model is a semi-parametric and from a statistical perspective this was ideal and logical for Friis-Moller et al to select Cox regression in their statistical methods (78). This explains methodological considerations that other cohorts outside the HIC will have to consider in their subsequent use of the D:A:D model. One South African study by Mashinya et al looked at CVD risk in a rural SSA population in the Limpopo using the full D:A:D score. Mashinya et al study does not categorically state the use or effect of stavudine as this drug was out phased. They found a 5-year CVD risk of <1%. From Mashinya et al study it would have been important to know the exact contribution of PI's to CVD risk. This

could have been done by comparing the reduced D:A:D model to the full D:A:D model if Mashinya et al had considered that analysis. It is important to take into account the contribution of stavudine on CVD risk as a major part of the population used this drug in the past.

The reduced D:A:D risk score can be used when cART history is not easily available. In our cohort cART history relied on participants self-reported data, which might have been inaccurate. In view of the possibility of cART history being inaccurate we decided to calculate the reduced D:A:D score, and subsequently analyze whether the use of stavudine or PI showed an association with CVD risk.

The use of cART and association with CVD is debatable in literature with some authors suggesting that PIs cause lipohypertrophy. On the contrary some studies have demonstrated that all cART classes are associated with lipohypertrophy to the same extent (36). Mashinya et al in their analysis could not check for the effect of cART on the CVD risk thus creating a gap in CVD risk factors that needs to be interrogated in the rural SSA HIV-positive population. Mashinya et al does not report the percentage of HIV-positive patients exposed to stavudine, abacavir or indinavir (18). The collection of cART data in rural LMIC may be inaccurate in most distant settings and for generalizability we need to look into a different rural SSA population.

The controversy that surrounds the effect of cART as a CVD risk factor needs to be reconsidered because there are multiple factors that are in the pathway for CVD endpoints, which may not have been considered by the authors of the D:A:D risk model. Confounders and intermediates need to be considered in causal relationships in studies such as D:A:D risk score modeling. Some of the confounders such as educational level and micro-albuminuria or ACR in a rural setting have to be considered as well.

Opponents in the controversy of cART causing CVD could be correct if the effect of other confounders in the causal pathway of CVD is not interrogated. Therefore the gap in literature around that controversy can be addressed by using the reduced D:A:D risk score and selecting models with biologically plausible predictor variables that can predict the CVD risk in rural HIV-positive populations. The D:A:D study by Friis Moller et al did not consider urine micro-albuminuria or ACR and the effect of educational levels in that population from HIC giving a gap that might need to be evaluated with respect to CVD risk estimation in LMIC.

To handle more distal CVD risk factors such as educational level as a proxy to socioeconomic status which can also be extrapolated to indicate social deprivation we propose to adjust for educational level into models of CVD risk as this researcher has taken into consideration.

The D:A:D risk score equation has been compared with FRS by d'Agostino et al (79) and its performance assessed by internal and external cross validation (80). In the D:A:D study triglycerides, lipodystrophy and BMI were excluded from the equation as a result of them showing non-significance. Clark et al has shown the burden of cardio metabolic risk factors such as BMI in HIV populations in SSA (14). In a cross-sectional survey they showed that BMI and waist circumference were significantly higher in women in the Agincourt district. There is need to assess whether BMI is related to a higher CVD risk in the SSA rural population though in the European and other countries the BMI was non-significant from the D:A:D study (78).

On the contrary, the FRS has been shown to over predict the CVD 10 year risk in other populations. For example the study in HIV populations in the Thais (19) and the Brazilians the FRS overestimated the 10 year CVD risk (81). The Brazilian study had 294 patients whilst the study from Thailand had 785 participants and the mean cART duration was 7.7 years. These studies from other regions do not assess the other risk factors such as level of education and urine micro-albuminuria or ACR. The study from Brazil has a smaller sample size whilst the study from Thailand, the cART regimens histories was complex with interruptions and changes in the treatment schedules thus introducing bias. Edward-Jackson N et al suggest that the results of their study is mainly applicable to HIV populations with similar background of cardiovascular risks and propose that the D:A:D risk score is a more suitable CVD risk equation to use in HIV populations (19).

Therefore on the basis of data from literature there are advantages to calculate the CVD risk score in HIV-positive individuals using reduced D:A:D risk score equation. From the conclusion of Edward-Jackson et al it is scientifically correct to choose the D:A:D risk score equation for a different environment such as the Limpopo province of South Africa, a rural low resource setting.

Reports have shown that there is an increase in CVD risk related to a significant increase in the occurrence of CHD, fatal/nonfatal MI, and vasculopathy in HIV-positive patients (8, 10, 26, 27, 82-86). For this reason, the reported increase in incidence of cardiovascular complications plus their untimely manifestation, make prevention a key issue in HIV-positive

populations. In addition an assessment of CVD risk in HIV-positive rural people has to be done to achieve the various preventative measures to be undertaken especially in poor resource environments.

To sum up, based on these findings, multiple factors contribute to the pathogenesis of coronary complications in HIV infection. These factors could be socio-economic, lifestyle, lipid parameters, gender, genetics, immune activation, viral levels, inflammation, and adverse effects of antiretroviral medications. There is a need to assess the CVD risk in HIV positive populations using the D:A:D risk score equation. It is also vital to determine the effect of HIV treatment and other risk factors on CVD risk in HIV-positive population in resource limited settings based on the gaps and paucity of data highlighted in the literature review.

1.6 Research Question

What is the D:A:D CVD risk score of HIV patients in a resource limited setting and what factors besides the effect of current and previous cART are associated with the D:A:D risk score?

1.7 Aim and objectives

1.7.1 Aim

To assess the CVD risk using the validated D:A:D CVD risk score and determine the effect of cART and other risk factors in HIV-positive participants getting cART on CVD risk in the Ndlovu Cohort Study, Moutse area, Limpopo province, rural South Africa

1.7.2 Study Objectives

1) To calculate and describe the D:A:D CVD risk score in HIV positive participants in the NCS in Limpopo province of South Africa.
2) To compare the socio-demographic, clinical characteristics and the D:A:D risk score stratification by sex of HIV-positive participants in the NCS in Limpopo province of South Africa.
3) To determine the effect of cART duration on the CVD risk score among HIV-positive NCS participants considering current, previous cART and potential confounding factors like educational level, BMI, ACR and other CVD risk factors.

2 CHAPTER 2 - METHODS

This chapter describes the study design, setting, population, sampling, and data collection of the primary study. The methods used to collect and manage data in the primary study are fully explained.

Baseline data used was from a primary study, which was a prospective cohort study that is on-going. Participant's demographics, social and medical history from study questionnaires captured in the primary study are described (72).

The data management and the secondary data statistical analysis performed in the study for each of the study objectives are also described including the details of final model selection. It concludes with the ethics of the primary study and the ethics approval for this research.

2.1 Study design

This was a cross-sectional study design. It was a data analysis using baseline data from HIV-positive study participants of the Ndlovu Cohort study (NCS).

2.2 Study setting

The NCS is conducted at the Ndlovu Medical Center (NMC, www.ndlovucaregroup.co.za) in Elandsdoorn, a rural township in the Moutse area, about 200 km from Johannesburg, Limpopo Province, South Africa.

2.3 Study population

The NCS consist of HIV-positive participants and HIV negative participants aged 18 and older who were consecutively enrolled between November 2014 and August 2017 with an intended follow up of 10 years. At completion of recruitment approximately 1000 HIV-negative and 885 HIV-positive men and women from the Moutse area, Limpopo Province, South Africa was in the NCS database. Table 1 outlines the NCS inclusion criteria.

Table 1: Inclusion criteria into the Ndlovu Cohort Study

HIV- positive group	HIV- negative group
Age ≥18 years	Age ≥18 years
HIV-infected patients from the NMC, Elandsdoorn, South Africa, Approx. 50% men and 50% women	Negative HIV serology test result at baseline Approx. 50% men and 50% women
Provided written Informed Consent ,	Provided Informed Consent
Long-term follow up commitment	Long-term follow-up commitment
NMC- Ndlovu Medical Center, HIV-Human immunodeficiency virus	

2.4 Sampling of primary study

All HIV-positive participants of the NCS were included in the cross-sectional study for secondary data analysis. Sample size calculation for the primary study was based on carotid intima-media thickness (CIMT) using a mixed model approach (72). A simplified approach was used in order to evaluate the power to detect very small differences between groups, as there were no reasonable estimations for CIMT progression over time. Power was evaluated for a given sample size of 1000 HIV-positive and 1000 HIV-negative patients for a significance level of 0.05 and a constant difference in mean CIMT over time of 0.006, 0.012, 0.018 and 0.024mm for an increasing correlation between measurements with time from 0.00 to 0.75 and a standard deviation of 0.09 mm. A minor difference of 0.012 mm will still be detectable with 95% power and a correlation of 0.60. For larger differences, the power exceeds 0.95, even with a correlation of 0.75. This sample size can also detect meaningful differences in other outcomes, such as prevalence of CV risk factors and pulse wave velocity (PWV).

2.5 Data collection of primary study

2.5.1 Socio-demographic and clinical characteristics

Baseline data collection in the NCS was undertaken from November 2014 to August 2017. At enrolment, every participant was interviewed. An Inclusion/exclusion criterion was checked and signed informed consent obtained. The following information was collected at baseline in the NCS: age, gender, demographics, general health and HIV status. A full medical history,

current medical condition(s) and chronic medication use was obtained. Detailed information on HIV treatment (time between diagnosis and treatment initiation and specific medication prescribed) and responses to treatment (latest plasma HIV-1 viremia and latest CD4 T-cell count) were recorded. Information on CVD risk factors, family history of cardiovascular disease in first-degree relatives, smoking and alcohol use was obtained with a modified version of the World Health Organization (WHO) STEPS instrument. The International Physical Activity Questionnaire (IPAQ) was used to assess physical activity (72). Validated questionnaires were used for employment, income position and household support (National Income Dynamics Study [NIDS] Wave 3 2012 Adults Questionnaire), food security and diet was from South African National Health and Nutrition Examination Survey (SANHANES). In the case of HIV infection data on adherence was collected with a structured questionnaire (72).

2.5.2 Physical measurements

Measurements were undertaken according to standardized procedures (72). These were height, weight, and hip and waist circumference. Hip circumference was measured in centimeters at maximum posterior extension of the buttocks and waist circumference was measured in the standard procedure. Blood pressure was measured in the sitting position after 5 minutes of rest with a sphygmomanometric device on both arms, and repeated on the side with the highest values. Blood pressure was measured at every participant's scheduled clinic visit and the average of all measures was used for further analysis. The definition of operational terms that were then used in the secondary data analysis is outlined in Table 2 below. In order to assess and describe CVD specific variables and to determine cut off values for categorization and analysis standard definitions from literature and World Health Organization definitions were used. To describe central obesity (87), hypertension (88), cholesterol levels (89), CIMT , cART and HIV (72) proper specific scientific definitions were applied. As an example for central obesity the cut of values at any given level of BMI the waist-hip ratio cut-off values where defined as 0.9 and 0.85 for males and females respectively.

Table 2: Definition of operational terms

Overweight/Obesity	Overweight (BMI 25-30) /Obesity (BMI=≥30) kg/m^2
Central obesity	At any given level of BMI,increased waist to hip ratio, (WHR)
Metabolic syndrome	WHO definition glucose ≥7.0mmol/L (110 mg/dL) and ≥2 of the following HDL-C <0.9 mmol/L (men); <1.0 mmol/L (women), or TG ≥1.7 mmol/L, or Obesity Waist/hip ratio >0.9 (men) or >0.85 (women) or BMI ≥30 kg/m^2, hypertension ≥140/90 mmHg
Hypertension	Standard definition ≥140/90 mmHg, on antihypertensive medication
Diabetes	Fasting plasma glucose ≥7.0 mmol/L or HbA1c ≥6.5, nonfasting serum glucose ≥11.1 mmol/L, or use of oral blood glucose – lowering drugs or use of insulin as per standard definition.
Low HDL-Cholesterol, High Total Cholesterol (TC), High triglycerides (TG), High TC/HDL-C, High TG/HDL-C, CRP, Urine micro-albumin,ACR	Standard definitions as per guidelines low HDL-C <0.9 mmol/L (men); <1.0 mmol/L (women), or TG ≥1.7 mmol/L and lipid lowering medications
Age	16-85 years for D:A:D 30-74 years for FRS 45 years and older for Reynolds
Gender	The same as the definition used in the NCS
Smoking	Tobacco use either through smoking, sniffing or chewing. Same as the definition in the primary study.
HIV	Human immunodeficiency virus infection as per the antibody-based point-of-care test (ADVANCED QUALITYTM Rapid HIV Test [InTec Products, China]), which has a sensitivity of 98.8% and a specificity of 100%.
cART	Combination anti-retroviral therapy as per standard definition and as used in the D:A:D study
CIMT	Carotid intima-media thickness-the thickness of the carotid intima-media measured using a standardized ultrasonographic protocol .
ACR-Albumin creatinine ratio, BMI-Body Mass Index, CIMT-Carotid Intima-media thickness, HbA1c-glycated hemoglobin, HDL-C-High density lipoprotein cholesterol, TG-Triglycerides, WHO-World Health Organization, WHR-Waist Hip Ratio, cART- combination antiretroviral therapy.	

2.5.3 Blood and urine samples

At baseline the following markers were measured in all study participants: Full blood count, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, random glucose, HbA1c and C-reactive protein, viral load and CD4 cell count. Approximately 50 mL of blood was collected during the baseline visit. This comprised two ethylenediaminetetraacetic acid (EDTA) tubes, one serum separator tube (SST), one fluoride oxalate tube and one heparin tube. A urine sample was collected for measurement of urine creatinine, micro albumin and the calculation of the albumin/creatinine ratio.

2.5.4 Data management

STATA version 14 was used to check for the integrity of the NCS dataset by performing data cleaning, checking for the outliers, duplicates. Necessary steps in STATA such as recoding, generating new categorical variables to suit the analysis were done. See table 2 for operational terms and the references within the table for the purposes of how recoding was then done in STATA.

2.6 Statistical analysis plan

2.6.1 Objective 1

To calculate and describe the D:A:D CVD risk score in HIV positive participants in the NCS in Limpopo province of South Africa.

The D:A:D risk score was calculated for each participant. The reduced D:A:D specified risk score equation modeled for HIV populations, which are shown below, was used (78).

Reduced D:A:D model (78)

Xb = (3.1777*ln(age) + 0.343856*male + 0.7311945*diabetes + 0.329772*famgp + 0.8157995*currsmk + 0.2394822*exsmk + 1.0925460*ln(chol) - 0.5194359*ln(hdl) + 1.517874*ln(syst) - 0.1137227*ln2(cd4)) - (3.1777*ln(40.412) + 0.343856*0.7442 + 0.7311945*0.0308 + 0.3297720*0.0840 + 0.8157995*0.5217+0.2394822*0.1670 + 1.0925460*ln(5.001) - 0.519436*ln(1.214) + 1.517874*ln(123.651) - 0.1137227*ln2(482.099))

Predicted risk score = $1 - 0.9853^{\exp(Xb)}$

Where:

famgp = family history of CVD

currsmk = currently smoking

exsmk = ex-smoker

chol = total cholesterol level

syst = systolic blood pressure

The CVD risk was estimated using the risk score build in STATA. As suggested by Friis-Moller et al replacement of the D:A:D mean values of predictors in the risk equation with the mean values from the NCS HIV-participants was done. The B-coefficients as published in the paper by Friis-Moller et al were used. The study is cross-sectional and therefore it is not possible to recalibrate and change the coefficients in the model. The natural logarithm for all continuous covariates as recommended in the paper was used (78).

The calculation of the mean in STATA for each prognostic factor in the reduced D:A:D risk model was the first step in order to compute the D:A:D risk score. This is based on the recommendation by the original authors of the D:A:D equation, which actually states that a second cohort could replace the D:A:D mean values of CVD predictors in the risk equation (78). Therefore the mean of all prognostic factors from the NCS was calculated in STATA and used to get the Xb in the reduced D:A:D formula. As for the binary prognostic factors for example family history of CVD the proportion was considered similar to Friis Moller et al D:A:D risk equation model to come up with the Xb (78).

The D:A:D model was computed in STATA in stages by first generating Xb to get to calculate the predicted risk as $1\text{-}0.985^{\exp(Xb)}$ according to the reduced D:A:D risk equation. Firstly, combination of all continuous prognostic factors from the formula to generate Xb using the coefficients with the corresponding logarithms for biological plausibity was done. For example 3.1777*ln(age)…+1.0925460*ln(chol)-0.1137227*ln2(cd4) was computed as 3.1777*logage+…1.092546*logchol -0.1137227(log2cd). The logage, logchol and log2cd were generated in STATA following the laws of logarithms. To consider ln2(cd4) as an example, to generate log2cd the log CD4 NCS result was multiplied by log(2) to fulfill the natural logarithms concept that Friis-Moller et al postulated to be consistent with previous analysis (78). To convert from natural logarithms to logarithm to base 2 the concept is to divide by ln2.

After using the means for all continuous prognostic factors to get Xb replacement of the generated Xb in STATA step by step for each binary variable was done. As an example we

added 0.7311945 to Xb if diabetes was present and subsequently for all binary prognostic variables such as current smoker, ex-smoker and CVD history in the family (78). Finally after getting Xb the last step was to generate the Predicted risk as given by $1\text{-}0.985^{\exp(Xb)}$ whereby STATA was used to get the reduced D:A:D risk score per participant using what Friis Moller et al state as D:A:D risk score=$1\text{-}0.985^{\exp(Xb)}$.

The assumptions for multiple linear regression model were checked to see if they hold for the calculated D:A:D risk score. The distribution of the logarithmic transformation showed a normal distribution pattern in STATA. Since the logarithmic transformation of the calculated reduced D:A:D risk score fulfilled the assumptions of multiple linear regression the same regression was used to then check for the predictors of CVD.

2.6.2 Objective 2

To compare the socio-demographic, clinical characteristics and the D:A:D risk score stratification by sex of HIV positive participants in the NCS in Limpopo province of South Africa.

Descriptive statistics - frequency and summary statistics by gender are presented as baseline characteristics in this cross-sectional study. Counts and proportions are reported for all categorical variables. The calculated D:A:D risk score was summarized for the 5-year risk (90, 91) according to the reduced D:A:D risk equation score (16) and described by gender. The mean and the standard deviation were reported for normally distributed data whilst median with interquartile range (IQR) were reported for skewed data. The Student T- test was used for continuous variables with a normal distribution, the Wilcoxon Ranksum test was used for continuous variables with non-normality and the Chi-square was used for categorical variables.

2.6.3 Objective 3

To determine the effect of cART duration on the CVD risk score among HIV-positive NCS participants considering current, previous cART and potential confounding factors like educational level, BMI, ACR and other CVD risk factors.

The main determinant was duration of ART use especially use of stavudine, current use of abacavir and protease inhibitors. Almost all participants were on first line cART and used the regimen as recommended by the South African Department of Health (DOH) guidelines.

Potential confounders were adjusted for with the main aim of investigating whether CVD risk differs with cART treatment duration that was tested by use of regression analysis. Predictor variables for objective 3 such as socioeconomic status or educational level, CRP, urine micro albuminuria, ACR, BMI were considered. These predictor variables were selected on the basis of evidence from literature to likely predict the D:A:D cardiovascular risk score and were available in the NCS dataset. The outcome (dependent) variable for the multiple linear regression was D:A:D risk score.

2.6.4 Model Selection

Multiple linear regression for log D:A:D risk score was used to determine the significant biologically plausible predictors of the D:A:D CVD risk score. The logarithmic transformation (logdad) was chosen as that fulfilled the criteria for the outcome variable assumptions of normality as per linear multiple regression. Multiple linear regression assumptions were checked with the residual analysis. As recommended by Vittinghof et al Chapter 10 (92), in order to fit a parsimonious model that adequately predicted the outcome variable, the backward elimination method was employed to select variables that were significant at a liberal p value of p=0.2 for the selection of the appropriate variables for the final model. The candidate variables considered were the following CRP, BMI, educational level, sex, stavudine, abacavir, PI, ACR, (see Table 4 on uni-variable analysis). The selection of these candidate variables was addressed in the research objectives considering the literature evidence around the CVD and biological plausibility. Sex was adjusted for in the model despite it being in the original D:A:D model which was developed from HIC. In order to deal with confounding the biological plausibility that logically the BMI consist of height squared was considered in the regression model and the fact that the cross sectional study is from a different LMIC HIV population. Generally height is affected by the sex category. For instance males generally being taller than females gender was adjusted for in the determination of factors that predict a higher D:A:D score from that biologically plausible perspective. The other reason gender was adjusted for was that the D:A:D score was modeled from HIC and in the original study design as stated in the literature review only 26% were women which is approximately one quarter. In SSA HIV-positive population, 58% are women. In our study population of 885 participants, 59.6% were women. It was logical to then adjust for gender given the discrepancy in comparison of 26% from HIC and 59.6% or 58% from LMIC. In addition adjusting is important for scientific clarity and reasoning since the D:A:D model we are using in a setting with 58% women and yet is designed from HIC cohort which does not

fully represent women with only a quarter considered in the model build up. The level of significance for statistical analysis was set at less than 0.05. Multi-collinearity was checked as well as interaction terms between sex and BMI and education level. The comparison of the standard errors using robust standard estimation for the final model was checked to authenticate the final model selected (see Appendix 3). The statistical software package used for the analysis was STATA version 14.2.

2.7 Ethics

The NCS received ethics approval from the Human Research Ethics Committee at the University of Pretoria (227/2014) and all participants gave written informed consent before enrollment into NCS. Ethics approval for secondary data analysis for this study was sought from the University of the Witwatersrand Research Ethics Committee (Medical) and approved (Clearance certificate No. M171194).

3 CHAPTER 3 - RESULTS

The chapter describes the socio-demographic characteristics of the NCS HIV positive patients by gender. The D:A:D risk score between females and males is also described. Finally the uni-variable and multivariable linear regression statistical analysis is described.

3.1 Baseline characteristics

885 participants (59.6% females) were included for data analysis. The mean age was 42 years (standard deviation (SD) 10.3). 186(21%) were cART naïve and 699(79%) were already on cART. Median duration of cART use was 2.4 years (interquartile range (IQR) 0.0-6.8). There was a statistically significant difference between females and males with respect to the following baseline characteristics: current smokers (n,%) females 44(8.4) males 159 (44.5) $p<0.001$; BMI (kg/m^2) (mean, SD) females 25.3 (6.3) males 21.7 (9.1) $p<0.001$; total cholesterol females 4.4(1.0) males 4.0(1.0) $p<0.001$; 39 (4.4%) were diabetic whilst slightly less than a quarter 203 (23%) were current smokers.

3.2 Objective 1

To calculate and describe the D:A:D CVD risk score in HIV positive participants in the NCS in Limpopo province of South Africa.

The median D:A:D score was 2.2 (1.1-5.0) whilst by categorization of being on HAART was 2.4% (IQR 1.2-5.2) for participants on cART and 1.4% (0.7-3.5) for those not on cART ($p<0.001$). A statistically significant difference between females and males with respect to the D:A:D score (%)Median (IQR) females 1.4 (0.8-2.9), males 4.5 (2.2-8.5) $p<0.001$ was noted.

3.3 Objective 2

To compare the socio-demographic, clinical characteristics and the D:A:D risk score stratification by sex of HIV positive participants in the NCS in Limpopo province of South Africa.

The level of education and smoking differed significantly between females and males. More females 43(8.2%) had attained college or university education levels compared to males 16(4.5%) $p<0.001$) and more males 108(30.3%) only had primary education compared to females 90(17.1%). There were no statistically significant differences between females and

males with respect to hypertension, diabetes mellitus, metabolic syndrome, and central obesity (Table 3).

3.4 Objective 3

To determine the effect of cART duration on the CVD risk score among HIV infected NCS participants considering current, previous combination antiretroviral therapy (cART) and potential confounding factors like educational level, BMI, ACR and other CVD risk factors.

From Table 4 the uni-variable and multivariable analysis is illustrated. Of note CRP was marginally significant (p=0.06) as a predictor of CVD.

Table 3: Baseline demographics, CVD risk factors of HIV participants by gender

Factor	Female (n=527)	Male (n=358)	All (n=885)	P value
Overall (n, %)	527 (59.6)	358 (40.4)	885	
Age (years) Mean (SD)	40.0 (9.9)	40.9 (10.4)	42.0(10.3)	<0.001
Education (n, %)				
None	24 (4.6)	15 (4.2)	39 (4.4)	
Primary	90 (17.1)	108 (30.3)	198 (22.4)	<0.001
Secondary	370 (70.2)	218 (61.1)	588 (66.5)	
College/University	43 (8.2)	16 (4.5)	59 (6.7)	
Diabetes Mellitus (n,%)				0.273
No	506 (96.2)	336 (94.6)	842 (95.6)	
Yes	20 (3.8)	19 (5.4)	39 (4.4)	
Current Smokers (n, %)				
No	482 (91.6)	198(55.5)	680 (77.0)	<0.001
Yes	44 (8.4)	159 (44.5)	203 (23.0)	
Ex-smoker (n,%)				
No	472 (89.7)	288 (80.7)	760 (86.1)	<0.001
Yes	54 (10.3)	69 (19.3)	123 (13.9)	
Family history of CVD (n,%)				0.008
No	469 (89.0)	337 (94.1)	806 (91.1)	
Yes	58 (11.0)	21 (5.9)	79 (8.9)	
Hypertension (n, %)				
No	461 (87.5)	303 (84.6)	764 (86.3)	0.228
Yes	66 (12.5)	55 (15.4)	121 (13.7)	
BMI (n,%)				
Underweight	51 (9.8%)	89 (25.4%)	140 (16.0)	<0.001
Normal weight	244 (46.7%)	207 (59.0%)	451 (51.7)	
Overweight/Obese	227 (43.5%)	55 (15.6%)	282 (32.3)	
Metabolic syndrome (n,%)				
No	521 (98.9)	352 (98.3)	873 (98.6)	0.497
Yes	6 (1.1)	6 (1.7)	12 (1.36)	
Central obesity (n,%)				
No	315 (99.7)	233 (98.7)	548 (99.3)	0.191
Yes	1 (0.3)	3 (1.3)	4 (0.7)	
Total cholesterol (mmol/L) Mean (SD)	4.4 (1.0)	4.0 (1.0)	4.3 (1.0)	<0.001
HDL cholesterol (mmol/L) Mean (SD)	1.5 (0.4)	1.4 (0.5)	1.4 (0.4)	0.084
LDL cholesterol (mmol/L) Mean (SD)	2.5 (0.9)	2.1 (0.8)	2.4 (0.9)	<0.001
TG (mmol/L) Mean (SD)	1.1 (0.6)	1.3 (1.0)	1.2 (0.8)	<0.001
HyperChol (n ,%)				
No	390 (74.3)	300 (84.3)	690 (78.3)	<0.001
Yes	135 (25.7)	56 (15.7)	191 (21.7)	
HyperTG(n,%)				
No	455 (86.7)	279 (78.4)	734 (83.3)	0.001
Yes	70 (13.3)	77 (21.6)	147 (16.7)	

Table 3: Baseline demographics, CVD risk factors of HIV participants by gender (cont)

Factor	Female (n=527)	Male (n=358)	All (n=885)	P value
CRP Median (IQR)	5 (2-10)	5 (2-14)	5 (2-11)	0.197
Albumin/creatinine ratio	1.03(0.62-2)	0.83(0.52-1.97)	0.97(0.56-1.97)	0.022
Urine microALB Median (IQR)	11.0 (6.6-23.3)	10.8 (6-22.6)	10.9 (6.5-22.7)	0.933
HIV (n, %) POT PNOT	410 (77.8) 117 (22.2)	289 (80.7) 69 (19.3)	699 (79.0) 186 (21.0)	0.294
CD4 cells / mm^3 Mean (SD)	540 (257)	434 (236)	497 (254)	<0.001
Viral Load Undetectable Detectable	208 (39.5) 319 (60.5)	166 (46.4) 192 (53.6)	374 (42.3) 511 (57.7)	0.041
cART duration (years) Median (IQR)	2.4 (0-6.4)	2.4 (0.2-7.3)	2.4 (0-6.8)	0.336
PI use (n,%) No Yes	516 (97.9) 11 (2.1)	354 (98.9) 4 (1.1)	870 (98.3) 15 (1.69)	0.273
D4T use (n, %) No Yes	520 (98.7) 7 (1.3)	357 (99.7) 1 (0.3)	877 (99.1) 8 (0.9)	0.106
ABC use (n, %) No Yes	517 (98.1) 10 (1.9)	351 (98.0) 7 (2.0)	868 (98.1) 17.0 (1.9)	0.951
D:A:D score (%) Median (IQR)	1.4 (0.8-2.9)	4.5 (2.2-8.5)	2.2 (1.1-5.0)	<0.001
BMI-Body mass index, IQR- interquartile range, SD- standard deviation, POT-participants on treatment, PNOT-participants not on treatment, PI-Protease inhibitor, ABC-Abacavir, D4T-stavudine, CRP-C-reactive protein,				

Table 4. Results of univariable- and multivariable analysis to log D:A:D

	Univariable		Multivariable	
Factor	**Estimate (95% CI)**	***p***	**Estimate (95% CI)**	***p***
Constant			0.81 (0.629;0.981)	
Sex Female Male	 0 (base) 1.023 (0.887; 1.165)	 <0.001	 0(base) 0.987 (0.851; 1.123)	 <0.001
Art duration Per year increase	0.020 (0.001; 0.038)	0.038	0.011 (-0.004; 0.027)	0.139
Education-None Primary Secondary/Matric College/University	0.373 (0.013; 0.733) 0 (base) -1.001 (-1.017; 0.833) -1.104 (-1.40; -0.803)	0.002 <0.001 <0.001	0.538 (0.209; 0.867) 0 (base) -0.827 (-0.981; 0.673) -0.854 (-1.129; -0.580)	0.001 <0.001 <0.001
BMI Underweight Normal weight Overweight/Obesity	 0.213 (-0.004; 0.430) 0 (base) 0.239 (0.098; 0.379)	 0.054 0.153	 -0.006 (-0.187; 0.174) 0 (base) 0.235 (0.091; 0.380)	 0.947 0.001
Albumin/Creatinine Ratio mg/g Per unit increase	0.002 (0.00035; 0.0029)	0.012	0.002 (0.00044; 0.0025)	0.005
CRP Per unit increase	0.004 (-0.00015; 0.0077)	0.06	0.001 (-0.0023; 0.0042)	0.558
On PI (yes)	0.05 (-0.55; 0.66)	0.86	0.25(-0.26; 0.77)	0.334
On D4T (yes)	-0.71 (-1.50 0.08)	0.08	-0.43(-1.10; 0.25)	0.216
On ABC	0.26 (-0.23; 0.86)	0.26	0.20(-0.25; 0.66)	0.387
BMI-Body mass index,CI- confidence interval, CRP-C-reactive protein, p-p value, PI-Protease inhibitor, D4T-stavudine, ABC-Abacavir				

3.4.1 Determinants associated with high D:A:D score

The following uni-variable analysis duration of cART, sex, educational level, BMI, Albumin creatinine ratio, were associated with an increase in 5 year CVD risk according to the D:A:D score. Only CRP was marginally significant with p value of 0.06. Male gender (0.987; 95% CI 0.851-1.122; p<0.001), no formal education (0.538; 95% CI 0.209-0.867; p<0.001), BMI (Overweight/obesity) (0.235; 95% CI 0.091-0.380; p<0.001) and Albumin creatinine ratio (0.0015; 95% CI 0.00044-0.0025; p=0.005) remained independently associated with an increase in the D:A:D score in the whole Ndlovu cohort.

4 CHAPTER 4 - DISCUSSION

In this chapter, the results of the research are discussed in relation to findings from previous national and international studies on CVD and within the context of rural South Africa and low resource settings. The strength and limitations of the study was also discussed to further guide the interpretation of our results.

4.1 Objective 1

In this cross sectional analysis of all HIV-positive participants of the Ndlovu Cohort Study cardiovascular disease risk according to the D:A:D score was low (2.2%). The D:A:D risk score was higher in participants on cART 2.4% (IQR 1.2-5.2) and 1.4% (0.7-3.5) for those not on cART ($p<0.001$).The other studies from SSA to the best of our knowledge did not compare the D:A:D risk score between HIV individuals on cART and those not on cART (18, 68) for us to draw up robust conclusions pertaining to the effect of cART on CVD.

The study by Mashinya et al from three rural clinics in the Limpopo province of South Africa has used the full D:A:D risk score to estimate the 5-year risk score and also they determined the various CVD risk factor prevalence. The sample size in their study was 214 participants and the CVD risk was also low (<1%) (18). Besides the sample size being small compared to 885 participants from our study, other factors noted from Mashinya et al such as different food behaviors, all participants' viral load<50 copies/ml and, age 44.8 ±11.8 years could explain the D:A:D risk score differences compared to our study.

According to Mashinya et al 75.8% were physically active to account for a low D:A:D but the authors point that they had a different data collection tool which could introduce measurement bias to explain variations in the physical activity with other studies for generalization. In addition most participants in Mashinya et al study had low intake of vegetables and fruits due to unavailability and unaffordability according to the authors.

Other studies have also compared the D:A:D risk score with the FRS and concluded that it is better to use the D:A:D risk score in HIV populations than the FRS which over-predicts CVD risk (19, 81). From the study by Edward-Jackson in 785 Thai subjects they found that the prevalence of a D:A:D score being less than 10% was 0.8% which was also consistent with our results (19).The result of 294 outpatient study participants from South America in Brazil also showed a low D:A:D risk score whilst the study by Krikke et al from Netherlands also confirmed low CVD D:A:D risk score from 997 HIV-infected patients (37, 81).

The overall low D:A:D score from our study plus advocating for using the D:A:D risk score in rural HIV populations contributes some important advise to physicians working with HIV populations world-wide. For example the fact that higher D:A:D risk score was found in HIV subjects on cART than those not on cART means physician's care in HIV clinics should look into this HIV subpopulation with a view to possibly alter their CVD future risk. Our study hopefully leads to a reduction in the overestimation of CVD risk, which in turn can contribute to less drug-drug interactions, and drug-related adverse events especially in the daily clinical practice.

Our study was focused on South Africa, a country with high prevalence of HIV subtype C. Given that our study revealed a low D:A:D score in a region with HIV subtype C and different risk factors compared to HIC risk factors, more research with respect to risk profiles is therefore advocated.

4.2 Objective 2

In this study comparing the socio-demographic, clinical characteristics and the D:A:D risk score stratification by gender of HIV positive participants in the Limpopo province of South Africa there were statistically significant differences in some CVD risk factors. For example for smoking, males had higher percentages for both current (44.5%) and ex-smokers (19.3%) in contrast to females 8.4% and 10.3% respectively. In this SSA population this is an expected usual finding in rural areas where more males are smokers.

The overall percentage of smoking was 23 % from our study and 22% from Mashinya et al's study, which shows percentage similarities. On the contrary from the CREATE 1 study done in the United Kingdom the smoking prevalence was 37% and in the D:A:D study 53.3% (16, 71). These differences show pronounced gender and ethnic disparities, which come about with variation of smoking prevalence between HIC and LMIC.

There were also gender statistically significant findings in the BMI with 43% females in the overweight/obesity category compared to 15% males. 32.3% of the whole NCS participants were in the category of overweight and obesity. Mashinya et al did not show any statistically significant difference between males and females with reference to obesity and abdominal obesity (18).

Another SSA rural population study by Clark et al looked at various cardio metabolic factors in the subgroup of the Agincourt population taking cART (14). However, Clark SJ et al could

not assess BMI as the HIV participants had low BMI because national policy guidelines in the period they carried their study required a CD4 less that 200 cells/mm^3 for initiation of cART. This means that HIV participants were more likely underweight and very ill in their study at time of cART commencement precluding an assessment of BMI as a cardio-metabolic risk factor in HIV-positive persons in that rural SSA South African Agincourt district (14).

Hypercholesterolemia and hypertriglyceridemia were 21.7% and 16.7% respectively from our study. Consistent with our findings almost similar percentages were observed among a rural population with 33.2% for hypercholesterolemia and 16.8% for hypertriglyceridemia (18). Law M et al found out that dyslipidemia was frequent in the HIV population (93).

We also noted differences in the lipid level spectrum with the LDL, which was higher in females at 2.5mmol/L compared to 2.1mmol/L, $p<0.001$ in males, whilst the triglycerides were higher in males at 1.3mmo/L compared to 1.1mmol/L $p<0.001$in females. These differences could be due to the effect of disparities in smoking and the overweight/obesity levels between females and males.

The level of education was low in this rural population with 93.3% educated up to secondary level. 27% were only educated to primary level or not educated at all and 66.5% were educated up to secondary level. Females generally had higher education levels than males and this was statistically significant. 70.2% females and 61.1% males were educated up to secondary level in our study. The study by Mashinya et al showed that only 48.5% females and 34.9% males were educated up to secondary level, which was lower compared to our findings. Given that CVD can be a result of chronic stress, a low level of education coupling with stigma related to HIV infection may predispose the HIV infected individuals to high levels of stress (94). The percentage variation in level of education between our study and Mashinya et al may also explain differences in the D:A:D score as reported in this study.

Other studies from SSA have used the Pooled Cohorts Equation for atherosclerotic CVD (ASCVD), the FRS (2008) and the D:A:D score but did not highlight the issue of no formal education as a CVD risk (18, 68). The determinant of education on CVD risk from HIC was not investigated in literature from HIV populations in these countries thus precluding comparisons to be made between HIC and LIMC.

Females (11%) from our study reported more family history of cardiovascular disease compared to males (5.9%) and this was statistically significant, $p=0.008$. This may be

accounted possibly by a reporting bias from the fact that females had higher education therefore were able to positively report CVD family history.

Females had higher CD4 counts compared to males and the explanation of this finding is likely due to the fact that females have more health seeking behavior than males. In addition services extended to women such as antenatal care could also influence females to start cART at a higher CD4 than males thus explaining the statistically significant difference in the CD4 levels by gender.

There was a statistically significant discrepancy in the detectable viral load, which was higher in females than males. The viral load would have been expected to follow the same observed trend of CD4 levels by gender. However this discrepancy cannot be explained, as it should have been consistent with the CD4 level by gender values and for it to be physiological correct. The trend in viral load does not necessarily follow the trend of CD4 as previously thought.

4.3 Objective 3

Male gender, no formal education, BMI and ACR were found to be factors significantly associated with a higher CVD risk in determining the effect of cART duration on the CVD risk score among HIV infected NCS participants considering current, previous cART and potential confounding factors. This was on the basis of using the D:A:D model in multivariable analysis.

The cART duration was found to increase the D:A:D risk score with 0.02 (CI; 0.001; 0.038) per year accumulated on cART before adjusting for other confounders. However we cannot suggest that for HIV care providers this means every year the CVD risk goes up as this was based on univariable analysis. Our findings on multivariable analysis were not consistent with Aboud M et al in terms of the duration of cART increasing the CVD risk though in the CREATE 1 study they used the Framingham (1991) risk score (71). Using the Framingham risk score, they found that variables such as cholesterol and duration of cART were key risk factors of CVD risk. Therefore during cART in HIV patients, we posit CVD risk assessment is to be done regularly in the HIV clinic especially paying close attention to the duration of cART regimens. This clinical evaluation in the HIV populations will assist to reduce the individual CVD risk.

Our results also showed that with no formal education the D:A:D risk score increases 0.538 (CI; 0.209; 0.867) times p=0.001 .The effect of being a male 0.987 (CI; 0.851; 1.123), p<0.001 increases the D:A:D risk score approximately twice more than the effect of no formal education. These results suggest that education offered to male HIV-positive individuals may influence them to make well-informed better lifestyle choices.

The effect of being in the overweight or obesity BMI category was found to increase the D:A:D risk score by 0.235 (CI; 0.091; 0.380) p=0.001 in this NCS HIV patients. No formal education (0.538) has approximately a double effect on the CVD risk as compared to the effect of the overweight/obesity category i.e. (0.235). In the care of HIV populations the focus on reducing CVD risk factors is important to bear in mind in order to ensure a reduction in CVD.

From our data none of the drugs were significantly related to the D:A:D score in the final model. Stavudine had an effect of reducing the CVD risk -0.71 (CI; -1.50 0.08) contrary to what literature suggests and this was marginally significant on uni-variable analysis with p value of 0.081. However on multivariable analysis the type of the ARV was found not to be related with a high D:A:D risk score. The explanation for this could be the fact that in the NCS there were small percentages on the individual drugs with d4T 8(0.9%), PI 15(1.69%), and ABC 17(1.9%) and the fact that in this cross sectional data analysis cART is only baseline exposure.

Some studies have suggested that ARVs could be contributory to the increased CVD risk in HIV populations (9). This could be probably acting via fat accumulation. In our study the past medical history medication exposure would have explained this effect if it had been captured at baseline. There is need to access more data on d4T exposure effect on CVD risk.

Our results showed that ACR was a statistically significant predictor of CVD risk (0.0015 (95% CI 0.00044; 0.0025); p=0.005. A value of 0.0015 increase in 5 year CVD risk may not mean a lot with respect to clinical relevance to a patient. Though statistically significant we think clinical significance may be of minimal contribution as a CVD risk factor given the magnitude of 0.0015. At public health level the added value of CVD risk from ACR may be negligible according to our results. However there seems to be a relationship between ACR and CVD risk from the final model of our study.

Wensink et al also showed that in a rural South African population CVD risk factor variables and HIV specific factors were related to albuminuria. These factors were hypertension (aOR 1.59; 95% CI 1.05–2.41; $p<0.05$) and total cholesterol (aOR 1.31; 95% CI 1.11–1.54; $p<0.05$), which remained independently associated with albuminuria (ACR>30mg/g) in the whole cohort. Though our study suggest a negligible effect of ACR on CVD risk from an etiological perspective on atherosclerosis it concurs with the study findings of Wensink et al (62). Wensink et al suggests that improving the CVD risk prevention in addition to achieving viral suppression is important to stop the vicious circle of renal impairment and CVD resulting in a good long term prognosis in HIV infected populations.

Given the different methodology and analysis compared to our study, Wensink et al findings and ours emphasize the value of not ignoring micro-albuminuria or ACR in the care of SSA HIV-positive populations.

Males with high ACR, no formal education belong to a particular CVD risk category according to our study. This observation from our study will assists in alerting clinicians detect HIV patients that need close follow up in the management of reducing their CVD risk. Other risk factors such as CRP that has been investigated in CVD in HIV populations of course may need to be considered as well.

In our study, univariable analysis showed that CRP was marginally significantly related to CVD risk, 0.0038 (-0.00015; 0.0077) $p=0.06$. However, the multivariable analysis found CRP not to be contributory as a predictor of CVD risk in our study. This is consistent with the findings of Vos et al who in their systematic review showed that proinflammatory markers were not associated with surrogate markers of CVD like CIMT (95). Vos et al conclusions were based on a systematic review including forty articles of which four out of eight studies frequently assessed CRP in relation to the occurrence of CVD. In our case we do not regard that the D:A:D risk score can be equated to be called a surrogate marker to be consistent with the findings of Vos et al.

Vos et al point out that no effect estimate could be analysed because there was heterogeneity in the studies. A longitudinal follow up of the NCS is continuing as proposed by Vos et al and this will probably yield or rule out a true association between CRP and CIMT in view of our findings from univariable analysis. CRP, D-dimers and interleukin 6 have also been evaluated using clinical endpoints in the SMART study and Interleukin-6 was found to be a stronger predictor of fatal events by Borges et al (96).

4.4 Strengths

Though few studies in SSA have addressed the issue of CVD risk factors and risk scores (18, 68) our study is distinctive in that we used a specific risk score designed for HIV patients to calculate the 5-year CVD risk. Thereafter we used multivariable regression analysis with plausible variables to bring out a parsimonious model with specific CVD risk factors.

Unlike other studies from South Africa (18) our analysis strength is that it is derived from a well set up cohort study of substantial sample size with an intended follow up duration of several years. In addition our data analysis is strengthened by the availability of the information that was carefully collected on HIV specific risk factors, biomarkers of inflammation, clinical data on conventional CVD risk factors (72).

The other strength is that our study setting is also ideal and clearly representative of a rural SSA HIV population for our results to permit generalization.

4.5 Limitations

Our study has limitations that deserve to be mentioned. Due to the cross-sectional study design we can only say something about associations, and not about the predictive value of a variable on CVD. The validation of an existing CVD risk score like the D:A:D requires a large cohort with sufficient follow-up time for cardiovascular events to occur. Only then a risk score can be validated and adapted to a local population but this was not possible in our study design.

In our study no measurements of cardiac diseases were performed upon enrolment and as a result previous cardiac disease might be under-reported for example. According to Soliman et al HIV positive patients might have baseline electrocardiogram (ECG) abnormalities. Although ECG is not the gold standard to assess previous cardiac ischemia it can show evidence of myocardial ischemia (MI) (97). Having no baseline ECG or Echocardiogram in the Ndlovu Cohort Study could mean some of the participants might have reported no history of CVD condition in the past when it actually existed at baseline for example silent myocardial infarction which might have occurred in the past which is picked on ECG as an old infarct.

The individual HIV positive participant's previous medical history of CVD is not included in the D:A:D risk equation. The unavailability of baseline ECG from our study participants

could lead to underestimating the NCS participants' cardiovascular risk in general, as previous history of CVD is important in CVD risk assessment. Despite that obtaining each individual HIV positive participants an ECG was not feasible in the rural population due to resource limitations.

Our study being cross sectional we analyzed baseline parameters and variables, which obviously may vary as time progresses. Taking that limitation into consideration, the prospective design of the NCS will permit the follow up of the HIV participants progressing into the future especially the variables that change with progression in time.

Another limitation could be the aspect of recall bias that participants might have on initial questionnaire assessment and the issue of incomplete information with regards to previous cART use and the date of commencement of ART. This probably might be a limitation especially in view of the low level of education from our analysis. Chronic HCV status was not checked in the NCS and individuals with chronic HCV infection have been reported to have lower CRP (98). There might be HCV co-infection in the study participants, which might be contributing to lower values of CRP thus leading to CRP not getting into the model on the multivariable analysis yet on univariable analysis was marginally significant at p value of 0.06.

The D:A:D risk score was developed in the HIV populations that are not from the African continent. The demographics and CVD risk profile between HIC and LMIC are different. This makes it debatable if the D:A:D can be utilized outside HIC context. The D:A:D score is the only score developed specifically for HIV populations. It makes sense to utilise the D:A:D score in the HIV-positive populations as it has a better biological and plausible explanation of CVD in the HIV-positive populations.

5 CHAPTER 5 - CONCLUSIONS AND RECOMMENDATIONS

The conclusions and recommendations to assist HIV positive patients, HIV clinicians and policy makers are presented. Future research opportunities are also highlighted.

5.1 Conclusion

In conclusion 5-year CVD risk according to the D:A:D score is low in this young HIV-infected rural African population. The risk is higher in participants on cART compared to those not on cART. Determinants of CVD risk are male gender, no formal schooling, BMI and ACR. These factors have to be considered for preemptive interventions focused on the HIV infected population in order to reduce CVD morbidity and mortality.

5.2 Recommendations

Given that the final aim is improvement in CVD risk prevention of which our study has helped to identify the risk factors of CVD we recommend clinicians to carefully evaluate HIV patients with risk factors such as male gender, no formal education, ACR, overweight and obese patients in order to reduce their CVD risk.

In the rural setting in SSA the care and management of HIV–positive individuals should ensure that there is adequate monitoring and evaluation of risk factors for CVD especially for the BMI overweight/obesity category. Therefore as part of HIV management when a patient falls into the category of overweight or obese, the correct advice to manage weight will be appropriate as our model shows an increase in CVD risk in those BMI categories.

Taking into consideration predictors of CVD risk found to be statistically significant from our model we recommend that this can assist the financial resource budget distribution to target areas of need with respect to the rural health care delivery in HIV populations. In this rural population the involvement in upgrading the social status of the HIV population by giving basic educational programs and building more schools with secondary level will reduce the CVD risk. Furthermore, campaigns such as smoking cessation and anti-obesity lifestyle modifications will need to be implemented, as that can reduce the CVD risk.

The overall D:A:D risk score showed that CVD is low in this cross sectional study, but the population is still young. The burden of CVD is high, and we recommend that the HIV care

platform could be used to integrate HIV and CVD care, in order to prevent a double burden when the HIV infected population is aging.

It is now well known in medical literature that lowering the CVD risk and halting the renal disease progression involves therapeutic interventions that are well established. These could be lowering the cholesterol levels using lipid lowering agents such as statins, treating hypertension adequately and lowering albuminuria with appropriate medicines like angiotensin 1 converting enzyme (ACE) inhibitors. The effect of ACR on CVD was demonstrated from the multiple linear regression in this cross sectional study. It is prudent to recommend introduction of cheaper generic medications such ACE inhibitors in these HIV-positive populations by the government, which could reduce morbidity and mortality.

This research lays a foundation for the prospective cohort design of the NCS. We recommend further longitudinal studies to evaluate CVD endpoints and the effect of CRP, D-dimers, and interleukin 6 on CVD in rural populations. Furthermore we recommend future intervention studies to address issues such as therapeutic efficacy and causality in CVD could be done as a follow up of our findings. For instance interventions such as loss of weight in BMI category of overweight/obesity, adjuvant antithrombotic and anti-inflammatory treatments in HIV patients, ACE inhibitor and angiotensin receptor blockers (ARBs) for HIV patients with albuminuria would be ideal to be evaluated.

The NCS is within the Ndlovu Care Group, which is an excellent environment for the prospective follow up of the HIV positive individuals. A need still exists to further facilitate the identification of specific HIV-individuals who will be at an elevated risk in the daily routine management practice in the SSA HIV populations. More studies need to be undertaken in the future on a CVD risk prediction score that will encompass the baseline characteristics of African populations.

In view of the fact that the D:A:D score was developed in European setting our study guides and provides valuable information for the prospective development of an ethnic/race specific CVD risk score for use in SSA. This can be developed and validated in SSA HIV positive populations. We recommend those future opportunities to be utilized to create data allowing validating or revising the CVD risk score in a HIV infected population in SSA.

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APPENDICES

APPENDIX 1: Ethical clearance certificate for the research

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr WF Mudombi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M171194

NAME: (Principal Investigator)	Dr WF Mudombi
DEPARTMENT:	School of Public Health Medical School
PROJECT TITLE:	Effect of HIV treatment and other risk factors on cardiovascular disease risk in rural South Africa
DATE CONSIDERED:	24/11/2017
DECISION:	Approved conditionally
CONDITIONS:	The date of birth on the data sheet is an identifier; please remove it and store the information on a separate linked file, if it is required
SUPERVISOR:	Professor K Klipstein-Grobusch & Dr A Vos
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	06/12/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially reviewed in **November** and will therefore be due in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature — Date: 6 DECEMBER 2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 2: NCS letter from gatekeeper

NDLOVU RESEARCH CONSORTIUM
NDLOVU CARE GROUP
Community Program with a Difference

P.O. Box 1508,
Groblersdal, 0470

Groblersdal, 31 October 2017

To whom it may concern

This letter serves to confirm that Dr. Wisdom Forward Mudombi, MSc Epidemiology & Biostatistics student (student number 1239321) at the School of Public Health, University of the Witwatersrand, Johannesburg, will be granted access to data from the Ndlovu Cohort Study for his MSc research project entitled: Effect of HIV treatment and other risk factors on cardiovascular disease (CVD) risk in HIV-positive people in rural South Africa.

On behalf of the Ndlovu Research Consortium

Dr. Hugo Tempelman

Tel: +27 (0) 13 262 9000 • Fax: +27 (0) 13 262 3498 • info@ndlovu.com • www.ndlovucaregroup.com
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APPENDIX 3: Analysis of standard errors similarity

Results of multivariable analysis to log D:A:D showing standard errors by vce robust

	Multivariable vce robust		Multivariable final model	
Factor	**Estimate (95% CI)**	***SE***	**Estimate (95% CI)**	***SE***
Constant			0.805 (0.629; 0.981)	
Sex Female	0 (base)	0.071	0(base)	0.069
Male	0.987 (0.847; 1.127)		0.987 (0.851; 1.123)	
Art duration Per year increase	0.011 (-0.004; 0.027)	0.007	0.011 (-0.004; 0.027)	0.007
Education-None	0.538 (0.290; 0.786)	0.126	0.538 (0.209; 0.867)	0.167
Primary	0 (base)		0 (base)	
Secondary/Matric	-0.827 (-0.978; 0.676)	0.078	-0.827 (-0.981; 0.673)	0.079
College/University	-0.854 (-1.111; -0.596)	0.131	-0.854 (-1.129; -0.580)	0.140
BMI Underweight	-0.006 (-0.205; 0.192)	0.101	-0.006 (-0.187; 0.174)	0.092
Normal weight	0 (base)		0 (base)	
Overweight/Obesity	0.236 (0.093; 0.378)	0.072	0.235(0.091; 0.380)	0.074
Albumin/Creatinine Ratio mg/g Per unit increase	0.0015 (0.0010; 0.0019)	0.0002	0.0015 (0.00044; 0.0025)	0.0005
CRP Per unit increase	0.0097 (-0.0014; 0.0034)	0.0012	0.00097(-0.0023;0.0042)	0.0017
On PI (yes)	0.255 (-0.110; 0.619)	0.185	0.25(-0.26; 0.77)	0.264
On D4T (yes)	-0.426 (-0.944 0.092)	0.264	-0.43(-1.10; 0.25)	0.344
On ABC	0.208 (-0.23; 0.86)	0.213	0.20(-0.25; 0.66)	0.232
CI- confidence interval, SE- standard error,CRP-C-reactive protein, PI-Protease inhibitor,ABC-Abacavir, BMI-Body mass index.				